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The proteome quest
to understand
biology and disease

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Cancer Proteomics

abstracts

OP001 - A QUANTITATIVE PROTEOMIC INVESTIGATION OF PAIRED COLORECTAL CANCER AND NORMAL TISSUE SAMPLES

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Colorectal cancer (CRC) is third most common cancer worldwide. The five year survival rate significantly drops to 11% for metastatic stage. Since current tumor markers lack sensitivity and specificity, there is an urgent need for more reliable biomarkers.Tumor tissues are rich sources of candidate biomarkers and their comparison with adjacent normal tissues can identify potential disease markers.

In this study we performed proteomic analysis of CRC tumor tissues (n=8) and their corresponding paired normal tissues (n=8). All CRC tissues were adenocarcinomas obtained from both male and female pateints varying in age (40-75 yrs), site (sigmoid, transverse etc.) and stage (I-IV). Samples were grouped according to their EGFR expression [EGFR+/- = 4/4]. Membrane proteins were extracted by Triton-X114 phase partitioning, in-gel trypsin digested into ten fractions and subjected to LC-MS/MS analysis by LTQ orbitrap XL.

Proteomic analysis revealed approximately 1000-1300 proteins in each CRC sample. Paired t-test analysis on each samples (tumor vs. normal) identified 201 differentially expressed proteins (p1) and 128 down regulated (fold change

In conclusion, this study provides a quantitative proteomic analysis of the CRC pathogenesis by comparing paired normal and disease tissues. This provides valuable insights into CRC-associated proteins and pathways advancing our mechanistic understanding of the disease.

OP002 - PROTEOMIC INVESTIGATION OF INTRA-TUMOR HETEROGENEITY

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Introduction and objectives

The surge of targeted cancer therapies necessitates understanding of intra-tumor heterogeneity at the molecular level. High-throughput technologies have revealed substantial intra-tumor heterogeneity at the DNA and transcript level. Although antibody-based studies have confirmed intra-tumor heterogeneity, it remains unclear to what extend protein levels vary between different tumor sites. Here, we present an analysis of intra-tumor heterogeneity using quantitative proteomic analysis.

Methods

Normal tissues and two prostate carcinoma subtypes (i.e. acinar and ductal) were histo-pathologically characterized in samples from three patients. Three or six punches (core diameter 1mm) were sampled from each non-tumorous and tumorous sections. Altogether, we obtained 30 tissue punches, each of which was divided into two aliquots and analyzed using pressure cycling technology-SWATH-MS (PCT-SWATH). The resultant SWATH data were interpreted using the OpenSWATH software tool and a SWATH library for human prostate tissues. In order to robustly quantify inter-punch (i.e. intra-tumor) variability of protein levels, we developed a customized analysis pipeline.

Results and Discussion

We built a SWATH assay library for human prostate cancer tissues containing over 6000 Swiss-Prot non-isoform proteins, with which we acquired relative quantitative data for 3101 proteins in the 30 tissue punches. Technical replicates displayed high correlation (Pearson coefficient of correlation >0.9) and minimal batch effects. Our analysis pipeline estimates the biological variation between punches as the difference between the total variance between punches and technical variance. We could show that computational filtering for the most reliable peptides per protein resulted in very robust and reproducible variance measures. Further, we detected highly significant inter-punch variation and our analysis revealed that specific classes of proteins are particularly affected by intra-tumor variation.

Conclusions

The application of latest mass-spectrometric technologies combined with rigorous statistical methods enables the quantification of intra-tumor variation for thousands of protein species.

OP003 - PROTEOMIC PATTERNS UNRAVEL A NEW LUMINAL A BREAST CANCER MOLECULAR SUBGROUP

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Background: Breast cancer is a heterogeneous disease including a variety of entities with different genetic background. Estrogen receptor-positive, HER2 negative tumors (ER+) usually have a favorable outcome, although some patients eventually relapse, which suggests some heterogeneity within this category. In this study, we analyzed the proteome of a retrospective series of formalin-fixed, paraffin-embedded breast cancer tissues applying label-free quantitative proteomics in order to deepen the molecular architecture of breast cancer.

Methods: Protein expression was obtained by LC-MS/MS from tumors of 106 patients with lymph-node positive, Her2 negative breast cancer, who had received anthracycline-based adjuvant chemotherapy, using MaxQuant. Differential protein expression analyses between ER+ and triple-negative (TNBC) samples was performed using Significance Analysis for Microarrays (SAM). Findings were verified using gene expression data from 1,141 patients included in public repositories.

Results & Discussion: A total of 3,239 protein groups were identified in FFPE breast cancer samples, of which 1,095 presented at least two unique peptides and detectable expression in at least 75% of the samples in at least one type of sample (either ER+ or TNBC samples). SAM revealed 224 proteins differentially expressed between ER+ and TNBC samples. Hierarchical clustering analyses showed that some ER+ samples had a protein expression profile similar to that of TNBC samples and a clinical outcome similar to those with TNBC disease. A TNBC-like predictive protein signature was built and validated using gene expression data from independent datasets. The signature had prognostic value in patients with luminal-A breast cancer. This prognostic information was independent from that provided by standard genomic tests for breast cancer.

Conclusions: A group of ER+ breast tumors with molecular characteristics of TNBC was identified (TNBC-like). Patients with this luminal-A, TNBC-like breast cancer type had a poor outcome. This prognostic information was complementary to that offered by genomic tests such as OncoType.

OP004 - **A SYNTHETIC LETHAL INTERACTION BETWEEN APC/C AND TOPOISOMERASE POISONS UNCOVERED BY PROTEOMIC SCREENS**

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Introduction

The Anaphase-promoting complex/cyclosome (APC/C) cofactor Cdh1 is a major regulator of cell cycle and a critical modulator of cell transformation by targeting multiple cell-cycle regulators for ubiquitin-dependent degradation. Despite its importance, little is known about the relevance of substrates targeted by Cdh1 involved in the maintenance of genomic stability.

Methods:

To identify Cdh1 substrates, we performed a quantitative mass spectrometry-based proteomic approach in two in vitro and one in vivo Cdh1-null systems. To this end we used several strategies based on metabolic and isobaric labeling followed by multidimensional LC-MS/MS analyses.

Results:

Our approach allowed us to identify 308 proteins showing upregulation in the absence of Cdh1 in asynchronous mouse embryonic fibroblasts (MEFs). We then established synchronous MEFs in G0 phase, where Cdh1 has its peak of activity, and found 21 proteins being upregulated. In addition, we analyzed brains from Cdh1-/- mice and found 31 proteins upregulated. From all these analyses, nine proteins were consistently upregulated in at least two of the three systems. Among them Eg5 and Top2a were significantly accumulated in the absence of Cdh1 both in vivo and in vitro and constitute two potential targets of interest in cancer therapy. These two proteins are ubiquitinated in a Cdh1-dependent manner and its upregulation in Cdh1-null cells results in differential response to therapeutic agents.

Conclusion:

This work identifies, using different proteomic screenings, in vivo targets of the mammalian APC/C-Cdh1 complex and reveals synthetic lethal interactions of relevance in anticancer treatments.

OP050 - PUTTING THE PROTEOME TO WORK: NEW ADVANCES FOR CANCER RESEARCH

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Gene activity in thirty-six glioma stem cell lines has been measured through untargeted deep sequencing and proteomics. With the recent integration of the ENCODE data into our proteomic workflow, we are positioned to identify the unknown human proteins at an accelerated pace. By use of databases that contain predicted sequences of known and novel proteoforms, we identified nsSNPs in proteins derived from GSCs that may be associated with genomic instability and invasivity, novel fusion proteins derived from chromosomal (somatic) rearrangements, and novel alternative spliceforms derived from fusions between known and previously unknown exons.

The identification of three different nsSNPs at a single locus in kallikrein 8 (KLK8), a protein not previously associated with glioma, is under investigation as a protein that may play a role in GSC invasivity. Five newly identified nsSNPs in DNA ligase 1 (LIG1), a DNA repair protein, will be studied to determine how these proteoforms may affect genomic instability and resistance to radiation therapy. The integration of RNA-Seq data and proteomics has allowed us to study the somatic-proteomic landscape of GSCs, thereby determining the roles of novel fusion proteins in GSC pathobiology. Bioinformatic searches of ENCODE data translated into a searchable database for proteomics (proteoENCODE db) yielded eighty-one chimeric peptides, the result of fusion known and unknown exons.

The identification of novel proteins and proteoforms in GSCs will allow further studies of their role in pathogenesis, tumor recurrence, and resistance to chemotherapy and radiation. The next step is to validate and quantify their expression by SRM across 36 GSC lines, at baseline conditions and following standard-of-care treatments. We expect that among the newly identified proteins, new therapeutic targets and biomarkers will be established.

OP051 - HIGHLY MULTIPLEXED IMAGING OF TUMOR TISSUES WITH SUBCELLULAR RESOLUTION BY MASS CYTOMETRY

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Tissues are assemblies of multiple cell types that communicate with each other to achieve physiological states. In cancer, malignant cells and cells of the tumor microenvironment (TME) facilitate tumor progression, and drug resistance. For an understanding of the underlying processes it is essential to comprehensively investigate the components and their relationship within the TME. This necessitates imaging approaches that can simultaneously measure dozens of biomarkers to define cell types, their functional and signaling states, and spatial relationships.

For highly multiplexed tissue imaging at subcellular resolution, we have coupled immunohistochemical (IHC) methods with high resolution laser ablation and mass cytometry (MC)1. In MC, metals are used as reporters on antibodies. Analysis of metal abundances using the mass cytometer, an atomic mass spectrometer, allows determination of biomarker expression. Here, tissue sections were prepared for antibody labeling using IHC protocols. After antibody labeling, the tissue was ablated spot by spot using a laser at 1 micrometer resolution, and the resulting particles were analyzed in the mass cytometer. Finally, the 32 isotope signals were plotted and a high-dimensional image of the sample was generated. Single-cell features were computationally segmented and the single cell marker expression data were extracted for downstream bioinformatics analyses.

Imaging MC provides high-dimensional analysis of cell type and state at subcellular resolution to study tissues and adherent cells. The imaging approach enabled the simultaneous visualization of 32 proteins and protein modi¬fications, with the potential to map up to 100 markers on a single tissue section. Application of imaging MC to breast cancer samples allowed delineation of cell subpopulations and cell-cell interactions, highlighting tumor heterogeneity and new routes to patient classification. It has the potential to yield novel insights of the TME by exploiting existing large collections of FFPE tumor samples and associated clinical information.

1 Giesen et al. Nat. Methods. 2014.

OP052 - SUITABILITY OF A CANCER PROTEOMICS SAMPLE

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The Clinical Proteomic Tumor Analysis Consortium (CPTAC) of the National Cancer Institute aims to proteomically characterize 100 tumors each in colorectal, breast, and ovarian cancer. Each tumor has been genomically characterized by The Cancer Genome Atlas (TCGA), the results of which are informing the proteomic analysis. Some pre-analytical variables of the samples, such as ischemic time and optimal cutting temperature compound (OCT) embedding, could obscure the proteomics data. Consequently, CPTAC is collecting a confirmatory sample set to validate findings in the TCGA collection. In the process, CPTAC is optimizing a pipeline for clinical sample collection for proteomics.

CPTAC is collecting human tumor samples using a protocol optimized for proteomics. Working with a group of surgeons, biostatisticians, cancer biologists, and proteomics experts, CPTAC developed a protocol for each tumor type that minimized ischemic time while maintaining clinical practicality. Using a network of Tissue Source Sites, each sample is shipped to a Biospecimen Core Resource (BCR). At the BCR, the sample will undergo a series of quality checks involving pathology and molecular analysis. The samples will be genomically sequenced, and CPTAC centers will proteomically characterize each sample.

Several requirements of a proteomics analysis limit the suitability of a clinical sample including: tumor size, ischemic time, and the presence of OCT. Additionally, tissue heterogeneity can obfuscate the protein content of a tissue of interest. Finally, the genomics community has a number of quality checks to determine a sample's suitability for genome sequencing. What quality checks might indicate suitability for proteomics?

Clinical studies in cancer proteomics often rely on samples of convenience. This study seeks to define acceptable criteria at the pathology, genomic, and proteomic levels to establish optimal parameters for a clinical proteomics sample collection. If successful, the study will demonstrate the feasibility and utility of proteomic analysis on clinical samples.

OP053 - AN INTER-CANCER STUDY OF HUMAN PLASMA PROTEINS BY GLYCOSWATH MASS SPECTROMETRY

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In the current project we aimed to perform a comparative analysis of plasma samples of 5 early-stage carcinomas. Specifically, the study included samples from colorectal; pancreatic; lung; prostate; and ovarian cancer patients. The aim of the study was to assess the acute state of molecular pathways of patients with early-stage cancer; to outline the plasma signature of the common cancer metabolism and also link the specific markers individual to each carcinoma. Targeted proteomics data extraction based on the SWATH technology was used to monitor a panel of plasma glycoproteins.

Peptides that were N-glycosylated in the intact proteins were generated from plasma samples from 5 different cancers and control samples were isolated using established protocols. We randomly processed and analyzed the samples to minimize experimental biases. The peptide samples were analyzed by SWATH-MS. The N-glyco spectral libraries were generated by shotgun sequencing of natural enriched glycoproteins of pool samples combining both carcinomas and controls, and the sequencing libraries of synthetic glycopeptides. For targeted SWATH-data extraction and quantification, the open source software openSWATH(Rost et al., 2014) was used. For statistical analysis and data visualization we used the R-statistical software and integrated MSstats package(Surinova et al., 2013).

Of the 337 identified glycoproteins, 156 were present in in all MS-runs and were reproducibly quantified among samples. Unsupervised hierarchical clustering of data was done based on fold changes of all the glycoproteins significantly expressed in the five cancer types, compared with the controls. Enhanced clustering between same cancer types indicated specificity of cancer-associated proteins. Colorectal, lung and prostate early-stage carcinoma exhibited significantly up-regulated Thrombospondin-1.

The carcinoma types specificity was reflected at proteomic level. Thrombospondin-1 up-regulation could be the sign of protective mechanism against the early onset of carcinoma since the protein is part of the p-53 signaling pathway directly involved in angiogenesis and metastasis inhibition.

P-1.00
OVEREXPRESSION OF AVß6 INTEGRIN ALTERS THE COLORECTAL CANCER CELL PROTEOME IN FAVOR OF ELEVATED PROLIFERATION AND A SWITCHING IN CELLULAR ADHESION THAT INCREASES INVASION

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Many proteins enhance cancer progression toward life-threatening metastases. These include linking proteins called integrins that mediate cell adhesion to the extracellular matrix (ECM), consequently altering both function and phenotype.

Specific neoexpression of the ß6 integrin subunit correlates with the epithelial-to-mesenchymal transition, metastasis, and poor overall patient survival. While ß6 is implicated in these processes, exactly how it affects signaling and/or proteolytic pathways in metastasis remains unclear.

A membrane-enriched peptide immobilized pH gradient isoelectric focusing (IPG-IEF) shotgun proteomics study was undertaken in which subclones of the SW480 colorectal cancer cell line transfected with a vector inducing unregulated ß6 integrin overexpression were compared with the "empty" mock vector control cell line. ß6 overexpression induced a significant change in 708 proteins and was found to be localized across most intracellular locations, some involving cellular processes and pathways underpinning cancer progression.

Proteomics data have been deposited to the ProteomeXchange with identifier PXD000230. ß6 expression increased cell proliferation 4-fold while decreasing cell adhesion to many integrin ECM substrates. ß6 expression also enhanced cell invasion and promoted the expression/repression of many established cancer-related pathways.

P-2.00

CIRCULATING PROTEOLYTIC PRODUCTS OF TUMOR-RESIDENT ENZYME AS POTENTIAL BIOMARKERS FOR EARLY DETECTION OF BREAST CANCER

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Background: It is feasible that we might take advantage of the fact that secreted proteases/peptidases in the tumor microenvironment generate proteolytic products, also referred to as "circulating peptides", which are detectable in bloodstream and provide ample information about the body, "coded" in the patterns and quantity of these peptides. Herein we clearly link the catalytic activity of Carboxypeptidase N (CPN) to its proteolytic products during breast tumor progression in mouse model and clinical samples.

Methods: The activity of CPN was evaluated using an ex-vivo peptide cleavage assay, in which synthesized C3f peptide is incubated in interstitial fluids of breast tumor and adjacent normal breast tissues in mice with orthotopic implantation of the human cell line MDA-MB-231. Circulating fragment profiling, by an approach combining nanopore fractionation and mass spectrometry, revealed the nature and extent of cleavage by CPN. These results correlated with the level of CPN-catalyzed peptides in blood specimens taken from the tumor-bearing mice, healthy women and breast cancer patients. CPN expression in the same set of samples was further examined by immunohistochemistry and immunoblotting.

Results: In both the mouse and clinical patient samples, the amount of CPN was clearly increased in tumor tissues compared to that seen in normal breast tissue, while its counterpart in blood remained constant. The amount of 6 CPN-catalyzed peptides predominantly increased in sera taken from the mice (n=8) at 2 weeks after orthotropic implantation and in the patients' plasma as early as the first pathologic stage of breast cancer.

Conclusions: Cumulatively, our results represent a first demonstration, to our knowledge, that clearly links the proteolytic activity of CPN, particularly at tumor sites, to the cleavage patterns of its catalytic substrates in the blood, by means of a rapid, reproducible, sensitive, precise and non-invasive approach.

P-3.00
CHARACTERISATION OF THE UPAR·AVß6 INTERACTION IN COLORECTAL CANCER CELL LINES

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Introduction and objectives: Urokinase plasminogen activator receptor (uPAR) and the epithelial integrin avß6 heterodimer play critical roles in cancer metastasis. Importantly, both these interacting molecules have been implicated in epithelial-mesenchymal transition (EMT) changes that facilitate escape of metastatic cells from tissue barriers. The aim of this study is to characterise the uPAR·avß6 interaction in cellulo, in vitro and in silico approaches.

Methods: Three orthogonal methods were employed to identify uPAR·avß6 interaction. First, proximity ligation assays (PLA; in cellulo) was used to identify the interaction in a ovarian cancer cell line (OVCA429) and four different colon cancer cell lines with varying levels of ß6 integrin subunit expression (SW480Mock, SW480ß6OE, HT29Mock and HT29ß6AS). Second, uPAR peptide arrays (in vitro) were utilised to determine the uPAR·avß6 potential binding sites and as well as interactions with other known uPAR partners (e.g., uPA and vitronectin) and individual integrin subunits (i.e., av, ß1, ß3 and ß6). Thirdly, uPAR·avß6 potential interaction sites were studied by in silico structural analysis.

Results and Discussion: PLA successfully demonstrated the uPAR·avß6 interaction in various cancer cell lines with their manner of ß6 expression. This interaction was further validated by uPAR peptide arrays and potential six binding sites were identified. Peptide array also suggests that interaction with uPAR requires expression of the complete aß heterodimer rather than either individual subunits (i.e., av, ß1, ß3 or ß6). Finally, using in silico structural analysis in concert with our studies, it appears the most likely unique site/s of interaction of avß6 with uPAR are located in uPAR domain II and domain III.

Conclusion: Functional consequences of disrupting uPAR·avß6 as a novel therapeutic target to ablate metastasis are suggested for further study.

P-4.00

MEMBRANE PROTEOME ANALYSIS OF TGFB STIMULATED COLORECTAL CANCER CELL LINES

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Introduction and objectives: It is now widely understood that Transforming Growth Factor-β (TGFβ) signalling is a potent regulator of cell growth, differentiation, migration and tumor suppression. However, during colorectal cancer (CRC) progression it is known to promote tumour growth. The mechanism of this switch to tumor promoter is poorly characterized. We think this is due to TGFβ signaling cross talk with other proteins (like integrin $\alpha v\beta 6$, uPAR). Studies have shown that integrin $\alpha v\beta 6$ (activator of latent TGFβ) is up-regulated in various cancers leading to increased levels of active TGFβ and providing inroads for cancer progression.

Methods: We used cell lines with changed expression in integrin $\beta 6$ and uPAR. The cells were stimulated with 10ng/mL active TGF$\beta 1$ and the membrane proteome analysis was done using iTRAQ. Protein identification was done using protein pilot and further analysis performed on Ingeunity pathway analysis (IPA) software.

Results and Discussion: We identified 2666 proteins, out of which 510 proteins were from the plasma membrane. The IPA analysis with significantly altered proteins revealed cellular assembly and organization, cell-to-cell signaling and interaction as the top-ranked altered biological function. We identified various proteins including integrins ($\alpha 3\beta 1$, $\alpha 4\beta 1$, $\alpha 6\beta 1$, $\alpha 5\beta 1$, αv), ERK1/2, TGFβ and MAPK1/2 through IPA network analysis. All these molecules have been previously implicated in major cancers including CRC.

Conclusion: The results show that TGFβ signaling is enhancing the ability of the cancer cells towards progression and metastasis. Therefore, it is very important to study the protein-protein interactions during CRC which will serve to identify novel key players for metastasis, and serve as biomarkers and/or drug targets to improve CRC therapy.

P-5.00
MAPPING THE FUNCTIONAL PROTEIN COMMUNICATION BETWEEN DIFFERENT CELL TYPES IN THE TUMOR MICROENVIRONMENT OF PANCREATIC CANCER

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DKFZ/Functional Genome Analysis

Pancreatic cancer is the fourth most frequent cause of death among all cancer types in Western countries. The dismal prognosis is due to its late presentation, high invasiveness and metastatic potential, and its resistance to therapy. There is clear evidence that the state of the tumor desmoplasia (stroma) is important to the course of the disease. In pancreatic ductal adenocarcinoma (PDAC), which is the most common type of pancreatic cancer, stroma accounts for up to 90% of the tumor mass and is made up of various cell types, such as acinar, stellate and inflammatory cells.

Along with the tumor cells, they secret a highly dynamic and interactive set of proteins that induce and regulate accelerated tumor growth, proliferation and invasion. Since stromal cells do not share the genetic alterations commonly observed in their cancer counterpart, a therapeutic targeting of pancreatic stroma may be an effective route of combating pancreatic cancer. However, this approach is still hampered by the lack of knowledge about the specific functions that each cell type has within the stroma. In particular, the nature of the cellular proteome, the secretome composition, and the resulting molecular cross-talk between the stromal and tumor cells is still poorly understood.

Our study aims at a comprehensive and at the same time in-depth analysis of the functional proteome of the tumor microenvironment and how the molecular communication between the different cellular components of the pancreatic microenvironment works. In addition, the investigation highlights new and important signaling pathways that support stromal formation in the pancreas during the course of inflammation and cancer.

P-6.00

THE B6 INTEGRIN ENHANCES PLASMINOGEN AND LATENT-TRANSFOR MING GROWTH FACTOR-B ACTIVATION, PROMOTING THE METASTATIC PHENOTYPE IN COLORECTAL CANCER CELLS

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Expression of the αvβ6 integrin, urokinase-type plasminogen activator receptor (uPAR) and transforming growth factor-β (TGF-β) have long been studied as crucial proteins involved in the progression of colorectal cancer (CRC) towards metastasis. Commonly upregulated in multiple epithelial cancers, the αvβ6 integrin is suggested to physically interact with uPAR and the latency-associated peptide of TGF-β and has been demonstrated to enhance metastatic cell phenotypes such as proliferation, invasion, adhesion and the epithelial to mesenchymal transition (EMT). We suspect that these interactions enhance the metastatic potential of αvβ6 expression by promoting the activation and sustained cross-reactivity of αvβ6 with the uPAR, TGF-β and mitogen-activated protein kinase (ERK) signalling pathways.

In the present study, we investigated whether αvβ6 expression enabled CRC cells to activate zymogen members of these proteolytic and cell signalling pathways, inducing proteomic shifts that facilitate phenotypic changes necessary to facilitate pro-metastatic transformation. Implementing a combination of cell-based assays, signalling activity studies and Triton X-114 phase partitioning, we determined that treatment with recombinant latent-transforming growth factor-β and/or plasminogen significantly increased CRC cell proliferation, migration, invasion and ERK1/2 signalling activity in an αvβ6-dependent mechanism.

This study suggests that through either mechanical force or proteolytic activation, αvβ6 expression implements these zymogens, resulting in significant phenotypic changes required for mediating metastatic progression.

P-7.00

IMPROVING THE CHROMATOGRAPHIC SEPARATION OF DMB-LABELED SIALIC ACIDS FOR THE COMPARISON OF BIOSIMILARS TO REFERENCE MATERIALS

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Introduction:
Sialic acids affect the bioavailability, function, stability, and metabolism of glycoproteins. Two forms of sialic acid are commonly present in therapeutic glycoproteins: N-acetylneuraminic acid (NANA) and N-glycolylneuraminic acid (NGNA). One of the most common quantification methods involves releasing sialic acids from the glycoprotein, derivatizing NANA and NGNA with 1,2-diamino-4, 5-methylenedioxybenzene (DMB), and analyzing by C18-HPLC with fluorescence detection. This procedure is subject to interference from peaks originating from excess reagent and other derivatized impurities, limiting sensitivity and reproducibility. The objectives of this study were to develop a significantly improved HPLC-fluorescence method for DMB-NANA and DMB-NGNA, and to apply this method to compare two candidate biosimilar therapeutic proteins to their respective reference materials.

Methods:
LC optimization was performed using standard mixtures of DMB-NANA and DMB-NGNA and an HPLC with a fluorescence detector. Four columns of the same dimensions were evaluated: C18, F5, RP-Amide (reversed-phased columns), and bare silica (HILIC column). Sialic acids were released from glycoproteins, derivatized with DMB, and then analyzed with the optimum chromatographic conditions. Data were fit to external calibration curves.

Results and Discussion:
Among the screened columns, the RP-Amide column provided the best selectivity. The optimized HPLC separation with the RP-Amide column resolved the DMB-NANA and DMB-NGNA as well as excess reagent and other derivatized impurities. The new method takes 12 minutes and uses a simple mobile phase and gradient (6-20% B). This is an improvement over the existing 25-minute isocratic method using a C18 column and a more complicated mobile phase composition (water/acetonitrile/methanol). DMB-NANA and DMB-NGNA are now baseline resolved, and the analytes are free from interfering peaks.

Conclusion:
An improved HPLC method has been established for analysis of DMB-NANA and DMB-NGNA. The method employs a RP-Amide column and offers improved run time, selectivity, sensitivity, ruggedness, and resolution.

P-8.00
A NOVEL NEGATIVE ENRICHMENT METHOD FOR PROTEOME SCALE C-TERMINAL PEPTIDES ANALYSIS AND ITS APPLICATIONS

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Analysis and detection of proteome C-termini are biologically important since it not only provides information about protein isoforms, but also facilitates the understanding of protein proteolytic events. Herein, we developed a negative enrichment strategy to achieve global C-terminal proteome analysis. In this method, protein samples were firstly digested by LysC to generate C-terminal peptides with ¦Á-amino groups and internal peptides bearing both ¦Á- and ¦Å-amino groups. Subsequently, site-selective dimethylation was performed to block ¦Á-amino groups, followed by labeling the ¦Å-amino groups of internal peptides using glyceraldehyde-3-phoshate with high efficiency.

The resulting phosphate containing internal peptides were depleted by TiO2, leaving exclusively the fraction of N-dimethylated C-terminal peptides for LC-MS/MS analysis. By such a strategy, we identified 1208 C-terminal peptides in E.coli lysate, which is the largest C-terminal dataset for the studied organism by so far. After enrichment, the ratio of C-teminal peptides over all peptides identified increased from 9.4% to 65.3%, which demonstrated the high selectivity of developed method. Moreover, the developed method was applied to the profiling of GluC substrates and cleavage sites using partially digested yeast lysate.

After enrichment, the number of identified GluC generated neo-C-terminal peptides and substrates increased from 132 to 449 and from 83 to 259 respectively. Finally, such a strategy was used for the differential analysis of C-terminal proteome in mouse heptacarcinoma cell lines with high and low metastatis rate. Of all 1200 identified proteins, 29 proteins have different C-termini in the two cell lines, among which several proteins were related to cancer or cellular movement.

All these results demonstrate that the developed strategy for C-terminal peptides enrichment might be a promising tool to facilitate the in-deep understanding of in vitro and in vivo proteolytic events.

P-9.00

IDENTIFICATION OF SIGNALING PATHWAYS ASSOCIATED WITH PROSTATE CANCER RADIORESISTANCE USING THE LABEL-FREE LC-MS/MS APPROACH

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Aim: Radioresistance is still a major problem in current prostate cancer (CaP) radiation therapy (RT). This study aimed to identify signaling pathways potentially responsible for CaP radioresistance by a LC-MS/MS proteomic approach.

Methods: Three prostate cancer radioresistant (RR) cell lines (PC-3RR, DU145RR and LNCaPRR:CaP-RR) and its parental cell lines (PC-3, DU145 and LNCaP:CaP-control) were analyzed by the LTQ Orbitrap Velos ETD (Thermo Scientific, US) and Progenesis LC-MS software (Non-Linear Dynamics, UK). The proteins related with the identified signaling pathways were validated by western blotting.

Results: Total 791 proteins were found to be statistically significant differences ($p \leq 0.05$, fold change>3, >80% power, false discovery rate q

Conclusions: Significant signaling pathway proteins were found in CaP-RR cells These identified pathway proteins are useful therapeutic targets to improve CaP radiosensitivity for future clinical radiotherapy.

P-10.00

QUANTITATIVE PROTEOMIC PROFILING IDENTIFIES DPYSL3 AS PANCREATIC DUCTAL ADENOCARCINOMA-ASSOCIATED MOLECULE THAT REGULATES CELL ADHESION AND MIGRATION BY STABILIZATION OF FOCAL ADHESION COMPLEX

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Introduction and Objectives

Pancreatic cancer is the fifth leading cause of cancer death in Japan with more than 24,000 annual deaths, while lung cancer is another hard-to-cure cancer with the highest death tolls of more than 70,000 lives a year. Widespread metastasis and/or massive local invasion are commonly present, when they are diagnosed, making long-term survival of these cancers remain unsatisfactory. Thus, it is evident that elucidation of the underlying mechanisms of invasion and distant metastasis is crucial to improve the current dismal outcome.

Methods

We employed combined proteomic technologies including mass spectrometry and isobaric tags for relative and absolute quantification peptide tagging to analyze protein profiles of surgically resected human pancreatic ductal adenocarcinoma tissues.

Results and Discussion

We identified a protein, dihydropyrimidinase-like 3, as highly expressed in human pancreatic ductal adenocarcinoma tissues as well as pancreatic cancer cell lines. Characterization of the roles of dihydropyrimidinase-like 3 in relation to cancer cell adhesion and migration in vitro, and metastasis in vivo was performed using a series of functional analyses, including those employing multiple reaction monitoring proteomic analysis. Furthermore, dihydropyrimidinase-like 3 was found to interact with Ezrin, which has important roles in cell adhesion, motility, and invasion, while that interaction promoted stabilization of an adhesion complex consisting of Ezrin, c-Src, focal adhesion kinase, and Talin1. We also found that exogenous expression of dihydropyrimidinase-like 3 induced activating phosphorylation of Ezrin and c-Src, leading to up-regulation of the signaling pathway.

Conclusions

The present results indicate successful application of combined proteomic approaches to identify a novel key player, dihydropyrimidinase-like 3, in pancreatic ductal adenocarcinoma tumorigenesis, which may serve as an important biomarker and/or drug target to improve therapeutic strategies.

P-11.00

HYPOTHESIS DRIVING PROTEOMIC STUDY ON THE EFFECT OF DIETARY UNSATURATED FATTY ACIDS ON PROSTATE CANCER

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Introduction and Objectives
Prostate cancer (PCa) is one of the most common cancers in men. In most PCa cases, tumors progress very slowly but in a small portion of patients, PCa develops into aggressive stages and becomes lethal. A line of evidence has suggested that early inference and dietary prevention are beneficial in PCa patient care. Fish oil (FO), which contains mostly omega-3 fatty acid, is one of the most widely studied candidate supplements for PCa prevention; however, the molecular mechanism is not thoroughly understood. A preliminary study indicated that fatty acid synthase (FASN) plays critical roles in FO metabolism. The goal of this study is to evaluate the regulatory role of FO on PCa through a global proteomic analysis.

Methods
An LC/MS-based label-free global protein quantification method was carried out on PC3 cells treated with vehicle, FO or oleic acid (OA) and were harvested on different days post-treatment.

Results and Discussion
Sequestosome-1 (SQSTM1), which is required by autophagy [13], was found to be over-expressed at in FO treated group. When cross-comparison of day 2 with day 6 was performed, we found that SQSTM1 level in FO group decreased through treatment. This result strongly suggests that autophagy may play an important role in FO induced cell death. The following proteins were also over-expressed in FO group: Isoform 4 of non-specific lipid-transfer protein, Macrophage migration inhibitory factor, Fascin, Isoform Beta-1B of Integrin beta-1, Calnexin. All of these candidates will be further validated by biochemical methods.

Conclusions
Overall, this study shows that FO and OA both suppress FASN activity but only FO induces cell death. The proteomic study result indicates a possible role of autophagy in this process. Moreover, our study suggests a regulatory association between FASN and COX2, both of which are important in PCa development and FO treatment.

P-12.00
PROTEOMICS IDENTIFIED OVEREXPRESSION OF SET ONCOGENE PRODUCT AND POSSIBLE THERAPEUTIC UTILITY OF PROTEIN PHOSPHATASE 2A IN ALVEOLAR SOFT PART SARCOMA

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Alveolar soft part sarcoma (ASPS) is an exceedingly rare sarcoma that accounts for fewer than 1% of all soft tissue sarcoma. While ASPS patients can often achieve prolonged survival, standard chemotherapy and radiation therapy have no significant survival advantage, and most eventually succumb to the disease as a result of late metastasis. Although several molecular targeting drugs have been applied for soft tissue sarcomas, their clinical significance has not yet been established, and novel therapeutic strategies have long been required in order to improve the clinical outcome.

The aim of this study was to identify proteins aberrantly regulated in ASPS, and to reveal their clinical significance. Protein expression profiling of tumor and non-tumor cells in tumor tissues from 12 ASPS patients was performed by two-dimensional difference gel electrophoresis (2D-DIGE) and mass spectrometry. Among the proteins with unique expression in tumor tissues, we further investigated the functional roles of SET protein, which was up-regulated in ASPS tumor tissues, and has multifunctional roles in cancer progression in the other cancer types. Western blotting and immunohistochemistry confirmed overexpression of SET in primary tumor tissues of all 15 ASPS cases examined, as well as the other sarcomas but not in normal tissues. Gene silencing of SET significantly decreased cell proliferation, invasion and migration against a background of induced apoptosis. SET is known to be an inhibitor of phosphatase 2A (PP2A), which functions as a tumor suppressor by inhibiting the signal transduction pathways and inducing apoptosis.

We found that a PP2A activator, FYN720, which is used for treatment of multiple sclerosis, inhibited cell proliferation through apoptosis in ASPS cells. Together, the results of our proteomics study may suggest the possible contribution of SET to the progression of ASPS, and the clinical utility of the PP2A activator, FYN720, for treatment of ASPS.

P-13.00
DISCRIMINATION BETWEEN PATIENTS WITH VARIOUS STAGES OF CERVIX CARCINOMA BY SERUM ANALYSIS OF DIFFERENTIALLY EXPRESSED HEAT SHOCK PROTEIN 90 FOUND BY LASER MICRODISSECTION

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Laser microdissection (LMD) allows the capture of different cell types or well-defined regions of tissue. We compared tumor, normal epithelium and stroma of the cervix from eight patients with cervical squamous cell carcinoma by capturing approximately 2500 cells. Cellular proteins were digested with trypsin and peptides were analyzed by high-resolution mass spectrometry with or without prior chromatographic separation. By comparing the proteomes from cancer tissue, normal epithelium and stroma, we found a number of differentially expressed proteins of the heat shock protein (HSP) family.

HSP90 gave a strong positive response upon western blotting of 10000 laser microdissected cells in tumor tissue from a different set of cervical squamous cell carcinoma patients while it was negative in stroma and epithelial tissue from healthy controls. The specific expression pattern of HSP90 was further confirmed by immunohistochemistry (IHC) in tissue from a set of different patients.

To translate our findings from tissue to an accessible body fluid for assay development, we designed a targeted LC-MS/MS method for HSP90 in serum and compared healthy subjects to patients with early- and late-stage cervical squamous cell carcinoma. In a validation set of 50 early-stage patients, 28 late-stage patients and 42 healthy subjects sensitivity and specificity was 62% and 64% for early-stage carcinoma, respectively, while for late-stage carcinoma sensitivity and specificity were 71% and a 67%. By combining squamous cell carcinoma antigen (SCC-ag) measurements with HSP90, sensitivity increased from 62% to 68% for early-stage carcinoma and from to 71% to 100% for late-stage carcinoma.

Adding HSP90 to the currently used SCC-ag biomarker assays thus increases sensitivity notably for late-stage cervical squamous cell carcinoma.

P-14.00

SNAIL11 SUPPRESSES DIFFERENTIATION IN 3T3-L1 FIBROBLAST AND MMSC BY REPRESSION OF NOVEL SNAIL-MODULATED TRANSCRIPTION FACTORS

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Snail1 transcription factor is a major inducer of the epithelial-to-mesenchymal transition (EMT) during embryonic development and cancer progression. Snail1 is not expressed by tissue-resident fibroblasts and is only detected in activated fibroblasts; still its role in stromal fibroblasts requires further studies. Here, we have studied the proteomic alterations induced by Snail expression in fibroblasts capable to differentiate to terminal mesenchymal phenotypes like 3T3-L1 and bone marrow-derived murine mesenchymal stem cells (mMSC).

Methods
We generated 3T3-L1 and mMSC cells stably over expressing Snail1 and control cells. Due to the characteristics of the cells, we used two different quantitative proteomic analyses, stable isotopic metabolic labeling (SILAC) for 3T3-L1 and isobaric labeling with tandem mass tags (TMT) for mMSC. We focused the proteomic analysis on the nuclear fraction where take place the major effects of Snail1 as transcription factor. Tryptic peptides were scanned and fragmented with a linear ion trap- Orbitrap Velos (ThermoScientific).

Results
We quantified 2250 protein with 554 proteins deregulated in 3T3-L1 and 1132 proteins quantified with 311 proteins deregulated in mMSC. Among the repressed proteins in both analyses, we identified deregulated proteins involved in nuclear transport as RAN pathway, proteins related to SRC famliy, mTOR and cytokine signaling. Most notorious effect of Snail1 overexpression was the down-regulation of several TFs , we observed a direct repression of Prrx1, Nr2f6 and Asc1 by Snail in preadipocyte 3T3-L1 and mMSC-Snail cells. A Luciferase and ChIP analysis confirmed the recruitment of Snail in these promoters.

Conclusions
Snail1 might be a master regulator and play a central role in the expression of a cascade of multiple transcription factors that control adipogenic gene expression at different levels. This work provided insight into novel proteins with potential roles in the regulation of differentiation to adipocytes of the 3T3-L1 and mMSC cells as Nr2F6, Asc1 or Prrx1.

P-15.00
PROTEOMIC PROFILING OF AN INDUCIBLE MODEL OF ACUTE MYELOID LEUKAEMIA REVEALS NOVEL INSIGHTS INTO LEUKAEMOGENESIS.
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The Mixed Lineage Leukemia (MLL) gene located on chromosome 11q23 is a frequent site of translocation that results in a number of oncogenic fusion proteins. MLL-translocations are present in a variety of paediatric and adult leukaemias and are associated with poor survival outcome. Despite intense research efforts the mechanisms underlying the leukaemogenic activity of MLL-translocations are poorly understood. Our aim was to identify proteins involved in leukaemogenesis that are regulated by MLL-translocation gene expression through quantitative proteome analysis using a leukaemia model. Our model of acute myeloid leukaemia (AML) is controlled by the regulated expression of a common MLL-translocation gene, MLL-AF9.

Initially, surface captured proteins were analysed using a SILAC quantitative proteomics workflow and high-resolution mass spectrometry to generate a profile of proteins that are expressed in the presence or absence of MLL-AF9 expression. We extended this analysis by also profiling changes to the entire leukaemia proteome during MLL-AF9 regulation.

The results from these two techniques have not only confirmed several known targets in AML but highlighted novel pathways and targets that are upregulated when MLL-AF9 is expressed. These pathways include epigenetic regulators, cell signalling and cell-cell or cell-extracellular matrix interactions.

The results from this study are not only helping to identify the mechanisms by which MLL-AF9 regulates leukaemogenesis, but are also demonstrating the potential for identification of biomarkers and novel drug targets.

P-16.00

MEMBRANE PROTEINS ASSOCIATED WITH INVADOPODIA STRUCTURES IN GLIOBLASTOMA MULTIFORME

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Glioblastoma multiforme (GBM) is the most aggressive and prevalent malignant brain tumour in adults. GBM is diffusely infiltrative, making complete surgical resection difficult. Tumour invasion of neighbouring tissues is facilitated by cell migration and degradation of the extracellular matrix (ECM). Invadopodia are actin-rich organelles that protrude from the ventral side of the plasma membrane in direct contact with the ECM and play an important role in tumour invasion.

The aim of the current study was to characterize the 'invasive potential' of a panel of established GBM cell lines using an invadopodia assay and perform comparative membrane proteomic analyses of highly invasive vs. less invasive cells.

Nine GBM cell lines were characterized based on their ability to produce invadopodia using a QCM gelatin invadopodia assay (Millipore). Fluorescence microscopy revealed areas devoid of Cy3-gelatin indicating degradation by GBM cell invasion. The membrane proteomes from GBM cells were enriched by differential ultracentrifugation and compared using isobaric tags for relative and absolute quantitation (iTRAQ) labeling. Samples were fractionated off-line using electrostatic repulsion-hydrophilic interaction chromatography (ERLIC) and subjected to liquid chromatography and tandem mass spectrometry (LC-MS/MS).

All 9 GBM lines produced invadopodia with a significant difference between the most invasive (U87MG) and least invasive (LN229) cells (65%, percentage of total cell area; $p=0.0001$). Overall, 1667 proteins were identified from duplicate runs, of which 76% mapped to membrane structures using the David bioinformatics database (http://david.abcc.ncifcrf.gov). The differential abundance of 38 proteins significantly correlated with the degree of invasion ($r2 > 0.45$ or $r2 <- 0.45$; $n \geq 9$; $p < 0.05$).

Cell surface proteins were involved in cell morphology and cellular movement. Fluorescence microscopy demonstrates co-localization of novel proteins to invadopodia structures and siRNA knockdown of a target protein confirmed its role in invadopodia-formation. Invadopodia-associated membrane proteins could be novel targets for anti-invasive GBM therapies.

P-17.00
DIFFERENTIAL DIAGNOSIS OF PANCREATIC CYSTS USING MASS SPECTROMETRY-BASED APPROACH

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Background: The widespread use of advanced imaging technologies is increasing an identification of pancreatic cysts. However, an accurate differential diagnosis between mucinous and non-mucinous neoplasm is still challenging. Thus, it is necessary to develop novel biomarkers for the diagnosis of pancreatic cysts.

Methods: Integrated proteomic analysis were used to screen the whole proteome of cystic fluids and verify biomarker candidates on 56 pancreatic cysts (17 pseudocysts, 18 mucinous and 22 non-mucinous neoplasms) using GeLC-Stable Isotope Dilution-Multiple Reaction Monitoring (GeLC-SID-MRM) and immunoassay. Statistical analysis was performed to prioritize the useful differential markers for detecting mucinous neoplasms.

Results: A total of 223 proteins(false discovery rate (FDR) < 1%) were identified using Nano-LC-MS/MS. Of them, 9 candidates, including Polymeric immunoglobulin receptor (pIgR), Neutrophil gelatinase-associated lipocalin (NGAL), IgGFc-binding protein (FCGBP), Lithostathine-1-alpha (REG1A), Afamin (AFM), Chymotrypsin-C (CTRC), Pancreatic alpha-amylase (PA), Galactin-3-binding protein (G3BP) and Elastase-3A (ELA3A) were successfully evaluated as a diagnostic marker for mucinous neoplasm. Three markers (AFM, REG1A, pIgR) were statistically significant to discriminate mucinous cystic lesion from benign cystic lesion (P < 0.05).

Conclusions: We suggest a diagnostic panel (AFM, REG1A, pIgR) to support a differential diagnosis of mucinous neoplasms from non-mucinous neoplasms combined pseudocyst and benign non-mucinous (AUC, 0.929).

P-18.00

PHOSPHOPROTEOMICS REVEALED TRPV4 CALCIUM PERMEABLE CHANNEL TO BE A NOVEL EFFECTOR OF BREAST CANCER METASTASIS

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Methods: Phosphoproteomic profiling of breast cancer metastasis was conducted using 4G10 affinity purification combined with iTRAQ-based LC/MS/MS. Bioinformatics was performed and a novel candidate was selected for validation, a battery of cell-based assays and elucidation of its mode of action in metastasis.

Results and Discussion: Forty nine proteins were found to be hyperphosphorylated in one or more cell lines with increasing metastatic potential. Bioinformatics revealed that the dataset was predominantly associated with intra/extravasation, an important event during metastasis. Transient Receptor Potential Vanilloid subtype 4 (TRPV4), a calcium-permeable channel was validated to be upregulated metastatic but not non-metastatic breast cancer cells. Silencing TRPV4 expression significantly abolished the invasiveness and the ability of murine mammary breast cancer metastatic cells to transmigrate through endothelial cells, but not their proliferation. In-vivo studies demonstrated that knockdown of TRPV4 significantly reduced the number (but not the size) of metastatic nodules in the lungs of mouse xenografts.

Mechanobiology techniques revealed that the effects of TRPV4 knock down were associated with increased rigidity of the cancer cells, reduced blebbing and abolishment of Ca2+ influx. Mapping of TRPV4-mediated signaling revealed a Ca2+-dependent activation of AKT and downregulation of the expression of E-cadherin possibly through a combination of proteosomal degradation and transcriptional regulation. Exogenous expression of constitutively active AKT mutant rescued the reduction of transendothelial migration of breast cancer cells caused by silencing of TRPV4 expression. The data generated from clinical specimens also provided evidence that TRPV4 plays a role in multiple other human cancers.

Conclusion: Phosphoproteomics remains a very powerful tool in signal transduction and cancer research for many years to come.

P-19.00

ANALYSIS OF MEMBRANE PROTEIN EXPRESSION PATTERNS FOR CELLS CULTURED USING CANCER-TISSUE ORIGINATED SPHEROIDS

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Introduction and objects: Cancer cell lines are often quite different from the primary cell cultures derived from the original tumors, since they have been selected by their ability to proliferate under culture conditions. Recently, a new culturing method for primary human cancer tissues, called the □gCancer Tissue-Originated Spheroids (CTOSs) method□h, was described. The present study reports results of a proteomic analysis of the CTOSs method in which expression patterns of membrane proteins following spheroid versus standard plate culture of primary cancer cells were analyzed.

Methods : CTOS preparation: CTOSs were prepared from mouse xenografts of human lung cancer. Organoid fractions were collected using cell strainers, discarding fractions that dissociated into single cells after dissociating tumor specimens mechanically and enzymatically.

Proteomic analysis: Membrane proteins were collected and purified using streptavidin affinity beads after biotinylating the surface-exposed cell membrane proteins. The proteins were then subjected to reduction, alkylation with iodoacetamide and tryptic digestion. Extracted tryptic peptides were concentrated by centrifugal lyophilization and analyzed by LC-MS/MS.

Results and Discussion: Three CTOSs were made from a xenograft murine model with CTOS derived from different patients. Proteomic analysis identified more than 600 membrane proteins under each culture condition. Approximately 2/3 of these proteins were present under both culture conditions, while the remaining 1/3 showed distinctive protein profiles for cells cultured under these two conditions. Several proteins derived from flat cultured CTOS were expressed in all three of the CTOSs.

Conclusions: Proteomic analysis revealed that cells cultured under spheroid conditions have expression profiles that are distinctly different from those of flat-cultured cells. Because CTOSs have been shown to form xenograft tumors that retained features of the cancer in patients, their expression profiles would be expected to indicate proteins responsible for maintaining the characteristics of cancer cells during in vitro cell culture conditions.

P-20.00

PROTEOMIC ANALYSIS REVEALS FAM120A AS A KEY REGULATOR OF IL13-TRIGGERED LIVER METASTASIS IN COLORECTAL CANCER

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Introduction and Objectives

Interleukin-13 receptor α2 (IL13Rα2) is a IL13 receptor expressed in a variety of cancer cells, including colorectal cancer. Although IL13Rα2 was supposed to be a decoy receptor, current evidence shows that it can trigger activation of signaling pathways, including the activation of PI3K and ERK1/2. In SILAC assays performed with poorly or highly metastatic colorectal cell lines, IL13Rα2 was found amongst the more upregulated proteins in highly metastatic cells. Also the presence of this receptor in cancer cells promotes cell invasion and metastasis and it is a marker of poor prognosis. However the mechanisms involved in metastasis triggered by IL13Rα2 are unknown. Our objective was to elucidate these mechanisms.

Methods

We performed iTRAQ mass spectrometry quantitative screen of IL13-treated colorectal cancer. Lysates were trypsin digested and resulting peptides were labeled with iTRAQ reagents. Then, the samples were mixed, fractionated by isoelectric focusing and analyzed by nanoLC-MS/MS using a linear ion trap-Orbitrap Velos mass spectrometer. Also we performed co-immunoprecipitations, which were resolved in gels, digested with trypsin and the peptides were analyzed as before.

Results and Discussion

By means of proteomic analyses we identified 256 proteins associated to IL13Rα2, including FAM120A, which was the sole protein identified involving in signal transduction. Proteomic analysis of FAM120A co-immunoprecipitates revealed that this protein is forming a signaling complex with Src, FAK, PI3K, ERK1/2, etc. FAM120A-silencing impaired IL13-triggered activation of both FAK and PI3K, cell invasion and metastasis in xenografts. We also carried out an iTRAQ of IL13-treated cells. Surprisingly, no differences were found, indicating that IL13 only has short term effects in activation of proteins promoting cell invasion, but no effect in protein expression.

Conclusions

IL13Rα2 exerts its action through the adaptor FAM120A, which is the core of a signaling complex able to activate the colorectal cancer cell invasion and metastasis.

P-21.00
DATA-INDEPENDENT MSE WITH ION MOBILITY IDENTIFIES A GPX4 AS A NEW TARGET FOR ANTI-CANCER DRUGS

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Recently, a new non-apoptotic cell death process termed ferroptosis was described involving the production of iron-dependent reactive oxygen species. In this work, we identified a protein target of a small molecule (RSL3) that is an inducer of ferroptosis and a potential targeted anti-cancer agent. The experimental design was a comprehensive label-free chemoproteomics approach with multiple biological and technical replicates of RSL3 bound to affinity beads and controls with an inactive RSL3 analog, as well as competition controls with active compound. A protein target of RSL3 was identified using data-independent acquisition (DIA) (MSE) with traveling wave ion mobility spectrometry (TWIMS).

Proteins were extracted from human fibroblast cells that had been treated with an RSL3-fluorescein affinity reagent. Most detected proteins were not differentially bound to fluorescein-RSL3 compared to control affinity preparations. Only one protein, glutathione peroxidase 4 (GPX4_HUMAN) exhibited significant ($P < 0.01$) enrichment and the highest fold-change (26-fold in the RSL3-affinity preparation compared to the inactive analogue control and 13-fold compared to the control of preincubation with free RSL3).

The identification of GPX_HUMAN as a target of RSL3 was subsequently validated by an exhaustive series of confirmatory experiments (see Yang, et al., 2014. Cell 156: 317-331). These included western blots with strong detection of GPX4 in fresh samples of affinity preparations of fluorescein-RSL3 and undetectable signals in inactive and competitor series of controls. GPX4 overexpression and knockdowns modulated cell death response to a number of compounds that induced ferroptosis, but not for other compounds with action mediated by other cell death mechanisms. GPX4 regulation of ferroptosis was seen in a mouse tumor model, and sensitivity profiling of cancer cell lines indicated that some lymphomas and carcinomas were susceptible to GPX4-regulated ferroptosis. These studies confirmed GPX4 as an essential regulator of ferroptotic cancer cell death identified by data-independent profiling with TWIMS.

P-22.00
SHOTGUN PROTEOMICS RECOGNIZES CELL LINES USING EXOME DATA: NCI-60 PANEL CASE STUDY

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In this work we constructed a sequence database for search of publicly available proteome data of NCI-60 cancer cell lines (Moghaddas Gholami et al, 2013) against recent results of exome sequencing of the same cell lines (Abaan et al., 2013). The primary objective of this analysis was to test the possibility of cancer cell line recognition based on the sets of variant peptides identified for each of the lines.

A database of mutant protein variants was generated using NCI-60 cell line exome sequences. Results of bottom-up "shotgun" proteome analysis of the same cell lines were processed using X!Tandem search engine. For in-home validation of the approach, cancer cell lines were provided in blinded manner. Proteomes were subjected to gel separation and proteins were digested by trypsin and analyzed using QExactive analyzer.

A percentage of identified variant peptides corresponding to the own cell line exome was used as a metrics of correct identification. Proteome data contained "deep proteome" and "full proteome" data depending on the fragmentation method (HCD or CID) and the number of fractionation steps. In order to recognize correct cell line, for a particular cell line the identified "correct" variant peptides were matched against all the other lines. The lists of "correct" peptides were always matching best the corresponding exome in all 9 "deep proteome" cell lines. Similar results were obtained for "full proteome" with 50 out of 59 correct matches.

The ability of shotgun proteomics to identify cancer cell lines by variant peptide signatures was further validated using in-home experiments with blinded set of cell lines and algorithms used for previous analysis.

In this study, we have demonstrated that shotgun "deep" proteomics data are sufficient for unambiguous recognition of the cancer cell lines. The task of recognizing unknown cancer tissues is, in some cases, useful for clinical practice.

P-23.00

DEVELOPMENT OF PROTEOMIC BASED DIAGNOSTIC BIOMARKERS FOR EARLY DETECTION OF LIVER CANCERS

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Introduction and objectives: Proteomics approach opens a new way for discovering novel biomarkers that can be used to diagnose, predict susceptibility and monitor progression of diseases. á-fetoprotein, the classic marker of HCC is of limited value as a result the diagnosis of HCC is usually made in the late stages of the disease when treatment options are severely limited. It is therefore pertinent to identify novel proteins in liver tissue and in serum samples of hepatocarcinoma patients that may be useful for the development of early diagnostic biomarkers.

Methods: One-dimensional electrophoresis (1D), two-dimensional electrophoresis (2DE) and liquid chromatography mass spectrometry (LC–MS/MS) and western blot were used to screen the serum proteins of liver cancer induced in animals.

Results and discussion: We describe the development of a hepatotcarcinoma model by modified Solt-Farber protocol in Wistar rats. The protein profiles of both serum samples and liver tissue have been confirmed by 1D SDS PAGE and 2D electrophoresis and correlated to disease progression to monitor specific changes in protein expression. Histopathology of the liver tissue was performed to confirm liver damage. The proteomic analysis has revealed a few proteins that are differentially expressed in diseased animals. Besides, some minor novel proteins were expressed during tumor promotion. These proteins were characterized by MALDI-TOF and other techniques to identify specific proteins that can be used as biomarker(s) for early detection and/or prediction of liver cancers.

Conclusion: These biomarkers would aid clinicians in diagnosing liver cancer during the early stages, eliminating the need for liver biopsy and allowing early treatment, thereby preventing the progression of liver cancer.

P-24.00

MRM VALIDATION OF TARGETED NONGLYCOSYLATED PEPTIDES IN UNDEPLETED HUMAN PLASMA USING SIMPLE SAMPLE PREPARATION METHOD

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For clinical use, we have developed a rapid, simple, and reproducible method to effectively monitor biomarker candidates, nonglycosylated tryptic peptides adjacent to N-glycosylation sites, from a large number of plasma samples. Glycoproteins have been major targets in biomarker discovery because in protein glycosylation are known to be associated with a variety of diseases. We have proved that nonglycosylated tryptic peptides adjacent to N-glycosylation sites differed in concentration between normal and hepatocellular carcinoma (HCC) plasma due to differences in steric hindrance of the glycan moiety in N-glycoproteins to tryptic digestion.

To increase the feasibility and applicability of clinical validation of biomarker candidates (nonglycosylated tryptic peptides), the simple sample preparation method was optimized to effectively monitor nonglycosylated tryptic peptides from a large number of plasma samples undepleted the most abundant proteins and to reduce the total analysis time with maximizing the effect of steric hindrance by the glycans during digestion of glycoproteins.

The AUC values of targeted nonglycosylated tryptic peptides from LC-MRM analyses were 0.955 for GQYCYELDEK, 0.880 for FEDGVLDPDYPR and 0.907 for TEDTIFLR, indicating that these could be effective biomarkers for hepatocellular carcinoma.

This strategy, therefore, provided the throughput necessary to monitor protein biomarkers, as well as quantitative accuracy in human plasma analysis

P-25.00

DIFFUSE LARGE B CELL TYPE NON HODGKIN LYMPHOMA OF PANCREAS IN A PATIENT WITH MS; A CASE REPORT FROM IRAN

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Introduction: Multiple sclerosis (MS) is a frequent chronic autoimmune demyelinating disease of the central nervous system (CNS) .It was shown that multiple sclerosis (MS) was linked to reduced rates of cancer .herein we report a case of multiple sclerosis with Diffuse large B cell type Non Hodgkin lymphoma in head of pancreas, according to best of or knowledge this is the first case in this era.

Case Presentation: The patient is a 47 year old man a Known case of MS for 6 years. In a routine follow up in outpatient clinic the patient had complain of a mild abdominal pain, and weight loss. Abdominal CT scan was performed for the patient which was infavour of lymphoma and fine needle aspiration (FNA) was recommended by the radiologist. FNA was done and according to the microscopic evaluations and immune histo chemistry study, diffuse large B cell type Non Hodgkin lymphoma was diagnosed for him.

Discussion: Recent epidemiological and immunological studies provide evidence for an association between Epstein-Barr virus infection and multiple sclerosis, suggesting a role of Epstein-Barr virus infection in disease induction and pathogenesis. Chronic suppression of cell-mediated immunity for treatment of MS may be associated with increased risk of malignancy in MS patients.

Key words: Multiple sclerosis, malignancy

P-26.00

LATE ONSET MULTIPLE SCLEROSIS IN SOUTHERN IRAN

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Introduction: Multiple sclerosis (MS) is the most common demylinating disease of central nevus system. After trauma multiple sclerosis is the most common cause of neurological disability in young adults, but in a subgroup of patients the first clinical symptoms presented after age 50 years. This late clinical presentation is defined as late onset multiple sclerosis (LOMS). The incidence and prevalence of MS including LOMS varies geographically. The study's purpose is to determine epidemiological characteristic of late onset MS in southern Iran.

Patients and Methods: patients over 50 year old and known to have definite Multiple Sclerosis according to MC Donald's criteria, and were members of Shiraz University Multiple Sclerosis Database (SUMSD) were evaluated in this study.

Results: From 1705 patients with clinically definite Multiple Sclerosis or CIS(clinically isolated syndrome), 138 patients (7.2%) identified as late onset M.S (time at onset of presentation over 50 years) which 60 patients (3.1%) identified as very late onset MS (VLOMS). Patients mean age with LOMS at the time of diagnosis was 58.81 years (with SD 2.6 year and 95% C.I 57.5 – 59.6). The oldest patient had 72 years old. 31 patient (20.8%) were male and 107 (79.2%) were female with female to male ratio of 3.4. 89.1 % of patients received Beta interferon as a disease modifying treatment including Avonex (16.3 %) cinovex (32.6%) Rebif(14.1%)and Betaferon (26.1 %).

Discussion: LOMS is a subgroup of MS that is not rare in south of Iran (Fars province). Incidence rate of 1-6 % was reported in some studies in other countries. In our study 7.2 % of all MS patients identified as LOMS. In this subgroup history and para clinical data should be thoroughly evaluated to exclude common conditions like cerebro vascular disease.

Key Words: late onset Multiple sclerosis , southern Iran

P-27.00

ALPHA 1-ANTITRYPSIN AND ALPHA 2HS-GLYCOPROTEIN, TWO TUMOR-ASSOCIATED ANTIGENS (TAA) DETECTED IN MEXICAN PATIENTS WITH EARLY-STAGE BREAST CANCER

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Introduction: The breast cancer is the most frequent cancer among women; every year, around 1.5 million new breast cancer cases are diagnosed in women throughout the world and around 0.5 million will die by this cancer. The prognosis of breast cancer is clearly related with the stage at which is diagnosed. Therefore, there is an important need to improve the screening and diagnosis of early invasive and noninvasive tumors. At present, a pre-symptomatic approach is crucial in breast cancer screening and diagnosis with potential to reduce mortality caused by this disease. Several studies have demonstrated that serum of patients with cancer contains specific antibodies that react with a group of autoantigens called Tumor-Associated Antigens (TAAs). Specific antibodies can be detected prior to clinical diagnosis; thus, they would be ideal biomarkers for early detection of cancer using only a few microliters of a patient's serum. The objective from present study was determining the presence of AAT and antibodies in the serum from patients with breast cancer in early stage.

Methods: In this study, we used an immune proteomic approach, combining two-Dimensional-(2D) electrophoresis, Western-blot, and Matrix-associated laser desorption/ionization-Mass spectrometry (MALDI-MS) methods to identify TAAs in patients serum with early stage breast cancer. The Fisher's exact test and X2 were used for the results analysis.

Results and discussion: Sera were obtained from 36 newly diagnosed patients with stage II breast cancer and 36 healthy volunteers were evaluated for the presence of the TAA. Serum antibodies against Alpha 2HS-Glycoprotein (AHSG) and A1AT were detected in 91.7% and 96% of the patients and only 8.3% an10% of controls respectively.

Conclusions: Our results strongly suggest that the presence of serum autoantibodies against TAAs: AHSG and A1AT proteins may be useful as serum biomarkers for early-stage breast cancer screening and minimally invasive diagnosis in Mexican populations.

P-28.00

DIAGNOSTIC PROTEIN BIOMARKERS OF WELL-DIFFERENTIATED THYROID MALIGNANCIES IN THAI PATIENTS

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Malignancy of the thyroid gland is most prevalent in the endocrine system, with 298,000 newly diagnosed cases accounting for 2.1% of all cancer cases worldwide in 2012. Even though fine needle aspiration (FNA) cytology remains to be the procedure of choice for the diagnosis of thyroid cancer, FNA does not definitively diagnose papillary thyroid carcinomas (PTC) and follicular thyroid carcinomas (FTC).

PTC and FTC account for more than 80% and 10-15% of all thyroid cancers, respectively, with variants which complicate diagnoses and FTC is considered to be more aggressive than PTC. Thus, there is a need for a biomarker(s) that can distinguish between the two-well differentiated thyroid carcinomas. In this study, classical proteomics was used to identify potential biomarkers for papillary thyroid carcinoma (BC-PAP) and follicular thyroid carcinoma (FTC-133) cell lines.

These proteins were further validated using immunoblotting against five papillary thyroid carcinoma tissues and five follicular thyroid carcinoma tissues, with their adjacent normal tissues from Thai patients. Using statistical t-tests with p-value

Keywords: biomarker, papillary thyroid carcinoma, follicular thyroid carcinoma, proteomics

P-29.00

UNDERSTANDING LAPATINIB ACTION BY CHEMICAL –AND PHOSPHOPROTEOMICS

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The small-molecule, ATP-competitive inhibitor Lapatinib is FDA approved for the treatment patients with advanced or metastatic breast cancer that overexpress HER2 and have been unsuccessfully treated with other therapies. Despite its clinical success, the downstream pathways are still not fully understood. In addition, most patients ultimately become resistant to the drug.

Here we used chemical proteomic kinase affinity purifications and label free quantitative proteomics as well as SILAC based phosphoproteomics to study the cellular response of a HER2 overexpressing breast cancer cell line to Lapatinib treatment.

Our chemical proteomics selectivity screen against more than 200 protein kinases revealed that Lapatinib very selectively inhibits Her2 and EGFR, confirming the compounds narrow target spectrum. By combining chemical proteomic and phosphoproteomic data, new and known effectors were assigned to the Lapatinib response pathway based on the dose dependent change of activity regulating phosphorylation sites. Surprisingly, despite inhibitor treatment, several signaling molecules, for example members of the Src family of kinases, showed sustained and dose-dependent activation suggesting the release of feedback loops and the presence of adaptive resistance pathways. Moreover, from the data it seems that Lapatinib exerts its cytotoxic function at least in part by disruption of the protein translation. The cumulative experimental results were summarized and visualized within a pathway map providing a rich resource for further studies. Currently, follow up studies evaluating possible synergistic effects of drug combination treatments and protein knock-outs, based on the here identified signaling molecules, are performed.

Taken together, preliminary results show that the applied methods are complementary and identify known and new effectors of the cellular response to Lapatinib, providing a starting point for the rational and hypothesis driven evaluation of drug combination treatment.

P-30.00

UNCOVERING AQUIRED RESISTANCE MECHANISMS TO EGFR-SPECIFIC KINASE INHIBITORS USING CHEMICAL PROTEOMICS

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The epidermal growth factor receptor (EGFR) is upregulated and activated in a large variety of human cancers. Despite the fact that EGFR-specific tyrosine kinase inhibitors (TKIs) often show a good initial response in patients with activating EGFR mutations, most aquire resistance against these agents. Here we describe a kinase affinity enrichment (Kinobeads) and intensity-based label-free mass spectrometry approach for the elucidation of aquired resistance mechanisms to EGFR-inhibition in cancer cells.

Cell lines that developped in-vitro resistance by long term exposure to EGFR inhibitors and their non-resistant counterparts were lysed to get total cell lysates. Immobilized kinase inhibitors (Kinobeads) were used for kinase affinity enrichment to subsequently analyze kinase abundance by intensity-based label-free mass spectrometry. Kinases overexpressed in resistant cells were tested for their function in triggering the resistance process by combination of kinase inhibitors and cell based assays.

Our methodology was able to identify previously described acquired resistance mechanims such as MET and IGFIR amplification in gefitinib resistant cell lines. Moreover, EphA2 has been found overexpressed (fold change=12, p

We conclude that chemical proteomics is a valuable generic tool for discovery of drug resistance mechanisms. We project that combinations of kinase inhibitors will prove valuable to circumvent resistance development in cancer cells.

P-31.00
AFFINITY PROTEOMICS IN PLASMA FROM CANCER BIOBANKS

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New possibilities to study protein biomarkers of disease are driven by the growth in categorized patient collections hosted in disease or population biobanks. For a systematic exploration of larger numbers of cancer patients, assays based on suspension bead arrays and antibodies from the Human Protein Atlas (HPA, www.proteinatlas.org, [1, 2]) are applied. This single-binder approach has yet revealed candidates for prostate cancer [3] or neuroendocrine tumors [4], and is being used for larger scaled, hypothesis-free disease studies. This affinity proteomic setting provides a versatile path that reaches beyond a pure discovery and enables different verification options through replication, additional serum or plasma samples [5], as well as other specimen such as proximal body fluids [6], tissues or cells. Experimental challenges such as susceptibility to off-target binding are being addressed by the use of several antibodies towards a common target, identification of captured proteins by mass spectrometry [7, 8], and ultimately by developing sandwich assays for the target of interest [8]. The presentation will give an overview on current activities to profile different types of cancer by antibodies for serum and plasma analysis, and will discuss strategies for candidate verification with and for multiplexed affinity proteomics technologies.

P-32.00

A UNIQUE CELLULAR MODEL OF THE COLORECTAL ADENOMA-TO-CARCINOMA SEQUENCE REVEALS CONSISTENT SERIES OF PROGRESSION MARKERS IN TWO INDEPENDENT DIFFERENTIAL LABEL-BASED (ITRAQ-MALDI) AND LABEL-FREE (TRIPLE-TOF) MASS SPECTROMETRIC SETTINGS

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Introduction: Colorectal cancer (CRC) is the second largest cause of cancer-related deaths in much of the industrialised World. As proposed in the genetic model of colorectal tumorigenesis, sequential mutations lead to progression from single crypt lesions, to adenomatous polyps, and to malignant cancer. We here report on a differential gel-free proteomics study based on a cellular model of the adenoma-to-carcinoma sequence, the adenoma cell clone AA/C1 and the derived anchorage-independent, tumorigenic carcinoma cell clone AA/C1/SB10C, which had previously been studied in a gel-based approach.

Methods: Label-free Triple-TOF and iTRAQ-MALDI MS-based proteomics of differential protein expression was applied to reveal progression-associated markers and combined with westernblot validation.

Results and Discussion: Over 100 consistently regulated proteins were revealed in both independent mass spectrometric settings. In the two evaluated replicate iTRAQ analyses a total number of over 2400 unique proteins were identified, of which about 5% showed consistent regulation in three out of four ratios. In the label-free TripleTOF setting of more than 2900 unique proteins a comparable percentage showed consistent regulation and large overlap with results from the label-based setting. Progression-associated proteins were grouped into functional complexes according to their GO terms and assigned to affected cellular pathways. Among up-regulated proteins CEAM1 and CEAM6, CYTC and CYTN, enzymes of the steroid biosynthesis, kreatin kinase isoforms, 15-hydroxyprostaglandin dehydrogenase, proteins S100-P and S100-A, and Toll-interacting protein are highlighted. Among the down-regulated proteins annexin isoforms, beta-arrestin, the apoptosis regulator BAX, proteases, protein kinases, proteasomal subunits, Rho GTPase-activating proteins, sorting nexin isoforms, vacuolar protein sorting-associated proteins, and the tumor suppressor p53-binding protein were found and are discussed in the light of previous work.

Conclusions: High-quality data sets were generated on the basis of a unique cellular model of colorectal cancer development that allowed to identify a large series of mostly novel candidate marker proteins of progression.

P-33.00

PROTEIN BIOMARKERS FOR PROSTATE CANCER IN URINARY EXOSOME-LIKE VESICLES

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Introduction and Objectives: The identification of prostate cancer (PCa) biomarkers that can classify patients into high- and low-risk groups of disease progression and therefore help in the treatment decision-making is a major area of ongoing research. The aim of this project was to describe and validate a set of protein biomarkers candidates in urinary exosome-like vesicles (ELV) to improve PCa patient stratification.

Methods: Protein biomarker candidates for PCa were initially identified from a discovery phase done in urinary ELV, isolated by ultracentrifugation, from urine obtained after digital rectal examination. Specifically, label-free LC-MS/MS protein quantitation was performed on 24 samples: 8 benign samples, 8 low-risk PCa samples (Gleason=7(3+4)), and 8 high-risk PCa samples (Gleason>7). Proteins significantly changing in abundance were selected for further selected reaction monitoring (SRM) validation. Only one peptide per protein was selected for the validation phase based on the following criteria: (1) similarity between peptide and protein fold change (FC), (2) how significant is the FC using the information of a single peptide, (3) how intense is the peptide. Using these criteria 64 peptides were selected and monitored in 53 urinary ELV samples from PCa patients and 54 urinary ELV samples from benign counterparts.

Results and Discussion:
With our strategy, several proteins showing significant changes in abundance were identified in urinary ELV during the discovery phase, including prostate-specific antigen (PSA) and Transmembrane protease serine 2 (TMPRSS2). Moreover, classical exosome markers, such as CD63, were also identified. These PCa protein biomarker candidates were further validated in a large cohort of patients using SRM.

Conclusions
Our results provide a set of promising protein biomarkers that need to be quantified in a larger cohort of samples. In the future, this could improve current PCa detection and patient stratification and, therefore, avoid PCa related over-diagnosis and over-treatment.

P-34.00

IDENTIFICATION OF NOVEL TWIST1 TARGETS IN HUMAN FIBROBLASTS BY ITRAQ-BASED QUANTITATIVE PROTEOMICS.

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Introduction and Objectives

Twist1 is known to be a key controller of the epithelial-to-mesenchymal transition (EMT) with other transcription factors such as Zeb and Snail. To date many studies have been focused on the characterization of Zeb and Snail regulation and mechanism of action, however little is known about the downstream targets of Twist1. In this work we aimed to characterize proteins regulated by Twist1 in human fibroblasts using MS-based quantitative proteomics.

Methods

We conducted an iTRAQ quantitative assay using BJhTert fibroblasts stably transfected with a lentiviral vector encoding Twist1 or a control empty vector. Proteins contained in whole cell extracts or alternatively, in nuclear fractions were trypsin digested and resulting peptides were labeled with iTRAQ reagents. Following iTRAQ labeling, the samples were mixed in equal proportions, fractionated by isoelectric focusing into 24 fractions and analyzed by nanoLC-MS/MS using a linear ion trap-Orbitrap Velos.

Results and discussion

We obtained a total of 198 proteins whose expression was directly or indirectly controlled by Twist1 overexpression, including several transcription factors and co-factors. We found that HMGA1, HMGA2, NR2F2 and LTF were downregulated whereas RXRA, STAT1, MYT1L and PRRX1 were upregulated. We used the Genomatix MatInspector software to find binding sites for these transcription factors in the promoter regions of the proteins found to be deregulated by Twist1 to build up a potential regulatory network of these transcription factors. Further bioinformatic analysis using the Genomatix Pathway Systems software, showed TGF-β, cell proliferation, matrix metalloproteinase and angiogenesis among the top predicted biological networks of the proteins deregulated by Twist1 expression.

Conclusions

Our proteomic screen allowed the characterization of novel Twist1 targets potentially important for its role in promoting the EMT process, along with transcription factors that might be regulating the complex network triggered by Twist1 overexpression.

P-35.00
MOUSE MONOCLONAL ANTIBODIES AGAINST HUMAN URINARY PROTEIN SM-UP2IP OF SCHISTOSOMA MANSONI

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Introduction and objective: There are innumerable pathological problems associated with schistosomiasis which have necessitated various control programs. Successful control would naturally depend on effective rapid diagnosis in the field. However, the overlapping distribution of urinary and intestinal schistosomiasis in hyperendemic areas calls for differential diagnosis. This study was aimed at producing anti-Schistosoma mansoni monoclonal antibodies (MAbs) for utilization in detecting antigens in the urine of infected persons.

Methods: Antibodies were raised to less immunogenic urinary parasite antigens by immunizing BALB/c mice with Schistosoma mansoni soluble worm antigens (Sm-SWA) while urinary proteins (Sm-UP2IP), isolated from infected human urine samples, was used as a final booster. Hybridoma cells were obtained by the fusion of mouse myeloma and spleen cells from the immunized mice, which were screened by ELISA and then studied further to obtain anti-S. mansoni specific MAbs using western blot and dot-blots techniques. The MAbs were also screened against common tropical heamopathogens including P. falciparum and S. heamatobium.

Results and discussion: The MAbs analyzed presented IgM isotypes. Reactivity of anti-S. mansoni MAbs against Sm-UP2IP, 13/43 (30.2%) MAbs showed stronger reactivity. It was observed that one of the MAbs cross-reacted with antigen associated with S. haematobium urinary antigen (Sh-UP2IP) in dot-blot analysis. Nine (9/13, 69.2%) MAbs recognized glycoprotein antigenic epitopes of Sm-UP2IP and Sm-SWA. On the other hand, 4/13 (30.8%) MAbs recognized carbohydrate antigenic epitopes. Band size of 8.9 kDa associated with Sm-UP2IP was detected by 13 MAbs. With Sm-SWA, all the MAbs detected band sizes of 177.8 and 158.5 kDa. In addition, three MAbs recognized a 38.9 kDa band.

Conclusion: The generation of anti-S. mansoni species-specific MAbs offers opportunities to develop a specific MAb-based diagnostic tool for use in the field to detect Schistosoma mansoni. It is also essential to identify the proteins of the Sm-UP2IP using sensitive proteomic techniques.

P-36.00

COMPREHENSIVE PROTEOMIC PROFILING OF BEVACIZUMAB-RESISTANT GLIOBLASTOMA MULTIFORME

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Prognosis for patients diagnosed with glioblastoma multiforme (GBM) is poor and few survive longer than three years. The vast majority of GBMs recur after the standard frontline chemo-radiotherapy protocol and relapsed lesions are generally unmanageable, with median survival times of a few months post-recurrence. Drugs that impair tumour angiogenesis, i.e. therapeutic antibody anti-vascular endothelial growth factor, bevacizumab (BEV), are becoming standard therapy for recurrent GBM, despite having no impact on overall survival times. Resistance to BEV is fatal, and mechanisms are largely unexplored.

With access to exceedingly rare fresh-frozen serial GBM tumours, we performed comprehensive quantitative proteome analyses to identify important mechanisms of BEV escape and tumour recurrence. Tumour tissues from three patients [primary (n=2), recurrent (n=2) and post-BEV recurrent (n=3)] were homogenised, clarified (1,000 x g, 4ºC) and ultracentrifuged (100,000 x g, 4ºC) to isolate the soluble (SOL) proteome supernatant from the microsomal (MEM) pellet. Digested SOL and MEM proteomes were analysed by two independent quantitative MS/MS approaches; Label-free quantitation performed on spectra obtained in triplicate using an Orbitrap Velos (Thermo Electron) and 4-plex iTRAQ-labelling coupled ERLIC-RP MS/MS analysis using a 5600 TripleTOF® (AB Sciex; single run for MEM; duplicate run for SOL). Spectra were processed using Mascot Distiller, Progenesis, Scaffold and ProteinPilot™ softwares.

This multi-centre proteomics project has achieved a number of highly reproducible and comprehensive quantitative proteome datasets (average of 1760 MEM proteins and 2334 SOL proteins identified at 95% confidence levels) from precious serial GBM specimens.

Significant differentially abundant proteins include those involved in Rho regulation of actin-based motility and cytoskeleton and endocytosis signalling. Bioinformatics analyses with captured whole exome sequencing data are underway to define novel mechanisms of evasive resistance to BEV in recurrent GBM.

P-37.00
MELANOMA; THE UNFOLDED PROTEIN RESPONSE AND MEMBRANE-ASSOCIATED PROGESTERONE RECEPTOR COMPONENT 1

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Introduction and Objectives
Metastatic melanoma is a lethal neoplasm with rapid progression and systemic dissemination. The 5-year survival rate of metastatic melanoma is just 6%-18% for patients with distant metastases. This is due not only to the aggressive nature of the disease but also the current lack of effective therapies. Activation of the unfolded protein response (UPR), an endoplasmic reticulum stress response, in melanoma positively correlates with tumour progression, metastasis and poor outcome. The complete action of the UPR is yet to be determined, and its role in cancer progression is undefined.

Method
In the present study, proteomic analyses have been used to identify proteins involved in the UPR and to elucidate its role in melanoma. Subcellular proteomes of human melanoma cell lines (Mel-RM and WMM1175) treated with thapsigargin (TH), an inducer of ER stress, were labeled by iTRAQ and anaylsed using 2D LC-MS/MS. Differentially abundant proteins were validated using selected reaction monitoring (SRM) mass spectrometry and Western blotting. Membrane-associated progesterone receptor component 1 (PGRMC1) was labeled with Cy5 conjugated antibody and the sub-cellular trafficking with TH treatment monitored by fluorescent microscopy. Finally knock-downs of PGRMC1 using siRNA were performed and changes in cell proliferation/viability observed.

Results and Discussion
Following ER-stress and activation of the UPR, 80 differentially abundant proteins common to both cell lines were identified by mass spectrometry. These proteins are involved in stress response, cell migration and adhesion, and apoptosis/survival. PGRMC1 is up-regulated and translocated to mitochondria as a result of activation of the UPR. PGRMC1 has been linked with cancer progression and chemotherapeutic resistance.

Conclusions
The UPR affects several important oncogenic proteins and pathways, such as Akt and MAPK pathways, showing the importance of the UPR in melanoma progression. The differentialey abundant proteins identified here extend our understanding of how the UPR contributes to melanoma tumourigenesis.

P-38.00

PROTEOMIC DYNAMICS IN UTERINE CERVICAL CANCER TUMORS FROM THE CANCER CELL LINES HELA AND SIHA.

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Cervical-uterine cancer (CuCa) is the third most common malignancy affecting women worldwide and is the major cause of morbidity and mortality in developing countries. During the last years, a significant effort has been made using high-throughput transcriptomics profiling technologies in order to identify genes associated with carcinogenesis of CuCa and there are some studies in the literature to describe the expression levels of proteins, but there is no report published to date, about the dynamic proteomic changes in tumor development in CuCa.

The proteomic expression during tumor development can be tackled with the induction of tumors, by xenotransplantation of cervical cancer cells in the mouse model nu/nu. Under a controlled environment, the changes of the proteome can be followed over time, and then establish those adaptations that are need over the progression from one stage to the next one in CuCa.

Using 2D-PAGE followed by MALDI-TOF-MS we have identified differentially expressed proteins over tumor development of CuCa tumors, from inoculated female nude mice with the cancer cell lines HeLa and SiHa. Once we established the proteomic dynamics over this period of time, we select the GSTP1 and GSTM3 proteins because of their strong expression and biological function. GST´s are detoxification enzymes and conjugate the tripeptide glutathione to a wide variety of endogenous metabolites, xenobiotics, and chemotherapeutics.

We made the knockdown of these proteins in six different tumor cell lines (HeLa, CaLo, SiHa, Caski, Colo-205 and MDA-231) and we found that the tumor development decreased in 4 tumors cell lines with the GSTP1 knockdown and in 3 tumor cell lines with the GSTM3 knockdown.

We are further investigating if these knockdowns have an effect in the signaling pathways of the kinases p38, JNK and ERK. Results will be presented and further discussed. Part of this work was supported by PAPIIT-UNAM grant-IN206113

P-39.00

TARGETED PROTEOMIC IMMUNOASSAYS OF CANDIDATE PLASMA BIOMARKERS FOR STAGE A-D CRC

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Introduction and objectives

Colorectal cancer (CRC) is one of the top three most common cancers worldwide. Cancer specific biomarkers are found in low concentrations (pg- ng/mL) in plasma as a result of structural changes in the microenvironment of cancer tissues followed by dilution in plasma. The current methods widely deployed for CRC screening are grossly inadequate on sensitivity and specificity ground. This study was designed to meet the longstanding unmet clinical need for earlier CRC detection by validating plasma biomarkers of CRC onset and progression.

Methods

Clinically stage CRC (Duke's A, B, C, D) and control (i.e., unaffected) EDTA plasma samples were obtained from 15 patients (age, sex matched with stringent exclusion/ inclusion criteria). Expression of 92 human proteins was measured from 1uL of plasma deploying Proseek® Multiplex Oncology I kit with high throughput (96 samples x 96 analytes) and real-time PCR. A duplicate set of samples were analyzed utilizing Bio-Plex Pro™ human cytokine 27-plex immunoassay kit. Finally the expression values were statistically analyzed and compared between stages

Results and Discussion

Similar expression pattern for 9 common antigens endorses the efficiency of Proseek® Multiplex Oncology I as an immune based assay for biomarker research. The PEA technology confirms that expression of CEA (a diagnostic biomarker for CRC) is significantly high in malignant stage ie. it is not an early stage biomarker. In addition, PDGF subunit B and IL 8 have significant level of expression between control, benign and malignant stages.

Conclusion

Existing immune assays are efficient tools for shape recognition, which can lead to false positive results. Knowledge about location of epitopes is important in order to design multiple epitope based targeted immune assays. Multiplexing technology provides suitable opportunities for accurate quantitation of biobanked samples.

P-40.00

PROTEOMIC ANALYSIS OF NXS2 NEUROBLASTOMA CELL LINE TREATED WITH FASTING IN COMBINATION WITH DOXORUBICIN

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Introduction and objectives
Neuroblastoma (NB) is the most common extracranial solid tumor in childhood whose prognosis is grim in a half of patients. In NB mouse models, cycles of fasting (short-term starvation-STS) plus chemotherapy, suppressed tumor growth resulting in long-term cancer-free survival.
Aim of this study is to analyze the proteomic profiles of NB cells in order to understand the molecular pathways modulated by STS in combination with doxorubicin (DXR).

Methods
The in vitro effect of DXR in combination with STS was tested in the murine NB cell line NXS2 by Trypan Blue, CFSE, BrdUdr and Annexin V staining.
Proteomic analysis was performed with a quantification strategy based on a label free approach (LFQ), that can provide robust and precise relative protein expression. The critical task of interpreting the proteomics data is allowed by a statistical data analysis, applying tools like PCA, T-Test, ANOVA, Clustering, Gene Ontology Enrichment and Perseus.

Results and Discussion
The in vitro experiments confirmed that STS in combination with DXR maximally reduced cell viability and triggered an increased of apoptosis compared to DXR alone. More than 6000 proteins derived from NXS2 cell line were identified in each group (control, STS, DXR, STS+DXR). Each group of proteins showed a significant segregation in PCA analysis.
Our results demonstrated that the combination of STS+DXR differentially modulated several proteins involved in proliferation, apoptosis and metabolism pathways, as compared to STS and DXR as single agents.

Conclusions
Our study provides evidence that LFQ based on high resolution mass spectrometry is an useful and specific technique to define the modulation of signaling pathways by STS plus chemotherapy.

P-41.00
QUANTITATIVE MS-BASED ANALYSIS OF HISTONE MODIFICATION PATTERNS AS MARKERS FOR RESPONSE TO HDAC INHIBITORS IN BREAST CANCER

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Introduction and objectives
Abnormalities in histone post-translational modification (hPTM) patterns are frequently implicated in the development of cancers and could represent biomarkers for drug response and patient stratification. Histone deacetylases (HDACs) are a class of enzyme that deacetylate histone lysine residues and have emerged as attractive targets for the therapy of various diseases, including breast cancer. Starting from our observation that primary breast cancer cells in culture display different sensitivities to HDAC inhibitors we sought to identify the epigenetic biomarkers that determine cellular responses to inhibition of HDAC in breast cancer by using mass spectrometry approaches.

Methods
We performed proliferation assays to identify groups of breast cancer cell lines that are either sensitive or resistant to HDAC inhibitors and employed a novel analytical platform that combines ultra-high pressure liquid chromatography with high resolution mass spectrometry analysis on a Q Exactive instrument to carry out a comprehensive analysis of their hPTMs patterns. We combined this approach with stable isotope labeling with amino acids in cell culture (SILAC), using a super-SILAC set up where a mix of heavy-labelled breast cancer cells served as spike-in reference for comparative analysis with unlabelled cells.

Results and Discussion
We profiled 24 distinct modifications at 14 different sites on histone H3 and H4, covering well-characterized histone marks, in addition to identifying novel or poorly characterized modifications. Comparison of breast cancer cells that are sensitive or resistant to HDAC inhibitors in the presence and in the absence of the compounds revealed hPTM patterns that could be associated with the cellular response to the drugs and pinpointed the modifications affected by HDAC inhibitors with different specificities.

Conclusions
The robust method that we have developed for the identification of hPTM in breast cancer cell lines could be easily applied to the analysis of primary samples and formalin-fixed-paraffin embedded breast biopsies.

P-42.00
FFPE TISSUE PROTEOMICS: WAKING SLEEPING BEAUTY

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Introduction and objectives

With their vast collections of diseased tissues and associated clinical records describing diagnosis, prognosis, therapy, and outcome, formalin-fixed, paraffin embedded tissue repositories represent an invaluable resource for performing retrospective proteomic investigations. Unfortunately, however, the formalin fixation process applied to tissues in order to enable microscopic diagnosis procedures leads to the establishment of highly stable intra- and intermolecular crosslinks, making protein extraction a significant challenge.

Methods

In the latest years, our research group concentrated its efforts on the development of sample treatment procedures enabling extraction of high-quality, full-length proteins from the fixed specimens, leading to the development of an optimized protocol with unprecedented performances.

Results and Discussion

Here, we illustrate the method workflow and summarize the results obtained with different tissue sample repositories. Specifically, human and animal cancers were investigated with established proteomic pipelines consisting of extraction, electrophoresis, mass spectrometry, and data processing by ontology and pathway analysis. Elevated performances were obtained with the different repositories, and proteins consistent with the disease biology were successfully identified in all cases.

Conclusion

In conclusion, the sample preparation workflow presented here offers the possibility to wake the sleeping retrospective potential that lies within fixed tissue repositories stored in hospitals worldwide, opening the way to biomarker discovery and validation studies on this powerful archive of cancer tissues and their associated clinical information.

P-43.00

IN-DEPTH QUANTITATIVE PROTEOMICS ANALYSIS OF SUBCELLULAR PROTEOME OF KM12 CELLS PROVIDES A GLOBAL VIEW OF COLORECTAL CANCER METASTASIS

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Introduction and objectives
Colorectal cancer (CRC) is the second deadliest cancer worldwide, because half of CRC patients develop recurrence and liver metastasis. We aimed to study mechanisms underlying CRC metastasis by quantitative proteomics using the KM12 cell system.

Methods
We used SILAC to compare highly-metastatic to liver KM12SM cells with low-metastatic KM12C cells. To increase the depth on the proteome coverage, forward and reverse SILAC experiments were fractionated in five subcellular fractions corresponding to cytoplasm (CEB), plasma, mitochondria and ER/golgi membranes (MEB), nuclear (NEB), chromatin-bound (NEB-CBP) and cytoskeletal proteins (PEB). After subcellular fractionation, proteins were in-gel digested and analyzed by LC-MS/MS using a LTQ Orbitrap Velos. To obtain a general view of the proteome alterations in KM12 cells associated to CRC metastasis, data was analyzed by bioinformatic approaches.

Results and discussion
We quantified 2247, 3837, 3604, 1738 and 1994 proteins in CEB, MEB, NEB, NEB-CBP and PEB fractions, from 2416, 4086, 3836, 1853 and 2117 identified proteins in the same compartments. Among them, we found a total of 1313 quantified proteins with at least 1.5-fold deregulation in KM12SM cells. Although some of these proteins were already described as implicated in metastasis, others might be useful CRC metastasis biomarkers. To obtain network enrichment, protein interaction partners and to get a general picture of CRC metastasis mechanisms, differentially-regulated proteins were analyzed with DAVID and Babelomics suite for functional profiling analysis. Several networks and protein interaction partners not previously associated to CRC metastasis were found, which might help to understand underlying mechanisms associated to CRC metastasis.

Conclusions
We have been able to quantify 1313 differentially expressed proteins implicated in CRC metastasis using SILAC combined with proteome subcellular fractionation. By bioinformatics, we have also been able to clarify mechanisms associated to CRC metastasis and identify key protein interaction partners as potential CRC-metastasis biomarkers.

P-44.00

DIFFERENTIAL PROTEIN EXPRESSION ANALYSIS IN HEPATOBLASTOMA BY 2D-DIGE AND LABEL FREE LC-MS

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Introduction

Hepatoblastoma (HB) is the most common malignant liver tumor in children, although little is known about the intrinsic biological heterogeneity and how it impacts on prognosis. Two prognostic HB subtypes, C1 and C2, have been described by unsupervised analysis of transcriptomic data (Cairo-Armengol, Cancer Cell, 2008). The aim of this work is to complete the characterization of the two HB subclasses at proteomic level by using both gel-based and gel-free approaches, and to identify key players of tumorigenesis as well as prognostic factors and signaling pathways related to tumor aggressiveness.

Methods

Protein was extracted from frozen tissues (8 control, 6 C1, 4 C2) using FastPrep (MpBio) and ultracentrifugation. 2D-DIGE was performed on pH 4-7 24cm strips (GE Healthcare) and 12% polyacrylamide gels. Differentially expressed spots were detected using REDFIN (Ludesi) and identified on an Autoflex MALDI-TOF (Bruker Daltonics). For label-free approach, in solution digested samples (5 individuals/condition) were analyzed on an LTQ-Orbitrap XL (Thermo) coupled to a nanoACQUITY (Waters). Progenesis LC-MS (Nonlinear Dynamics) was used for the detection of differential features. For functional annotation analysis, IPA software was used.

Results

The unsupervised analysis of proteomic data revealed the same two HB subclasses described at the transcriptional level. By supervised analysis, 129 unique proteins distinguishing control and tumor samples were identified. Additionally, 239 proteins that difference C1 and C2 tumors were characterized. A different degree of MYC, MAPK, TGF-β and ERBB2 signaling pathways activation was observed between these two classes.

Conclusions

Our results confirm previously described molecular characteristics of HB cells, and detect novel interesting protein signatures. The differential activation of MYC, MAPK, TGF-β and ERBB2 signaling pathways in the two subclasses suggest they could play a key role in the acquisition of a more malignant phenotype in HB.

P-45.00

PHOSPHOPEPTIDE ENRICHMENT ON A MONOLITHIC COLUMN CONTAINING SUPERFICIALLY IMMOBILIZED NANO TIO2

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Introduction and Objectives

Protein phosphorylation plays an important role in regulating a broad spectrum of key physiological processes like cell signaling, cell growth and cell differentiation. Prior to LC/MS analysis, enrichment of phosphopeptides is a crucial step because of their low stoichiometry in biological sample, longer retention on reversed phase columns, and lower ionization efficiency compared to non-phosphorylated peptides.

The use of metal oxides, most prominently of TiO2 enabled efficient and relatively simple phosphopeptide-enrichment. In this study a new monolithic column from BIA Separations packed with immobilized TiO2-nanoparticles was tested for its ability to enrich phosphopeptides. The TiO2-column was also tested for possible carryover originating from biological samples.

Methods

The enrichment procedure was conducted according to an altered and optimized protocol described by Mazanek et al., sample was loaded onto the column using a syringe pump and five fractions were collected. Briefly: the flow-through fraction, two wash and two elution fractions. The same sample treatment was performed using the "blank" monolithic column, i.e. without immobilized TiO2 nanoparticles.

Resulting fractions were re-injected onto the reversed phase nano separation column and detected with MS.

Results and Discussion

Results show that the "blank" column was unable to discriminate between the phosphorylated and unphosphorylated peptides.

In conclusion, tested monolithic TiO2 columns show significant binding ability for phosphopeptides and are considered as suitable for phosphopeptide enrichment. Additional optimization steps will be applied for better prevention of carryover and for more efficient peptide trapping by using different loading buffers.

P-46.00

THE IDENTIFICATION OF BIOMARKERS FOR LUNG CANCER USING PLASMA COLLECTED PROXIMAL AND DISTAL TO THE SITE OF THE TUMOUR.

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Introduction and Objectives
Lung cancer is the most common cause of cancer related mortality in the world, characterised by late clinical presentation and poor outcomes, with five-year survival in the UK at

Methods
Plasma was collected from pulmonary artery (prior entry into lung) and two pulmonary veins (tumour and non-cancer draining) at the time of surgical resection of squamous cell carcinoma of the lung (n=6 patients). The three sites of collection were than compared using an isobaric tagging based proteomic workflow.

Results and discussion
More than 800 proteins were identified in the three different plasma samples across the six patients, including proteins classified as tissue leakage proteins like CA-125, KRT5, KIT and MET. There was some variation between individuals, but differences in the sample collected from different sites were nonetheless identified. Some of these differences include reduced endothelial marker ESAM and increased MPO which has been validated using ELISA assays on the discovery set. A panel of proteins have been developed for testing in peripheral blood for their lung cancer biomarker properties using SRM and SWATH/MS.

Conclusion
Using a novel approach to sample collection looking for proteins draining out of a tumour specifically, new biomarkers have potentially been identified for diagnosis of lung cancer patients. This panel is now being tested in validation samples taken at the point of diagnosis in the clinic.

P-47.00

PROTEOMICS ARGUES TOWARDS MTOR/PI3K DOMINANCE IN PEDIATRIC MEDULLOBLASTOMA TUMORS WITH 17P DELETION

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Medulloblastoma (MBL) is the commonest pediatric CNS malignancy, for whom, following multimodal therapy with surgery, radiation and chemotherapy, 5-year survival rates approach >60%. Through the integration of genomics, bioinformatics and proteomics, the present study aims at shedding light at the proteomic-related molecular events responsible for MBL pathophysiology, as well as at providing molecular/protein/pathway answers regarding tumor-onset.

Methods and Results:. With 17p loss being the commonest chromosomal aberrance observed in the sample set analyzed, array-CGH was employed to first distinguish for 17p-positive cases, which were further processed. 2-DE coupled to MALDI-TOF-MS, fully exposed the MBL-specific protein profile. Protein profiles of malignant tissues were compared against profiles of normal cerebral tissues and quantitative protein differences were determined. Bioinformatics, functional and database analyses, characterization and classification and sub-network profiling, generated information on MBL protein interactions.

Key molecules of the PI3K/mTOR signaling network were identified via the techniques applied herein. Conclusion: 382 single-gene products were uniquely associated with the MBL phenotype. Quantitative assessment revealed 208 proteins to be up-regulated and 157 down-regulated, in MBL tissues compared to normal cerebral controls. IGF2, PI3K, Raf-1, Rictor, MAPKAP1 , S6K1 , 4EBP1 and ELF4A, as part of the IGF system (implicating PI3K/mTOR) were deemed to be de-regulated. The deciphering of the MBL protein content pinpointed that the mTOR/PI3K signaling activity is of dominant importance in 17p MBL cases.

P-48.00

METHOD DEVELOPMENT FOR THE QUANTITATION OF A HUMAN URINE BIOMARKER FOR ACUTE KIDNEY INJURY USING A QTRAP® MASS SPECTROMETER

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Introduction

Acute kidney injury (AKI) is a common complication in critically ill patients and a reason for increased morbidity and mortality. Neutrophil gelatinase-associated lipocalin (NGAL) is one of the most important inflammatory biomarkers for the early detection of acute kidney injury. Here we present the development of a method for the detection and quantitation of NGAL by mass spectrometry using a QTRAP® 6500 system.

Methods

A method for the detection and quantitation of NGAL in human urine by multiple reaction monitoring (MRM) as well as MRM3 was developed on a QTRAP® 6500 system (AB SCIEX, Framingham, USA). Sample preparation was optimized by spiking urine with increasing concentrations of NGAL. Two methods for protein purification were tested with regard to the reproducibility of quantitation and recovery of the potential biomarker: Protein precipitation with ice cold methanol and protein purification using centrifugal filter devices. The protein purification steps were followed by tryptic digestion and quantitation of selected NGAL peptides by MRM /MS3 on the QTRAP® system.

Results and Discussion

The detection and quantitation of protein biomarkers by mass spectrometry has advantages in comparison to antibody based assays due to the higher specificity and therefore lower risk of cross-reactivity. A multiple reaction monitoring method as well as a MRM3 method for six tryptic peptides of human NGAL was optimized. The evaluation of the two different sample preparation methods clearly showed that a purification using protein precipitation has higher protein losses with more influence on the low NGAL concentrations, which led to a wrong slope of the calibration curve. The CVs differ for the tested NGAL peptides. The best peptide candidates for a reliable quantitation were the NGAL peptides with the sequences SYNVTSVLFR and SYPGLTSYLVR. These peptides showed CVs < 12% for all concentrations, which reflect the reproducibility of the whole workflow.

P-49.00

COMPARISON OF EARLY STAGES OF COLORECTAL CANCER FFPE SAMPLES BY LABEL FREE PROTEOMICS

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Introduction and objectives: Colorectal cancer (CRC) is the second most frequent cancer in women and the third in men. Identification of the mechanisms of progression in these early CRC stages is important to develop new diagnostic and therapeutic tools. Formalin-Fixed Paraffin-Embedded (FFPE) specimens are materials that enable proteomic clinical research. Hence our aim was to address comparison of early stages FFPE samples from CRC patients using shotgun proteomic analysis.

Methods: We performed a retrospective study on 36 CRC tissue samples (T1N0M0, n=16 and T2N0M0, n=20) compared together and with 40 control tissue samples (20 patients with diverticulitis, using paired inflamed (I) and healthy tissue (HC). Each tissue slice was macrodissected to select only epithelial cells. We used FFPE-FASP for sample preparation and protein digests were analyzed using 2D-nanoAquity UPLC separation online with Q-Tof Synapt HDMSTM G2 using ion mobility as additional separation. We performed protein identification and differential analysis using TransOmics (Waters, Corp., Milford, USA) with global normalization and unique peptide quantitation.

Results and discussion: We selected 151 proteins differentially distributed between T1 and T2 CRC stages which are not significantly distributed between CRC and HC or I. Only 30 proteins were significantly more abundant in T1 versus T2 and 121 were distributed inversely, with a minimum fold ratio of 2. ATP synthase subunit beta, Aspartate-tRNA ligase, Haptoglobin and Kininogen were identified and previously pointed as potential CRC biomarkers. Others proteins which also require further validation, are implicated in molecular functions as binding, metabolism or are matrix structural proteins which might be characteristic of epithelium T1 to T2 CRC progression changes.

Conclusion: This FFPE retrospective study highlighted proteins previously identified as potential CRC biomarkers and some proteins that might be involved in early CRC stages physiopathological progression and which might be potential early CRC biomarkers.

P-50.00

DECIPHERING THE RESISTANCE MECHANISMS TO EGFR TYROSINE KINASE INHIBITORS IN NON-SMALL CELL LUNG CANCER: A QUANTITATIVE PROTEOMIC APPROACH

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Current targeted therapies prescribed in non-small cell lung cancer (NSCLC) such as gefitinib, are inhibitors of the epidermal growth factor receptor tyrosine kinase activity (EGFR-TKI). As most patients developed resistances, many efforts were made to understand the resistance mechanisms and were focused mainly on gene mutations. Last year, two studies were published regarding respectively the effect of gefitinib treatment on the level of expression of proteins involved in angiogenesis and invasion process (Box et al. 2013) and the changes in phosphorylation on tyrosine residues (Tzouros et al. 2013). But there is still a lack of a global proteomics study. Thus, this study aimed to identify the proteins involved in the mechanism of resistance to apoptosis in NSCLC cell line resistant to EGFR-TKI, such as H358.

To achieve this goal, we used iTRAQ quantitative proteomics approach to determine the proteins, which expression was modified upon gefitinib treatment. Practically, H358 cells, were treated or not with gefitinib for 96h. Apoptosis assays were performed by a flow cytometry analysis of active caspase-3 (immunostaining). Proteomics approach was carried out by performing trypsin digestion on extracted proteins before labelling the resulting peptides by iTRAQ reagent. Then, peptides were fractionated by nano-HPLC and analysed by MALDI TOF/TOF in MS & MS/MS modes. The data were analyzed by Protein Pilot software in an iTRAQ relative quantitation approach.

Using such approach, we identified 1217 proteins with 150 proteins whose expression was modified upon gefitinib treatment. We performed an ontology enrichment and network analysis and found some anti-apoptotic proteins significantly upregulated after gefitinib treatment. Interestingly, the main processes identified as dysregulated were related to mRNA processing, cell cycle and apoptosis regulation.

These results will highlight the resistance mechanism to gefinitib in NSCLC, and therefore, could identify new targets to overcome this resistance.

P-51.00

PROTEOMIC ANALYSIS OF SECRETOME AND TOTAL CELL EXTRACT FROM NORMAL AND MALIGNANT CERVICAL CELL LINES

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Introduction and objectives

Cervical cancer represents the second most common malignancy in women worldwide, and HPV (human papilloma virus) is its main cause. Malignant transformation is caused mainly by infection from HPV16 or HPV18, combined with a constitutive expression of viral oncoproteins E6 and E7. However, the eventual steps leading to cancer remain to be elucidated. The cell secretome represents the collection of the entire macromolecules secreted by a cell, and constitutes a vital aspect of cell-cell communication. During carcinogenesis, the secretome composition is altered, reflecting the acquisition of hallmarks of cancer and probably contributing to the individual stages of carcinogenesis. Also the differences between the total cell extract and secretome appear to be significant during the progression of malignant transformation. In this study, we evaluated systematically four cervical cancer cell lines in order to reveal putative biomarkers, deriving from both total cell extract and secretome.

Methods

The total cell extract and secretome of normal human keratinocytes HCK1T, was compared to different cervical cancer cell lines [C-33A (HPV-), SiHa (HPV16+), HeLa (HPV18+)]. Proteins were analysed by 2D-electrophoresis. Spot quantification was performed by the PDQuest8 software. Differentially expressed protein spots were identified by MALDI-TOF/TOF MS.

Results and Discussion

The secreted proteins displayed a fold enrichment factor of 3, 2.6 and 5 times higher for SiHa, HeLa and C-33A respectively in comparison to the proteomic analysis of total cell extract. Cofilin-1, a protein related to cytoskeletal remodeling was significantly upregulated in HeLa and C-33A cells, compared to HCK1T. Cathepsin D, a secreted protease was upregulated in cancer cell lines compared to control HCK1T. These proteomic results were further validated by Western Blot.

Conclusions

The proteomic analysis of total cell extract and secretome of cervical cancer cell lines, provide putative biomarkers, such as Cofilin-1 and Cathepsin D for further clinical validation.

P-52.00
MULTIPLEXED MRM FOR THE VERIFICATION OF PLASMA PROTEINS BIOMARKER: APPLICATIONS TO HEPATOCELLULAR CARCINOMA DEVELOPMENT AND METASTASIS

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Hepatocellular carcinoma is one of the most highly aggressive and lethal of all solid malignancies. Plasma protein biomarkers have been valuable in the early diagnosis of hepatocellular carcinoma and the use of multiple biomarkers has been suggested to overcome the low specificity of single ones.

For biomarker development, Multiple reaction monitoring (MRM)-mass spectrometry (MS) with stable isotope-labeled standards (SIS) has proven adept in rapidly, precisely, and accurately quantifying proteins in complex biological samples. In this study, we used this approach to quantitate 14 candidate hepatocellular carcinoma marker proteins in human plasma by using ultra-high performance LC and an AB SCIEX 6500 triple quadrupole mass spectrometer.

The proteins have been reported to be associated to hepatocellular carcinoma development and metastasis. We implemented measures to minimize the analytical variability, improve the quantitative accuracy, and increase the feasibility and applicability of this multiplexed MRM - based method. Further, quantification and validation these proteins in huge sample herd will facilitate protein biomarker panels for clinical use.

P-53.00

MASS SPECTROMETRY-GUIDED DEVELOPMENT OF A LUMINEX-BASED DIAGNOSTIC TEST FOR THE REDUCTION OF UNNECESSARY PROSTATE BIOPSIES

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Prostate cancer (PCa) is the most frequently diagnosed cancer in men. Prostate specific antigen (PSA) has a low specificity in the detection of PCa, which results in high numbers (about 70%) of negative prostate biopsies. ProteoMediX' biomarkers were discovered using a genetics-guided discovery approach focusing on the PI3K/PTEN cancer pathway (Schiess et al.(2009), Mol Oncol 3,33-44; Cima et al.(2011), Proc Natl Acad Sci U S A 108,3342-7 and Kälin et al.(2011), Eur Urol, 60,1235-43). Our objective was to develop immunoassays for biomarkers previously identified by label-free mass spectrometry and verified by targeted proteomics (SRM).

Proprietary reagents including protein reference standards and antibodies were generated for the individual biomarker candidates. Subsequently, sandwich immunoassays were developed and validated according to bioanalytical FDA guidelines. The concentrations of the biomarker candidates were determined in a patient sample cohort consisting of 397 patients that underwent prostate biopsy.

Four Luminex-based immunoassays for OLFM4, ASPN, CTSD and ICAM1 were tested in a subset of 132 patients with a total PSA (tPSA) level between 2.0 and 10.0 ng/ml. This range is considered as a "diagnostic grey zone", where increased PSA may be caused by benign conditions and therefore, large numbers of false positive cases occur. Urologists might use percent free-to-total PSA (%fPSA) as an additional aid to decide if a biopsy is necessary, if tPSA is at such an intermediate level.

The ProteoMediX` biomarker panel in combination with tPSA, %fPSA and age revealed a significantly higher area under the receiver operating characteristic (ROC) curve (AUC) of 0.868 (95% CI=0.807-0.929) compared to %fPSA with an AUC of 0.772 (95% CI=0.691-0.852). In addition the specificity was increased to around 60% at a sensitivity of 90%.

The implementation of a multiple biomarker test in clinical practice has the potential to significantly lower the rate of unnecessary biopsies by more than half.

P-54.00

MS-BASED IMMUNOASSAYS FOR TRANSPORTER QUANTIFICATION IN XENOGRAFT-BASED TUMOR MODELS

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Nmi

Multidrug resistance (MDR) describes the resistance against structurally diverse substances that affect cells by different mechanisms. MDR can develop during chemo-tumor-therapy and is conveyed by several mechanisms e.g. up-regulation of ABC transporters which mediate increased efflux of xenobiotics. These transporters are essential to maintain barriers in several organs (blood-brain-barriers, placenta-barrier…). Additionally, transporters are a component of the xenobiotics metabolism. However, transporters cannot be detected by conventional bioanalytical methods such as sandwich immunoassays because it is very difficult to generate good antibodies against these hydrophobic multi-transmembrane proteins. For this reason, we developed a test system to quantify the expression level of transporters via mass spectrometry-based immunoassays.

Hydrophobic proteins are measurable by this method because samples are digested with trypsin and subsequently analyzed on peptide level. From each protein, one peptide which can be assigned unambiguously, is identified via tandem MS and quantified by means of an isotope labeled reference. Prior to MS-read-out the peptides are enriched by antibodies which recognize a very short c-terminal epitope. These epitopes are selected in such way that they are common in peptides derived from several transporters and therefore allow the analysis of transporter groups with few antibodies. Currently, we have generated 29 antibodies covering 23 ABC and SLC transporters in eight different species - in total we target 108 proteins.

The assays were used to analyze xenograft tissue derived from different human tumors and drug-resistant cell lines. Results show tumor-specific transporter profiles. More data for cell derived and tumor derived xenografts will be gathered for setting up a quantitative drug- and tumor-related transporter profile. That might allow the prediction of drug candidates´ potential to mediate transporter-based drug resistance in future. Finally, the detection of specific transporters in the tissue of tumor patients might allow treatment decision if data about transporter substrate specificity is available.

P-55.00

CAN PROFILING FOR PLASMA PHOSPHORYLATION BE A USEFUL TOOL IN AIDING THE DIAGNOSIS OF ALZHEIMER'S DISEASE?

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Introduction and Objectives: A blood-based biomarker of Alzheimer's disease (AD) would have great utility to identify subjects at risk prior to disease symptoms and could be easily adapted for population screening. There is an urgent need to discover a cost-effective and reproducible test to aid an early diagnosis, initiate disease-modifying treatment, as well as monitor disease progression or treatment end-points.

Post-translational modifications (PTMs) play an important role in the physiological functions of proteins. The most prevalent modifications in plasma include glycosylation, acetylation as well as phosphorylation. These modifications have all been associated with the pathogenesis of AD. One of the classical pathological hallmarks of AD is intracellular neurofibrillary tangles predominantly comprised of hyperphosphorylated Tau. Despite its critical involvement in AD pathogenesis and substantial evidence of phosphopeptides in plasma changes in protein plasma phosphorylation has been not been investigated in relation to disease. We thus hypothesise that a more detailed profiling of phosphorylated proteins in plasma may reveal biomarkers with specific modified isoforms in AD.

Methods: Peripheral biomarker discovery was undertaken using two common phosphorylation enrichment strategies in combination and as stand-alone strategies; Titanium dioxide (Ti02) and Fe-NTA IMAC columns. Phosphorylated peptides were measured using an Orbitrap Velos Pro (Thermo Scientific) mass spectrometer and identified using Proteome Discover 1.3.

Results and Discussion: Results will attempt to confirm plasma phosphorylation sites observed in previous studies and demonstrate the utility of combing two enrichment strategies for plasma phosphorylation identification. We will also present preliminarily data for a subset of subjects to identify a phosphorylation signature of AD in blood.

Conclusion: Phosphorylation in plasma is a regular and measurable event that could be utilised for biomarker research. The phosphorylated proteins measured in this study will need to be tested in larger cohort to assess their predictive ability of Alzheimer's disease.

P-56.00

MALDI TOF/TOF DETERMINATION OF SERUM PLASMINOGEN SIALYLATION PROFILE FROM PATIENTS WITH GASTRIC PRECANCEROUS LESIONS

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The current diagnosis of gastric mucosa lesions relies almost exclusively in endoscopy followed by biopsy, which is both invasive and costly to apply for screening strategies. Therefore, biomarkers which can aid in the screening and identification of individuals with silent gastric pathologies are highly needed. Glycosylation is a common post-translational modification of proteins with more than 50% of eukaryotic proteins thought to be glycosylated. In gastric pathologies the glycosylation alterations include aberrant expression of simple mucin type carbohydrate antigens, namely sialyl-Tn (Neu5Acα2-6GalNAcα-O-Ser/Thr). We have recently identified Plasminogen as a circulating protein carrying sialyl-Tn in the serum of patients with gastritis and intestinal metaplasia (1).

In order to characterize the plasminogen glycosylation sites containing sialyl-Tn, sialoglycopeptides from plasminogen of the different clinical groups were enriched by titanium dioxide chromatography. Using this method we have detected the three O-glycosylation sites and one N-glycosylation site previously described in human plasminogen. Based on this approach we found 1 sialyl-Tn-containing glycopeptide in healthy controls, 5 in gastritis, 4 in complete IM and 8 in incomplete IM.

Structural analysis was performed in order to validate immunoblotting assignments regarding the expression of sialyl-Tn in plasminogen. The protein purified by affinity chromatography using Lysine-sepharose, further separated by SDS-PAGE electrophoresis was chemically de-O-glycosylated in gel. The released O-glycans from plasminogen were then permethylated to avoid desialylation by MALDI in-source and metastable decay and analyzed by MALDI-TOF/TOF. Further analysis of enriched for sialic acid containing glycoproteins from gastritis, intestinal metaplasia, and carcinoma patients confirmed sialyl-Tn reactivity of plasminogen in an independent set of samples.

Reference:
1) Gomes C, ..., Osório H (2013). Glycoproteomic analysis of serum from patients with gastric precancerous lesions. J Proteome Res, 12, 1454-1466.

P-57.00

DECIPHERING ALTERED CELL SIGNALING PATHWAYS IN B-LYMPHOCYTIC CHRONIC LEUKEMIA (B-CLL) BY FUNCTIONAL PROTEOMICS APPROACHES

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In post-genome era having sequence the human genome, one of the most important pursuits is to understand the function of gene-expressed proteins. The overwhelming size and complexity of human proteome requires very high-throughput techniques for rapid analysis. Despite significant advancements in molecular biology and genetic tools, this demand has not been satisfied and only a small fraction of the proteome has been understood at the biochemical level. Systems Biology and Proteomics strive to create detailed predictive models for molecular pathways based upon quantitative behavior of proteins.

Understanding these dynamics networks provides clues into the consequence of aberrant interactions and why they lead to B-chronic lymphocytic leukemia. Historically, methods capable of collecting quantitative data on biochemical interactions could only be used for one or a few proteins at the time.

Protein microarrays allow hundreds to thousands of proteins to be analyzed simultaneously, providing an attractive option for high-throughput studies such as protein-protein interaction, differential protein profiles,… A novel bead suspension array system, designed and developed by Prof. Lund-Johansen, based on color coded beads which are compatible with flow cytometry, allows measuring many proteins simultaneously because this novel approach offers the advantage that hundreds of different proteins or antibodies can be codified in specific color combination; in addition, it is combining with size resolution chromatography and subcellular fractionation, by this way, it is possible to determine protein complexes and/or specific protein identification.

We will present differential protein profiles (such as BCL2, SOS, LYN,… among others) obtained from normal B cells and aberrant B-cells from chronic lymphoid leukemia (with different cytogenetic alterations and well-characterized immunophenotype).

P-58.00

KNOCKDOWN OF C-FOS SUPPRESSES THE ACTIVATION AND ECM PROTEINS PRODUCTION OF PANCREATIC STELLATE CELL

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Pancreatic cancer represents the fourth leading cause of cancer-related deaths in Western Europe and North America. Although the last years have seen much progress in understanding pancreatic cancer biology, the main question is still awaiting an answer: what is making pancreatic cancer such an aggressive disease? Evidence has suggested a primary role of reactive stroma as the main contributor to pancreatic cancer lethality. Recent observations have shown that the most common marker of reactive stroma in pancreatic cancer is the transformed some of cells to myofibroblast-like cells.

This cell type is known as activated pancreatic stellate cells (PSCs), and identified by their ability to express α-smooth muscle actine (α-SMA) and increased responsiveness to proliferative and fibrogenic cytokines. The current study aimed to demonstrate that c-Fos is required for TNF expression and for activation of pancreatic stellate cells. Here we have investigated whether inhibition of c-Fos expression in PSCs would result in reduced TNF-α expression and suppression of growth and activation in PSCs in vitro.

We transfected PSCs with c-Fos small interfering RNA (siRNA) or with the corresponding control siRNA. Our data demonstrated that siRNA-c- Fos significantly suppressed both PSCs activation, and TNF-α production. Further, we established that siRNA-c- Fos inhibits the progression of experimental fibrogensis of PSCs. knock down of c-Fos inhibited the production of collagen type I, fibronectin factors that play important roles in tumor – stromal interactions in pancreatic cancer.

Overall, our results demonstrate that blockade of TNF-α production by siRNA-c-Fos effectively suppressed activation and ECM proteins production in vitro.

P-59.00

PROTEIN EXPRESSION PROFILES IN B-CLL PATIENTS WITH CYTOGENETIC AND MOLECULAR ALTERACTIONS

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B-cell chronic lymphocytic leukemia (B-CLL) is the most common leukemia in the Western world, and more than 80% B-CLL cases show genomic alterations. The effect of cytogenetic and molecular abnormalities on the proliferation of B cells in B-CLL is still unknown. Biological factors, such as Zap70, CD38 expression or TP53 alterations, have been associated with the development of the disease. Nevertheless, the clinical heterogeneity is not yet entirely explained. Recent studies have highlighted the importance of chromosomal structural changes and mutational status for stratifying patients.

The most common alterations are related to mutational status of NOTCH1 and immunoglobulin heavy-chain variable-region genes IGHV, and cytogenetic disorders involving del(13q14), del(17p13), and trisomy 12.Here, we propose the employment of expression protein microarrays for the screening of protein patterns in genomic-altered B-CLL patients compared with normal counterpart. For the isolation of cytoplasmic protein fraction, a two-fraction lysate extraction protocol was performed in CD19+ CD5+ B-lymphocytes from B-CLL patients (with known genomic alterations) and healthy donors both isolated from peripheral blood. IgG-depleted soluble proteins were labeled with Cy3 or Cy5 at dye/protein ratio > 2 and incubated in Panorama® Antibody Microarrays. Conventional statistical packages (Cy-Z score, reference design and balanced-block ratios), and array software analyses (MeV, Cluster 3.0, Qlucore) were applied. Different assays were performed to screen the proteins and the related pathways involved in NOTCH1, RB1, TP53, del(13)(q14), and trisomy 12 alterations.

Our primarily studies show differences between the B-CLL and the normal counterpart, and between genomic aberrations. The different regulation of the proteins was studied by Ingenuity Pathway Analysis revealing the alteration of proliferation, migration, or apoptosis pathways. Specifically, interleukin production and signaling of numerous molecules were altered in patients with genomic alterations.

This study could provide information for the diagnosis, prognosis and treatment as well as improvement in the knowledge of B-CLL.

P-60.00

OPTIMIZED NONLINEAR GRADIENTS FOR REVERSED-PHASE LIQUID CHROMATOGRAPHY IN SHOTGUN PROTEOMICS

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Reversed-phase liquid chromatography has become the method of choice for separating peptides in most of the mass spectrometry-based proteomics workflows of today. Typically, the peptides are released from the chromatography column by gradually adding an organic buffer according to a linear function.

When applied to complex peptide mixtures, this approach leads to an unequal spread of the peptides over the chromatography time. To remedy this shortcoming, we propose to substitute the conventional linear functions with non-linear ones, customized for each setup at hand. We developed an algorithm to generate such optimized gradient functions for shotgun proteomics experiments, and evaluated it for a dataset consisting of four replicates of a human complex mixture.

Our algorithm calculates two distinct gradients, denoted in silico-optimized and MS1-optimized. To generate the in silico-optimized gradient, we perform an in silico digestion of the proteome, use a predictor to estimate the retention time of the resulting peptides, and then compute the gradient that gives an equal spread of these peptides over the separation time. For the MS1-optimized gradient, we extract the most abundant MS1 ions observed in a run using a linear gradient, and then calculate the gradient corresponding to a uniform spread of these ions.

Our data showed that the optimized gradients produced the expected retention time distributions for the theoretical peptides and the abundant MS1 ions, respectively. Furthermore, the optimized gradients yielded increased numbers of peptide identifications at a fixed false discovery rate. The peptide identifications obtained using the non-linear gradients differed considerably from the ones generated by the linear ones. In addition, the peptide identifications based on the non-linear gradients were as reproducible as the ones generated by linear gradients. Furthermore, a substantial proportion of the peptides identified only with the optimized gradients eluted in the most crowded areas of the linear runs.

P-61.00

SHOTGUN PROTEOMIC ANALYSIS OF PANCREATIC CANCER STEM CELLS

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Introduction and objectives
Pancreatic adenocarcinoma (PDAC) is initiated and sustained by a subpopulation of cells defined as cancer stem cells (PCSCs). PCSCs are responsible for pancreatic tumorigenesis, tumour progression, and resistance to chemotherapeutic treatment.
The aim of this work is the identification of new biomarkers or diagnostic/therapeutic targets of PDAC, through a proteomic comparison of a PDAC cell line and the derived PCSCs.

Methods
In this study, we performed an iTRAQ 8-plex/2D-LC-MS/MS analysis to compare the secretomes and whole proteomes of Panc1 and PCSCs cell lines.

Results and Discussion
The iTRAQ approach allowed us to identify 1146 proteins. Out of these, 111 proteins were found have higher abundance in the conditioned media as compared to the whole cell lysate of Panc1 and PCSCs cells. By using secretion prediction tools, we have found that 25% of the 111 proteins is exported by the classical secretory pathways, while the 75% is secreted by non-classical pathways. Then we performed a quantitative comparison of Panc1 and PCSCs secretomes, and whole proteome, identifying 71 and 162 differentially expressed proteins respectively.
The global implications of secreted and intracellular differentially regulated proteins were elucidated by the Ingenuity Pathway Analysis (IPA). The differentially expressed proteins are involved in cell proliferation, invasion and contribute to epithelial-mesenchymal transition (EMT).

Conclusions
Our results indicate that iTRAQ technology is a promising way to investigate the PCSC biological model and to find useful biomarkers for PDAC.

P-62.00

PROTEOMIC ANALYSIS OF CONNEXIN 43 REVEALS NOVEL INTERACTORS RELATED TO OSTEOARTHRITIS

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Introduction and objectives. We have reported that articular chondrocytes in tissue contain long cytoplasmic arms that physically connect two distant cells. Cell-to-cell communication occurs through connexin (Cx) channels termed Gap Junction (GJ) channels, which achieve direct cellular communication by allowing the intercellular exchange of ions or second messengers. Cx43 protein is overexpressed in several diseases including in articular cartilage from patients with osteoarthritis (OA). An increase in the level of Cx43 is known to alter gene expression, cell signalling, growth and cell proliferation. The interaction of proteins with the C-terminal tail of Cx43 directly modulates GJ-dependent and -independent functions. The goal of this research work was to identify the proteins that interact with the human native Cx43 isolated from primary chondrocytes from healthy donors and patients with OA.

Methods. Cx43 complexes were extracted from human primary chondrocytes using mild extraction conditions and immunoaffinity purification. The proteomic content of the native complexes was determined using LC-MS/MS, and protein associations with Cx43 were validated using western blot and immunolocalisation experiments.

Results and Discussion. We identified >100 Cx43-associated proteins including previously uncharacterised proteins related to nuclear functions, RNA transport and translation. We also identified several proteins involved in human diseases and cartilage structure as novel functional Cx43 interactors, which emphasised the importance of Cx43 in normal physiology and functional integrity of chondrocytes in cartilage. Gene Ontology (GO) terms of the proteins identified in the OA samples showed an enrichment of Cx43-interactors related to cell adhesion, calmodulin binding, the nucleolus and the cytoskeleton in OA samples compared with healthy samples. However, the mitochondrial proteins SOD2 and ATP5J2 were identified only in samples from healthy donors.

Conclusions: The identification of Cx43 interactors will provide clues to the functions of Cx43 in human cells and its roles in the development of several diseases, including OA.

P-63.00

CHARACTERISATION OF A NOVEL SPONTANEOUS MODEL OF EPITHELIAL TO MESENCHYMAL TRANSITION USING A PRIMARY PROSTATE CANCER CELL LINE USING QUANTITATIVE LABEL-FREE PROTEOMICS (SWATH™).

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Introduction and Objectives
Current evidence suggests cancer cells reactivate the latent embryonic programme of epithelial to mesenchymal transition (EMT) to acquire the invasive and migratory/metastatic properties that are associated with aggressive disease and its progressive in cancer. We have developed a spontaneous model of EMT using a primary human prostate cancer cell line (OPCT1), and used High Resolution Accurate Mass mass spectrometry and data independent acquisition (SWATH™) to quantify the secretome of the epithelial and mesenchymal clonal progeny.

Methods
The epithelial and mesenchymal model cell lines have previously been characterised on the basis of their expression of classic markers for epithelial/mesenchymal traits (including vimentin, E-cadherin, fibronectin, SLUG, SNAIL, TWIST, ZEB1, OCT, SOX2, NANOG) using qRT-PCR, western blot, immunofluorescence and other functional assays. For this study, supernatants from epithelial and mesenchymal model cell lines that had been grown to 70% confluency in a complete KSF media with and without serum were analysed using an AB SCIEX 5600+ TripleTOF instrument using SWATH™ methodology.

Results and Discussion
A total of 633 proteins (n=3 biological replicates) derived from the harvested secretome of epithelial and mesenchymal clones were quantified by SWATH from an ion library containing 740 proteins (1% FDR). Ontological information derived from UniprotKB classified these proteins as being non-secreted (30%), secreted (20%) or as being potentially involved in exosomal secretory pathway (50%). Twenty of these proteins were categorised as being significantly downregulated and 21 as being upregulated in the mesenchymal clone on the basis of a 1-log fold change and $p<0.05$.

Conclusions
Classical markers of mesenchymal and epithelial clones cell types, such as VIME, COCA1, TSP1, CO5A1, K2CB, MMP2, PCDH1 among others were identified as being the top differentially expressed proteins using quantitative mass spectrometry. This technique could therefore be used as a substitute for conventional laboratory-based techniques such as western blot.

P-64.00

FROM DISCOVERY PROTEOMICS TO FUNCTIONAL INVESTIGATION: STUDYING ABERRANT GLYCOSYLATION IN CLL CELLS

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Chronic lymphocytic leukemia (CLL) is characterized by the progressive accumulation of functionally incompetent CD5+ lymphocytes in lymphoid tissues. It is possible to distinguish good or bad prognosis patients, according to several clinical, genetics and biochemical parameters. Aims of this work is to identify prognostic factors for a more accurate patients' stratification and to possibly unravel the mechanisms of the aggressive behavior of the disease.

We applied a label-free, shotgun mass spectrometry-based proteomic approach on total protein extracts of CLL primary cells from patients selected for good or bad prognosis. Immunoblotting on a larger cohort was used to validate some of the differentially expressed proteins. Among them, proteins involved in the first step of glycosylation are particularly interesting as they might explain the aberrant glycosylation of surface IgM, characteristic of more aggressive CLL. We investigated this mechanism by inhibiting the intracellular transport and studying by FACS and immunoblotting the changes in surface IgM expression.

Starting from as little as 35000 cells, we have identified and quantified more than 1200 proteins, 20 of them are consistently and significantly differentially expressed in bad vs good prognosis patients. Bioinformatics analysis shows their involvement in many important cellular and biological processes. Ribophorin1 (RPN1), oxoketoglutarate dehydrogenase (OGDH) and trifunctional enzyme subunit alpha (HADHA) have been validated as candidate prognostic biomarkers. We have demonstrated that highly mannosylated IgMs are transported through an alternative route from intermediate Golgi cisternae to the surface.

We have verified 3 proteins capable of discriminating between good and bad prognosis CLL patients. As one of them, RPN1, is involved in the glycosylation of nascent proteins, and progressive CLL is characterized by highly mannosylated surface IgM, we are focusing on the pathways leading aberrantly glycosylated IgM to the cell surface, to shed light on the mechanisms conferring the aggressive behavior to leukemic cells.

P-65.00

IDENTIFYING TARGETS SPECIFIC FOR METASTASIS VIA A WHOLISTIC STRATEGY COMBINING PROTEOMICS AND TRANSCRIPTOMICS

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Introduction and objectives

Cancer is one of the main causes of death in the western word, whereat not only the primary tumour is the superior problem but forming metastasis and spreading over the whole organism means the major challenge. Dealing with tumours always implies dealing with a huge heterogeneity of different cells. Additionally, there is the need to gain control over the remaining complexity of a wholistic experimental setup. In this study, the combination of a differential proteomic and a transcriptomic approach enabled us to identify metastasis targets in pancreatic tumor cells.

Methods

For the proteomic approach, the iTRAQ labelled multiplexed sample was analysed by IP-RP-HPLC hyphenated to ion trap-Orbitrap mass spectrometry. Differential transcriptomics was performed on the Affymetrix Gene Array system. Data analysis was performed with OpenMS using ConsensusID, the R package isobar, and Ingenuity Systems Pathway Analysis.

Results and Discussion

We identified about 350 potential targets for metastasis treatment which were cut down to a small subset of proteins after different validation steps. It could be demonstrated in cd1 nu|nu mice via knock-out clones that the two calcium binding proteins S100A8 and S100A9 as well as the Galactin-3-binding protein LGALS3BP not only play an important role in the EGRF signalling pathway, but are also relevant for STAT3|IL6 signalling.

Conclusion

Combining transcriptomics and proteomics for unbiased hypothesis generation becomes a valuable strategy for the target identification in an aggressive pancreatic metastasis cell line. Along with this innovative holistic shot-gun experiments, the validation of the effect of target proteins on the EGFR signalling pathway through knock-out cancer stem cells in vivo results in the elucidation of signalling pathways and future drug targets.

P-66.00

EVALUATION OF MODE OF ACTION AS WELL AS RESISTANCE MECHANISMS REGARDING THE NOVEL ANTICANCER RUTHENIUM COMPOUND KP1339 BY PROTEOME PROFILING

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Introduction and Aim

KP1339 (Sodium trans-[tetrachlorobis(1H-indazole)ruthenate(III)]) is a novel and promising anticancer drug candidate. However, the mode of action of this Ruthenium based compound has not been fully established yet. So far, redox-activities, DNA as biological target and induction of apoptosis have been reported as important key terms in relation to KP1339 activity. Our aim was to get more insight in the mechanism of action of this compound by using a proteomic approach applied to sensitive and resistant tumor cells. The cellular proteomes were evaluated to see whether resistance was an innate feature of cells. Furthermore, treatment of these cells with KP1339 was applied to evaluate acquired resistance mechanisms.

Methods

Cells were fractionated into cytoplasm and nuclei and processed for subsequent shotgun analysis. Comparative proteome profiling was performed using a label-free quantification method employing high resolution Orbitrap mass spectrometry and the MaxQuant software.

Results and Discussion

Quantitative assessment of 6975 distinct proteins allowed us to investigate differential expression of anti-apoptotic, redox-regulating, DNA repair and other stress-related proteins. Remarkably, neither anti-apoptotic nor DNA repair proteins were found affected, while the resistant cells displayed significantly increased concentrations of redox-regulating proteins including glutaredoxins and glutathione S transferases. Challenging the cells with KP1339 induced little new protein synthesis in the resistant cells, whereas many apoptosis-regulating proteins were induced in the sensitive cells.

Conclusion

While KP1339 positively induced apoptosis in colon and pancreas carcinoma cell lines, we found that resistance of other colon and liver carcinoma cells to KP1339 treatment correlated with high abundance of redox-regulating proteins.

P-67.00
IN-DEPTH PROTEOME PROFILING IN CHRONIC LYMPHOCYTIC LEUKEMIA – UNRAVELING NEW TREATMENT OPTIONS

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Introduction and Objectives
Chronic lymphocytic leukemia (CLL) is the most common type of leukemia in adults in the industrialized world. It primarily affects B lymphocytes so that these can no longer produce functional antibodies but accumulate and eventually cause repression of other blood cells. In spite of extensive research and steadily improving treatment options, CLL still remains incurable and the main pathomechanisms unsatisfactorily understood. After initial treatment success the disease usually relapses and evolves further to resist chemotherapy. While genomic approaches have already yielded substantial progress in CLL research, proteomics still remains a poorly exploited yet powerful tool for the revelation of the molecular background of chronic lymphocytic leukemia.

Methods
B lymphocytes were isolated from peripheral blood of healthy donors as well as CLL patients via magnetic-activated cell sorting. Comprehensive proteome profiles were generated via high-resolution orbitrap mass spectrometry and spectra subjected to MaxQuant software for label-free quantification.

Results and Discussion
Identification and relative quantification of more than 8100 proteins over all samples enabled us detailed insight into CLL cell proteome profile in comparison to normal B cells. On the one hand we were able to reproduce previously published data like strong regulation in cell adhesion, apoptosis and ECM-receptor interaction. On the other hand we identified previously undescribed down-regulation of several proteins of the interferon gamma pathway in CLL B cells. The down-regulation of this pathway within the CLL cells could represent a mechanism for CLL to avoid apoptosis and may present us an Achilles heel for future treatment options.

Conclusion
The novel finding of the down-regulation of several interferon gamma pathway proteins in CLL may reveal innovative treatment options for increased therapy success.

P-68.00

ASSESSMENT OF TUMOR-PROMOTING ACTIVITIES OF TUMOR-ASSOCIATED FIBROBLASTS

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Introduction and Objectives
While tumor-associated fibroblasts do not harbor genetic abnormalities, they may be functionally altered eventually resulting in tumor promotion. These undesired cell activities may become detectable by secretome analysis of such cells focusing on the quantification of survival, growth and other signaling factors. Therefore, quantification of the most bioactive compounds secreted by these cells provides a measure for their tumor promoting activities. Using targeted proteomics, we are screening for drugs inducing the down-regulation of these molecules.

Methods
Cells were isolated from clinical biopsy samples and cultured for two to three passages. Cell supernatants were collected after changing medium for serum-free condition and subsequent culturing for 6 hours. For screening of the most important biomolecules we employed the Thermo QEXACTIVE orbitrap and evaluated the data with MaxQuant software for label-free quantification. Furthermore, we established MRM assays for the tumor promoting molecules including growth factors, cytokines and chemokines. MRM assays were accomplished using an Agilent nano-flow Chip-HPLC coupled to the Agilent 6490 triple quadrupole mass spectrometer

Results and Discussion
Tumor-associated fibroblasts were found to display significantly altered secretion profiles including strongly up-regulated tumor promoters when compared to fibroblasts obtained from non-neoplastic background. Targeted proteomics proved to be a fast and reliable method to determine secretion characteristics of cells derived from individual donors. Furthermore, treating the cells in vitro with standard drugs currently used in clinical practice such as Dexamethasone and Thalidomide allowed us to directly assess drug effects.

Conclusion
Secretome profiling was successfully employed to determine tumor-promoting activities of primary human tumor-associated fibroblasts isolated from different tissue types. Furthermore, the same assays may allow us to screen for drugs specifically interfering with such undesired tumor-promoting activities of fibroblasts.

P-69.00

TERMINOMICS ANALYSES OF PRIMARY CELL CULTURE SUPERNATANTS IDENTIFY A ROLE FOR ADAM17 IN EXTRACELLULAR CATHEPSIN ACTIVITY

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Ectodomain shedding involves proteolysis of integral membrane proteins causing their release into the extracellular space and has emerged as a novel method of regulating biological processes. For example, shedding of several ligands is required for receptor engagement, and shedding of many receptors results in their inactivation and desensitization. One class of proteases implicated in shedding is the ADAM (a disintegrin and metalloproteinase) family, of which Adam17 is one of the best characterized. Adam17 is the main sheddase for epidermal growth factor receptor ligands. It also sheds tumor necrosis factor-alpha and several other cell surface proteins.

As a result, Adam17 has become a potential drug target for cancer and immune disorders. However, to fully comprehend its cellular functions, as well as the full impact of targeting Adam17 to treat a disease, all of the proteins processed by this enzyme, as well as their functions must be understood. To this end, we have collected cell culture supernatants from both wild type (WT) and Adam17-/- primary mouse embryonic fibroblasts and used stable-isotope dimethyl labeling to quantitatively compare these conditions.

At 1% FDR, 1051 proteins (5762 peptides) were identified and quantified with 15% exhibiting greater than 2-fold change. Interestingly, several lysosomal proteins such as cathepsins were found to be more abundant in the WT condition. Extracellular cathepsins have recently been implicated in cancer suggesting a role for Adam17 in this process.

Terminal amine isotope labeling of substrates has also been used to identify specific cleavage sites within several of these proteins.

P-70.00
A COMPREHENSIVE COMPARATIVE AND SEMI-QUANTITATIVE PROTEOME OF A VERY LOW NUMBER OF NATIVE AND MATCHED EPSTEIN-BARR VIRUS- TRANSFORMED B LYMPHOCYTES INFILTRATING HUMAN MELANOMA

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Introduction and Objectives: Melanoma, the deadliest form of skin cancer, is highly immunogenic and frequently infiltrated with immune cells including B cells. The role of tumor-infiltrating B cells (TIBCs) in melanoma is as yet unresolved, possibly due to technical challenges in obtaining TIBCs in sufficient quantity for extensive studies and due to the limited life span of B cells in vitro.

Methods: A comprehensive workflow has been developed for successful isolation and proteomic analysis of a low number of TIBCs from fresh, human melanoma tissue. In addition, we generated in vitro-proliferating TIBC cultures using simultaneous stimulation with Epstein Barr Virus (EBV) and the TLR9 ligand CpG-oligodesoxynucleotide (CpG ODN). The FASP method and iTRAQ labelling were utilised to obtain a comparative, semi-quantitative proteome to assess EBV-induced changes in TIBCs. Real-time PCR was used for validation of the proteomic data.

Results and Discussion: By using as few as 100,000 B cells (~5μg protein)/sample for our proteomic study, a total number of 6,507 proteins were identified.
EBV-induced changes in TIBCs are similar to those already reported for peripheral B cells and largely involve changes in cell cycle proliferation, apoptosis and interferon response while most of the proteins were not significantly altered, suggesting that EBV- induced TIBC cell lines may be a reliable surrogate for native B cells and may be employed for functional studies.

Conclusion: This study provides an essential, further step towards detailed characterization of TIBCs including functional in vitro analysis.

P-71.00

PROTEOME PROFILING OF HUMAN HEALTHY AND TUMOR COLON ORGANOIDS

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Introduction and objectives

In understanding cancer biology organoids exhibit a high potential as novel human-derived model system by using the characteristics of stem cells in vitro. Several research areas could benefit from organoid technology, like stem cell research, disease modeling and regenerative medicine. Here, we present the first comparative analysis of several patient-derived healthy and tumor colon organoids providing insight into the biology of colorectal cancer.

Methods

Organoids are epithelial in vitro cultures in which all differentiated cell types are present. They retain critical in vivo characteristics such as cell type composition and self-renewal dynamics, which makes them an ideal model system. The proteome of 6 patient-derived healthy and colorectal tumor organoids were analyzed and quantified. Dimethyl labeling was used to quantify the different expression levels of the proteins present in the healthy and tumor colon organoids. In order to reduce the sample complexity, a fractionation step was performed using SCX (strong cation exchange). Furthermore, to achieve the highest proteome coverage, two mass spectrometers were utilized, an ETD enabled Orbitrap Elite and a Q-Exactive (alongside nanoUHPLC) to analyze SCX fractions composing of distinct charge states. The same organoids were in parallel characterized by transcriptome analysis using an Affymetrix platform.

Results and discussion

From each patient approx. 6000 proteins could be quantified with more than 5500 proteins across all organoids. A number of statistical tools were applied to distinguish proteins relating to either the healthy or diseased types of organoids. From the combination of proteomics and transcriptomics a first genome-wide insight into this novel human-derived model system could be obtained.

Conclusions

Our data represent the first proteome profiling of patient-derived organoids, healthy and cancer colon organoids. We demonstrate that organoid proteomics provides an excellent tool for obtaining insight into cancer and intestinal stem cell biology.

P-72.00

DEVELOPMENT OF TARGETED PROTEOMIC METHOD FOR POSSIBLE BIOMARKERS FOR ORAL CANCER SCREENING IN HUNGARIAN POPULATION

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Introduction:
Oral squamous cell carcinoma (OSCC) accounts for about 90% of malignant oral lesions which is the 6th most common malignancy with increasing incidence and mortality rate especially in the younger generation. Hungarian population occupies the top places of statistics regarding OSCC incidence. Delayed detection is likely to be a primary reason for the low 5-year survival rate (around 50%) supporting the need for biomarkers to improve early detection.

Methods:
The nation-wide, multi-center, bench-to-bedside, prospective study involves patients with OSCC from four university departments in Hungary. 14 proteins previously reported in the literature as significantly elevated in saliva of OSCC patients either at gene or protein levels were examined. SRM-based targeted proteomic method was developed and the relative amount of the proteins was determined in the saliva of OSCC patients and controls. In case of those proteins where the available antibodies could be included into a Luminex-based multiplex kit the salivary concentrations were determined.

Results and Discussion:
The levels of six possible salivary biomarkers (IL-1 alpha, IL-1 beta, IL-6, IL-8, TNF-alpha and VEGF) were examined both with Luminex-based multiplex assay and SRM-based targeted proteomic method and the obtained results show good correlation. After several rounds of optimization and stable isotope containing peptide administration we could develop an SRM-based method for rapid salivary biomarker detection. Based on our preliminary results protein S100-A9, catalase, thioredoxin, IL-6, IL-8 and TNF-alpha seem to be useful biomarkers for OSCC detection in the Hungarian population.

Conclusion:
A noninvasive method to detect biomarkers useful for the early diagnosis of OSCC and precancerous lesions was developed and this seems to be an attractive strategy to decrease morbidity and mortality, to enhance survival rate and to improve quality of life.

P-73.00

PRELIMINARY PROTEOMIC STUDY OF EPITHELIAL –MESENCHYMAL TRANSITION INDUCED BY TGF-B1 AND EGF IN PANCREATIC CANCER CELL LINE

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Pancreatic cancer (PanCa) is considered one of the most aggressive adenocarcinomas and of difficult diagnosis. Since it develops silently and presents a high genetic complexity, diagnosis is often late, once it has already metastasized. Therefore, studying processes by which metastasis is triggered are relevant not only to understand the disease, but also to improve its treatment and diagnosis. In that regard, a mechanism associated to the gaining of migratory and invasiveness abilities is the Epithelial to Mesenchymal Transition (EMT), which promotes tumor progression and metastasis in adenocarcinomas.

With the purpose of study the EMT in PanCa from a proteomic point of view, we develop in-depth proteomic qualitative and quantitative analysis based on stable isotope labeling in cell culture (SILAC) followed by cellular and protein fractionation in order to elucidate the alterations promoted by the induction of EMT in pancreatic cancer cell lines. We induced EMT by treating SILAC-labeled PANC-1 cells for 72h with EGF and TGF-β1. EMT was confirmed after microscopy observation and Western Blotting of known EMT markers. Nuclear and cytoplasmic subcellular fractions were analyzed by gel-LC-MS/MS approach in a LTQ-ORBITRAP Velos and data processing was performed using Labkey Server. More than 2000 proteins were accurately identified and quantified, with false-discovery rate below 1%. Interaction networks comprising proteins altered were assessed by STRING and FATIGO.

Identification of regulated protein associated with oxido-reduction and stroma remodeling suggests that intense metabolic processes are occurring in association with phenotypic changes during PANC-1 EMT induction. Some known proteins associated with cancer aggressiveness such as AGR2, MUC16 and L1CAM have also been identified, among several others which represent potential targets for knock-down studies in order to evaluate they roles in tumor progression and metastasis. SUPPORTED BY FAPESP, CTC-CEPID AND CISBi-NAP.

P-74.00

ASSESSMENT OF EPITELIAL TO MESENCHYMAL TRANSISTION BY MRM

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Epithelial to mesenchymal transition (EMT) naturally occurs during embryogenesis and tissue repair but is also involved in cancer progression and metastasis. EMT induces complex cellular and microenviromental changes resulting in loss of epithelial phenotype and acquisition of mesenchymal properties, which promotes migratory and invasive capabilities to cells. EMT can be triggered by several factors, including TGF-β, HGF and PDGF or by overexpression of some transcription factors such as ZEB1, TWIST1, SNAIL and SLUG. We studied EMT induction using a targeted proteomics approach in different cancer tissues, represented by breast, ovarian, pancreatic and prostate cell lines.

Proteotypic peptides representing 12 relevant proteins were selected to compose our molecular signature of EMT: CDH1, CDH2, HE4, MMP2, MMP9, MSLN, OLFM4, PSA, SOD2, TIMP1, TWIST and VIME. Using a 15 minutes LC-MS/MS MRM method we quantified this 12-plex EMT molecular signature in adenocarcinoma cell lines that were induced to EMT by treatment with growth factors or overexpression of the transcription factor SNAIL. Fifty micrograms of total extracts and conditioned media of cell lines induced (or not) to EMT were processed to obtain tryptic peptide digest for subsequent MRM analysis.

The method reached a sensitivity of around 50fmol injected, allowing quantification of proteins present at nanograms/ml in samples. Our data not only validates previous experiments where some EMT markers were regulated in one ore more cancer cell lines, but also demonstrates important protein alterations that can be considered tissue specific. For instance, HE4, a promising biomarker for ovarian cancer was extensively modulated in several cell lines during EMT.

With this MRM method we expect to monitor large patient sample sets in order to validate EMT-related proteins as potential targets for cancer diagnosis. SUPPORT: FAPESP, CISBi NAP/USP and CTC-CEPID.

P-75.00

D3CLP: AN UNFOLDED PROTEIN RESPONSE ACTIVATOR IDENTIFIED THROUGH A PROTEOMIC APPROXIMATION.

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In the search for new drugs to fight cancer, the bioisosteres of drugs with proven activity has served to increase the spectrum of compounds in the clinic. Recently, cytotoxic bioisosteres of amsacrine (IC-50 9 µM) have been synthesized, being D3ClP, one of the compounds with highest cytotoxic activity (IC-50 7 µM). As a bioisostere of amsacrine, D3ClP was proposed to act as a DNA topoisomerase II poison, leading to DNA damage. D3ClP was found to induce the phosphorylation of histone γH2Ax, a marker of DNA double strand breaks, however, D3ClP did not inhibit DNA topoisomerase II in vitro or in vivo, leaving its primary target uncertain.

Methods and Results

In the search for clues of the mechanism underlying the cytotoxicity of D3ClP we used a proteomic approximation(2-D gel electrophoresis and mass spectrometry analysis) to identify changes following D3ClP treatment in HeLa cell cultures.

Significant changes in the content of proteins related to apoptosis (Cytokeratin18, prothymosine) and to the Unfolded Protein Response (UPR; Nascent Polipeptide Associated Complex, Calumenin) were found to occur. UPR involves cell mechanisms to cope with protein damage at various levels, and if the damage cannot be repaired, UPR turns on the apoptotic processes. To provide further proof of D3CLP-dependent UPR stimulation, we evaluated the activation of IRE1-α RNAse, trough excision of the 26 bp unconventional intron form XBP-1 transcript (by RT-PCR), and also the upregulation of the proapoctotic factor CHOP (immunoblot). Both markers are uniquely related to ER stress and were found elevated in D3ClP HeLa treated cells. Our finding are thus consistent with the UPR induction by this compound, although the primary molecular target of D3ClP remains unknown.

P-76.00

OPTIMIZATION OF A SILAC BASED QUANTITATIVE PLATFORM FOR TUMOR TISSUE PROTEOMICS

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Introduction: Despite advances in genomics, little is known about the proteome of medulloblastoma, a malignant brain tumor of childhood. We aim to use stable isotope labeling of amino acids in cell culture (SILAC) for accurate quantification of tumor tissue proteins to better understand the biology of this disease.

Methods: We created a SILAC reference atlas of proteins from 4 medulloblastoma cell lines and spiked it equally into cell lysates of primary short term medulloblastoma cultures. An LC-Orbitrap mass spectrometer was used to quantitate proteins relative to the atlas.

Results and Discussion: Accurate quantitation was achieved for an average of 1215 proteins per primary cell line. Technical replicates resulted in r2 values between 0.91 and 0.98. 94.2-98.6% of sample proteins were found within a 5 fold dynamic range relative to the reference atlas. For one line created from a tissue mince, the dynamic range shifted across passages as tumor clones outgrew contaminating stromal elements influencing the accuracy of protein quantification. As this issue is analogous to a major challenge in tissue based proteomic workflows, we designed an ELISA based methodology to correct for contamination. We found that nucleolin is consistently expressed in tumor lines and thus could be used to normalize tumor protein as a component of total protein. In converse, ELISA for glycophorin A was used to estimate red blood cell contamination. Together these were used to calculate the amount of reference atlas to achieve the correct ratio between sample and atlas proteins to maintain an optimal dynamic range.

Conclusions: SILAC reference atlases can be used to perform relative proteomic quantitation of clinical tissue specimens. Normalization to total protein is inaccurate in tissue based proteomics as it represents the conflation of tumor and stromal/blood proteins. Normalization to representative proteins is one method for dealing with this technical challenge.

P-77.00

DEVELOPMENT OF CANDIDATE BIOMARKERS FOR PANCREATIC CANCER USING MULTIPLE REACTION MONITORING

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Pancreatic cancer is the fourth most frequent cause of cancer mortality in the United States. Statistically, after diagnosis, most PC patients die within 1 year or have a 5-year survival rate of less than 4%. As a marker of detection and prognosis after surgery of PC, CA 19-9 has been used widely; however, its low sensitivity and specificity have been a cause of concern. Thus, more effective markers for the early stages of PC are urgently needed.Biomarker candidate proteins were selected via comprehensive global data mining, which included previous PC proteomic data and PC microarray data. PC-related candidates were analyzed not only relative quantities based on external standard peptide spiking but also absolute quantities based on stable isotope-labeled internal standard (SIS) peptides of those candidate proteins which were measured using multiple reaction monitoring (MRM) assay.

The optimized transitions were verified using high-abundance proteins depleted 100 plasma samples.Statistical analysis of the MRM results for the PC group versus the normal healthy group and PC group versus non-PC group indicated that 68 PC-related proteins showed an area under the curve (AUC) of >0.7. From those proteins which were quantified, relatively based on external standard peptide spiking, 12 proteins were validated its absolute quantities using stable isotope-labeled internal standard (SIS) peptides and they showed an AUC of > 0.7. Followed multimarker panels constructed by the combination of MRM-verified proteins were improved the classification power of PC versus control, with comparable efficacy as CA 19-9.

This study aimed to develop the pancreatic cancer markers using global data mining and MRM verification in individual plasma and construct a multimarker panel. As a result, multimarker panels determined using multivariate analysis can classify PC group more effective than CA 19-9 which has been the only molecular biomarker for pancreatic cancer diagnosis and prognosis

P-78.00
QUANTITATIVE PROTEOMIC ANALYSIS OF PERIPHERAL BLOOD MONONUCLEAR CELLS TO IDENTIFY NOVEL BIOMARKERS FOR PANCREATIC CARCINOMA

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Introduction and objectives: Peripheral blood mononuclear cells (PBMCs) are a critical component in the immune system to fight infection and adapt to intruders and have emerged in recent years as surrogate markers of several diseases .Here we using a combination of quantitative proteomic analysis to identify and validate novel biomarkers of peripheral blood mononuclear cells for Pancreatic carcinoma.

Methods: PBMC of forty blood samples of healthy people and pancreatic carcinoma patients were applied to iTRAQ-based proteomic profiling and SWATH analysis. Differentially expressed proteins chosen based on statistical analysis were further quantitated relatively and validated by MRM hr method and western blot. IPA analysis of validated proteins were applied and candidate biomarkers were further evaluated by ELISA.

Results and Disscussion :A total of 4977 proteins of PBMC were identified and 107 proteins were chosen to quantitate relatively and validate. Most different expressed proteins in PBMC were enriched in ECM-receptor interaction, TGF-beta signaling pathway et al. PIGR, NUMB,S100A9 were demonstrated to be promising novel biomarkers for pancreatic carcinoma.

Conclusions: We applied firstly a series quantitative methods to investigate and validate protein expression in PBMCs in pancreatic carcinoma.Many proteins were up and down regulated in cancer samples compared to healthy people. Via our study, PIGR, NUMB,S100A9 showed particular promising candidate for further study.

P-79.00

PROTEOMIC ANALYSIS OF SP600125-CONTROLLED TRKA-DEPENDENT TARGETS IN SK-N-MC NEUROBLASTOMA CELLS: INHIBITION OF TRKA ACTIVITY BY SP600125

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The c-Jun N-terminal kinase (JNK) has been well known to play an important role in cell death signaling of the p75 neurotrophin receptor. However, little has been studied about a role of JNK in the signaling pathways of the tropomyosin-related kinase A (TrkA) neurotrophin receptor.

In this study, we investigated JNK inhibitor SP600125-controlled TrkA-dependent targets by proteomic analysis to better understand an involvement of JNK in TrkA-mediated signaling pathways. PDQuest image analysis and protein identification results showed that hnRNP C1/C2, alpha-tubulin, beta-tubulin homologue, actin homologue, and eIF-5A-1 protein spots were upregulated by ectopic expression of TrkA, whereas alpha-enolase, peroxiredoxin-6, PROS-27, HSP70, PP1-gamma, and PDH E1-alpha were downregulated by TrkA, and these TrkA-dependent upregulation and downregulation were significantly suppressed by SP600125. Notably, TrkA largely affected certain posttranslational modification(s) but not total protein amounts of the SP600125-controlled TrkA-dependent targets. Moreover, SP600125 strongly suppressed TrkA-mediated tyrosine phosphorylation signaling pathways as well as JNK signaling, indicating that SP600125 could function as a TrkA inhibitor.

Taken together, our results suggest that TrkA could play an important role in the cytoskeleton, cell death, cellular processing, and glucose metabolism through activation or inactivation of the SP600125-controlled TrkA-dependent targets.

P-80.00

QUANTITATIVE PROTEOMIC ANALYSIS OF ACQUIRED SORAFENIB RESISTANCE OF LIVER CANCER CELLS REVEALS EXPRESSION SIGNATURES ASSOCIATED WITH METASTASIS

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Hepatocellular carcinoma (HCC) is one of the most common potentially lethal human malignancies worldwide. Sorafenib, a multi-kinase inhibitor against Raf kinase and vascular endothelial growth factor receptor (VEGFR), has become standard therapy for patients with advanced HCC. However, most of patients eventually develop acquired resistance.

The molecular basis for this resistance is only partly elucidated. To investigate the proteins differentially expressed between sorafenib-acquired resistance (HuH-7R) and parental HuH-7(HuH-7) cells, we applied quantitative protein profiling approach (SILAC) to analyze protein expression levels between HuH-7R and HuH-7 cells (in vitro), combining with iTRAQ quantitative analysis of HuH-7R & HuH-7 tumors (in vivo). In total, 2851 quantified proteins were simultaneously identified in SILAC and iTRAQ experiments, with 81 proteins showing > 2.0 fold-change. In silico analyses of 81 up-regulated proteins in the sorafenib-acquired resistance cells, we observed a major abundance of proteins involved in cellular movement, cancer and cellular growth/proliferation.

These finding indicate that the adaptive changes in the proteome of sorafenib-acquired resistance cells are characterized by activation of alternative signalling pathways, enhanced cell migration and metastasis, which provide valuable information for further development of novel targeted therapies for drug resistant HCC.

P-81.00
COMPARATIVE PROTEOMIC ANALYSIS OF MALIGNANT PLEURAL MESOTHELIOMA: FOCUSING ON THE BIPHASIC SUBTYPE

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Introduction and Objectives
Malignant mesothelioma is a neoplastic disease linked to asbestos exposure whose diagnosis is limited. Malignant pleural mesothelioma (MPM) is a rare and devastating primary tumor of the pleura. Its prognosis is very poor, the median survival after diagnosis is less than 12 months. In a previous study, we performed a comparative proteome analysis on tissue biopsies between epithelioid mesothelioma (E-PM) and hyperplasia. E-PM samples evidenced an altered expression of nuclear lamin and filament related proteins. We extended the comparative proteomic analysis to the biphasic mesothelioma (B-PM) characterized by a combination of elements of epitheliod and sarcomatoid subtypes.

Methods
Tissue biopsies were obtained from 25 patients who were subjected to a diagnostic thoracoscopy. After histological examination, the samples were classified as: B-PM, hyperplasia and E-PM. The samples were precipitated, pooled according to their classification, and submitted to 2-DE. Images were analyzed with Progenesis Same Spot software. Spots of interest were identified by NanoLC-ESI-MS/MS analysis and validated by western blot analysis or commercial ELISA kits.

Results and Discussion
27 proteins that showed a fold variation≥2 were identified. The different expression of prelamin, vimentin and calretinin previously found in E-PM was confirmed in B-PM samples with respect to hyperplasia. Moreover, other proteins resulted increased in B-PM when compared both to hyperplasia and E-PM: γ-enolase, peroxiredoxin-1, S100-A11, serum amyloid P component, and serum amyloidA1. A different expression of chloride intracellular channel protein-3 and CRYAB was observed in B-PM and E-PM vs hyperplasia. Research are in progress to understand if these proteins belong to the sarcomatoid component of B-PM.

Conclusions
Overall, our results suggest new potential biomarkers which can be added to those previously identified in order to differentiate B-PM. These findings might be important in the prognosis of B-PM patients and in the probability of response to therapy.

P-82.00

IDENTIFICATION OF MOLECULAR MARKERS OF CANCER REVERSAL AND CELL DIFFERENTIATION BY SELDI PROTEIN PROFILING

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INTRODUCTION AND OBJECTIVES.

Human glutaminase (GA) enzymes are the products of two genes, GLS and GLS2. GLS2 isoforms are associated with low proliferation rates and resting/quiescent cell states. While brain tumour cells usually repress GLS2 expression, its overexpression in glioma induces a marked change in cell transcriptome conducive to a less-malignant and more differentiated phenotype. The aim of the present study is to discover molecular markers of the differentiation process by profiling nuclear proteins in cancer cells with targeted GLS2 expression.

METHODS.

Human cancer cell lines: glioblastoma T98G (wild-type), T98G-pcDNA3 (sham-transfected), T98G-GAB (overexpressing GLS2 gene) and PMA-treated or untreated SHSY-5Y neuroblastoma cells. Proteins were incubated with strong-anion exchange (Q10) and weak-cation exchange (CM10) ProteinChip arrays and analyzed using SELDI-TOF/MS and ProteinChip Data Manager Client 4.0 software. Protein expression profiles for T98G-GAB were compared with those for T98G-WT/T98G-pcDNA3, whilst profiles for PMA-treated differentiated SHSY-5Y cells were compared with untreated cells. Putative markers were identified by nano-HPLC-ESI-MS analysis.

RESULTS AND DISCUSSION.

76 proteins were found to be differentially expressed between control and GAB-transfected T98G cells. Nine proteins with m/z between 5.5-29.7 K were identified to be highly expressed in T98G-GAB (p

CONCLUSIONS.

The potential of proteomic profiling to get a deep insight into the role of GLS2 in brain tumours and to assess its suitability as a novel anti-cancer therapeutic target has been proven. We noticed a trend towards low-to mid-mass peaks in the total number of peaks found. Also, profiling on CM10 surface produced a higher number of peaks compared to the Q10 surface.

P-83.00

PROTEOMIC INVESTIGATION OF BIOMARKERS USING HUMAN PLASMA AND SERUM SAMPLES FOR EARLY BREAST CANCER DIAGNOSIS.

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Introduction: Breast cancer (BC) is the most commonly diagnosed form of cancer and the leading cause of cancer deaths in women. The early detection of BC using blood biomarkers would significantly reduce mortality.

Aim: To identify and compare the proteins differentially expressed in serum and plasma in different stages of BC patients using different blood tubes by a label-free liquid-chromatography/tandem-mass spectrometry (LC-MS/MS) proteomic approach, for early cancer diagnosis.

Methods: The study cohort consisted of 20 BC patients with ductal carcinoma in situ (DCIS, n=6), invasive BC (IBC, n=8), metastatic BC (MBC, n=6), also patients with benign breast disease (BBD, n=6) and healthy control subjects (n=8). The blood was collected in 4 different blood tubes (2 serum/2 plasma). The results from BC patients were compared with those from BBD and normal controls. The findings were statistically analysed using Progenesis-LC-MS software with significance criteria applied p3.

Results: Over 100 differentially abundant proteins were identified from BC patients in the plasma and serum samples whereby a greater number of proteins were identified from the Gold-top serum tubes in both the low mass (3kDa) fractions. The systematic computer analysis of the data detected in both serum and plasma samples demonstrated that15 secreted proteins, with varied expression pattern across the different BC stages, are promising to define early stage breast cancer.

Conclusions: The extensive LC-MS/MS protein information obtained from human serum and plasma comprises of cell defense proteins, protein folding and structural proteins which contains the key information for diagnostics of BC. The interesting proteins (biomarkers) identified will be validated in BC cell lines and tissues in the following study.

Keywords: Breast cancer; serum; plasma; LC-MS/MS; DCIS; biomarkers.

P-84.00

PROTEOMIC VARIATIONS IDENTIFIED BETWEEN INVASIVE AND NONINVASIVE NONFUNCTIONAL PITUITARY ADENOMAS

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Introduction and Objectives: The incomplete surgery section of invasive nonfunctional pituitary adenomas (NFPAs) carries the increased risks of complications and requires adjuvant radiotherapy and medications. It is necessary to clarify the molecular mechanisms and markers of invasiveness to guide the management of NFPA patients. The study aimed to proteomic variations of invasive and noninvasive NFPAs and sought the protein markers for invasive NFPAs.

Methods: Invasive (n = 4) and noninvasive (n = 4) NFPA tissues were analyzed (n = 3-5/each tissue) with two-dimensional gel electrophoresis (2DGE) and PDQuest software. Twenty-four high-resolution 2DGE gels were quantitatively compared to determine differentially expressed proteins (DEPs) between invasive and noninvasive NFPAs. Mass spectrometry was used to characterize each protein. Bioinformatics and systems biology were used to determine the pathway network variations that function in the NFPAs.

Results and Discussion: Approximate 1200 protein spots were detected in each 2DGE map, and 103 differential spots (64 upregulated and 39 downregulated) were identified. Among those 103 differential spots, 57 DEPs (30 upregulated and 27 downregulated) were characterized with peptide mass fingerprint and tandem mass spectrometry. Gene-Ontology (GO) and Ingenuity Pathway analyses of those DEPs revealed pathway networks including mitochondrial dysfunction, oxidative stress, mitogen activated protein kinase (MAPK)-signaling abnormality, TR/RXR activation, proteolysis abnormality, ketogenesis and ketolysis, cyclin-dependent kinase C (CDK5) signaling abnormality, and amyloid processing that were significantly associated with invasive characteristics of invasive NFPA.

Conclusions: Those data demonstrate that proteomic variations exist between invasive and noninvasive NFPAs. 2DGE-based comparative proteomics is the effective approach to identify proteomic variations and pathway network variations. Those findings will serve as a basis to understand the molecular mechanisms of invasive NFPAs and to discover protein markers to effectively manage patients with invasive NFPAs.

P-85.00
ITRAQ TECHNOLOGY COMBINED 2DLC-MS/MS DIFFERENTIALLY EXPRESSED PROTEINS IN RENAL CELL CARCINOMA SCREENING

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Objective To screen differentially expressed proteins of prostate cancer by the pro-teomics analysis using isobaric tags for relative and absolute quantifica-tion(iTRAQ)combined with two-dimensional liquid chromatography-tandem mass spectrometry(2DLC-MS/MS).

Methods The patients undergoing renal cell carcinoma biopsies were classified in 3 groups: normal kidney tissue (n=10),tumor-adjacent tissue (n=10), and renal cell carcinoma (n=10).After quantification and enzymolysis of the protein extract from the specimens of the 3 groups,the iTRAQ regents 114,116 and 117 were used to label the peptides of the 3 groups respectively.Then,the mixture of the peptides was analyzed by 2DLC-MS/MS. The MS/MS data were searched against the International Swissprot(090210,Human)using the Protein Pilot software(version 3.0)for peptide identification and quantification.

The fold change cutoff ratio>1.50 or proteins, comparing with kidney normal tissues,21 proteins were significantly up-regulated(>1.5-fold)and 16 were significantly down-regulated in renal cell carci-noma

P-86.00

METABOLIC ADAPTATION IN NON-SMALL CELL LUNG CANCER

Alfonso Martín-Bernabé[1,2,3,4], Roldán Cortés[1], Sylvia G. Lehmann[2,3,4], Michel Seve[2,3,4], Marta Cascante[1], Sandrine Bourgoin-Voillard[2,3,4]

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While KRAS mutations in non-small cell lung cancer (NSCLC) are known to play a key role in resistance to EGFR-targeted therapies, therapeutic treatment to target mutant KRAS-driven cancer have been unsuccessful. Therefore, targeting lung cancer metabolism is a promising alternative strategy that could overcome drug resistance in such cancer. In this study, we used iTRAQ (isobaric Tags for Relative and Absolute Quantitation) quantitative proteomic analysis to evidence key enzymes involved in metabolic adaptations in NSCLC.

Proteomic analysis was performed on two KRAS-mutated NSCLC cell lines (A549 and NCI-H460) and a non-tumoral bronchial cell line (BEAS-2B). Extracted proteins from those cells were digested, then, labelled with iTRAQ tags (m/z 114, 116 and 117 for BEAS-2B, A549 and NCI-H460, respectively). The resulted labelled peptides were fractionated in two dimensions (OFFGEL/RP nanoLC) prior MALDI-TOF/TOF mass spectrometry analysis.

Among 1169 proteins identified and 834 proteins quantified, several enzymes involved metabolic pathways especially in glycolysis and pentose phosphate pathway (PPP) were up-regulated. Up-regulation of those proteins was confirmed by western blotting analysis. Moreover, the up-regulation of enzymes expression in PPP is correlated to their enzyme activity suggesting them as potential metabolic targets for the development of new therapeutic treatments and/or biomarker assays for NSCLC.

P-87.00

AN UNDERSTANDING OF METABOLIC ADAPTATION IN NON-SMALL CELL LUNG CANCER

Alfonso Martín-Bernabé[1,2,3,4], Roldán Cortés[1], Sylvia G. Lehmann[2,3,4], Michel Seve[2,3,4], Marta Cascante[1], Sandrine Bourgoin-Voillard[2,3,4]

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P-88.00

GLOBAL PROTEOME CHANGES INDUCED BY C-MYB SILENCING IN K562 HUMAN CHRONIC MYELOID LEUKEMIA CELLS

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Introduction and objectives

Myeloid leukemia is a malignant disease of the hematopoietic system in which cells of myeloid lineages accumulate to an undifferentiated state, due to the accumulation of mutations. In particular, it was shown that an aberrant expression of the c-Myb transcriptional factor is associated with the suppression of normal differentiation processes promoting the development of the hematopoietic malignancies. In this work, we originally describe a differential proteomic approach to facilitate the comprehension of the regulation of the protein networks exerted by c-Myb.

Methods

To shed light on the molecular mechanisms associated with aberrant expression of c-Myb in chronic myeloid leukemia, comparative proteomic analysis was performed on c-myb RNAi-specifically silenced K562 cells, sampled on a time-course basis. 2D-DIGE technology highlighted 37 differentially-represented proteins that were further characterized by nLC-ESI-LIT-MS/MS and validated by western blotting and qRT-PCR analysis.

Results and Discussion

Most of the deregulated proteins were related to protein folding, energy/primary metabolism, transcription/translation regulation and oxidative stress response. Our study reveals a complex network of proteins regulated by c-Myb. The functional heterogeneity of these proteins emphasizes the pleiotropic role of c-Myb as a regulator of genes that are crucial for energy production and stress response in leukemia. In fact, variations in glyceraldehyde-3-phosphate-dehydrogenase and α-enolase protein species suggest a possible role of c-Myb in the activation of aerobic glycolysis. Moreover, significant differences were found for heat shock 70 kDa protein and 78 kDa glucose-regulated protein known as direct c-Myb targets. Protein network analysis suggested that glycolysis, gluconeogenesis and protein ubiquitination biosynthesis pathways were highly represented, confirming also the pivotal role of c-Myc.

Conclusions

Data here presented, while providing novel insights onto the molecular mechanisms underlying c-Myb activity, highlight potential protein biomarkers useful for disease progression and translational medicine approaches in myeloid leukemia.

P-89.00

PROFILING URINARY PROTEOME FOR STRESS INDUCED FEMALE URINARY INCONTINENCE

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Recent genetic studies identified several genes encoding components of the extracellular matrix (ECM), which seem to be related to a urinary incontinence (UI) predisposition. ECM protein turnover plays a role in the development of UI, but much remains to be understood of this complex dynamic interplay of enzymes, proteins and molecules. Biomarkers offer a promising means of detecting this disease before clinical expression. Identifying risk factors for UI can facilitate prevention strategies with the aim to reduce UI prevalence among women. In particular, the identification of protein patterns specifically in stress urinary incontinence (SUI) would be a precious instrument, beside clinical and instrumental exams, in the diagnostic and therapeutic process.

Increased number of proteins involved in inflammation, angiogenesis, and collagen metabolism was identified in samples from patients suffering on SUI compared to the control group. We also report the analysis of urinary proteins by applying online digestion of urinary proteins using enzymatic reactor (IMER) to accelerate protein digestion, reduce manual sample handling, and provide reproducibility to the digestion process in clinical laboratory.

The use of IMER and online digestion enables significant,time reduction for protein digestion from overnight to one hour (including all sample preparation steps), and analysis and detection of secreted proteins without compromising the data quality when compared to classical overnight sample preparation.

Although no "marker" proteins have been clearly identified, we could narrow the search for clinically relevant proteins and significantly increase the sample throughput.

Marianne Koch2; Rosa Laterza2; Wei-Qiang Chen1; Miloš Barut3; Sonja Seyfert4; Heinz Kölbl2; Goran Mitulovic1

P-90.00

DISCOVERY AND LONGITUDINAL MEASUREMENT OF CANDIDATE BIOMARKERS OF BIOCHEMICAL RECURRENCE IN PROSTATE CANCER PATIENTS TREATED WITH CHRT

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Compared with hormonal therapy alone, combined hormone and radiation therapy (CHRT) has been demonstrated to give improved disease-specific survival outcomes for prostate cancer. However, many CHRT patients still succumb to recurrent disease. Earlier prediction and monitoring of patient response to CHRT would allow for more beneficial and personalized treatment. Although prostate specific antigen (PSA) is currently used for this purpose, transient increases in PSA can occur without concomitant disease recurrence and disease recurrence sometimes occurs without a rise in PSA. Here, patient samples obtained as part of an on-going non-interventional clinical trial (ICORG06-15), were used to identify and evaluate a potential serum protein signature of disease recurrence.

Label-free LC-MS/MS based protein discovery was undertaken on MARS14 depleted serum samples from patients diagnosed with biochemical recurrence (BR) (n=3) and time-matched disease-remission individuals (n=3). Samples were analysed on a Q-Exactive mass spectrometer with protein identification and quantification undertaken using PEAKS (version no.6) and MaxQuant (version no.1.4.1.2) software. Multiple reaction monitoring (MRM) assays were designed for longitudinal measurement of identified protein biomarkers on Agilent 6460 and 6490 triple quadrupole instruments.

LC-MS analysis led to the identification of 374 serum proteins. Of these, 120 showed a significant (p

In this proof of principle study we used samples accrued under strict clinical trial governance to identify and verify a potential serum protein signature of disease (biochemical) recurrence in patients treated with CHRT. Further validation studies using samples from other clinical trials will facilitate the development of a protein signature of significant clinical utility.

P-91.00
DAILY RHYTHMS IN THE CYANOBACTERIUM SYNECHOCOCCUS ELONGATUS PROTEOME AND PROTEIN COMPLEXES

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Introduction
Circadian rhythms are present in animals, plants, fungi and several bacteria. The central mechanism behind these 'pacemakers' and the connection to the circadian regulated pathways are still poorly understood. However, it is known that the cyanobacterium Synechococcus elongatus PCC 7942 (S. elongatus) has a highly robust central clock system, controlled by only three proteins, named KaiA, KaiB and KaiC. Moreover, this system has been extensively studied functionally, structurally and can be reconstituted in vitro. These characteristics together with the relatively small genome (2.7 Mbp) of S. elongatus, makes it an ideal model system for the study of circadian rhythms.
Our aim is to uncover the global dynamics of this system at the protein level, as well as on the abundance dynamics of protein complexes.

Methods
To uncover the variation in protein abundances during 48 hours, under light and dark cycles (12:12-hours), we applied quantitative proteomics approaches. We used TMT 6-plex isobaric labeling and a multi-dimensional separation method coupled to mass spectrometry analysis. When focusing on the protein complex dynamics, we combined native size exclusion chromatography (SEC) with a mass spectrometry based label free approach.

Results
We were able to find protein evidence of 82% of the genes in S. elongatus. Among the 1537 proteins quantified over the time-course of the experiment, 77 underwent significant cyclic variations., significantly lower than at the mRNA level. Moreover, we find evidence for in- and out-of-phase correlation between mRNA and protein levels pointing to the importance of post-transcriptional and/or post-translational regulatory mechanisms. For several protein complex members, such as photosynthetic and ribosomal proteins, we find agreement in abundance dynamics, which will be explored further.

Conclusions
Our data provides novel insights into the phasing of cyclic protein abundances relative to corresponding mRNA levels, namely out-of-phase cases. Moreover, abundance dynamics of several protein complexes is visualized.

P-92.00

PROTEOMIC ANALYSIS OF BENIGN AND MALIGNANT PROSTATE TISSUES BY 2-D DIGE COUPLED WITH MASS SPECTROMETRY TO IDENTIFY NOVEL DIAGNOSTIC MARKERS FOR PROSTATE CANCER

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Introduction and objectives

The worldwide incidence of prostate cancer (PCa) ranks third among cancers in men. Prostate-specific antigen (PSA) test is an established diagnostic tool for PCa detection but it has some major limitations that have led to a significant number of false positive results and unnecessary prostate biopsies. The low sensitivity and specificity of current non-invasive diagnostic methods for PCa underscores the need for improvement in this area.

Methods

We compared the proteome of benign prostate hyperplasia (BPH, n = 5) and prostate cancer (PCa, n = 5) tissues by two-dimensional differential gel electrophoresis and mass spectrometry to identify potential biomarkers which might distinguish the two clinical situations. Western blot was used to validate the findings on several proteins with the highest discrimination potential.

Results and Discussion

Proteomic data revealed 119 differentially expressed protein spots and 46 of them were up-regulated in PCa compared to BPH. Using principal component analysis and hierarchical clustering we could clearly separate tumor and normal tissue into two distinct tumor groups based on the differential protein expression pattern. For the MS identification, we selected a set of 39 proteins with the highest potential to discriminate PCa from BPH. These proteins are involved in many diverse pathways and have established roles in cellular metabolism. Ingenuity Pathway Analysis revealed highly significant interactions between the identified proteins and cancer. Investigation of the potential transfer of several up-regulated in cancer proteins into the urine and their validation in the larger and independent cohort is ongoing and might potentially lead to the novel diagnostic strategies.

Conclusions

Our study revealed a significant up-regulation of several proteins in PCa which have the potential to distinguish it from BPH. Some of the proteins identified in this study may be used as new targets for non-invasive PCa diagnosis.

P-93.00

THE ANTITUMOR NATURAL COMPOUND CURCUMIN PROMOTES PROSTATE CANCER CELL DEATH BY INDUCING ENDOPLASMIC RETICULUM STRESS.

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Recent advances have highlighted the importance of the endoplasmic reticulum (ER) in cell death processes. Natural compounds that effectively enhance tumor cell death through activating ER stress have attracted a great deal of attention for anti-cancer therapy. Curcumin, an active phenolic compound extracted from the rhizome of the plant Curcuma longa has been known to possess anti-inflammatory, antioxidant and antitumor properties.

A bio-evaluation on curcumin against highly metastasic prostate cancer PC3 and different cancer cell lines was performed through MTT assay. Furthermore and real time cell assay were used to evaluate the apoptotic induction of curcumin . The proteins modulated by purified curcumin were identified through a quantitative proteomics approach. Western blot and RT-qPCR were then utilized to confirm whether cur cumin induced apoptosis is mediated through activating ER stress pathway. Curcumin exhibited strong anti-tumor activity and significantly induced apoptosis in PC3 cells. Curcumin treatment showed a decrease in cell viability at 5ug/ml. TUNEL assay showed a marked decrease in cell viability when PC3 were exposed to curcumin treatment.

The latter compound significantly upregulated both the mRNA and protein expression levels of ER stress markers: Calreticulin, Protein disulfide isomerase (PDI), 78 kDa Glucose-regulated protein (GRP78) and apoptosis markers indicating that endoplasmic reticulum (ER) stress responses were induced, leading to apoptosis. Curcumin exhibited anti-tumor effects on PC3 cells via an ER stress-mediated mechanism. This anticancer compound could be further explored as potential candidates for chemoprevention and as an attractive therapeutic strategy in prostate cancer.

P-94.00
PROTEOMIC PROFILING OF THYROID NEOPLASMS IDENTIFIES CANDIDATE MARKERS OF TUMOUR MALIGNANCY
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Introduction. Thyroid nodules can be found by ultrasonography in about 50% of the general population and most of them are benign. However, their preoperative diagnosis, based on cytological evaluation of fine-needle aspiration (FNA) biopsies, results in up to 30% of indeterminate results. Of them, only 20-30% of cases are proven to be malignant, which means that many patients undergo unnecessary surgeries. Furthermore, protein markers of thyroid cancer recurrence are still needed.

Objectives. 1) Identify markers of malignancy among the three most common thyroid neoplasms: benign follicular adenoma (FA), follicular thyroid carcinoma (FTC) and papillary thyroid carcinoma (PTC); 2) Identify markers of recurrent PTC, the main type of thyroid cancer

Methods. We performed a comprehensive discovery-based proteomic study using iTRAQ labelling and mass spectrometry. Fresh frozen tumour tissues of 9 FA, 8 FTC and 10 PTC cases (five of them with recurrence) were employed. Lysates were labelled with 4-plex iTRAQ reagents, fractionated by SCX chromatography and analysed using data-dependent acquisition on a TripleTOF 5600. Expression of some candidate protein markers was validated by SRM and Western blot.

Results and discussion. 267 proteins were differentially expressed between PTC-FTC and 402 between PTC-FA. The comparison between FA and FTC demonstrates high similarity among these histotypes, with only 30 proteins differentially expressed. Nonetheless, we found that several extracellular proteins were commonly upregulated in the malignant follicular neoplasms and that a combination of some of them (e.g. IGFBP5 and TGFBI) allows their discrimination. Also, the distinction between papillary and follicular tumours can be made with different proteins such as fibronectin, galectin-3, HMGA2 and Annexin 1. Underexpression of biglycan was found in recurrent PTC. Further studies may support their use as ancillary diagnostic markers.

Conclusions. Several proteins can differentiate papillary and follicular neoplasms. FA and FTC can be distinguished by the expression of extracellular proteins.

P-95.00

QUANTITATIVE ANALYSIS OF N-LINKED GLYCOPROTEIN IN THE BRAIN OF APP/PS-1 ALZHEIMER'S DISEASE MODEL

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Alzheimer disease (AD) is a neurodegenerative disorder characterized pathologically by the accumulation of senile plaques and neurofibrillary tangles, and both these pathological hallmarks of AD are extensively modified by glycosylation.

To discover the molecular basis of Alzheimer's Disease, we analyzed the APP/PS-1 double-transgenic mouse model of Alzheimer's disease using quantitative glycoproteomic analysis. In this study, the glycopattern of brain proteins from the mouse model was profiled using lectin bloting. And then N-glycoprotein profiles in brain of diseased mouse and age-matched healthy one were quantitatively compared using iTRAQ labeling, sequential enrichment of sialic acid-containing glycopeptides using TiO2 chromatography followed by neutral glycopeptide enrichment using IP-ZIC-HILIC, and liquid chromatography tandem mass spectrometry (LC-MS/MS).

In the brain of APP/PS-1 Azheimer's Disease, the level of protein sialylation was signifcantly lower than control. Several differentially expressed sialoglycoprotein and neutral glycoprotein have also been quantified using this strategy. Further researches are going on in our laboratory.

The findings of aberrant glycosylations in AD may help understand the mechanisms of neurodegenerative diseases.

P-96.00
SPATIOTEMPORAL UPAR EXPRESSION IN STAGE B AND C RECTAL CANCER CORRELATES DIFFERENTIALLY WITH PATIENT SURVIVAL

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Introduction and objective: Over the last two decades, urokinase plasminogen activator receptor (uPAR) has be extensively studied as a potential prognostic indicator of colorectal cancer (CRC) survival, reflected by >800 uPAR oncology publications. However, uPAR expression in CRC remains controversial, especially with regards to cell type on which uPAR is overexpressed (e.g., tumour epithelial (uPAR-E) or stroma-associated cells (uPAR-S)). Here, two epitope-specific anti-uPAR MAbs discriminate uPAR-E from uPAR-S and are correlated with patient survival in a large Stage B and C rectal cancer (RC) study.

Methods: Using immunohistochemistry, anti-uPAR MAbs #3937 and R4 were utilised to discriminate uPAR-E from uPAR-S in 343 Stage B and C RC tissues in both the central and invasive front tumour spatial regions. Intensities of antigen expressions were evaluated by two independent assessors and Kaplan-Meier curves used to examine correlation with survival.

Results and discussion: This study conclusively revealed that expression of uPAR occurred in both tumour epithelial and stromal compartments in RC. Our study strongly correlated uPAR-E adversely with overall survival in Stage B RC patients (p=0.006) reproducing many previous studies. Fascinatingly, uPAR-S correlated with better Stage C survival (p=0.008), also reflecting previous studies demonstrating macrophage accumulation at tumour margins is associated with better survival. The study demonstrates that uPAR epitopes should be considered as spatiotemporally expressed in human cancer (i.e., detectable by different epitope-specific MAbs in different cell types at different cancer Stage).

Conclusions: Our finding will benefit Stage B CRC patients as it allows clinical discrimination and more informed therapeutic decision making regarding patients who do well from those that do not post-resection. Understanding the uPAR-E and uPAR-S interactomes is anticipated to reveal how this spatiotemporal expression difference occurs.

P-97.00

CALIBRATION FREE CONCENTRATION ANALYSIS (CFCA) OF PROTEINS IN COMPLEX SERUM SAMPLES USING SURFACE PLASMON RESONANCE

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Introduction: In complex biological samples such as serum, the accurate protein quantification based on its interaction with binding partner, which is independent of a standard calibration curve would be valuable for many biological applications. Calibration free concentration analysis (CFCA) is a label-free approach, where concentrations are derived without reference to a calibration curve. Assay is based on calculating concentrations using known diffusion coefficient of proteins and observed changes in binding rates at two different flow rates under diffusion-limited conditions. Here, we demonstrate the application of CFCA method for quantifying different proteins in complex serum samples.

Methods: The reaction surfaces appropriate for CFCA were prepared by immobilizing antibodies on CM5 chips via amine coupling, giving 5000-12000 RU. The CFCA was performed for Beta-2 microglobulin (B2M) and Serum amyloid A (SAA) in healthy, infectious and non-infectious disease serum samples. Serum dilutions were prepared and injected in duplicates at flow rates of 5 and 100 μl/min over active and reference flow cells followed by regeneration of the surface.

Results: Beta-2 microglobulin could be detected to less than 15 ng/ml of protein in 2000-fold serum dilution of infectious samples using Biacore T200. The accuracy and sensitivity of the results was successfully determined by simultaneously analyzing pure B2M and B2M spiked in serum samples, through CFCA. We also measured the serum concentration of Serum Amyloid A (SAA) in healthy and severe/non-severe infectious and non-infectious disease samples using Biacore T200 and significant fold changes were observed. The fold changes of SAA calculated from CFCA correlated effectively with mass spectrometry/ proteomics analysis of these samples.

Conclusion: The reproducibility and consistency of CFCA assay in various serum samples made the interpretation of assay simple and reliable. This study illustrates a significant step forward in monitoring the active protein concentration in serum samples, which has utility for therapeutic applications.

P-98.00
STUDY OF GRADE-SPECIFIC PROTEOMIC ALTERATIONS IN GLIOMAS

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Introduction and objectives

Gliomas are brain tumors originating from glial cells. WHO classified human gliomas into four different grades based on the mitotic activity and microscopic characteristics of the tumor cells. Grade-IV glioma is also known as glioblastoma multiforme (GBM) and the median survival period of these patients is around 12 months. The protein expression levels vary in various cancerous tissues with respect to normal tissues. Objective of our study is to find out the grade-specific proteomic alterations in various grades of gliomas.

Methods

We studied proteomic alterations in various grades of gliomas using gel-based (2D-DIGE) and gel free (iTRAQ) proteomic approaches. Brain tissue proteins from normal and glioma grade-II, III & IV tumor tissue was extracted using Trizol protein extraction method. Protein extracts from 6 patients each of normal, grade-II, III & IV were subjected to 2D-DIGE analysis. For iTRAQ experiments, protein samples were made LC-MS compatible by performing buffer exchange with 0.5M TEAB buffer followed by in-solution digestion and iTRAQ labeling. The iTRAQ labeled samples were fractionated using off-gel and subjected to LC-MS/MS analysis.

Results and Discussion

Our study showed down-regulation of various proteins involved in oxidative phosphorylation and pentose phosphate pathway in higher grades of gliomas. Some enzymes involved in lactate metabolism were also found to be up-regulated. Some of the identified proteins like nicotinamide phosphoribosyltransferase, protein disulfide-isomerase, alpha-1-antitrypsin and hemopexin showed positive correlation with increase in the grade of tumor, whereas tubulin polymerization-promoting protein, brain acid soluble protein, and contactin showed negative correlation with increase in the grade of tumor.

Conclusion

We have performed a comprehensive quantitative proteomic analysis of human gliomas and identified grade-specific proteomic alterations in gliomas. Further, the pathway analyses suggests modulation of various pathways and signaling networks in different grade of gliomas and enhanced our understanding of gliomas pathobiology.

P-99.00

QUANTITATIVE SERUM PROTEOMIC ANALYSIS OF MENINGIOMAS FOR THE IDENTIFICATION OF SURROGATE PROTEIN MARKERS

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Introduction and Objectives: The pathophysiology of meningiomas is still unclear and often diagnosis can be misleading, especially when radiology is equivocal and locations of their occurrence are unusual. This study aims to investigate serum proteome alteration in different grades of human meningiomas; grade I, grade II and grade III to obtain insights about disease pathogenesis and identify grade-specific surrogate protein markers.

Methods: For comparative analysis three complementary quantitative proteomic approaches; 2D-DIGE along with MALDI-TOF/TOF MS, iTRAQ-based and label-free MS using ESI-Q-TOF mass spectrometer were employed, and results were validated by ELISA and western blotting. The differentially expressed proteins ($p < 0.05$) identified in different grades of meningiomas were subjected to functional pathway analysis using Protein Analysis Through Evolutionary Relationships, Database For Annotation Visualization and Integrated Discovery and GeneTrail functional annotation tools for understanding their biological contexts.

Results and Discussion: In silico analysis involving the identified 157 differentially expressed proteins revealed modulation of different vital physiological pathways, including complement and coagulation cascades, metabolism of lipids and lipoproteins, immune signaling, cell growth and apoptosis and integrin signaling in meningiomas. The ROC curve analysis demonstrated apolipoprotein E and A-I and hemopexin as efficient predictors for meningiomas (AUC > 0.70). Identified proteins like vimentin, alpha-2-macroglobulin, apolipoprotein B and A-I and antithrombin-III, exhibited a sequential increase in different malignancy grades of meningiomas. Additionally, few targets exhibited differential expression only in a particular grade.

Conclusion: To the best of our knowledge, this is the first comprehensive investigation describing serum proteomic alterations in different grades of human meningiomas. Our results provide better understanding of the underlying mechanism of the disease pathogenesis and tumour progression in human meningiomas.

P-100.00

IMPROVING SELECTIVITY AND SENSITIVITY IN CLINICAL ASSAYS USING PARALLEL REACTION MONITORING

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Targeted proteomics analyses in biomarker evaluation studies are routinely performed on triple quadrupole mass spectrometers in selected reaction monitoring (SRM) mode. However, the low resolution of quadrupole mass filters has limited selectivity for the analysis of complex samples, characterized by a high background interfering with the signal of analytes. Hybrid mass spectrometers with high resolution and accurate mass capabilities overcome this limitation, and opened new avenues in quantitative proteomics. Targeted analyses of clinical samples were performed using parallel reaction monitoring (PRM) on a quadrupole-orbitrap mass spectrometer to demonstrate the gain in selectivity.

Plasma samples were collected from patients diagnosed with lung cancer (at various disease stages) as well as healthy controls. Analyses were performed on a quadrupole-orbitrap instrument (Q-Exactive, Thermo Scientific). The quantification of peptides, based on isotope dilution strategy, was performed by extracting post-acquisition the ion traces of specific fragments. The confirmation of the identity of peptides was performed by spectral matching.

Targeted LC-MS/MS analyses were first performed on a pool of control plasma samples to acquire full MS/MS spectra of the peptides of interest. The signals of fragment ions were extracted post-acquisition using narrow mass window (typically 10 ppm), which dramatically reduced background interferences and improved the selectivity of the assays. The trapping capability of the instrument proved beneficial for the enrichment of precursor ions of peptides present in tiny amounts.

A total of 80 peptides corresponding to 40 proteins, including lung cancer candidate markers, were monitored in triplicated PRM analyses of 10 patient (at different stages of the disease) and 10 control samples. The analyses were replicated in SRM mode on triple quadrupole instrument.

The PRM analyses showed better quantification performance and confident measurements of the low abundance endogenous peptides, which translated in a clear discrimination between the control and patient samples, and even different disease stages.

P-101.00

MULTIPRONGED QUANTITATIVE TISSUE PROTEOMIC ANALYSES INDICATE MODULATION OF VARIOUS SIGNAL TRANSDUCTION PATHWAYS IN MENINGIOMAS

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Introduction and Objectives: Meningiomas are the most common non-glial tumors of the brain and spine. There is inadequate information available regarding the mechanism for meningioma tumorgenesis, and their etiology, disease pathobiology and epidemiology. This study was conducted to investigate the tissue proteomic alterations in different grades of human meningiomas (benign, atypical and anaplastic) and modulations in various signaling networks to identify the molecules associated with the vital processes involved in meningioma tumorgenesis.

Methods: Comparative tissue proteomic analysis of control and different grades of meningiomas were performed by iTRAQ-based quantitative proteomics using ESI-Q-TOF and Q Exactive Orbitrap mass spectrometers as well as gel-based 2D-DIGE, and employing ELISA results were validated. The differentially expressed tissue proteins (fold-change > 1.5; $p < 0.05$) identified in different grades of meningiomas were subjected to functional pathway analysis.

Results and Discussion: In 2D-DIGE analysis, we have indentified differential expressions of 98 and 44 protein spots ($p \leq 0.05$) in benign (grade I) and atypical (grade II) meningiomas, respectively. Further, quantitative proteome analysis revealed differential expression (1% FDR) of nearly 3000 tissue proteins in different grades of meningiomas. Interestingly, in silico functional analysis involving the identified differentially expressed proteins revealed the aberration of diverse vital physiological pathways and signal transduction pathways, including integrin, EGF and FGF receptor, Ras, VEGF, Wnt and FAS signaling pathways, which play crucial roles in cell differentiation, proliferation and cell death in meningiomas.

Conclusion: Findings obtained from our proteomics and subsequent pathway analyses evidently suggest the existence of aberrant signaling networks in different grade of meningiomas, which may lead to the uncontrolled proliferation, survival and invasiveness. Identified targets indicate the rationale for employing combination of different signaling inhibitors for therapeutic interventions.

P-102.00

PROTEOMIC ANALYSIS OF G PROTEIN-COUPLED ESTROGEN RECEPTOR 1 (GPER) BY MALDI-TOF/TOF MS

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Introduction and objectives:
Numerous physiological processes in mammals are influenced by estrogens and estrogen receptors. Estrogen is implicated in the development or progression of a number diseases, such as human cancers, endometriosis, fibroids, and cardiovascular diseases. The biological actions of estrogen are mainly mediated by classical estrogen receptors, ERα and ERβ that belongs to the nuclear receptor superfamily. In recent years, a class of membrane-associated estrogen receptors are found to mimic the functions of classical ERs. A member of the GPCR family, GPER (formerly known as GPR30), mediates rapid biological responses to estrogen in diverse normal and cancer cells, as well as transformed cell types. It may play a significant role in the development of tamoxifen resistant breast cancer.
The principle objective of the study is to isolate, identify and characterize GPER in order to understand the mechanisms underlying complex pathways and identify potentially new drug targets.

Methods:
Whole protein lysate from 4 different cancer cell lines were used. A unique protocol with the use of hydroxyapatite spin column was developed to isolate and enrich GPER from protein lysate. The eluate was subjected to immunoblot analysis. Later, the eluate was digested with different proteases, in different experimental conditions and analyzed in MALDI TOF/TOF MS.

Results and Discussion:
In the present study, the immunoblot confirmed the presence of GPER when probed against GPER (N15) antibody. MALDI MS profiling identified the presence of GPER after intensive data analysis. According to UNIPROT, GPER has 4 potential post-translational modifications (3-glycosylations & 1-disulfide bond). We manually validated 2 out of 3 glycosylation sites and predicted possible glycans from the
corresponding MS/MS data.

Conclusion:
This approach is the first of its kind to identify GPER and its post translational modifications by mass spectrometry. The proposed protocol is simple, robust and unique with great reproducibility.

P-103.00

DISCOVERY OF COLORECTAL CANCER BIOMARKER CANDIDATES BY MEMBRANE PROTEOMIC ANALYSIS AND SUBSEQUENT VERIFICATION USING SELECTED REACTION MONITORING AND TISSUE MICROARRAY ANALYSIS

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Recent advances in quantitative proteomic technology have enabled the large-scale validation of biomarkers. We here performed a quantitative proteomic analysis of colorectal cancer tissue using membrane protein extraction with PTS followed by iTRAQ labeling to discover biomarker candidates, and then extensively validated the candidate proteins identified. A total of 5566 proteins were identified in six tissue samples, each of which was obtained from polyps and cancer with and without metastasis.

GO cellular component analysis predicted that 3087 of these proteins were membrane proteins, while TMHMM algorithm predicted that 1567 proteins had a transmembrane domain. Differences were observed in the expression of 159 membrane proteins and 55 extracellular proteins between polyps and cancer without metastasis, while the expression of 32 membrane proteins and 17 extracellular proteins differed between cancer with and without metastasis.

A total of 105 of these biomarker candidates were quantitated using selected (or multiple) reaction monitoring (SRM/MRM) with stable synthetic isotope-labeled peptides as an internal control. The results obtained revealed differences in the expression of 69 of these proteins, and this was subsequently verified in an independent set of patient samples (polyps (n=10), cancer without metastasis (n=10), cancer with metastasis (n=10)). Significant differences were observed in the expression of 44 of these proteins, including ITGA5, GPRC5A, PDGFRB, and TFRC, which have already been shown to be overexpressed in colorectal cancer, as well as proteins with unknown function.

The expression of some of the biomarker candidates were also verified using a multi-cancer tissue microarray, which included 1150 cores from 14 cancer tissues. Our methods for biomarker discovery and subsequent validation using SRM/MRM will contribute to the identification of useful biomarker candidates for various cancers.

We are currently investigating if our identified biomarker candidates could be detected and quantitated in the serum/plasma of colorectal cancer patients.

P-104.00

IDENTIFICATION OF AN EXOSOMAL PROTEIN INVOLVED IN INVASION OF BREAST CANCER CELL AND STUDY ON CLEARANCE OF THE EXOSOMES FROM THE CIRCULATION

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Exosome are nanoscale (50–100 nm) membrane vesicles, released by fusion of multivesicular bodies with the plasma membrane from most cell types. Emerging evidences suggested that tumor-derived exosomes may promote invasion of tumor cell. To identify exosomal proteins involved in invasion of breast cancer cell, proteomic analysis of exosomes from MCF-7 and MDA-MB-231 cell lines are employed. The functional activity and the clearance mechanism from the circulation of the protein are further studied.

Exosomes from MCF-7 and MDA-MB-231 cell lines were isolated and invasion assays were performed for identification of invasion factors. Because exosomes from MDA-MB-231 cell line increased invasion ability of MCF-7 cells, exosomes from MCF-7 and MDA-MB-231 were analyzed by LC-MS/MS to identify differently expressed proteins. We focused on a protein and studied knockdown and over-expression of the protein X on exosomes. For clearance study of exosomes in the circulation, uptake assays were performed with fluorescence-labeled exosomes and human umbilical vein endothelial cells (HUVECs).

Here we identified a number of proteins from results of proteomic analysis. Among the proteins up-regulated in exosomes from MDA-MB-231, several proteins relevant to cancer development were selected. We focused on a protein X and showed that this protein is involved in invasion of cancer cell. The mechanism of invasion through protein X on exosomes and the mechanism of exosomes clearance were studied. These findings suggest that exosomal proteins play an important role in cancer invasion and these circulating exosomal proteins could be potential biomarkers for early diagnosis of breast cancer.

We identified a new function of exosomal protein X in invasion of breast cancer and clearance mechanism of these exosomes from the circulation.

P-105.00
THE CANCER-DERIVED SECRETORY/RELEASING PROTEOME: A VALUABLE RESOURCE FOR DISCOVERING TUMOR MARKERS

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Introduction and objectives: The circulating plasma is the most difficult protein-containing specimen to characterize because of the high-abundance resident proteins; whereas most of the potential tumor markers are presented at very low abundances. To discover the ¡®secretory/releasing¡¯ proteins derived from solid tumors in the circulating plasma, a novel system has been developed.

Methods: Primary cultures were established with surgically resected tumor and corresponding adjacent normal tissues; and the serum-free conditioned medium (CM) samples were collected and processed for proteomic analysis. The total proteins released into the CM were identified by liquid chromatography-tandem mass spectrometry. Following protein database searching and bioinformatic analyzing, the resulting proteins were validated both in the tumor tissue and circulating plasma samples by immunochemical stain and enzyme-linked immunosorbent assay (ELISA), respectively.

Results and Discussion: This proteomic study system has been applied into 5 human solid tumors, and the secretory/releasing protein profiles for these malignancies have been generated. The numbers of non-redundant proteins identified from the tumor-derived CM samples were, 828 for lung cancer, 1129 for ovarian cancer, 1149 for hepatocellular carcinoma, 459 for laryngeal carcinoma and 579 for pancreatic cancer. Panels of the ¡®CM proteins¡¯ were validated by ELISA with human circulating plasma specimen, as potential tumor markers, which are CD98, Fascin, pIgR/SC and 14-3-3 eta for lung cancer; MMP1, OPN and PSG9 for hepatocellular carcinoma; NID1, TIMP2 and VCAN for ovarian cancer; and KLK6 for laryngeal cancer.

CONCLUSIONS: The strategy adopted in this approach is effective in discovering the low abundant proteins released into the blood by solid tumors. It may lead to identification of novel protein markers and provide valuable information for establishing tumor biomarker profiles, for guiding diagnosis, prognosis or therapeutic monitoring of human cancers.

P-106.00

ANALYSIS OF THE EXOSOME UNDER Γ IRRADIATION.

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More and more information is positioning RNA in the sphere of the signalling pathways. For instance, microRNAs or lncRNAs are being involved in the regulation of the expression of several genes and therefore in the expression of specific proteins in a specific cellular context.

The surveillance of the RNA molecules is an essential step in keeping the correct homeostasis of RNA levels in the cell. The deregulation of the RNA levels can lead to a collapse in the cell, from altering transcription till deregulating the expression of specific proteins. Therefore, the maintenance of the correct RNA levels is a crucial step in the cellular homeostasis.

The exosome is a multiprotein complex involved in a myriad of cellular functions by its activity in RNA degradation and processing. Different kinds of RNA can be degraded by this complex thanks to its ribonucleolitic activity. The exosome is highly conserved throughout evolution from yeast to humans. The exosome subunits in Drosophila melanogaster have been resolved and are very similar to their human counterparts. In D. melanogaster, the catalytic activity of the exosome is exerted by two subunits: RRP6 and DIS3.

In our work, we have studied the protein network associated to the subunit RRP6 in control cells and in cells exposed to γ irradiation, mimicking the effects of a radiotherapy context. Immunoprecipitation of the RRP6 protein has been complemented with a mass spectrometry assay to determine the proteins bound to RRP6 after irradiation.

In our results, several RNA and DNA binding proteins are found as possible interactors of RRP6 in irradiated cells. This analysis has been complemented with the specific investigation of the function of a RRP6 mutant that is catalytically inactive.. This work will provide information about the role of RRP6 under irradiation.

P-107.00

BREAST CANCER TUMOUR TRANSFORMATION FROM PRIMARY TUMOUR TO SECONDARY SITE

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Introduction and objectives

Breast cancer is a very heterogeneous disease and some patients are cured simply by surgical removal of the primary tumour while other patients suffer from recurrence and spreading of the disease. A number of treatment predictive factors have been identified such as tumour size, estrogen (ER) and progesterone (PgR) receptor status and human epidermal growth factor receptor 2 (HER2) status. The predictive factors assessing the nature of the tumour are all based on the status of the primary tumour. However, it could be anticipated that the cancer cells undergo a molecular transformation allowing the spreading to a secondary site. If the lymph nodes are positive for cancer cells or if distant metastases are identified, this disease would likely be more successfully treated by assessing predictive markers characterizing the cells having undergone spreading.

Methods

We are analysing a set of 14 primary breast cancer tumours with matched axillaries positive for breast cancer cells and a set of 9 primary tumours with matched distant metastases to further understand the molecular changes during the spreading and identify novel predictive markers. Protein glycosylation is predominant in both membrane proteins and secreted proteins. Importantly, changes in glycosylation of these proteins have been shown to correlate with cancer states. Glycopeptide capture was used in this study to selectively isolate and quantifies N-linked glycopeptides from mixtures of glycoproteins. The captured glycopeptides were subjected to mass spectrometry analysis.

Results and Discussion

Protocols for glycoprotein and glycopeptide capture were tested and optimized and it was concluded that the glycopeptide capture gave the most satisfactory results with 1145 proteins identified in total, all samples combined.

Conclusions

Glycopeptide capture is a highly sensitive and specific method that will allow IHC analysis of the tissue but also allow affinity capture to be used to analyze for biomarkers in serum.

P-108.00

ALPHA-ACTININ 4 IS ASSOCIATED WITH CANCER CELL MOTILITY AND IS A POTENTIAL DIAGNOSTIC BIOMARKER IN NON-SMALL-CELL LUNG CANCER

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Introduction and objectives: Alpha-actinin 4 (ACTN4) is differentially expressed and secreted between lung cancer CL1-0 and CL1-5 cells from previous proteomics investigation. The aim of this study is to investigate functional properties of ACTN4 protein in non-small-cell lung cancer cells (NSCLC) and evaluate its clinical value.

Methods: We used RNA interference to knock down ACTN4 protein expression and evaluated its effect to cancer cell invasion and migration as well as microscopic cellular morphology. Furthermore, we examined ACTN4 protein expression in a total of 84 tissue samples at different stages of lung cancer by immunohistochemistry. We also compared ACTN4 protein levels of blood plasma samples collected from 185 patients with histologically confirmed lung cancer and 147 healthy controls

Results and Discussion: CL1-5 cell motility was significantly suppressed by the ACTN4 protein knockdown. The morphology of CL1-5 cells changed from a dominant mesenchymal-like into a globular shape in response to ACTN4 protein knockdown. Quantitative immunohistochemical assessment of the lung cancer tissues revealed that ACTN4 protein expression levels were considerably higher in cancerous tissues than the adjacent normal ones. The area under curve (AUC) of the receiver operating characteristic (ROC) curve was 0.736. Using ELISA assay, plasma levels of ACTN4 protein were significantly different between cancer patients and healthy controls while ROC analysis resulted in an AUC of 0.828. Notably, the plasma levels of ACTN4 protein were significantly elevated in early stages of NSCLC.

Conclusions: In this study, we demonstrate that ACTN4 protein knockdown inhibited invasion and migration of lung cancer cells, but did not alter their growth. Our results suggested that ACTN4 protein is associated with lung cancer cell motility. The protein level in cancerous tissue is associated with stages of lung cancer, while its level in plasma is an early diagnostic marker.

P-109.00

IMMOBILIZATION OF ANTIBODIES ON FE3O¬4@POLYDOPAMINE CORE¨CSHELL MICROSPHERES FOR SELECTIVE ENRICHMENT OF LYSINE-ACETYLATED PROTEINS AND PEPTIDES

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Protein lysine acetylation is a dynamic and reversible post-translational modification and plays an important role in life processes. However, the low amounts of the acetylated peptides or proteins could hardly be detected before enrichment.

In this study, for the first time, antibody-immobilized Fe3O4@polydopamine core¨Cshell microspheres were developed for selective enrichment of acetylated proteins and peptides. Covalent strategy through schiff base reaction and covalent-orientated strategy through the Fc region of protein A were both used in the process of antibody immobilization. At first, standard proteins composed of acetylated bovine serum albumin, myoglobin, ¦Á-casein and ovalbumin were used as model proteins to compare and verify the enrichment efficiency of these two strategies. Then, the synthesized acetylated peptide was employed to confirm the selective enrichment behavior of the covalent-orientated antibody immobilization microspheres.

The results turned out that covalent-orientated antibody immobilization strategy performed better than covalent strategy. With the help of covalent-orientated antibody immobilization microspheres, the S/N ratio of acetylated peptide was significantly improved even the molar ratio of acetylated peptide and myoglobin was 1 : 100. Moreover, the covalent-orientated antibody immobilization microspheres were successfully applied to analyze mouse liver tissue acetylated proteins.

P-110.00

THE EFFECT OF MUTATIONS IN BETA-AMYLOID ON ZINC ION COORDINATION

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Alzheimer's disease (AD) is characterized by severe accumulation of proteinaceous plaques, which mostly consist of aggregated amyloid-beta peptides. Mutations in amyloid beta are associated with a familial early-onset form of AD. Two of such mutations (Tottori D7N mutation, English H6R mutation) are located in the metal-binding domain and the mechanism of their high pathogenicity is unclear. It is assumed that they might affect the zinc chelation mechanism of amyloid-beta.

To determine the zinc coordination sphere of the amyloid-beta peptide and its mutated forms mass-spectrometric approaches were used. Using electron capture dissociation (ECD) and collision induced dissociation (CID) methods fragmentation mass spectra of the zinc-bound complexes have been analyzed. Zinc ion coordinators were deduced from the intersections of fragments with zinc ions fragments carrying zinc in CID spectra.

Most of the scientists have agreed earlier that the main three zinc ion chelators in the native amyloid beta molecule are the three histidine residues. The identity of the fourth chelator is of some debate, and many possible candidates are named. According to our data the fourth chelator is E11, and the7th aspartate is not involved in any way in the process, and its substitution for a glutamine has no effect on zinc ion coordination. Thus the pathogenic effect of the Tottori mutation is not associated with changes in zinc ion coordination and needs to be further investigated. As for the English mutation, substitution of one of the main chelating histidines for an incapable of such action arginine leads to a dramatic change of the whole coordination scheme.

As a result the zinc ion is coordinated by residues E3, D7, E11 and H14, what leads to a very even and highly symmetrical chelator distribution along the molecule, what in turn promotes dimerization and further aggregation, explaining the high pathogenicity of this mutation.

P-111.00

IMPROVED METHOD FOR SEQUENCING OF IMMUNOGLOBULIN MOLECULES; COMBINING NEXT GENERATION SEQUENCING AND MS/MS.

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Introduction and objectives

Antibody specificity is generated by somatic recombination and mutations in the immunoglobulin genes. Antibodies are composed of heavy and light chains. Monoclonal antibodies generated by cell fusion of B-cell with an immortal myeloma cell line. After screening and sub-cloning, cells that produce a monoclonal antibody are obtained. We wanted to develop a method for obtaining the encoding sequence of the antibody and verify its sequence by MS/MS.

Methods

RNA was extracted from the antibody producing cells, and total mRNA was sequenced with a HiSeq Systems. CLC-Bio was used for assembly of sequences. The monoclonal antibody was cultivated serum free. The antibody was purified using Protein-G. The antibody was digested with trypsin or chymotrypsin. MS analysis was performed on a QExactive LC-MS/MS with a 30 minutes gradient. Spectra were analysed on a mascot server using the deduced protein sequences of the mRNA.

Results and Discussion

The mRNA sequences were de novo assembled in CLC-Bio. A blast search was performed with a heavy and light chain nucleotide sequence. Approximately 2% of the total mRNA was from the immunoglobulin genes, and coverage was 40,000 times. From the deduced amino acid sequence the tryptic fragments were calculated. Specifically in the variable regions, fragments of more than 30 amino acids were observed, but several chymotrypsin sites were present. Therefore, digestion was performed with each of the enzymes. A near to 100% coverage was obtained with MS/MS.
Searching the MS/MS spectra against the complete de novo assembled sequences also identified the immunoglobulin sequences.

Conclusions

The combination of next generation sequencing and MS/MS gave an extremely valid characterization of a monoclonal antibody. The method is independent of the species from which the monoclonal antibody is obtained, and is generally applicable for samples from which mRNA and de novo assembly can be done.

P-112.00
OPTIMIZATION OF A WORKFLOW FOR FAST AND DEEP PROTEOME SEQUENCING

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Proteomics has evolved to the point near complete proteome coverage can be achieved. This comes at the cost of time – typically takes days of MS running time to achieve deep coverage of the proteome. To release the power of MS to the masses, we need a workflow that enables deep proteome coverage fast so that MS can become affordable for biomedical researchers.

We report a workflow capable of measuring up to 8,000 gene products (GPs) at 1 % protein FDR in less than 12 hours of MS running time. We first adapted a small-scale 2D reverse-phase-chromatography using different pH (sRP-RP at pH10 and 2) to profile the proteome in 6 MS runs of 1 hr each to achieve a coverage of 6,000 GPs; we next utilized TiO2 to enrich phospho-peptides and the above sRP-RP in 4 MS runs of 45 mins each to acquire 3,000 GPs in 3 hrs; we finally applied affinity isolation of transcriptional factors and DNA binding proteins using a transcription factor response elements (TFRE) coupled with sRP-RP in 2 MS runs of 45 minutes each to obtain another 2,000 GPs in 1.5 hrs. In most of cases, such combination led to a proteome coverage up to 8,000 GPs in 10.5 hours of MS running.

Such a workflow allows a peek into the proteome of important regulatory components – transcription factors and kinase substrates in a short time. We demonstrated that such a workflow is compatible with label-free quantification, and thus can be applied to many situations of biomedical research.

The day has come that a pair wise comparison of two proteomes at the depth of 8,000 GPs covering most of the regulatory factors using MS as a sole tool. This has lay down the foundation for releasing the power of MS to the masses.

P-113.00

MS- AND AB-BASED PROTEOMICS TO UNDERSTAND BIOLOGY AND DISEASE OF KIDNEY

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Patients with advanced stage of chronic kidney disease (CKD) are finally treated with dialysis or kidney transplantation. Primary lesion of CKD is the glomerulus in the kidney, and the causes (pathogenesis) and progression mechanisms (pathophysiology) of the disease are almost unknown. Therefore, no radical therapies have been developed yet. Proteomics to analyze kidney tissues and urine samples of the patients may break through this deadlock situation and make it possible to understand the pathophysiology and pathogenesis and to predict drug targets for development of radical therapies and clinically-beneficial urine biomarkers.

Normal human kidney compartment and human urine were analyzed by mass spectrometry (MS). The proteins identified were verified and localized by antibody (Ab)-based proteomics, such as immunohistochemistry (IHC) and the data were combined into the database of human kidney and urine proteomes. Then, proteomes of the glomerulus, microdissected from CKD kidney biopsy samples, were examined by MS and IHC. The MS-identified proteomes were quantitatively compared to normal glomerulus proteomes by the normalized spectral index (SIN) method and analyzed by a pathway analysis tool (Ingenuity Pathway Analysis).

Combining MS- and Ab-based proteomics of normal human kidney compartments provided both quantitation and localization data of proteins in normal human kidney tissue and possible sources of urinary proteins. Bioinformatics analyzing the proteomics data demonstrated unique networks and pathways in the kidney compartments. The proteomics of human kidney biopsy samples and its bioinformatics analysis disclosed several pathways, which were either enhanced or suppressed or unique in the CKD glomerulus, suggesting crucial pathways in the disease progression.

MS- and Ab-based proteomics and following bioinformatics analysis of human kidney tissue and urine samples provided new insights into the kidney biology. Furthermore, development of radical therapies and new clinically-beneficial urine biomarkers for CKD was expected to be innovated from these approaches in the near future.

P-114.00

IDENTIFICATION OF DISEASE SPECIFIC PATHWAYS THROUGH SILAC PROTEOMICS IN HBX-TRANSGENIC MICE

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The hepatitis B virus X-protein (HBx), a multifunctional viral regulator, participates in the viral life cycle and in the development of hepatocellular carcinoma (HCC). However, the underlying molecular mechanism remains obscure. Here we applied a SILAC strategy to define the underlying pathological mechanisms in the occurrence and development of liver cancer in the HBx transgenic mouse.

By systematically comparing a series of samples from the control, 12- and 24-month old HBx transgenic mice and their non-transgenic littermates representing the inflammation and HCC stage respectively, we identified 22 and 99 proteins with significant amount change from 4744 totally quantified proteins. Bioinformatics analysis suggested that the lipid metabolism and cytoskeleton remodeling pathways had been heavily affected by the transferred HBx gene. The protein-protein interacting proteomics study revealed that HBx directly interact with multiple proteins in these two pathways, further confirmed this result.

These results were validated by immunoblotting and immunohistochemistry in independent sets of samples from HBx transgenic mice. More importantly, we found some of these proteins specifically accumulated in HBV-associated HCC patient samples (HBV-HCC). The amount of these proteins and the severity of HBV related liver disease are positively correlated. The studies reported here provide evidence that the dysregulation of cytoskeleton remodeling and lipid metabolism induced by HBx leads the occurrence and development of liver cancer.

P-115.00

A QUANTITATIVE PROTEOMICS TOWARDS THE TISSUE INTERSTITIAL FLUID FROM AOM-DSS MOUSE MODEL OF COLORECTAL CANCER

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Introduction and objectives

Tissue microenvironment impacts the development and progression of colorectal cancer (CRC). It is generally accepted that tissue interstitial fluid (TIF) contains a number of metabolites and signaling molecules, which may exert influence to or be affected by tumor. Besides, a mouse model of AOM-DSS inducement is well accepted in CRC study because it offers an ideal sample comparable with human with identical genetic background. Herein, we initiated quantitative proteomics to monitor the TIF protein changes in response to CRC development and aimed at finding more potential biomarkers related with CRC.

Methods

Azoxymethane-dextran sodium sulfate (AOM-DSS) induced mouse model was modified for recapitulate ulcerative colitis. TIFs were prepared from the tissues collected from middle and distal colon of the mice, which were broadly divided to four different stages, control, cycle 1, cycle 2, and cycle 3. The quantitative proteomics in the TIFs was implemented through iTRAQ and the potential biomarkers of CRC were further verified by MRM in individual mouse TIFs and serum.

Results and Discussion

A total of 647 proteins were identified with more than 2 unique peptides in the TIFs, and 161 proteins were found the abundance changes (fold change>1.5) responding to tumor development. The development-related proteins were divided to five groups, constantly increased (n=58), constantly decreased (n=20), cycle 1-related (n=35), cycle 2-related (n=22) and cycle 3-related (n=10). In the group with constantly increased or cycle 3-elevated, 24 targets were selected for MRM verification in individual TIF samples, resulting 21 proteins that remained the same abundance changes as acquired from iTRAQ. Finally, 16 targets were employed into MRM assay in the serum samples, and 11 proteins were found their abundance responses to CRC in serum similar in TIF.

Conclusion

A fast and informative approach of quantitative proteomics was established in exploration of protein biomarkers in TIF.

P-116.00

THE VITREOUS PROTEOME IN YOUNG AND MATURE NEW ZEALAND WHITE (NZW) RABBITS

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Introduction: The vitreous humor (VH) is a gel-like matrix containing proteins and water with known age-related clinical alterations. Different compartments of vitreous exist which are either in contact with the lens/ciliary body anteriorly or posteriorly with the retinal surface. The vitreous proteome of the NZW rabbit has not been described nor is there information regarding protein profile in the vitreous compartments or age-related changes.

Methods: VH was dissected and separated from the ciliary body and retina into anterior (AC) and posterior compartments (PC) respectively. Specimens were collected from young (YR, n=4) and mature (MR, n=4) NZW rabbit eyes. Tryptic digests of total proteins were analyzed by liquid chromatography followed by tandem mass spectrometry (LC-MS/MS). Protein groups were identified using PASS/ UniProt database for all mammalian sequences. Spectral counting determined the relative abundance of proteins in each sample group. G test followed by Holm-Sidak correction determined differences in protein abundances between compartments and age groups.

Results/Discussion: More than 500 distinct proteins were identified in each sample. Common retinal proteins such as vimentin, aldehyde dehydrogenase, retinol binding protein and retinaldehyde binding protein 1 were 2-5 fold higher in the PC than AC of both YRs and MRs. Spectrin and glycogenin were 5.5 and 4 fold higher in AC of the YR compared to the MR. ApoE and aminolevulinate dehydratase were 2 and 3 fold lower in the AC of the YR compared to the MR respectively. Spectrin and APOE were 5 and 2 fold lower in the PC of the YR compared to the MR.

Conclusion: The protein profiles of the VH showed age and compartment related differences. The differential protein profile provides a baseline for understanding the vitreous compartmentalization and will help in the identification of future biomarkers for various vitreoretinal diseases with respect to age and the specific location.

P-117.00

CHARACTERISTIC PATTERNS OF PROTEIN AND GENE EXPRESSION AS MARKERS FOR DIAGNOSIS AND PROGNOSIS OF MALIGNANT MELANOMA

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Introduction and objectives

Malignant melanoma is a highly heterogeneous cancer that arises from the transformation of melanocytes. The diagnosis and prognosis of malignant melanoma is largely based on histological evaluation of biopsies of skin lesions. However, many of the histopathological features described so far are common in nevi and melanomas, remaining problematic diagnosis in a large number of patients with multiple pigmented lesions. The aim of our work was to identify a set of markers that can be used in the diagnosis and/or prognosis of malignant melanoma.

Methods

We performed a proteomic analysis to identify proteins differentially expressed between melanoma cell lines and primary melanocytes using two-dimensional polyacrylamide gel electrophoresis (2-D PAGE) and mass spectrometry (nLC-MS/MS). After validation of protein levels by Western blot, we analyzed the transcriptional levels of coding genes by RT-qPCR.

Results and Discussion

A hundred and thirty-three proteins were identified from 40 protein spots, which were considered differentially expressed between melanoma and primary melanocytes groups (with fold changes of at least ± 1.4 and p

Conclusions

The proteins identified could facilitate the diagnosis, predict the risk for metastasis in melanoma patients and help to develop more efficient therapies against this neoplasm.

P-118.00
COMPREHENSIVE ANALYSIS OF RADIOTHERAPY EFFECTS AFTER IRRADIATION WITH CARBON AND PHOTON USING GENOME-WIDE TRANSCRIPTOMICS AND QUANTITATIVE PROTEOMICS

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Introduction and objectives
Pulmonary microvascular endothelial cells are critically involved in the development of radiation induced acute inflammation and late fibrosis often limiting the delivery of sufficient dose to optimally treat lung cancer. Moreover, tumor-endothelium communication has emerged as a key target to augment radiotherapy by resensitizing endothelial specific survival signals. Therefore, we aimed to employ high-throughput genome-wide gene expression and whole proteome analysis to uncover the molecular mechanisms governing tumor-vessel communication and radiation induced normal tissue toxicity.

Methods
Human primary isolated pulmonary microvascular endothelial cells (HPMEC) were irradiated with 2 Gy carbon and 6 Gy photon, respectively. Cells were harvested two hours and six days after irradiation and proteins as well as RNA were extracted. Gene expression analysis was performed using 47k Illumina microarray (HumanHT-12 v4) platform. Mass spectrometry on an LTQ-Orbitrap instrument was employed for protein identification and relative quantification using label free quantification techniques. An IMAC/TiO2 enrichment step to obtain phosphorylated peptides was performed and analysed by mass spectrometry as well. The regulation of candidate radiation regulated genes and proteins were confirmed in vitro and in irradiated lung specimen (C57BL/6 mouse) by real time qRT-PCR, western blot and ELISA.

Results and Discussion
At the early timepoint a strong induction of genes involved in apoptosis and DNA-damage response such as GADD45A, PARP3 and DDB2 was identified. In contrast to acute radiation response, cell adhesion (Integrins), pro-inflammatory (Interleukines), pro-angiogenic and matrix-remodelling related genes (MMPs, PDGFs, VEGFs) were induced late after irradiation. Integrative quantitative analysis of protein expression, phosphorylation and gene expression analysis further revealed novel insights to an intricate gene/protein regulatory network underlying late radiation response in microvascular endothelium.

Conclusion
The here identified genes and proteins involved in late radiation response constitute attractive targets for modulation of radiotherapy effects in tumor-endothelium and prevention of undesired radiotherapy-induced late side effects.

P-119.00

CHEMOKINE RECEPTOR CXCR7 PROMOTES HEPATOCELLULAR CARCINOMA CELL GROWTH AND METASTASIS IN VITRO AND IN VIVO VIA ACTIVATING MAPK PATHWAY

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CXC chemokine receptors have been posited widely to have significant roles in many primary tumors and metastasis. However, the function of chemokine receptor CXCR7 in hepatocellular carcinoma (HCC) progression remains to be elucidated. In this study, CXCR7 levels were measured in 9 cell lines of HCC and in surgical specimens of 48 patients who received curative resection for HCCs.

We found that CXCR7 was overexpressed in highly invasive cell lines compared with poorly invasive ones. CXCR7 expression was significantly upregulated in metastatic HCC samples compared with the non-metastatic specimens (P = 0.0004). CXCR7 overexpression enhanced cell growth and invasiveness in vitro, and tumorigenicity and lung metastasis in vivo. By contrast, CXCR7 stable knockdown markedly reduced these malignant behaviors. Moreover, alterations in CXCR7 expression were positively correlated with the phosphorylation levels of MAPK pathway proteins. And we demonstrated that 26 and 19 differential cytokines in CXCR7-overexpression cell cultured media and CXCR7-depletion cell supernatant, respectively, using antibody array technology. CXCR7 expression was further proved to regulate circulating levels of VEGFA and galectin-3. Thus, elevated expression of CXCR7 contributes to HCC growth and invasiveness via activation of MAPK and angiogenesis signaling pathways.

Targeting CXCR7 may prevent metastasis and provide a potential therapeutic strategy for HCC.

P-120.00

A NOVEL PANEL OF PROTEINS CORRELATING WITH LYMPH NODE METASTASIS OF LOW-GRADE BREAST CANCER AS IDENTIFIED BY COMBINED PROTEOMICS AND TRANSCRIPTOMICS APPROACH

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Introduction and objectives
Current prognostic factors are not sufficient for precise risk discrimination in all groups of breast cancer patients. This is evident in the case of low grade breast tumors the small percentage of which, in disagreement with favorable prognosis, forms early lymph node metastasis. New molecular targets and biomarkers of this phenomenon are thus of high clinical need.

Material and Methods
We focused on identification of low grade specific, metastasis correlating proteins in the set of 48 small (T1) grade 1 luminal A primary breast tumors. Another set of 48 high grade tumors of various subtypes was analyzed to investigate the specificity of identified gene products. 4405 proteins were identified (FDR < 5%) within iTRAQ-2DLC-MS/MS proteomics analysis. Expression of 95 selected genes was analyzed at transcript level. Levels of top 5 proteins were analyzed also using immunohistochemistry. Connection of gene expression and patient survival was investigated in completely independent set of 214 breast tumors.

Results and Discussion
The panel of identified proteins correlating with lymph node positivity within low grade breast tumors at protein and transcript level, supported by similar trends in immunohistochemistry and patient survival data, involved activators of NF-êB pathway, secreted protease and cytoskeletal proteins. Analysis of biomarker selectivity divided the potential biomarkers into two selectivity groups: (i) Gene products up-regulated specifically in lymph node positive low grade tumors, (ii) those that had, in addition, higher levels in another groups of aggressive tumors (high grade and triple negative breast cancer).

Conclusions
The group of identified functionally relevant biomarkers in now in the process of further validation and deeper characterization of their role in breast cancer metastasis.

Acknowledgements

P-121.00

COMPREHENSIVE CHARACTERIZATION OF CELL SECRETOME VIA LC MS/MS ANALYSIS OF CONDITIONED MEDIUM AND SECRETORY PATHWAY ORGANELLES.

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Introduction and objectives: Secretome is defined as the rich, complex set of molecules secreted from living cells through classical (signal peptide), non-classical or exosomal pathways. The objective of the current study was to develop proteomic methods for the comprehensive analysis of secretome of bladder cancer cell lines differing in metastatic potential. Two different approaches were employed; based on the analysis of cell culture conditioned medium and targeting the secretory pathway organelles (ER/Golgi).

Methods: T24 and T24M cells were cultured in serum-deprived conditions for 24h. Conditioned medium was concentrated using Amicon ultrafiltration centrifugal filters. ER/Golgi were enriched using differential centrifugation. Total proteomic cell extract was in prarallel prepared to determine enrichment efficiency. FASP followed by LC-MS/MS analysis was in all cases applied. Proteins were designated as secreted according to UniprotKB, Gene Ontology and online tools (SignalIP, SecretomeP)

Results and Discussion: The selected strategies for secretome enrichment were highly complementary and reproducible (>60% of overlapping identifications in replicate runs). Of the proteins identified, 21% of the secretome, 9% of the ER/Golgi and 7% of the total cell extract were predicted to be secreted.

Conclusions: Even though contamination of the secretome by intracellular proteins is inevitable, the enrichment efficiency and complementarity of the applied methods is clearly shown. Current studies target validation and further investigation of selected findings in human clinical samples.

P-122.00

ANTIBODY-BASED PLASMA PROFILING FOR THE IDENTIFICATION OF PROTEIN SIGNATURES IN LYMPHOMA

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Lymphoma is a cancer of the lymphatic system. According to WHO it can be classified into four large groups of which one is Hodgkin's Disease. At present diagnostics are mainly based on biopsies. Thus, there is a need to establish new diagnostic tools that are fast and minimal invasive.

Affinity proteomics are a suitable tool for the quantitative analysis of biomarkers in blood-derived samples. For the identification of new potential biomarker candidates, antibody suspension bead arrays offer an approach that allows for the screening of a large number of analytes and samples.

In order to identify new potential biomarker candidates, 60 samples from a Swedish cohort covering three lymphoma subgroups, namely Hodgkin's Disease, Diffuse large B-cell carcinoma, and healthy controls, were analysed on more than 10.000 antibodies. Based on significant group differences, 370 antibodies from this initial screen were selected for further analysis.

In the second phase of the study, 60 additional samples from the same cohort were included, and a set of four antibody targets was found to differentiate between Hodgkin's Disease and healthy controls. The analysis of samples from a second cohort from Denmark confirmed these findings. Additionally, the most promising target was verified applying immunocapture and subsequent analysis by mass spectrometry.

P-123.00

CHARTING THE TEMPORAL SIGNALING NETWORK OF EGFR UPON EGF STIMULATION

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EGFR is a member of the receptor tyrosine kinase (RTK) family that upon receptor binding, is activated through homodimerization or heterodimerization with other surface receptors. Tyrosine phosphorylated EGFR engages scaffold proteins to recruit downstream signaling molecules and activate multiple biological pathways to promote proliferation, survival, migration, differentiation and resistance to apoptosis.

EGF binding also triggers rapid endocytosis of activated receptors, resulting in either recycling of receptors or their degradation by lysosome. While canonical endocytotic pathway is an important negative feedback mechanism for down-regulation of EGFR signaling, accumulating evidence strongly suggests that signaling can continue from endosomes, implicating the importance of endocytosis and trafficking in EGFR oncogenic pathway.

Here we report the comprehensive mapping of the dynamic EGFR signaling network stimulated by EGF. We developed a streamlined workflow of in-situ formaldehyde crosslinking that allowed us to isolate membrane-bound protein complexes that are normally dissociated during biochemical manuplation. Using MS-based proteomics, we quantitatively analyzed EGFR-associated complexes following EGF stimulation, and monitored its dynamic change as EGFR undergoes intracellular trafficking.

Bioinformatics analysis allowed us to cluster the large number of EGFR-interacting proteins to functional categories that implicate their functional roles in distinct intracellular organelle. Furthermore, we identified proteins that are not previously reported in EGFR signaling, which include kinases, phosphatases and phospholipid binding proteins. Our work thus establishes a temporal flow of signals mediated by EGFR, and may provide potential new therapeutic targets for EGFR tumorigenic pathways.

P-124.00

COMBINING BOTTOM-UP AND MIDDLE-DOWN APPROACHES TO MAP HYPER-MODIFIED HISTONE PEPTIDES USING UHPLC SEPARATION AND QEXACTIVE INSTRUMENT.

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Introduction and objectives

High-resolution (HR) Mass spectrometry (MS) is a powerful tool in epigenetic field for the analysis of Histone post-translational modifications (hPTMs). However, major restrains remain in the study of hyper-modified histone peptides using standard analytical HPLC-MS/MS platforms. In the present study we assess a novel analytical strategy combining ultra-high pressure liquid chromatography (UHPLC) with HR-MS analysis to study complex pattern of histone modified regions from macrophage-like cells at steady-state and upon inflammatory stimulus induced by Lipopolysaccharide (LPS). We also apply this approach in combination with Asp-N digestion to carry out a middle-down analysis to dissect "long-distance" connectivity among hPTM.

Methods

Histone cores were purified from both LPS treated and untreated cells and then subjected to different protein-digestion protocols: Arg-C, "Arg-C like" and Asp-N. Peptides were analysed both with HPLC and UHPLC device coupled with LTQ-Velos Orbitrap and QExactive instrument, respectively. Raw data were processed with MaxQuant. Dynamic changes of hPTMs were profiled using an intensity-bases approach.

Results and Discussion

Our results show the benefit upon introduction of UHPLC, that outperforms pre-existing HPLC configuration in identifying and quantifying nearly isobaric peptides, bearing distinct combination of modifications. Moreover, UHPLC analysis of "Arg-C like" digestion efficiently separates up to thirteen forms of the H3 (27-40), whereas Arg-C in solution digestion performs better in dissecting the acetylation pattern of H4. Furthermore, Asp-N digestion allowed us to identify more complex modification patterns, such as the five acetylations and methylations on R2, K20 and R23 on the N-terminal tail of H4. Once assess the performance of the platform on these case-study peptides, we carried out the label-free quantitative profiling of hPTMs changes upon LPS.

Conclusions

This analytical workflow based on UHPLC and HR-MS is suited for the systematic mapping and quantification of complex patterns of hPTMs.

P-125.00

NEW POTENTIAL BIOMARKERS FOR PROSTATE CANCER DIAGNOSIS AND PROGNOSIS USING 2D-DIGE

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Introduction and objectives: Prostate cancer is the most frequent cancer among men. To distinguish between indolent and clinical relevant prostate cancer is currently not possible. Aim of this study was to identify and validate potential new biomarkers for diagnosis and prognosis of prostate cancer.

Methods: Prostate cancer tissues of patients with and without relapse as well as tumor free tissue samples were analyzed using 2D-DIGE (two-dimensional Difference in Gel Electrophoresis) and mass spectrometry. Additionally Multiple Reaction Monitoring (MRM), immunohistochemistry and Western blotting were used for validation of promising candidates.

Results: Comparison of tumor free (n= 14) versus tumor samples (n=23) lead to 14 proteins differentially regulated in the 2D-DIGE experiment and identified by mass spectrometry. Furthermore a comparison of prostatectomy samples from prostate cancer patients without biochemical relapse (n=12) those with relapse (n=11) resulted in 29 deregulated protein spots that could be identified using MALDI-MS and LC-MS/MS.
2D-DIGE revealed down regulation of secernin-1 (p
Secernin-1 and vinculin might serve as new biomarkers for prostate cancer diagnosis and prognosis, respectively.

Conclusion: New markers in the field of prostate cancer research coulc be detected and identified by 2D-DIGE and mass spectrometry. Vinculin, galectin-3 and PAP concentrations were successfully measured in urine using MRM, so that this technique shows promising results for future implementation in clinical diagnostics.

P-126.00
RELATIVE QUANTITATION OF N-LINKED GLYCANS USING CARBONYL-REACTIVE TANDEM MASS TAG™ (TMT™) REAGENTS

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Introduction and Objectives:
Protein glycosylation is one of the most abundant and one of the most difficult post-translational modifications to study. Accurate quantitation of glycans remains elusive due to the lack of a comprehensive selection of standards, poor ionization efficiency of carbohydrates relative to other classes of biomolecules, and broad structural heterogeneity of glycomic samples. Recently, we have introduced a set of isobaric carbonyl-reactive Tandem Mass Tag (TMT) reagents, Thermo Scientific™ aminoxy TMT™ Reagents, which enable efficient relative quantitation of carbohydrates, improve ionization efficiency and increase analytical throughput. In this work we explore the use of these reagents in quantitative glycomics by combining our multiplexed TMT-based approach with HILIC LC-MS technique for more sensitive analysis with improved glycome coverage.

Methods:
N-glycan pools from several monoclonal antibodies and human serum samples were labeled with a set of isobaric aminoxyTMT reagents and the samples were analyzed by ESI mass spectrometry in multiplex experiments. Samples were analysed using a Thermo Scienitific™ Velos Pro™ dual-pressure linear ion trap and a Thermo Scientific™ Orbitrap Fusion™ Tribrid™ mass spectrometers. Combination of multiple-stage trap-HCD/CID fragmentation of the fully and partially protonated precursor ions was used to obtain both quantitative and structural information for sample glycans.

Results and Discussion:
We have established a protocol for derivatization of N-glycan mixtures, including all necessary quenching and clean-up steps. Twenty-fold improvement in signal intensity at the MS-level was observed for native labeled glycans ions for all precursor types. A combination of trap-HCD and CID MS analysis enabled both the quantitation by measuring reporter ion peak intensities at MS2 level and confirmation of the corresponding glycan structures using the diagnostic ions. Both neutral and acidic glycoforms are amenable to this strategy.

Conclusions:
The use of isobaric aminoxyTMT reagents enabled more sensitive relative quantitation of N-glycans with better precision and increased throughput.

P-127.00

GC–MS-BASED SEMI-QUANTITATIVE ANALYSIS OF TARGETED METABOLITES IN HUMAN PLASMA

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Introduction and objectives

Targeted quantitation of metabolites is generally considered to be more sensitive and accurate than global profiling. Here, we present a method for semi-quantitative analysis of targeted metabolites using Agilent 7890 gas chromatography (GC) coupled with 5975C single quadruple mass spectrometry (MS) in single ion monitoring (SIM) mode.

Methods

We analyzed 50 human plasma samples for quantitative analysis of 35 metabolites we selected for this study. Metabolites were extracted using a buffer containing six deuterated internal standards (Myristic Acid D27, L-phenylalanine-phenyl-D8, L-alanine-D4, L-tyrosine-D2, glycine-D5, and L-glutamic acid-D5) and trimethylsilyl derivatized. A quality control (QC) sample pooled from multiple plasma samples was run in both full scan and SIM modes to determine appropriate retention time (RT) points for the selected metabolites. We performed RT locking and used a mixture of fatty acid methyl esters (FAMEs) for retention index calibration. For each of the 35 target metabolites, we selected three ions (one target ion and two qualifier ions). We grouped these ions into seven RT intervals to balance the dwell time and the number of data points across the peaks.

Results and Discussion

We detected the six spiked deuterated internal standards in all 50 samples with coefficients of variation (CVs) ranging from 1-6% based on their log transformed intensity. Among the 35 metabolites, we detected all except one with an average CV of 3% ($\pm$ 1%) across the 50 samples. The intensity ratios between the target and the qualifier ions were highly consistent. Also, the ratios were highly comparable with those in the Fiehn's library.

Conclusion

This study demonstrates the reliability of GC-MS in the SIM mode for semi-quantitative analysis of targeted metabolites. We believe that this method has the potential to be one of the platforms for biomarker discovery studies to evaluate changes in metabolite levels in biological samples.

P-128.00

LC–MS/MS-BASED SERUM PROTEOMICS FOR IDENTIFICATION OF BIOMARKERS FOR HEPATOCELLULAR CARCINOMA

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Introduction and objectives

Hepatocellular carcinoma (HCC) is the third leading cause of cancer mortality worldwide. Survival rates of HCC patients can be significantly improved if the diagnosis was made at earlier stages, when treatment is more effective. Alpha-fetoprotein, the serologic biomarker in current use, lacks the desired sensitivity for detection of HCC. The objective of this study is to identify additional protein markers in serum for detection of HCC in high risk population of patients with liver cirrhosis.

Methods

We analyzed sera from HCC cases and cirrhotic controls in Egyptian cohort (40 cases and 49 controls) and US cohort (57 cases and 59 controls). Following depletion of high-abundant proteins by Agilent MARS Hu-7, we acquired LC-MS/MS data on a 3000 ultimate nano-LC system interfaced to an LTQ Orbitrap Velos mass spectrometer. In addition to the patient samples, we included in-between runs technical replicates by injecting peptide calibration mix from Bruker and analytical replicates by using aliquots of a sample pooled from the patients' sera.

Results and Discussion

Analysis of the LC-MS/MS data from the technical replicates by MaxQuant led to pairwise correlation coefficients ranging from 0.972-0.999. The correlation coefficients for the analytical replicates were between 0.937-0.995. We identified 278 and 258 protein groups in the Egyptian and US cohorts, respectively, considering only those with at least two peptides identified. From these, we found 28 and 38 protein groups with statistically significant difference between cases and controls (adjusted p-values

Conclusions

Analysis of LC-MS/MS data acquired from sera of 97 HCC cases and 108 cirrhotic controls led to identification of 58 candidate protein biomarkers. Targeted quantitation by MRM is currently underway for these candidates and other previously reported HCC biomarkers.

P-129.00

BIOMARKERS BREAST CANCER IDENTIFICATION THROUGH ITRAQ LABELING AND TANDEM MASS SPECTROMETRY

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Introduction. Breast cancer is the most common type of cancer in women worldwide and the principal cause of death by cancer in females. This disease is a serious health concern in the world that needs to be diagnosed opportunely. One of the most important tools used for the identification of this and other diseases are biomarkers. The mass spectrometry based proteomics has a key role in the identification of biomarkers useful for the diagnosis and prognosis of many diseases including breast cancer. There are few reports of quantitative proteomics applied to several breast cancer cell lines using iTRAQ and tandem mass spectrometry. For this, we determined the expression profiles of breast cancer cell lines MCF7, MDA-MB-231, SK-BR-3 and T47D in comparison to a normal cell line MCF 10A.

Methods. The total protein extract were digested with trypsin and labeled with isobaric tags. All samples were pooled, prefractionated by isoelectrofocusing, separated through a C-18 reverse phase and analyzed in a QSTAR ESI mass spectrometer. The proteins were identified and quantified by ProteinPilot software. Results. We identified 1020 proteins correctly labeled with at least one peptide with 95% in confidence. We found 78 over-expressed and 128 sub-expressed polypeptides in all cancer cell lines. These proteins were categorized with PANTHER into biological processes, being the metabolic pathways the most affected. In the interaction protein-protein networks obtained with STRING, over-expressed proteins were involved in DNA topology, regulation of translational initiation, degradation and nuclear export and sub-expressed proteins in the oxidative phosphorylation process, principally. The selection of potential biomarkers was done through searches in PubMed.

Conclusions. The metabolic pathway was the most affected process. Over-expressed proteins involved in processes such as DNA topology and regulation of translational initiation might be associated with the high rate of division and proliferation of breast cancer cell lines.

P-130.00

SERUM PROTEOMIC ANALYSIS OF PATIENTS WITH THE CERVICAL HIGH-GRADE SQUAMOUS INTRAEPITHELIAL LESION

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In the present study, was to identify serum proteins from women with intraepithelial neoplasia grade III and control healthy women to identify potential biomarkers to detect lesions with greater probability of cervical transformations.

Five serum samples from HPV 16(+) and histopathology diagnosis of CIN 3 women and five control healthy female HPV(-) were used for this study. The proteins separations was performed on 2-DE electrophoresis, stained with colloidal Coomassie blue. Quantitative analysis was performed with the ImageMaster 2 Platinum software. Peptide sequence identification of was done in a LC-ESI/TOF-TOF. The proteins with the highest score in MASCOT (Complement C3, A1BG, Haptoglobins and Apoliproteins). Only Complement C3 and A1BG were validated by Western Blot and the densitometry analysis of the corresponding bands from both groups were compared in 30 sample sera from control and 25 samples for CIN 3 with HPV(+). The relative density of the bands from both proteins was greater in all of the serum samples from the women with cervical intraepithelial neoplasia CIN 3 compared to the control group.

This study allowed us to identify two proteins, whose expression is elevated in women with CIN 3, which opens the possibility that they could serve as biomarker of CIN 3 lesion, but before will be interesting to expand the study in order to investigate its expression to throughout the different premalignant lesions.

P-131.00
PROTEOMIC MAPPING OF GLIOMA ENDOTHELIAL CELL SURFACE PROTEINS

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Plasma membrane proteins of vascular endothelial cell (EC) in the glioma are critically related to the glioma functions. However, the profile or difference from other vascular endothelial cell membrane proteins have not been well examined yet. Our aim was to elucidate proteome of glioma EC plasma membrane proteins and compare to that of normal brain EC.

We isolated these membrane fractions from rat glioma model and normal brain directly by the cationized colloidal silica beads coating method, which is based upon the ionic interaction of cationic colloidal silica with negatively charge endothelia membranes, and analyzed them by mass spectrometry. Specific labeling of vascular ECs were revealed by light microscopy and transmission electron microscopy. After homogenization and density ultra centrifugation, the EC plasma membranes with beads were enriched and the enrichment and purity were certified by Western blot analysis using antibody against EC-specific protein, caveolin 1. By MS, we identified 780 glioma EC plasma membrane proteins and 1101 normal brain EC plasma membrane proteins.

Some difference in the profile betweens the glioma and normal brain EC plasma membrane protein were identified.

We conclude that glioma and normal brain EC plasma membranes were isolated directly and mapping of the vascular EC-surface proteome may provide further knowledge of glioma EC functions .

P-132.00

EMILIN-1 AND TALIN-2 ARE MATRIX METALLOPROTEINASE-9 DEPENDENT MECHANISMS OF STIFFNESS IN THE AGING HEART

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How extracellular matrix (ECM) composition and structure evolve during cardiac aging is poorly understood. Matrix metalloproteinases (MMPs) are a family of proteases that collectively degrade all ECM proteins. Of the MMPs, MMP-9 has the strongest link to the development of cardiac dysfunction. Aging is linked to increased MMP-9 expression in the left ventricle (LV) as well as a decline in cardiac function. Accordingly, we investigated the effect of MMP-9 deletion on the cardiac ECM proteome in aged animals.

We used wild type (WT) and MMP-9 null mice (n=6/genotype/age) from 2 age groups, middle-aged (10-16 month old) and old (20-24 month old). LVs were decellularized to enrich for ECM proteins and analyzed by shotgun proteomics.

Elastin microfibril interface-located protein (EMILIN)-1, an adhesive glycoprotein, decreased in WT with age (p

In summary, we identified changes in the cardiac ECM proteome that are associated with age and are MMP-9 dependent. MMP-9 deletion blunted those changes suggesting MMP-9 as a possible therapeutic target for the aging patient.

P-133.00

A QUANTITATIVE TELOMERIC CHROMATIN ISOLATION PROTOCOL (QTIP) TO CHARACTERIZE TELOMERE COMPOSITION CHANGES

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Introduction and objective
Telomeres protect linear ends of eukaryotic chromosomes by preventing chromosomal end-to-end fusions and telomere attrition. They consist of repetitive DNA sequences, telomeric repeat containing RNAs (TERRAs) and proteins. Telomeres play a crucial role in chromosome stability, cancer biology, and ageing. With each cell division, telomeres shorten, as the replication machinery of the cell is not capable to fully copy the very terminal part of chromosomes. After a certain number of cell divisions, telomeres get critically short and elicit signals that stop the proliferation. A cell can circumvent these signals by activating mechanisms that elongate telomeres (telomerase expression). In this case, cells gain the ability to divide indefinitely, which can, in combination with other events, eventually lead to cancer.
Measuring the poorly defined compositional changes during tumorigenesis, aging and in telomere syndromes remains one of the biggest challenges in the telomere field. Here, we develop a quantitative telomeric chromatin isolation protocol (QTIP) for human cells that allows purification of telomeric chromatin and its analysis by mass spectrometry.

Methods
QTIP involves three main steps: in vivo crosslinking, immunopurification with specific antibodies and analysis by mass spectrometry. Quantification is performed by SILAC.

Results
QTIP specifically enriches telomeric DNA and all shelterin components. We identify and validate known and novel telomere associated polypeptides including all THO subunits, SMCHD1 and LRIF1. When applied to cells with long and short telomeres as model for different telomeric states QTIP detects increased density of SMCHD1 and LRIF1 confirmed by ChIP experiments and increased association of the shelterin components TRF1, TIN2, TPP1 and POT1 with long telomeres.

Conclusion
Our results validate QTIP as a powerful method to study composition and modular changes of a variety of chromatin-associated complexes which might be applicable to other chromatin interacting complexes such as chromatin modifiers or transcription factors.

P-134.00

STEAROYL-COA DESATURASE AS A POTENTIAL NOVEL THERAPEUTIC TARGET IN HEAD AND NECK SQUAMOUS CELL CARCINOMA

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Introduction and objectives:
The practice of chewing tobacco is common in certain parts of Southern Asia particularly in the Indian subcontinent. It is estimated that India accounts for 80% of global tobacco chewers. Although prolonged chewing of tobacco is associated with the development of oral cancer, the exact mechanism of carcinogenesis induced by smokeless tobacco is not understood at the molecular level. The first contact with tobacco products occurs in the mouth which necessitates studies designed to investigate the functional consequences of tobacco chewing. Thus, it is necessary to establish a cellular model that mimics the chronic in vivo tissue exposure to chewing tobacco.

Methods:
Non-neoplastic oral keratinocytes, OKF6/TERT1, were chronically treated with chewing tobacco. In vitro cellular assay including proteomic analysis were performed using the OKF6/TERT1-parental and tobacco-treated cells to understand the effects of chewing tobacco on cellular transformation.

Results and discussion:
Chronic exposure to chewing tobacco resulted in a significant increase in cellular proliferation, invasion and colony forming abilities of the OKF6/TERT1cells. High resolution Fourier transform based quantitative mass spectrometry analysis resulted in the identification of 3,645 proteins (177 overexpressed and 219 downregulated proteins). Among the overexpressed proteins, stearoyl-CoA desaturase(SCD), an enzyme implicated in fatty acid synthesis, was 2.5-fold overexpressed in cells exposed to chewing tobacco. siRNA-mediated silencing of SCD resulted in decreased cellular proliferation, decreased invasive and colony formation abilities of not only the OFK6/TERT1-tobacco treated cells, but across a panel of head and neck squamous carcinoma cell lines. Similar results were observed using CAY10566, pharmacological inhibitor specific to SCD.

Conclusions:
Our study reveals that chronic tobacco chewing leads to cellular transformation and SCD is one of the key regulators of this process. Our data further suggest that SCD could serve as a potential therapeutic target in head and neck cancer, especially in patients using tobacco.

P-135.00

QUANTITATIVE PROTEOMICS OF SERUM IDENTIFIES INTER-ALPHA-TRYPSIN INHIBITOR HEAVY CHAIN H4 (ITIH4) AS A POTENTIAL BIOMARKER OF GASTRIC CANCER

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Introduction and objectives:

Gastric adenocarcinoma is an aggressive cancer associated with poor prognosis and high mortality. It is often diagnosed at an advanced stage resulting in limited therapeutic options. Identification of early detection biomarkers has the potential to improve clinical outcome of this disease. Towards this, we studied the differential expression of proteins in serum samples of gastric adenocarcinoma patients compared to non-diseased healthy individuals.

Methods:

We used an iTRAQ-based quantitative proteomic approach to identify differentially expressed proteins in the sera of patients with gastric cancer. Abundant serum proteins including, human serum albumin, transferrin, haptoglobin, Immunoglobulin G, Immunoglobulin A, aplha-1-antitrypsin, fibrinogen, alpha-2-macroglobulin, alpha-1-acidglycoprotein, complement C3, Immunoglobulin M, apolipoprotein AI, apolipoprotein AII, and transthyretin were depleted by immuno-affinity chromatography prior to labeling with iTRAQ.

Results and Discussion:

Using high resolution Fourier transform mass spectrometry, we identified 643 proteins, of which 62 proteins were differentially expressed in serum of gastric cancer patients when compared to the controls (51 proteins were in higher abundance and 11 in low abundance). Our study led to the identification of multiple novel secreted proteins associated with gastric cancer which includes inter-alpha-trypsin inhibitor heavy chain H4 (ITIH4), leucine-rich repeat protein 1 (LRR1) and sex hormone-binding globulin (SHBG). ITIH4 was further validated across gastric cancer sera using multiple reaction monitoring (MRM). In agreement with earlier reports, we also detected a higher abundance of some previously known markers for gastric cancer, including serum amyloid A protein (SAA1), alpha-1-acid glycoprotein 1 (ORM1), extracellular superoxide dismutase [Cu-Zn] (SOD3).

Conclusion:

Our study led to the identification of several novel secreted proteins from sera of patients with gastric cancer including ITIH4, LRR1 and SHBG. Further validation of these markers in a larger cohort of samples will be required to translate these findings to a clinical setting.

P-136.00

MACROPHAGE MIGRATION INHIBITORY FACTOR - A THERAPEUTIC TARGET IN GALLBLADDER CANCER

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Introduction and objectives
Gallbladder cancer is a prevalent malignancy of the biliary tract and the fifth most common cancer of the gastrointestinal tract. It is often detected at an advanced and unresectable stage. The prognosis is dismal with a survival of less than 5 years in 90% of the cases. Poor prognosis in gallbladder cancer is mainly due to late presentation of the disease and a lack of reliable biomarkers for early diagnosis. There is an urgent need to identify potential therapeutic targets in addition to early diagnostic markers to improve the outcomes of this deadly disease.

Methods
Proteomic analysis of four gallbladder cancer cell lines based on the invasive property (non-invasive to highly invasive) was carried out using the isobaric tags for relative and absolute quantitation (iTRAQ) labeling-based quantitative proteomic approach. In vitro cellular assays were carried out on a panel of gallbladder cancer cell lines to test the effect of targeting macrophage migration inhibitory factor (MIF), a molecule identified in this study, using a pharmacological inhibitor and an siRNA against MIF.

Results and Discussion
Our quantitative proteomics experiment led to the identification of 3,183 proteins of which 627 were overexpressed and 327 were downregulated more than 2-fold. Of the overexpressed proteins, macrophage migration inhibitory factor (MIF), a pro-inflammatory cytokine, was found to be 3-fold overexpressed in the invasive GBC lines. Inhibition of MIF using a specific pharmacological inhibitor, ISO-1, led to a significant decrease in their cell viability, colony forming ability and invasive property in a panel of gallbladder cancer cell lines. Similar results were obtained upon silencing of MIF using a specific siRNA.

Conclusions
Our data suggests that MIF plays an important role in tumor aggressiveness of gallbladder cancer and implicates MIF as a novel potential therapeutic target in gallbladder cancer.

P-137.00

INVESTIGATION OF DIFFERENTIALLY EXPRESSED PLASMA MEMBRANE GLYCOPROTEINS IN ORAL SQUAMOUS CELL CARCINOMAS

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Oral squamous cell carcinoma (OSCC) is the sixty most frequent cancer worldwide and affects the mucosa between the lip and the palate including oral tongue, floor of the mouth, buccal mucosa, alveolar ridge and retromolar trigone. Despite intense research and recent advances in diagnosis and therapeutic strategies, the five-year survival rate has not significantly changed in the last decades.

Proteomics have provided powerful tools to study the biology of many cancers. Protein glycosylation is a very common post-translational modification. Most cell surface receptors are glycosylated and there are many evidences that N-glycans modulate their conformation and activity. Aberrant protein glycosylation has been associated with several diseases including cancer.

The objective of the present study was to search for differentially expressed glycosylated membrane proteins in OSCC that can be used as biomarkers for prognosis and monitoring of the disease progression. The study was carried out on primary tumors and normal surgical margins using liquid chromatography and Orbitrap mass spectrometry. Among the glycosylated membrane proteins identified, Desmocollin-3, Desmoglein-3, Translocon-associated protein subunit alpha and Solute carrier family 2, facilitated glucose transporter member 1 were observed with higher expression in tumors relative to margins. Kininogen-1 and Solute carrier family 4, anion exchanger, member 1 showed lower expression in tumors relative to margins.

These proteins are involved in many important biological processes related to cancer, including cell adhesion and proliferation, apoptosis and inflammatory response. The results require experimental validation and functional assays.

P-138.00
MEASURING WNT-PATHWAY ACTIVITY IN CANCER MODELS

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The Wnt-signaling pathway is often found to be hyperactive in colorectal, hepatocellular, and other cancers. Therefore, its modulation is in the focus of basic and pharmaceutical research. The activity of the pathway is reflected by concentration changes of beta-catenin. The homeostasis of beta catenin is regulated by the ubiquitin proteasome system. Mutations encoding either proteins of the degradation machinery or the N-terminal region of beta-catenin lead to accumulation of beta-catenin in the cell. Accumulated cytosolic beta-catenin is translocated into the nucleus and its function as transcriptional co-activator is elevated. Since the protein plays also a role as adaptor molecule in cell adhesion processes by bridging cadherins with cell skeleton proteins the analysis of the pathway activity on protein level is challenging.

We have combined three biochemical methods - sandwich immunoassay, co-immunoprecipitation and protein-protein interaction assay - in one suspension bead assay panel (10 plex) for the relative quantification of total beta-catenin, the extent of phosphorylation at multiple sites and the ratio of complexed and free beta-catenin. We investigated the dynamic effects of chemical inhibitors on proteins abundance, on function and on posttranslational modifications. A detailed quantitative and time-resolved analysis of the pathway activity is possible and gives insights into the mechanisms of natural Wnt-ligands and pathway inhibitors.

The assay was used to test the impact of Wnt-signalling inhibitors on pathway activity in an in vivo PK/PD study. Human colon tumor cell lines were subcutaneously injected into athymic mice and mice were treated with Wnt pathway inhibitors. Modulation of the Wnt pathway activity in the tumor xenografts was tested by using the beta-catenin assay panel with ex vivo lysates.

P-139.00

SOMASCAN™, A TOOL FOR UNCOVERING THE MECHANISM OF ACTION OF ONCOLOGY DRUGS: DIFFERENTIAL PROTEIN SIGNATURES IN ERLOTINIB SENSITIVE AND RESISTANT LUNG CANCER CELL LINES IN VITRO AND IN VIVO

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SomaLogic, Inc.

Tarceva™ (erlotinib) is a first-line treatment for non-small cell lung cancer (NSCLC). NSCLC cell lines sensitive to erlotinib undergo G1/S cell cycle arrest. With SOMAscan™, we investigated proteomic changes in NSCLC HCC827 (sensitive) or H1299 (resistant) cells exposed to erlotinib, and NOD/SCID mice implanted with HCC827 cells exposed to erlotinib.

SOMAscan uses novel SOMAmer™ affinity binding reagents (Slow Off-rate Modified Aptamers). SOMAmer applications include SOMAscan™ quantitative multiplex proteomics discovery assay (>1,100 human proteins, median LLOQ 100 fM and median CVs

Cells were grown in RPMI 1640 with 10% BSA. HCC827 cells were treated with erlotinib at 5 nM, 10 nM, 20 nM and 40 nM, and H1299 cells were treated with erlotinib at 2.5 µM, 5 µM, 10 µM and 20 µM, all in triplicate. Cell lysates and media were collected at 0, 6, 12, and 24 hours, and each sample analyzed by SOMAscan.

We observed that erlotinib sensitivity and resistance correlate with cyclin A and p27KIP1 protein changes in HCC827 and H1299 cell lines, and observed additional changes in kinases, growth factors and other regulatory proteins that correlate with erlotinib sensitivity and resistance. HCC827 cells implanted in NOD/SCID mice dosed with erlotinib showed similar protein changes observed in the HCC827 cell lysates.

1129 proteins were quantified from lysate and media of HCC827 and H1299 cells, and tumor xenografts exposed to erlotinib. Concentration-dependent changes were seen for many proteins, including p27KIP1 and cyclin A (consistent with G1/S arrest). The many protein changes may have implications for treating patients that develop resistance to erlotinib in the clinic, and provide new targets for drug development.

P-140.00

PROTEOMIC STUDY OF CHROMATOME DYNAMICS REVEALS REGULATORY SWITCHES OF EPIGNOME IN HYPOXIA-INDUCED ONCOGENESIS

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Environmental factors such as tumour hypoxia microenvironment are critical triggers that change the epigenomes of cells undergoing development and in disease progressions. Recent focus on epigenetics have been on the identification of epigenetic marks containing cytosine methylation or histone modifications that associates with biological or disease states. Chromatin-associated proteome (chromatome) which responds to environmental stimulus to program the epigenome has been principally overlooked. The understanding of this interaction could very well provide molecular information that may potentially controls and reverse the undesirable effects of disease-related epigenetic changes. In pursue of the master regulatory switches that changes the cancer epigenome during cancer progression, we have developed a proteomic method to profile the dynamic changes of chromatome that have been induced by hypoxia microenvironment.

We used partial DNase I digestion together with iTRAQ-based quantitative proteomics to analyze the chromatome of A431 cancer cells that had been subjected to hypoxia and re-oxygenation stress. Profiling of both the solubilized fraction (euchromatin-associated proteins) and undigested fraction (predominantly heterochromatin-associated proteins) uncovered novel effects of hypoxia on chromatin association topology in growing cancer cells.

Using this proteomics strategy, we identified a total of 1446 proteins at a high level of confidence, including 819 proteins that were observed to change their chromatin association topology under hypoxic conditions. These hypoxia-sensitive proteins included key mediators of chromatin organization, transcriptional regulation and DNA repair. Furthermore, our proteomic data mining and functional experiments revealed a novel role for the chromatin organizer protein HP1BP3 in mediating chromatin condensation during hypoxia, leading to increased tumor cell viability, radio-resistance, chemo-resistance and pluripotency. Our findings indicate that HP1BP3 is a key mediator of tumor progression and cancer cell acquisition of therapy-resistant traits by modulating chromatine structure and epigenome, and may thus represent a novel therapeutic target in a range of human malignancies.

P-141.00

OVARIAN CANCER: HUNTING BIOMARKERS BY MASS SPECTROMETRY IMAGING AND TISSUE PROTEOMIC

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Introduction and objectives

Early diagnostic and disease management is one of the most important challenges facing modern medicine. The lack of effective assays measuring multiple blood-based biomarkers is absent in many types of cancer. Moreover, transforming a biomarker into a useful clinical diagnostic test is a complex process, which starts with identification, proceeds through validation. Identification can be carried out by various means (gene arrays, purification procedures, proteomics), that focus on observed changes of the marker correlated with the disease progression. As the markers are identified within extracts or in a non-spatial context, further validation is always required. Several choices are available, such as establishing specific antibodies, using protein microarrays or including more refined techniques.

Methods

A major new alternative that combines both biomarker identification and validation in a single step is now possible at the tissue level with the development of MALDI Mass Spectrometry Imaging coupled to tissue micro proteomic.

Results and Discussions

We present here data obtained on ovarian cancer (OVC) using such a technology. Our findings reflect that a global molecular profile is more associated with the pathological states observed in serous ovarian cancers compared to the benign stage, based on MALDI MS profiling studies. We established from 500 patients that the Cterm part of PA28 is a specific maker of serous OVC. It can be found at very early stages of the pathology. We then investigate the serous ovarian cancer origin, its resistance and invasion strategies. We also found proteins issued from alternative ORF as potential markers. We then demonstrate that PACE4 is implicated in OVC development and confirm that OVC is originated from fimbria.

Conclusions

From our study we have now 7 novel biomarkers for serous OVC e.g. C-term PA28, MUC 9, HLA-G, 4 alternative proteins. All of them are now under validation through multi-centers.

P-142.00

N-LINKED GLYCOPROTEIN PROFILING FOR BIOMARKER DISCOVERY IN COLORECTAL CANCER

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Colorectal cancer (CRC) is a common cause of death in the industrialized countries, and for this reason the discovery of new diagnostic biomarkers is required. Post-translational modifications, such as glycosylation, are known to play an important role in cancer progression and can be used as biomarkers for the detection and monitoring of the disease. Hence, we profiled N-linked glycoproteins in 19 CRC tissues using a quantitative proteomic technique based on (18)O stable isotope labeling in order to identify differentially expressed proteins in CRC compared with adjacent normal colorectal tissue.

First, N-linked glycopeptides were isolated from healthy and tumor tissue samples by Solid Phase Extraction of N-linked Glycopeptides (SPEG). Afterwards, glycopeptides were quantified by (18)O labeling and High Resolution Quadrupole-Orbitrap Mass Spectrometry. A total of 508 glycoproteins were identified. Of those, 54 glycoproteins were found up-regulated in CRC compared to normal tissue, and consequently potentially involved in the biological processes of tumorigenesis.

Of the 54 quantified glycoproteins, 22% and 19% were found to be respectively plasma membrane and extracellular proteins, which are known to be possibly shed into blood stream. More specifically, a subset of 9 glycoproteins were found up-regulated in the great majority (>80%) of the cohort. Of those, five (DPEP1, SE1L1, PAR1, LAMP3, OLFM4) have been previously described in CRC studies, whereas no association to CRC has been hitherto found for the remaining four glycoproteins.

In conclusion, our findings suggest that N-linked glycoproteins which are differentially expressed in CRC and potentially detectable into blood might act as potential biomarkers of CRC. Furthermore, a specific subset of these proteins might be employed for the diagnosis and prognosis of patients with CRC using targeted proteomic quantification in serum.

TEAR PROTEIN BIOMARKERS IDENTIFY LACRIMAL GLAND TUMORS

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Introduction and objectives:
Lacrimal gland tumors (LGT) are usually epithelial in origin with approximately 50% classified as benign and 50% as malignant. As the aqueous secretion of the lacrimal gland is the major source of tear proteins and fluid, identification of protein biomarkers in tears is useful for diagnosis of the tumor.

Methods:
Tears were collected using Schirmer strips from a total of 18 patients with different lacrimal gland diseases (malignant LGT: 5 cases; benign LGT: 6 cases; LG inflammation: 7 cases). iTRAQ together with nanoLC-MS/MS was used to quantitatively compare the tear proteomic profiles among three groups as well as the lacrimal gland tissue samples between malignant and benign LG tumor. Gene expression of lacrimal gland tumor tissues was examined using cDNA microarray (Affymetrix Gene 1.0 ST array). ELISA, Immunofluorescent staining and transmission electron microscopy (TEM) were used to confirm the proteomic results.

Results and Discussion:
iTRAQ quantitative proteomics results of tears showed that markedly reduced levels of LG secreted proteins (LCN-1, LYZ, LFT, PIP and PRR4, ect.) and elevated MMP-9 level in tears from malignant LGT compared to two other groups. iTRAQ results of LG tissues also showed that these LG secreted proteins are down-regulated in malignant LGT. TEM showed largely decrease of the number of secretory granules in malignant LGT tissues. ELISA (~100 fold higher in malignant LGT) and immunofluorescent staining results confirmed proteomics results. There is a strong correlation among lacrimal-preferred genes, lacrimal gland secreted proteins in lacrimal gland tissues and tear fluids. They all showed a significant down-regulated expression in lacrimal gland malignant tumor.

Conclusions
The extreme low level of lacrimal gland secreted proteins in tears and the markedly elevated MMP-9 tear level unique to the malignant LGT would provide a useful diagnostic tool to identify individuals with lacrimal gland malignant tumors.

P-144.00

SYSTEM-WIDE CHARACTERIZATION OF DYNAMICS OF CYTOSOLIC MACROMOLECULAR PROTEIN COMPLEXES IN ONCOGENE-INDUCED CELL TRANSFORMATION

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Introduction and objectives
The majority of human cancers are caused by the malignant transformation of epithelial cells, one of the most frequently occurring malignancies being colorectal carcinoma (CRC). Recent studies suggest that the use of B-Raf inhibitors in BRAF mutant CRC cells yield less uniform responses than in melanoma reflecting the heterogeneous character of CRC. These studies highlight the need for a better understanding of normal and mutant B-Raf signalling at the molecular level, in particular a solid understanding of B-Raf-dependent interaction networks, their structure and regulation.

Methods
We studied direct B-Raf-dependent as well as global alterations in protein interactions upon oncogene expression. (1) Using quantitative AP-MS differences between interaction partners of B-RafWT and B-RafV600E were characterized. (2) In an unbiased approach SEC-PCP-SILAC was used for high-throughput screening of changes in protein interactions. Quantitative proteomics data was linked to large-scale image cytometry data.

Results and Discussion
We hypothesized that B-Raf-dependent oncogenic cell transformation leads to global alterations in protein-protein interactions driving tumor development. To address this we used human epithelial colorectal adenocarcinoma cells (CaCo-2) inducibly expressing oncogenic or wild type BRAF as CRC model system. We were able to identify several thousand protein-protein interactions including several that demonstrate differences between the wild type and oncogenic form of B-Raf. Combining image analyses with MS-based proteomics provided a platform for identification of potentially new multimeric proteincomplexes.

Conclusions
Thus, these global unbiased image and MS-based proteomics investigations of B-Raf-dependent and -independent protein-protein interactions elucidate new players in malignant cell transformation that represent potential new therapeutic drug targets.

P-145.00

PROTEIN CARBONYLATION IN HEPATOCELLULAR CARCINOMA DERIVED FORM VIRUS C AND METABOLIC SYNDROME. TWO DIMENSIONAL FLUORESCENCE DIFFERENCE IN GEL DETECTION.

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Metabolic syndrome (MS) is becoming the leading cause of chronic liver diseases worldwide. Hepatocellular carcinoma (HCC) derived from MS is peculiar compared to HCC derived from viral infections. Carbohydrate and lipid metabolic imbalance in MS increase reactive oxygen species damaging proteins. Herein, we analized the protein oxidative damage (carbonylation) in human HCC derived from virus C infection (VHC) and from MS (MS_HCC) as the only subjacent cause.

A patient cohort of 10 non-tumoral and 10 tumoral liver resections for each study group: virus C and MS HCC was selected based on clinical patient history and histological parameters. Protein samples were labeled to saturation using CF 647-hydrazide™ dye (carbonyl labeling), followed by Cy3NHS™ staining/labeling for protein detection. An internal standard control was prepared and labeled with Cy2NHS™. Protein carbonylation was normalized using the protein Cy3NHS signal to correlate the total amount of oxidative damage with the total amount of protein within each spot.

A total of 1184 spots with 36 differentially expressed proteins and 47 spots differentially carbonylated between VHC and MS_HCC (fold change >1.5, p

Our results indicate that MS and Virus C derived HCC had different specific protein oxidative damage that might be responsible for difference in hepatocellular carcinoma development.

P-146.00

PROTEOMIC ANALYSIS OF THE HETEROGENEITY OF IN BREAST CANCER

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Introduction and objectives

About 70% of all breast cancer express the oestrogen receptor and are dependent on oestrogen for growth. Chemotherapy inhibits the effects of oestrogen but the major obstacle for curative treatment is recurrence of resistance to anti-oestrogen and expansion of subpopulations of cancer cells.

In a luminal-like oestrogen-dependent growing xenograft model, four phenotypically different subpopulation of cancer cells (defined by their expression of two stem cell markers) were monitored after oestrogen withdrawal. Proteomic analysis of signalling pathways in these four subtypes may help understanding the tumorigenic process.

Methods

Nude - Foxn1nu mice were inoculated with the luminal-like MAS98.06 human breast cancer carcinoma and Oestradiol was supplied. When tumours reached 200-500 mm3, oestradiol was removed. Fifteen days later tumours were collected.

FACS, protein extraction, in gel tryptic digestion and phospho-peptide enrichment were performed on samples followed by Eksigent 2D NanoLC/LTQ OrbitrapXL analysis. All peptide features with MS/MS data were selected for quantitation and identification.

Results and discussion

After oestradiol withdrawal, xenografts demonstrated reduced growth and significant decrease in tumour volume compared to control xenografts. From all four subpopulations, 3045 proteins were identified and quantified. The results from PCA and ANOVA analyses implied that the differences in protein expression were most pronounced between the non-tumorigenic subpopulation and the three tumorigenic subpopulations.

Conclusions

In the non-tumorigenic subpopulation, cytoskeleton remodelling was the most significantly enriched pathways. Interestingly, regulation of translation had a higher score in double positive indicating that differences in translational control and cytoskeleton remodelling might confer less tumorigenic and oestrogen-responsive phenotype. Different subpopulations presenting with the same level of receptor respond so differently to restricted supply of the ligand. Also our phospho-enrichment analysis detected several phosphorylated proteins involved in growth factor signalling, indicating the possibility of ligand independent activation of ER signalling pathways in the populations surviving after oestrogen withdrawal.

P-147.00

VALIDATION OF POTENTIAL BIOMARKERS FOR AJCC STAGE III MELANOMA

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Background: The present AJCC (American Joint Committee on Cancer) staging system for Stage III melanoma stratifies patients into heterogeneous groups, with widely varied outcomes and responses to available therapies. Average 5-year survival after surgical treatment is about 40%. Biomarkers that more accurately predict prognosis are urgently needed to stratify patients according to risk as we move toward personalized management.

Methods: Based on previous discovery experiments in our lab (Mactier, S. et al.) and a literature review Schramm, S.J. et al., a total of 55 potential prognostic markers were validated by selected reaction monitoring (SRM) mass spectrometry using melanoma lymph node metastases, surgically excised from Stage III patients with poor (n = 14, < 1 year survival) and good (n = 19, > 4 years) survival outcomes.

Results and Discussion: Several proteins previously were validated as potential biomarkers for survival in Stage III melanoma. Patients with poor prognosis were characterised by an increased abundance of proteins associated with metabolism/folding, nucleic acid metabolism, angiogenesis, deregulation of cellular energetics and methylation processes, and decrease in proteins involved in apoptosis and immune responses.

Conclusions: The proteins validated in this study may represent novel prognostic markers for Stage III melanoma patients. Predicting patient outcome utilising the biomarkers identified in this study will allow for tailored treatment and management according to an individual patients survival and risk.

P-148.00

INTEGRATIVE ANALYSIS OF ACUTE AND LATE RADIOTHERAPY EFFECTS USING GENOME-WIDE TRANSCRIPTOMICS AND QUANTITATIVE PROTEOMICS

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Introduction and Objectives

Pulmonary microvascular endothelial cells are critically involved in the development of radiation induced acute inflammation and late fibrosis often limiting the delivery of sufficient dose to optimally treat lung cancer. Moreover, tumor-endothelium communication has emerged as a key target to augment radiotherapy by resensitizing endothelial specific survival signals. Therefore, we aimed to employ high-throughput genome-wide gene expression and whole proteome analysis to uncover the molecular mechanisms governing tumor-vessel communication and radiation induced normal tissue toxicity.

Methods

Human primary isolated pulmonary microvascular endothelial cells (HPMEC) were irradiated with 0 and 6Gy. Cells were harvested two hours and six days after irradiation and proteins as well as RNA were extracted. Gene expression analysis was performed using 47k Illumina microarray (HumanHT-12 v4) platform. Mass spectrometry on an LTQ-Orbitrap instrument was employed for protein identification and relative quantification using label free quantification techniques. An IMAC/TiO2 enrichment step to obtain phosphorylated peptides was performed and analysed by mass spectrometry as well. The regulation of candidate radiation regulated genes and proteins were confirmed in vitro and in irradiated lung specimen (C57BL/6 mouse) by real time qRT-PCR, western blot and ELISA.

Results and Discussion

At the early timepoint a strong induction of genes involved in apoptosis and DNA-damage response such as GADD45A, PARP3 and DDB2 was identified. In contrast to acute radiation response, cell adhesion (Integrins), pro-inflammatory (Interleukines), pro-angiogenic and matrix-remodelling related genes (MMPs, PDGFs, VEGFs) were induced late after irradiation. Integrative quantitative analysis of protein expression, phosphorylation and gene expression analysis further revealed novel insights to an intricate gene/protein regulatory network underlying late radiation response in microvascular endothelium.

Conclusion

The here identified genes and proteins involved in late radiation response constitute attractive targets for modulation of radiotherapy effects in tumor-endothelium and prevention of undesired radiotherapy-induced late side effects.

P-149.00

MOLECULAR MECHANISMS RELATED TO IRON HOMEOSTASIS AND DISTURBED IN BREAST CANCER

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Introduction and Objectives. We have recently provided evidence that in patients with breast cancer hepcidin and ferritin light chain level are significantly higher than in healthy population. Studies in patients with multiple myeloma and Hodgkin lymphoma show that hepcidin is upregulated by mechanisms governed by IL-6 and bone morphogenetic protein (BMP) which act synergistically to induce hepatocyte hepcidin synthesis.
 This study is aimed at exploring the pathogenic cascade leading to upregulation of hepcidin synthesis.

Methods. In the experiments, we explored gene and protein expression patterns of molecules involved in iron metabolism in breast tumors and matched normal tissues. We then integrated these data with measures of other iron-related parameters in plasma of matched and non-matched subjects to unravel the relationship existing between breast tissue pathways and changes in plasma hepcidin concentration.

Results and Discussion. We obtained evidence that increases in hepcidin concentration in plasma of breast cancer patients may be consequence of a complex signaling network primarily involving Jak/STATs signaling pathways in response to inflammatory and erythropoietic stimuli. Another main pathway of hepcidin regulation is depending upon signaling through the bone morphogenetic protein/Smad (BMP/Smad). All these signaling are active in breast cancer tissues and peritumoral tissues contributing to increase local and systemic mediators acting to a liver level and able to stimulate liver production of hepcidin. Hypoxia and WNT pathways, disregulated stromal proteases and iron storage systems may play a role at different levels.

Conclusions. Data obtained from different methodological approaches together with current knowledge on iron metabolism homeostasis and molecular growth determinants in breast cancer converge to support the importance of iron in breast cancer biology.

P-150.00

A DISTRIBUTED SYSTEM FOR THE ANALYSIS OF HIGH THROUGHPUT PROTEOMICS DATA

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As data volumes in mass spectrometry based proteomics experiments keep increasing, it becomes increasingly demanding to thoroughly perform the corresponding analyses. Especially for labs that have several instruments or that may want to refresh by re-analysing (some of) their data sets at regular intervals, the demand on computing power quickly rises. Since the processing speed of CPUs has largely stagnated over the past years, the main course of action to allow analysis to keep up with data generation is to use parallelization. Indeed, the only viable mid-term solution is to distribute the work over many different computers, allowing the compute resources to expand as the need arises [1].

We have therefore built a fully distributed architecture for the analysis of proteomics data that can run on any collection of computers with shared storage or access to an FTP server. Searches are safeguarded through persistent failover, allowing for failed jobs to be re-launched automatically. Built around a grid-like architecture, the system can dynamically expand over time, providing for instance the ability to have idle desktop computers in the lab join the worker pool at night, and dropping out again in the morning.

As the complexity of proteomics experiments continues to increase, so will the computational needs of a typical proteomics lab. The only realistic and affordable mid-term solution to cope with the increased need for processing power in fields, such as proteomics, lies in distributed computing, preferably using cheap, idle computational capacity of a lab. Our framework provides a powerful solution to implement such an infrastructure around existing tools, and thus allows proteomics researchers to stay ahead of their data analysis challenges.

[1] Verheggen, K., Barsnes, H., Martens, L, Distributed computing and data storage in proteomics: many hands make light work, and a stronger memory. Proteomics 2014, 14, 367-377.

P-151.00

IMAGING MASS SPECTROMETRY (IMS) TO DISCRIMINATE BREAST FROM PANCREATIC CANCER METASTASIS IN FFPE TISSUES

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Introduction and objectives

Diagnosis of the origin of metastasis is mandatory for adequate therapy. In the past, classification of tumors was based on histology (morphological expression of a complex protein pattern), while supportive immunohistochemical investigation relied only on few "tumor-specific" proteins. At present, histopathological diagnosis is based on clinical information, morphology, immunohistochemistry, and may include molecular methods. This process is complex, expensive, requires an experienced pathologist and may be time consuming.

Currently, proteomic methods have been introduced in various clinical disciplines. MALDI IMS combines detection of numerous proteins with morphological features, and seems to be the ideal tool for objective and fast histopathological tumor classification.

Methods

To study a special tumor type and to identify predictive patterns that could discriminate metastatic breast from pancreatic carcinoma MALDI IMS was applied to multi-tissue paraffin blocks. A statistical classification model was created using a training set of primary carcinoma biopsies.

Results and discussions

This model was validated on two testing sets of different breast and pancreatic carcinoma specimens. We could discern breast from pancreatic primary tumors with an overall accuracy of 83.38 %, a sensitivity of 85.95 % and a specificity of 76.96 %.

Conclusions

Furthermore, breast and pancreatic liver metastases were tested and classified correctly.

P-152.00

PROFILING OF TRANSMEMBRANE PROTEINS IN HUMAN LYMPHOMA CELLS BASED ON MEMBRANE-EMBEDDED PEPTIDES

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Introduction and objectives: Transmembrane proteins (TMPs) carry out important functions such as membrane transport, cell adhesion and cell signaling. These roles make TMPs attractive targets for pharmacotherapy of human diseases including cancer. Yet, because of their low expression and due to their amphipathic nature, TMPs are largely underrepresented in proteomic analyses. Numerous methods aimed specifically at TMP enrichment have been developed. However, substantial contamination by abundant non-membrane proteins remains an issue. To overcome some of the obstacles, methods targeting selectively either hydrophilic (extra-membrane) or hydrophobic (membrane-embedded) domains of TMPs have been developed.

Methods: Hydrophobic alpha-helical domains protected by the phospholipid bilayer can be selectively isolated after complete proteolytic degradation of unprotected hydrophilic domains and all contaminating non-membrane proteins as demonstrated by Blackeler and Wu. We modified the original method and our current approach is based on a single-step enrichment of crude membrane fraction, followed by opening of membrane vesicles at high pH, and tryptic digestion of all accessible protein material. The "shaved" membranes are then solubilized, and transmembrane domains are re-digested with CNBr to generate shorter peptides. The sample is "on column" delipidated, and the hydrophobic transmembrane peptides are identified by LC-MS/MS.

Results and Discussion: We applied our modified method to the analysis of human lymphoma cells. We identified over 700 TMPs from plasma membrane and other organellar membranes. TMPs represented over two thirds of all identified proteins in our analysis. Most of the proteins were identified by peptides that overlapped with predicted transmembrane domains.

Conclusions: This method yields excellent enrichment and very high number of identified TMPs from various membrane compartments. Combined with a quantitative approach (such as SILAC) the method could complement standard proteomic methods and provide invaluable data on expression of TMPs in eukaryotic cells.

P-153.00

IDENTIFICATION AND SPATIAL LOCALIZATION OF PROTEINS FROM MOUSE BRAIN TUMOR USING A COMBINATION OF MALDI IMAGING AND LC-MALDI

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Introduction and objectives

MALDI MS imaging has generated a large body of information about spatial localization of proteins in different tissues. Identification of the proteins with interesting localization requires often more efforts and time than MALDI imaging experiments. One workflow that achieves spatial localization and protein identification correlates MALDI imaging data with bottom-up proteomics data from LC-MALDI analysis of peptides from a second digested section. We apply this workflow to study treated and untreated tumors in mouse brain.

Methods

Two consecutive coronal tissue sections of mouse brain (tumor treated, tumor untreated, healthy) were simultaneously subjected to on-tissue trypsin digestion. Peptides were extracted from one of the tissue slices with 0,1% TFA. The other tissue slice was sprayed with DHB matrix for MALDI-TOF MS imaging. The list of identified peptides, grouped by protein, from the LC-MALDI dataset was correlated to the peak list from MALDI MS imaging dataset using the ImageID utility in FlexImaging software, in which the correlated peptides grouped by protein were visualized.

Results and discussions

The combined workflow was used to identify and obtain spatial localization of proteins in tissue sections of mouse brain tumor treated with temozolomide, untreated tumor control and healthy mouse brain control. LC-MALDI-TOF/TOF identification yielded 741 proteins in untreated tumor control tissue, 568 proteins in treated tumor tissue and 649 proteins in healthy control tissue. Certain proteins are identified and localized exclusively in the tumor region of untreated tumor control tissue. One example is the voltage-dependent anion-selective protein 2Other proteins are localized in both treated and control brain tumor tissues. We are working on comparison of localization and identification data from untreated brain tumor control tissue with treated tumor and healthy tissues.

Conclusions

Using combined imaging and LC-MALDI workflow to determine spatial localization and identify proteins in treated and untreated mouse brain tumors.

P-154.00
A SILAC MOUSE-BASED QUANTITATIVE PHOSPHOPROTEOMIC APPROACH TO ANALYZE SRC ONCOGENIC SIGNALING IN XENOGRAFT TUMORS

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Introduction-Objectives

The tyrosine kinase SRC is a master regulator of cell proliferation. Hyperactive SRC is a potent oncogene and a driver of cellular transformation and carcinogenesis. Accordingly, SRC is frequently deregulated in human cancers, including colorectal cancer (CRC), and plays important roles during tumorigenesis. For example, SRC over-expression in human CRC cells favors tumor growth when engrafted in nude mice and is accompanied with a strong increase in protein tyrosine phosphorylation. However, our knowledge on the molecular mechanism by which SRC controls oncogenic signaling is incomplete due to the limited number of substrates identified so far. Here we applied a SILAC mouse-based quantitative proteomic approach to xenograft models in order to analyze SRC oncogenic signaling and identify SRC substrates in vivo.

Methods

We applied the SILAC mouse approach to nude mice engrafted with SRC-expressing human CRC cells by adding [13C6]-Lysine in the food. One month of this regimen was sufficient to efficiently label the tumor proteome in engrafted animals (>87%).

Results-Discussion

SRC signaling in tumors significantly differs from the one in cell culture as more than 60% of the SRC targets identified in vivo were not found in vitro. Particularly, we showed that a large cluster of vesicular trafficking and mRNA binding proteins are SRC targets in tumors, thus highlighting the potential novel role of SRC in mRNA splicing/stability and vesicular trafficking processes related to endocytic/secretory pathways in vivo. The specificity of this analysis was confirmed experimentally by the depletion of the vesicular trafficking protein and SRC substrate TOM1L1 that strongly compromised tumor growth in vivo, while it slightly affected cell proliferation in vitro.

Conclusions

Our data validate the SILAC mouse approach in xenografts as a valuable method to decipher tyrosine kinase oncogenic signaling in vivo and suggest that vesicular trafficking plays an important role in SRC-induced tumor growth.

P-155.00

EXPLORING THE DETECTION LIMITS OF ERG ONCOPROTEIN IN PROSTATE CANCER USING DIFFERENT SAMPLE TYPES SIMULATING CLINICAL SPECIMENS

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TMPRSS2-ERG gene fusions are common events in prostate cancer, occurring in about half of radical prostatectomy samples, and represent one of the most specific biomarkers that define a distinct molecular subtype of prostate cancer. However, quantification of the protein products of ERG gene rearrangements in pathologic specimens has not been reported.

Herein we applied both PRISM (high-pressure high-resolution separations with intelligent selection and multiplexing)-SRM (selected reaction monitoring) and immunoassays to determine the detection limit of ERG protein under conditions that simulate three different sample types (cell line, tissue, and post-DRE urine sediment). As a reference, NanoString and qRT-PCR were also applied for measuring mRNA levels in the same samples. Using PRISM-SRM, as low as 20 pg of recombinant ERG3 protein spiked in ~50,000 LNCaP cells can be detected; it also allowed for detection of ERG protein from ~10,000 VCaP cells spiked in 1,000,000 LNCaP cells (simulating tissue), and from as low as 600 VCaP cells spiked in 5 mL of female urine (simulating urine sediment).

The improved detection of the ERG protein in urine sediments compared to tissue samples is likely due to the reduced protein background in urine. For comparison, ELISA and Western blotting allowed for detection of ERG3 spiked in LNCaP lysates at ~50 pg and ~1 ng levels, respectively; NanoString allowed for detection of ERG from ~10,000 VCap cells spiked in female urine, respectively; qRT-PCR allowed for detection of ~200 VCaP spiked in female urine. The findings suggest that the sensitivity of PRISM-SRM is higher than ELISA, Western blotting (using our antibodies), and NanoString, but is lower than qRT-PCR.

The ability to detect ERG protein in different sample types and the quantitative comparison between ERG protein and mRNA detection as demonstrated in this study provides a rationale and sensitivity of ERG protein detection for potential clinical applications.

P-156.00

PROTEOGENOMIC ANALYSIS OF GLIOMA STEM CELLS: A POTENTIAL TOOL FOR THE DISCOVERY OF GBM BIOMARKERS

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INTRODUCTION:

Glioblastoma Multiforme (GBM) is the highest grade of glioma, being the most common and aggressive primary central nervous system tumor in adults. Despite maximal safe surgical resection followed by ionizing radiation and chemotherapy, median survival is only of 12-15 months due to tumor recurrence. This highlights the need of further GBM characterization to define biomarkers to detect risks of recurrence and to develop novel therapeutic approaches. Recent studies have demonstrated the presence of cancer stem cells in glioblastoma, which may be responsible for tumor recurrence and poor prognosis due to their high resistance to chemotherapy and radiotherapy. Therefore, glioma stem cells (GSCs) represent a crucial target to develop novel therapeutic strategies.

OBJECTIVES

To settle a comprehensive proteomic and transcriptomic analysis of several GSC lines derived from different GBM patients to gain further insight into the molecular biology of these cells and search for potential novel biomarkers.

METHODS

We first performed shotgun proteomic studies of several GSCs by LC-MS/MS to establish their global proteomic profile. To gain deeper knowledge of the specific expression of molecular markers, we next studied protein dynamics by quantitative proteomics using iTRAQ labeling.

RESULTS AND DISCUSSION

Based on results from both qualitative and quantitative proteomic experiments and from the literature, we have selected a list of targets that reflect the state of activation of specific signaling pathways as a signature of cell transformation. We are generating MRM transitions based on SRM databases and on our shotgun data to reproducibly quantify these targets in a large cohort of samples.

CONCLUSIONS

All this data is being integrated with transcriptomic analyses by RNA-seq to identify splice variants of proteins potentially related to GBM progression. This integral view of both transcriptional and proteomic profiles will provide us a powerful tool to elucidate potential GBM biomarkers in a more reliable way.

P-157.00

TRANSFORMING GROWTH FACTOR BETA REGULATES NOVEL PROTEINS IN A HUMAN TROPHOBLASTIC CELL MODEL.

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Trophoblast cells become part of the placenta and invade the maternal endometrium during pregnancy. The high but regulated proliferation and invasion abilities make trophoblast cells an interesting model to understand malignant development. Transforming growth factor beta (TGF-β) has been shown to have anti-proliferative and anti-invasive effects on trophoblast cells, being considered as a tumor suppressor. However in other cancer models, TGF-β exerts pro-metastatic effects. The study of changes on the trophoblast proteome in response to TGF-β would bring new keys about the regulation of trophoblast and the malignant development. The aim of this work was to identify TGF-β-differentially expressed proteins in a human trophoblast cell line model.

We stimulated HTR8/SVneo trophoblast cells with or without TGF-β (10.0 ng/ml) during 24 hours. Total proteome was separated by bidimensional electrophoresis, images were analyzed and 45 differential spots were identified by MS MALDI-ToF. 40 unique proteins were identified, out of which 33 were up-regulated and 7 were down-regulated in response to TGF-β.

Gene functional classification by DAVID 6.7 gave 2 groups: one with 11 proteins and terms as unfolded protein binding and nucleotide binding, other with 3 proteins and terms as focal adhesion and cell-substrate adherens junction. Highest up-regulated proteins included: Phosphoribosylformylglycinamidine synthase (PFAS), CTP synthase 1 (PYRG1), Neutral alpha-glucosidase AB (GANAB) , Kinesin-like protein (KIF11), Gelsolin and Serrate RNA effector molecule homolog (SRRT). On the other hand, Acidic leucine-rich nuclear phosphoprotein 32 family member A (AN32A) protein showed the highest down-regulation. STRING, VisANT and ChiBE programs were used to build-up highly connected networks from total, up- and down- regulated proteins. Interestingly, the down-regulated protein network revealed an interaction of Akt with HSP90 and estrogen receptor 1 linked to AN32A.

In conclusion, we found novel TGF-β-regulated proteins suggesting new regulatory effects in addition to the classical ones. Its significance is currently under assessment.

P-158.00

FUNCTIONAL PROTEOME ANALYSIS OF TRANSLATIONALLY CONTROLLED TUMOR PROTEIN (TCTP), A NOVEL NEUROFIBROMATOSIS TYPE 1 (NF1)-RELATED PROTEIN

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Neurofibromatosis type 1 (NF1) is an autosomal dominant disease that predisposes individuals to developing benign neurofibromas and malignant peripheral nerve sheath tumors (MPNSTs). Due to the lack of information on the molecular mechanism of NF1-associated tumor pathogenesis or biomarkers/therapeutic targets, a radical treatment for NF1 tumors has not been established.□

@By a unique integrated proteomics, comprising iTRAQ, 2D-DIGE, and DNA array, using MANGO/iPEACH (1, 2), we identified a novel NF1-related abnormal network, gTranslationally controlled tumor protein (TCTP)-mediated oncogenic signaling□h (3). Then, we investigate the role the TCTP in NF1-tumorigenesis. TCTP was found to be upregulated via activated MAPK/PI3K-AKT signaling in response to growth factors in NF1-deficient Schwann cells, and the TCTP expression level was correlated with their malignancy. The knockdown of TCTP suppressed cellular viability and dimension in NF1-associated tumor cells. To further analyze the function of TCTP in detail in NF1-associated tumor, we identified the TCTP-interacting proteins by proteomic approach in. We constructed the plasmid expressing Flag-tagged TCTP, and transfected it into MPNST cells. Using antibodies for flag-tag, TCTP complexes in the cells were purified, digested with trypsin/LysC, and analyzed by nanoLC-ESI-MS/MS and SWATH (sequential window acquisition of all theoretical spectra).

As a result, TCTP-interacting proteins significantly include the protein translation related molecules, such as translation elongation complex consisted of elongation factors, and valyl-tRNA synthetase which was found to be up-regulated in NF1-deficient cells.

 These findings suggest that TCTP is functionally implicated in the genesis and progression of NF1-associated tumors via the activation of protein translation machinery.

P-159.00

STATHMIN-1 SILENCING INHIBITS METASTATIC PROCESSES IN COLORECTAL CANCER

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Introduction and Objectives: Colorectal carcinoma (CRC) remains a concern in public health, given the poor survival that results primarily from metastatic complications to the liver. In search for molecular drivers that control liver-specific CRC metastasis, the proteome of HCT116 colon carcinoma cells was compared to its hepato-metastatic variant E1 by 2D-DIGE. Quantitative profiling of these isogenic cell lines revealed Stathmin-1 (STMN1) as one of the most up-regulated proteins that may be functionally important in the CRC metastatic cascade. This study thus aims to describe the role of STMN1 in CRC dissemination and ascertain its relevance in anti-metastatic therapy.

Methods: Stable STMN1 knockdown cells were generated and loss of metastatic phenotype was quantified by a series of cell-based assays. Proteome changes induced by STMN1 silencing were further profiled by an iTRAQ labeling approach on the AB SCIEX TripleTOF 5600 system. Validation of differential protein abundance was performed by western blotting, immunofluorescence and label-free SWATH MS. Strengthening of cell-cell contact was visualised by three-colour confocal microscopy.

Results and Discussion: STMN1 silencing significantly abrogated cell migration, matrix invasion and anchorage-independent colony formation, while increasing cell adhesion. These observations were supported by proteome changes that recapitulate metastatic inhibition and strengthening of cell-cell interactions. A large number of protein targets regulated by STMN1 silencing were confidently validated, while staining for desmosomal and hemi-desmosomal junctions were visibly more intense in STMN1 knockdown cells. These results indicate good coherence between data from proteomics and cell-based investigations, and demonstrate the advantage of applying proteomics to the study of cell biology.

Conclusions: Based on the quantitative identification of 4562 proteins, STMN1 silencing was found to exert a highly specific inhibitory effect on metastatic processes through modulation of 3% of the detectable proteome. This highlights the potential for a novel STMN1-based anti-metastatic therapy that may aid in reducing CRC-related mortality.

P-160.00

PROFILING EGFR KINASE INHIBITOR RESISTANCE PATHWAYS IN NON-SMALL LUNG CANCER CELL LINES

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INTRODUCTION

Tyrosine kinase inhibitors (TKIs) against the epidermal growth factor receptor (EGFR) have positive therapeutic effects in a subset of Non-Small Cell Lung Cancer patients; however, their clinical efficacies are limited due to the development of TKI resistance. To determine mechanisms of TKI resistance, two NSCLC model cell lines resistant to the EGFR TKI erlotinib were created. Thermo Scientific™ Tandem Mass Tag™ (TMT™) reagents in combination with phosphopeptide enrichment, high pH fractionation, and high resolution mass spectrometry (MS) were used to identify signaling pathways which may confer TKI resistance.

METHODS

Parental and erlotinib-resistant H358 NSCLC cell lines were treated with or without EGF for 2.5 minutes in the presence or absence of 10µM erlotinib. After reduction and alkylation, proteins from ten conditions were digested with trypsin and then labeled with Thermo Scientific™ TMT10plex™ reagents. The peptides were then combined phosphopeptide enrichment and high pH reverse phase fractionation. Unenriched and enriched samples were analyzed using nano-LC coupled to a Thermo Scientific™ Orbitrap Fusion™. Thermo Scientific™ Proteome Discoverer™ 1.4 was used to identify/quantify proteins from the MS spectra.

RESULTS AND DISCUSSION

To identify which signaling pathways may be responsible for TKI resistance, H358 parental and resistant cell lines were analyzed by mass spectrometry. More than 6500 protein groups were identified and quantified. Relative to the parental H358 cells, protein expression in erlotinib-resistant H358 cells showed a marked increase in expression of DNA replication proteins, translation/transcription factors, apoptosis inhibitors and some Ras-associated signal transduction pathway proteins. Cluster analysis of changes in relative protein abundance and phosphorylation state identified patterns in response to erlotinib/EGF treatment among conserved signaling pathways.

CONCLUSIONS

Comprehensive proteomic profiling of parental- and drug-resistant cancer cells using 10plex TMT reagents combined with high pH fractionation and phosphopeptide enrichment enabled the identification of multiple candidate proteins responsible for TKI resistance.

P-161.00
RELATIVE QUANTITATION OF N-LINKED GLYCANS USING CARBONYL-REACTIVE TANDEM MASS TAG REAGENTS

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Introduction:
Protein glycosylation is one of the most abundant andone of the most difficult post-translational modifications to study. Accurate quantitation of glycans remains elusive due to the lack of a comprehensive selection of standards, poor ionization efficiency of carbohydrates relative to other classes of biomolecules, and broad structural heterogeneity of glycomic samples. Recently, we introduced a set of isobaric carbonyl-reactive Tandem Mass Tag (TMT) reagents, Thermo Scientific™ aminoxy TMT™ Reagents, which enable multiplexed relative quantitation of carbohydrates, improve ionization efficiency, and increase analytical throughput. In this work we explore the use of these reagents in quantitative glycomics by combining our multiplexed TMT-based approach with HILIC LC-MS technique to enable more sensitive analysis with improved glycome coverage.

Methods:
N-glycan pools from monoclonal antibodies and human serum samples were labeled with a set of isobaric aminoxyTMT reagents and analyzed by ESI mass spectrometry in multiplex experiments. Samples were analysed using a Thermo Scienitific™ Velos Pro™ dual-pressure linear ion trap and a Thermo Scientific™ Orbitrap Fusion™ Tribrid™ mass spectrometers. Combination of multiple-stage trap-HCD/CID fragmentation of the fully and partially protonated precursor ions was used to obtain both quantitative and structural information for sample glycans.

Results and Discussion:
We have established a protocol for complete derivatization of N-glycan mixtures including all necessary quenching and clean-up steps. At least 20-fold improvement in signal intensity at the MS-level was observed for native labeled glycans ions for all precursor types. A combination of trap-HCD and CID MS analysis enabled both the quantitation by measuring reporter ion peak intensities at MS2 level and determination of the corresponding glycan structures using the characteristic diagnostic ions. Both neutral and acidic glycoforms are amenable to this strategy.

Conclusions:
The use of isobaric aminoxyTMT reagents enabled sensitive relative quantitation of N-glycans with precision and multiplexed analysis.

P-162.00

PROTEIN DEEP SEQUENCING IN MALIGNANT MELANOMA PATIENT SAMPLES

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Introduction and objectives. The aim of present study is to deepen knowledge about underlying mechanisms of malignant melanoma cancer through examining protein expression in several subtypes of malignant melanoma using high quality mass spectrometric approach in combination with appropriate sample preparation.

Methods. Fresh frozen lymph nodes tissues, containing metastasis of malignant melanoma were utilised in the present study. Sliced tissues were lysed with urea and subjected to a standard trypsin digestion protocol. Samples were fractionated using cation-exchange chromatography and the peptide fractions were eluted using increasing salt gradient. Both unfractionated and fractionated tryptic digests were measured on a hybrid quadrupole-OrbitrapTM Q Exactive and Q Exactive Plus mass spectrometers, both equipped with nano-LC pumps (Thermo Scientific, Waltham, MA) in a data-dependent mode. Data was analysed with Proteome Discoverer v 1.4 (Thermo Scientific, Waltham, MA) and TOPPAS (Open MS) customised workflow using a combination of search engines.

Results and Discussion. In order to obtain a more comprehensive view of the protein expression in isolated tissues the samples were analysed on two different LC-MS/MS platforms. The total number of proteins detected using Q Exactive instrument was 5944. A number of proteins detected in unfractionated samples was comparatively lower that that obtained for samples separated on cation-exchange column. Combination of Q Exactive Plus instrumentation with analysis performed at elevated constant column temperature allowed increasing number of assigned proteins and improved reproducibility. Substantial differences were observed in protein presence between pigmented and the high immune subsets, with only about half of proteins detected in both subsets.

Conclusions. A number of characteristic proteins differently expressed in two subsets of malignant melanoma identified previously in a genomics study, the pigmented and the high immune subsets, were identified using shortgun proteomics approach. These proteins can be further analysed as a potential clinical biomarkers for targeted drug treatment.

P-163.00

PROTEOMIC ANALYSIS OF NOVEL NGF-REGULATED TRKA-DEPENDENT TARGETS ASSOCIATED WITH PI3K SIGNALING AND JNK SIGNALING IN SK-N-MC NEUROBLASTOMA CELLS

Eun Joo Jung, Ky Hyun Chung, Choong Won Kim
Gyeongsang National University

Tropomyosin-related kinase A (TrkA) is a transmembrane protein tyrosine kinase and plays an important role in the regulation of cell survival, death, differentiation, and neurodegenerative diseases.

The ability of TrkA to exploit various cellular processes is enhanced by binding to the nerve growth factor (NGF), leading to the activation of its multiple signal transduction pathways including phosphatidylinositol 3-kinase (PI3K) signaling and c-Jun N-terminal kinase (JNK) signaling.

We have previously reported major targets associated with TrkA-mediated tyrosine phosphorylation signaling pathways in a TrkA-inducible SK-N-MC neuroblastoma cells. To further study about novel TrkA-dependent targets regulated by NGF, we investigated the effects of NGF in TrkA-dependent upregulated or downregulated protein spots by proteomic analysis using 2DE/Coomassie Blue stain/PDQuest/MALDI-TOF MS or MALDI-TOF/TOF MS/MS. As expected, upregulation or downregulation of the major protein spots associated with TrkA-mediated tyrosine phosphorylation signaling pathways were significantly enhanced by NGF treatment in TrkA-induced SK-N-MC cells. Moreover, we identified novel NGF-regulated TrkA-dependent targets associated with PI3K signaling and JNK signaling through the effects of PI3K inhibitor wortmanin and JNK inhibitor SP600125.

Our results suggest that these novel NGF-regulated TrkA-dependent targets could play a critical role in TrkA-dependent more elegant signal transduction pathways and cell metabolism.

P-164.00
COMPREHENSIVE IDENTIFICATION OF GENETIC VARIATIONS AT PROTEIN LEVEL WITH LARGE SCALE MS DATASETS

Tieliu Shi, Ruifang Cao, Juan Yang, Peng Li
East China Normal University

Introduction and objectives
Variations at genomic level in human, such as single nucleotide polymorphisms(SNPs) and genetic mutations, have proved strong correlations to phenotypic variations including diseases. The applications of high-throughput technologies to human genome have identified millions of genetic variations, those variations in coding regions will potentially affect the corresponding amino-acids, resulting in the variations at amino-acid level called single amino-acid polymorphisms(SAPs). Although studies have been conducted to globally identify SAPs, only a small number have been detected in each study due to inadequately inferred protein variation database(PVDB) and the low coverage of mass spectrometry experiments. Here, we built a comprehensive PVDB and identify SAPs with large scale MS datasets which include over 30,000 experiments, covering various tissues and diseases.

Methods
First, we created a PVD by integrating various genetic variations. We collected the known human mutation data from UniProt, Protein Mutation Database, HGMD, SysPIMP and OMIM, and SNPs from NCBI dbSNP, Ensembl and COSMIC. We extracted missense and nonsense SNPs in exonic regions from related databases. Then, we used Annovar to annotate the SNPs in the exonic regions in human Ensembl transcriptome. SNP-derived PVDB and the known disease mutant database were combined to form the in-house human PVDB. Next, we identified inferred SAPs with our high-throughput mass spectra data analysis platform ¨C Firmiana ¨C which incorporated TPP and MASCOT.

Results and Discussion
Interestingly, some SAPs appeared mostly in disease samples, when others were detected in both normal and disease states. Mutant proteins with significant effect in human diseases could be potential disease biomarker candidates.
Furthermore, we built spectra with good quality for SAPs into peptide spectral library, which could significantly improves the usage of MS spectra and increases protein identification.

Conclusions
Our protein variation database with identified SAPs provides a rich resource for variation detection at protein level.

P-165.00

PROTEOME PROFILING OF HUMAN CANCER CELL LINES USING DATA-INDEPENDENT ACQUISITION LABEL-FREE LC-MS

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Institute For Immunology, University Medical Center Of The Johannes Gutenberg University Mainz

Introduction and objectives

Cancer is a primary cause of mortality in industrialized countries. Due to molecular heterogeneity in cancer, often less than 25% of treated individuals profit from the approved therapies. Individualized medicine is regarded as a potential solution to low efficacies and high costs for innovation in drug development and health systems, but requires comprehensive data from individual samples. As a proof of principle, we analysed the proteome of ten human cancer cell lines derived from six different tissues (lung, skin, kidney, blood, uterus, ovary) using ion-mobility separation (IMS) enhanced data-independent acquisition (DIA) label-free LC-MS.

Methods

Tryptic peptides were generated from tumor cell lysates using a modified FASP protocol. Peptides (320 ng) were analysed by LC-MS using a nanoAcquity UPLC system coupled to a Waters Synapt G2-S HDMS instrument. MS analysis was performed in DIA mode with IMS using optimised collision energies (UDMSE). Rawdata processing and database search was performed in PLGS3.02, searching UniProtKB/Swissprot (human reference proteome). Post identification analysis including retention time alignment, EMRT (exact-mass-retention-time) and IMS clustering were performed using the in-house developed software package ISOQuant.

Results and Discussion

Using a novel DIA LC-MS approach, we established a reference proteome data set for ten cancer cell lines covering in total over 6.700 protein groups and their relative abundances. Only proteins with a minimum of 2 peptides at an FDR below 1% at peptide level were considered. Over 90% of proteins were detected in all five technical replicates. The dynamic range of identified proteins spanned up to five orders of magnitude with the majority of proteins (72%) identified by at least six peptides. 51% of proteins were identified by more than ten peptides.

Conclusions

To our knowledge, the present dataset is the most comprehensive cancer cell line proteome obtained by a DIA MS workflow without any pre-fractionation.

P-166.00

DECIPHERING PROTEOME CONTENT OF EXOSOMES SECRETED BY LIVER CANCER CELL LINES

Terence Chuen Wai Poon[1,2,3], Melody Man Ting Lam[2,3], Mian He[2,4], Wai Hung Lau[2,3], Eddy Wing Yin Ng[2,3], Arthur Kar Keung Ching[2,4], Kwok Wai Lo[2,4], Ka Fai To[2,4] Nathalie Wong[2,4]

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Introduction and objectives: Cancer-derived exosomes play various roles in carcinogenesis, and are potential tumor markers. In this study, we aimed to decipher proteome contents of exosomes secreted by human hepatocellular carcinoma (HCC) cell lines (HKCI-8, HKCI-10, HKCI-C3, MHCC97) and a non-tumorigenic immortalized hepatocyte cell line (MiHA).

Methods: Exosomes were harvested from culture medium by ultracentrifugation. The exosome proteins were first separated by SDS-PAGE. Proteins in consecutive 1mm gel slices were subjected to shot-gun proteomic profiling by Ultraflex-III MALDI-TOF/TOF MS at FDR <5%.

Results and Discussion: 44 proteins were found in the exosomes of both HCC cell lines and the immortalized cell line MiHA, including members of RAS oncogene family and annexin family, which may play important roles in conferring immortal property of hepatocytes. The presence of ANXA2 in the exosomes was confirmed by Western blot. Annexin A2 (ANXA2) was previously shown to be involved in HCC carcinogenesis and a potential serum tumor marker. 246 proteins were found in the exosomes from the HCC cell lines, but not MiHA. Gene ontology analysis showed that they were predominantly involved in cell adhesion and signal transduction. 39 proteins including MET proto-oncogene tyrosine kinase (MET) and caveolin-2 (CVA2) were identified only in the metastatic HCC line MHCC97. The specific expression patterns of MET and CVA2 were confirmed by Western blot. MET is well known to promote metastasis in many human organs, and CVA2 has been recently shown to be involved in the cancer cell proliferation, migration and invasion.

Conclusions: Unique proteins were found in exosomes released by liver cancer cells. Future studies are warranted to investigate diagnostic and prognostic values of circulating exosomes that contain these proteins.

P-167.00

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Terence Chuen Wai Poon[1,2,3], Melody Man Ting Lam[2,3], Mian He[2,4], Wai Hung Lau[2,3], Eddy Wing Yin Ng[2,3], Arthur Kar Keung Ching[2,4], Kwok Wai Lo[2,4], Ka Fai To[2,4] Nathalie Wong[2,4]
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Conclusions: Unique proteins were found in exosomes released by liver cancer cells. Future studies are warranted to investigate diagnostic and prognostic values of circulating exosomes that contain these proteins.

P-168.00

IMMUNOPROFILING OF CANCER – HARVESTING THE IMMUNE SYSTEM AS A SENSOR FOR CANCER

Carl Borrebaeck, Christer Wingren
Lund University

Introduction and Objectives: Unlocking the proteome and delivering biomarkers to the clinic will be critical for early and improved disease diagnosis, prognosis, and classification. While major efforts have been made to decipher cancer-associated biomarkers, the lack of validated, multiplexed biomarker panels still represents a major unmet clinical need. To this end, we have exploited the immune system as an early and specific sensor for cancer using affinity proteomics.

Methods: To harvest potential biomarkers, we have designed a set of high-performing recombinant antibody micro- and nano-array technology platforms for high-throughput protein expression profiling of crude proteomes, such as non-fractionated serum. The technology allows us to generate detailed molecular portraits, or fingerprints, of the target disease in a highly specific and sensitive manner.

Results and Discussion: Using recombinant antibody microarrays, we have deciphered candidate (and pre-validated) biomarker panels (5 to 25 markers) for diagnosis (e.g. pancreatic cancer, breast cancer), prognosis (e.g. breast cancer), classification (e.g. B cell lymphomas, prostate cancer), evidence-based therapy selection (e.g. glioblastoma multiforma), as well as providing novel means for studying the disease heterogeneity (e.g. B cells lymphomas) and potentially the cancer immunoediting process taking place (e.g. breast cancer). Hence, data demonstrating the use of the immune system as a sensor for cancer will be presented.

Conclusions: Harvesting the immune system as a sensitive and specific sensor for cancer will provide novel means for generating detailed molecular serum portraits, or fingerprints of cancer, paving the way for improved cancer diagnosis, prognosis, and classification.

P-169.00

DIAGNOSIS OF THE PRESENCE OF NON-SMALL-CELL-LUNG CARCINOMA (NSCLC) USING AUTOANTIBODIES

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Hong Kong Polytechnic University

Lung carcinoma is the second most commonly cause of cancer-related death in Hong Kong. Early diagnosis of NSCLC allows a window of opportunity for effective treatment. After prompt surgical removal and chemotherapy, 5 year remission rate is less than 20%. Hence, there is an urgent need for identifying reliable biomarkers for early diagnosis, especially in high-risk individuals.

With traditional comparative proteomic approach, we compared protein expression in NSCLC tissues from patients with that from their surrounding normal tissues. 12 proteins were found to be up-regulated. These proteins are sometimes called tumor-associated antigens (TAAs). It is because it was found that some patients produced autoantibodies to these TAAs. 8 of these TAAs were found to have corresponding autoantibodies in serum from NSCLC patients. It is known that patients' serum autoantibodies against TAAs could be used for diagnostic purposes. In contrast to finding minute amounts of TAAs in serum, detection of autoantibodies is technically more feasible. Firstly, TAAs-associated autoantibodies circulate in the blood much earlier and in higher amount than serum TAAs. In a published case of breast cancer, autoantibodies could be detected 5 years before clinical presentation of breast cancer. Secondly, presence of these autoantibodies is more persistent, stable and abundant than their TAAs.

With recombinant technologies, several of these TAAs were cloned, expressed, purified and bind to multiplex beads for fabrication of the diagnostic test. Expression levels of autoantibodies of some of these TAAs are significantly higher than that of normal controls. Results of fabrication of this diagnostic test will be presented.

P-170.00

IMMUNOCAPTURE LC-MS; A TOOL FOR ISOZYME DIFFERENTIATED MEASUREMENT AND CO-DETERMINATION OF CANCER MARKERS

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Cancer markers provide a dynamic and powerful approach to understanding pathological states, and measurement of these can aid in decision of clinical intervention. Disease tailored targeted methods have for this reason been developed, validated and utilized for cancer marker measurement in patient serum samples with aim to provide novel clinically relevant information. Two clinically complementary markers for neuroendocrine tumors; neurospesific enolase (NSE) and progastrin releasing peptide (ProGRP), were used as models in this research to demonstrate the potentials of this method design.

The method design is as following:
1.) Highly enriched and clean extracts are provided by immunocapture, involving use of monoclonal antibodies; mAb E146 for ProGRP and mAb E21 for NSE, immobilized on magnetic   beads
2.) Peptide bonds of the captured proteins are systematically cleaved by trypsin to produce peptides well suited for the LC-MS analysis
3.) Signature peptides (peptides specific for markers isozymes) are determined by ESI-SRM-MS

The method was validated upon use on patient samples and the following was shown:
- Protein isoforms of ProGRP were measured for the first time in patient samples, and the relative isoform ratios were shown to vary in the 60 serum samples analyzed
- Analyses of the same patient samples were compared with clinically used immunometric assays and absolute amounts were found to co-vary, but not to be identical
- The immunocapture LC-MS method enables differentiated measurement of isozymes of both ProGRP and NSE in one single analysis from one single patient sample

The demonstrated potential of immunocapture LC-MS is to allow for novel insight to pathological marker expression by enabling simultaneous evaluation of their variant presence in patient serum.

P-171.00

PARALLEL REACTION MONITORING ANALYSIS OF NIPPLE ASPIRATE FLUID FROM BRAZILIAN BREAST CANCER PATIENTS: LOOKING FOR MOLECULAR SIGNATURES

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Worldwide, breast cancer is the main cause of cancer deaths in women. In Brazil, the mortality rates remain high, possibly due to late diagnosis. Most deaths occur as a result of disease spread to distant sites (metastasis). During this progression, the epithelial-mesenchymal transition (EMT) phenomenon may be activated, being responsible for turning the focal lesions into aggressive/invasive ones. Some studies suggest that aggressive tumors, in particular the triple-negative breast cancer type (TNBC - characterized by the absence of the receptors of estrogen, progesterone and human epidermal growth factor 2), could be related to EMT. Breast cancer is a highly heterogeneous disease and most cases arise from mammary ductal cells that produce nipple aspirate fluid (NAF), a breast-proximal fluid closely related to the tumor microenvironment.

To evaluate the relation of hormonal/growth factor receptor status of the tumor and EMT, we quantified 23 target proteins in the NAF samples of 13 women using the parallel reaction monitoring (PRM) method on a Q-Exactive mass spectrometer. Using the Skyline software, we calculated the area under the curve of the target fragment ions, comparing the protein ratios between the breast with cancer and the contralateral healthy one from patients with aggressive and non-aggressive unilateral breast cancer. These results were further compared to the ratios of NAF samples from non-diseased subjects.

Preliminary results showed that MMP7, a small-secreted proteolytic enzyme known to be vital for invasion and metastasis, was under expressed in TNBCs, whereas vimentin, a protein relevant to EMT, was over expressed. In accordance with the literature, the invasiveness does not seem to be concomitantly stimulated by soluble activating molecules and EMT inducers.

We are presently analyzing the metabolic interaction panel of these proteins for the identification of cancer aggressiveness-EMT molecular signatures in breast cancer, which may be correlated to potential biomarkers in cancer diagnosis.

P-172.00

IDENTIFICATION OF MARKERS OF ENDOMETRIAL CANCER IN EXOSOME-LIKE VESICLES OF UTERINE FLUIDS USING A SUPER SILAC QUANTITATIVE PROTEOMICS APPROACH

Irene Campoy[1], Marina Rigau[1], Tamara Sequeiros[1], Elena Martínez[1], Lucia Lanau[1], Laura Devis[1], Antonio Gil-Moreno[1], Jaume Reventós[1] Eva Colás[1] Pierre Thibault[2]
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Endometrial cancers (ECs) are the most common gynecologic cancers with over 52,000 new cases diagnosed and 8,500 deaths occurring every year in the US alone. There is an urgent need to develop a non invasive test that can provide early detection of ECs to improve the prognostic and selection of appropriate therapies. Recent studies have identified several differential expressed genes and proteins in EC tissue and body fluids (e.g. serum and urine), but none of these had a direct impact on the clinical management of this disease. We surmised that neoplasic endometrium cells are likely secreting key proteins during disease progression, and that proximal uterine fluid could represent an appropriate source of biomarkers. In this study, we combined subcellular fractionation and quantitative proteomics to profile differentially abundant proteins in exosome-like vesicles (ELVs) of uterine fluids.

Protein extracts from purified ELVs were obtained following ultracentrifugation of uterine fluid from age-matched groups of control, endometrioid and serous EC patients (10 patients/group). The quality of isolated ELVs was monitored by Nanoparticle Tracking Analysis (NanoSight®), and immunoblots, and only ELVs of diameter 100±50nm showing exosomal markers (CD81, CD63 and TSG101) were selected for subsequent experiments. To profile protein abundance across different groups, we develop a super SILAC approach where ELV proteins from 3 different EC cell lines grown in heavy Lys and Arg amino acids were combined with ELV protein extracts of each patient. Proteins were separated by SDS-PAGE and gel-isolated bands were digested with trypsin and analyzed on a Q Exactive Plus mass spectrometer.

Our proteomics analyses identified key exosome markers including CD81, CD63, CD9, and TSG101, thus confirming the quality of subcellular fractionation. Quantitative proteomics in ELVs also enabled the identification of protein biomarkers previously identified at tissue level and novel biomarkers for endometrioid and serous EC diagnosis.

P-173.00

MAPPING THE METALLOPROTEASE DEGRADOME OF BREAST CANCER CELLS THROUGH A GLYCOPROTEIN CAPTURE SILAC PROTEOMIC ANALYSIS

Francesc Canals[1], Gemma Reverter-Branchat[1], Joan-Josep Bech-Serra[1], Marta Monge[1], Núria Colomé-Calls[1], Laura Villarreal[1], Luna Martín[1], Stephanie Kaspar[2] Pierre-Olivier Schmitt[3] Carsten Baessmann[2]
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ADAM10 and ADAM17 (TACE) are enzymes with metalloprotease activity and a disintegrin domain in their structure, localized in the cellular membrane. Several of their substrates have been implicated in the pathogenesis or progression of breast cancer. They are known to promote cancer progression by releasing HER/EGFR ligands that culminates in increased cell proliferation, migration and survival. A deeper knowledge of the substrate repertoire (degradome) of these proteases will be helpful to better elucidate their role in tumor growth and metastasis, and to evaluate their potential use as therapeutic targets.

The aim of this study is the characterization of the substrate array of metalloproteases in breast cancer, through the proteomic analysis of a panel of cell lines comprising different subtypes of breast cancer.

We have used a proteomic analysis methodology, previously set up in our laboratory, based on SILAC quantitative comparison of the secretomes of cell lines cultured in the presence or absence of a metalloprotease inhibitor. Glycoprotein products released to the medium by proteolytic cleavage are enriched by either covalent binding to immobilized hydrazide, or by a lectin affinity chromatography. Enriched glycoproteins are then digested, analyzed by LC-MS/MS, on a UHR-QTOF instrument (Impact, Bruker), and SILAC quantitation is performed with ProteinScape software (Bruker).

Using this methodology, we have been able to map the metalloprotease substrate profile to a wider extent as compared to previous approaches. Among the newly identified substrates, we find a relevant number of shed proteins involved in cell-to-cell signaling, cell adhesion and cell migration processes, whose function most probably could be affected by ADAMs activity. The results unravels potential new roles of those proteins in tumor development not deeply studied yet. On the other hand, the secreted products identified have the potential to be released to the blood and constitute potential new cancer biomarkers

P-174.00
TARGETED PROTEOMIC ANALYSIS OF POTENTIAL NEW THERAPEUTIC TARGETS FOR PSORIASIS

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Introduction
Psoriasis is a common inflammatory skin disease with a complex pathophysiology and limited treatment options. In order to identify new therapeutic targets in the psoriatic epidermis, a proteomic profiling analysis of skin from a pool of patients was analysed using iTRAQ. We found S100A8, S100A9 and the complement component C3 as the most upregulated proteins, identifying them as potential therapeutic targets. These results were compared to a similar study we performed in a psoriasis-like mouse model, revealing parallel results. In this work, we aimed to develop a methodology to measure the relative amounts of S100A8, S100A9 and C3 in a single assay by MS–based targeted proteomics in epidermis from psoriasis-like mice that had been treated with a potential new therapy for psoriasis.

Methods
Epidermis samples from sick mice treated with lentivirus-delivered shRNAs targeting C3 were digested using FASP method and analyzed by nanoLC-SRM on a 5500 QTRAP MS (AB Sciex). Unique peptides showing good fragmentation spectra were selected for SRM analysis and transitions were chose based on chromatographic co-elution, shape similarity and low level of interfering signals. The peak areas from each transition were normalized to a reference protein, GAPDH.

Results
Blocking C3 in the mouse model ameliorates the inflammatory skin disease, proving it to be a potential new therapeutic target for psoriasis.
SRM MS was used to measure relative C3 protein levels in ears of lenti-shC3 injected mice compared to scramble controls, showing a significant reduction of the complement levels, while S100A8 and S100A9 protein levels remained up-regulated.

Conclusions
Relative protein levels of C3, S100A8 and S100A9 in treated tissue were measured in a single experiment by SRM, with no need of previous enrichment and/or fractionation, proving it to be a fast, selective and sensitive methodology to measure changes of potential biomarkers after treatment of the disease.

P-175.00

MOLECULAR EVIDENCE FOR TUMOUR HETEROGENEITY IDENTIFIED BY MALDI TOF IMAGING AND INTACT CELL MASS SPECTROMETRY

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Molecular evidence for tumour heterogeneity relies at the moment in essence on determination of gene expression profiles from punches of different areas of the same tumour and its metastases. The information obtained is limited because gene expression is relatively unknown. Randomly selected tumour areas are examined containing an uncontrollable number of tumour and stromal cells, thus individual tumour cells are missed. Novel visualization methods assigning "fingerprints" of individual tumour cells are urgently required. From this the identification of biomarkers specific for metastasis-forming cells is to be expected.

To identify molecular fingerprints of cells, convolute cell layers cultured from patient tumour cells (primary tumour, metastasis) were trypsinized, washed and dissolved in MALDI matrix solution for intact cell mass spectrometry (ICMS). To localize primary tumours and metastases in whole tissue sections mass spectrometry imaging (MSI) and profiling (MSP) was performed on formalin fixed, sucrose embedded biopsies (500 nm slices). After trypsinization and matrix application the tissue samples were analysed at cellular level (7 µm). Multiple MSI (n=15), MSP (n=57) and ICMS experiments (n=65) were carried out and statistically evaluated.

Statistics showed that ICMS allows differentiating primary and metastatic cell cultures as well as cell cultures from different patients on the intact protein level with a high significance. 25 protein signals (m/z 5000-50000) were relevant for tumour/metastasis differentiation. MSI experiments at a lateral resolution of 7 µm and a vertical resolution of 500 nm allowed tissue analysis below the cellular level and results correlated nicely with high-resolution images of respective Tolouidin stains. Despite the very low amount of material (500 nm sections) trypsinized sections revealed a significant number of peptides from which 13 were identified to be significant for primary tumour/metastasis differentiation. Assignment of areas belonging to primary tumour tissue or metastasis-forming cells was achieved.

P-176.00
THE QUEST FOR ENDOMETRIAL CANCER PROTEIN BIOMARKERS IN UTERINE ASPIRATES

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Introduction and Objectives.
Endometrial cancer is the most frequent of the invasive tumors of the female genital tract accounting for 76,000 deaths every year worldwide. Uterine fluids are an attractive source of biomarkers for gynecological diseases, as they are easily accessible biofluids in direct contact with the endometrium. However, one of the main challenges to be addressed remains their inherent variable blood contamination that may jeopardize the search of disease-specific biomarkers. In this study we aimed to verify potential biomarkers that are not affected by the blood contamination of the uterine fluids.

Methods.
Uterine fluid and full blood samples were digested with trypsin and analyzed by LC-MS/MS (LTQ orbitrapVelos) in a data-dependent acquisition mode. The list of identified protein was compared to a list of 500 biomarker candidates obtained from an extensive literature review. To assess the effect of blood contamination on uterine fluids, increasing amounts of blood were spiked into the samples. The profiles of the candidates were monitored by SRM on a triple quadrupole instrument using stable isotopically labeled synthetic peptides as internal standards. The option of spiking a super-SILAC mix from 3 labeled endometrial cancer cell lines in the samples as internal standards was evaluated.

Results and discussion.
The shotgun analysis performed on uterine fluids and blood samples identified about 750 and 330 proteins, respectively. Among the proteins detected in uterine fluids, 168 of the 500 potential biomarker candidates were identified. Those candidates for which no signal increase was observed when diluted by blood were selected for further verification by SRM on a triple quadrupole instrument in a large series of uterine fluids from age-matched control and EC patients (50 samples/group).

Conclusions.
Uterine fluids are a promising source of biomarkers for an earlier and minimally invasive diagnosis of gynecological diseases such as endometrial cancer.

P-177.00
QUANTITATIVE ANALYSIS OF YEAST KINETOCHORE COMPLEXES USING SWATH-MS

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Kinetochores are large protein assemblies that mediate chromosome segregation in eukaryotes. The structure and organization of kinetochores is partially understood, therefore new approaches such as the SWATH-MS can help to decipher the organization of kinetochores.

The kinetochore complex is evolutionary conserved and subdivided into inner and outer kinetochore. The main function of the kinetochore is to maintain the correct chromosome segregation during cell division. A simple model system to investigate the architecture of kinetochores is a Saccharomyces cerevisiae, where a single copy of kinetochore is attached to a microtubule and its assembly is facilitated on a DNA.

SWATH-MS is a data independent acquisition method that brings, in a single measurement, a possibility to quantitatively investigate all peptides detectable in a sample such as yeast lysate. The SWATH-MS, which in contrast with SRM, is not based on targeted MS acquisition, but on targeted data extraction from maps consisting of MS2 ions from all precursors in the sample.

Here we have combined cell-cycle specific purifications of kinetochore assemblies with genetic deletions of individual subunits to determine the topology of kinetochores. To purify the inner kinetochore, a 6xFlag-tag was integrated at the c-terminus of the AME1 subunit and various deletion strains were constructed. The yeast strains were logarithmically grown; kinetochores were purified using antibody-coupled magnetic beads and analyzed by MS. SWATH-MS was used for label-free quantification of the kinetochore and quantitative changes in subunit composition following gene deletion. Using this approach, we confirmed and extended known interactions within kinetochore subunits, such as CHL4-IML3 and CTF3-MCM22-MCM16. Also up-regulation of the abundance of certain subunits was observed in the deletions, which could be due to functional substitution of the deleted protein.

We conclude that SWATH-MS method is an effective tool to quantitatively follow the changes in the kinetochore network and to discover novel interaction partners.

P-178.00

ECM PROTEOMICS AND CYTOKINE PROFILING IN 3D-CELL CULTURE MODELS

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As 2D-cell culture models do not represent the in vivo situation, 3D cell culture models are on the increase in drug discovery (Rimann M., Graf-Hausner U (2012) Curr Opin Biotechnol 23,803-809). Sophisticated 3D tumor- stromal models enhance the understanding of the tumor microenvironment (Linde N et al. (2012), PLoS One 7, e40058) and have high-potential to become valuable tools for stratified or even individualized screenings of anticancer drug efficacy. As such there is a need for well-characterized highly reproducible 3D-matrices for such complex tumor models. Defined 3D-Life biomimetic hydrogels built from inert dextran and EG can be polymerized in the presence of cells to generate 3D-cell cultures for drug screening (Rimann M et al. (2013), J Lab Autom 19, 191-197). As such hydrogels contain no extracellular matrix (ECM) components they offer the possibility to analyze cytokine and metalloproteinase profiles as well as the quantitative composition of the ECM synthesized and rebuilt by 3D-tumor-stroma models at the same time.

Therefore we established methods to degrade the hydrogels and to extract ECM analytes prior to tryptic digest and analytical characterization via mass spectrometry. Furthermore, multiplexed bead-based sandwich-immunoassays were used for detection and quantification of cytokines and matrix metalloproteinases produced by the tumor cells. Seven cytokines (MCP-1, IP-10, IFNã, IL-1â, IL-6, IL-8, TNFá) present in concentrations ranging from femtogram to picogram per ml could be determined either from enzymatically degraded hydrogels or from cell culture supernatants. Signature peptides for MS based detection of extracellular matrix proteins were defined.

The hydrogel model proved to be a valuable system for analysis of different kinds of tumor biomarkers. The easy degradability of 3D-Life gels enabling direct access to tumor cells and relevant ECM and cytokine analytes showed their application potential for improved biomarker based studies of tumor-stroma interactions in 3D co-culture models.

P-179.00
LPS- INDUCED COLORECTAL TUMOR PROTEINS

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INTRODUCTION

Surgical removal of colorectal cancer can paradoxically contribute to tumor recurrence and hepatic metastasis of colon cancer. This is due to the release of inflammatory mediators in response to surgical trauma, which can promote the metastatic ability of circulating cancer cells.

During surgery, bacterial products such as lipopolysaccharide (LPS) from commensal bacteria translocate across the bowel wall. LPS is a potent inducer of inflammatory response through Toll like receptor-4 (TLR4) recognition. LPS induced TLR4 signaling is mediated by the nuclear factor kappa B (NFκB) transcription factor.

To further understand the process of a LPS induced inflammatory response by colorectal cancer cells we set out to identify proteins that are secreted by the cancer cells in response to LPS stimulation.

METHODS

We treated six colorectal cancer cell lines: HT29, HCT116, SW480, CaCo2, Colo205 and LoVo with LPS to reproduce the surgical conditions, and we measured by Luminex 200 a 21 inflammatory cytokine panel in the soluble secretome of one of them.

The cells were incubated with LPS in a concentration of 1µg/ml for 18 hours in serum free medium. The conditioned medium was harvested from the six cell lines and the soluble secretome and the exosome fraction was isolated. These fractions were further processed for proteomic analysis by 1D GeLC-MS/MS, which is currently ongoing.

RESULTS AND DISCUSSION

In the Luminex approach we observed several cytokines up and downregulated, including Macrophage colony-stimulatory factor (MCSF), Macrophage migration inhibitory factor (MIF), Interleukin 18 (IL18), Stem cell factor (SCF) or Hepatocyte growth factor (HGF).

CONCLUSION

These results suggest inflammatory events take place upon LPS stimulation and recognition by TLR4, but other molecular processes, such as cell migration or adhesion have been suggested to be affected as well in response to stimulation with bacterial products and to be related to the metastatic process.

P-180.00

SECRETED PROTEINS IN URINE OF RENAL CELL CARCINOMA PATIENTS: INVESTIGATIONS USING THREE DIFFERENT PROTEOMIC APPROACHES

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INTROCUCTION AND OBJECTIVE: Renal cell carcinoma (RCC) represents 3% of all tumors. The most common histological type is clear cell RCC (ccRCC) that represent 70% of RCC. Based on the histological type, tumors are classified according to cellular differentiation degree (Fuhrman degree FI-FIV). FI and FII patients are classified as low-grade and present a good prognostic. Whereas in patients classified in FIII and FIV, which are high-grade tumors present poor prognostic. The only effective treatment is surgery, because this tumor does not respond to chemotherapy or radiotherapy. To improve the early diagnosis and accurate treatment a better understand of cell differentiation mechanism in RCC is necessary.

METHODOLOGY: Urine from ccRCC patients was collected and divided into two groups: good and poor prognostic (GP and PP), which were compared with control group (health individuals - CG). The urine samples from patients and CG were pooled, followed by 1D and 2D electrophoresis analyses or performed an in-solution digestion combined with both MALDI-TOF/TOF and LC-MS/MS (ESI-Q/TOF and SYNAPT HDMS).

RESULTS AND DISCUSSION: Initially, 1DE followed by ESI-Q/TOF analysis indicated the presence of non-common proteins in the GP, PP and CG groups. This was in agreement with the 2DE protein profile, which showed a total of 20 proteins differentially secreted among the three groups. Label-free analysis (SYNAPT - MSe) revealed 201 protein differentially secreted, such as Kinonogen1, ApoliproteinD and uromodulin which were down-secreted in the GP and PP groups. However, alpha-1 beta glycoprotein, ApoliproteinA and Fibrinogen were up-secreted in those same groups. Hence, label-free protein quantification validated the results observed in the 1DE and 2DE analyses and generates new information that deserves further attention.

CONCLUSION: Strategies used proved to be valuable in assisting the identification of potential biomarkers candidates for renal tumors, according to the histological type and cellular differentiation to which they belong.

P-181.00
ANALYSIS OF ERLIC TIP-BASED ENRICHMENT OF GLYCOSYLATED SERUM PROTEINS

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Introduction
Analysis of the blood glycoproteome represents a promising strategy for the discovery of disease-related protein biomarkers. This is supported by the fact that the majority of the clinically approved blood biomarkers are glycosylated (Schiess et al., 2009). Though, mass spectrometry (MS)-based analysis of blood samples is challenging due to the high concentrations range (Landegren et al., 2012). We report the optimisation of a workflow for purification of glycosylated peptides and their analysis with KYSS, a new software for assessment and visualisation of large scale proteomics datasets (Such-Sanmartín et al., 2014).

Methods
Serum samples were digested, glycopeptide-enriched with PolyWAX (PolyLC) phase packed in a micropipette tip format, and analysed by LC-MS/MS using the tribrid Orbitrap Fusion MS/MS system (Thermo Scientific). Precipitated serum samples enriched for the low mass proteome (Chertov et al., 2004) were used for optimisation of acquisition parameters for the Orbitrap Fusion. Data files were database searched with Proteome Discoverer (Thermo Scientific) using a comprehensive workflow adapted from Ahmad et al. (Thermo Scientific, poster communication). Datasets were exported as text files and visualised and compared with KYSS.

Preliminary   Data
We report the comparison of different protocols for improved protein digestion and optimised ERLIC enrichment of glycosylated peptides. We investigated various parameters for large-scale peptide sequencing in the Orbitrap Fusion applied to serum samples, employing precipitated serum samples for removal of medium and high mass proteins that include the dominating serum albumin and immunoglobulin proteins. We have assessed a recently reported comprehensive Proteome Discoverer workflow for database searching (Ahmar et al., Thermo Scientific) that uses several search engines and multiple PTM combinations. We further report the new iteration of the KYSS software (http://kyssproject.github.io), including more graphical representations for data visualization and comparisons that facilitate the analysis of modified peptides.

P-182.00
ENRICHMENT OF EGFR/PI3K/AKT/PTEN PROTEINS USING IMMUNOPRECIPITATION AND ANALYSIS WITH MASS SPECTROMETRY-BASED PROTEOMICS

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Introduction:
A major bottleneck in the verification of protein biomarkers in clinical research is the lack of methods/reagents to quantify medium to low levels of proteins of interest in human samples. Immunoprecipitation (IP) and mass spectrometry (MS) are complementary techniques that permit sensitive and selective characterization and quantitation of low abundance protein analytes in cell lines, tissue, and biofluids. IP provides both enrichment and high selectivity while the MS provides high sensitivity and multiplexing across a range of analyte concentrations in different matrices. The quantitative evaluation of protein expression and post-translational modification (PTM) status of EGFR-PI3K-AKT signaling pathway proteins enables the precise characterization of the disease at the diagnostic or prognostic level and to monitor its progression and treatment response.

Methods:
We evaluated immunoprecipitation with directly coupled antibodies or biotinylated antibodies with immobilized streptavidin resin. EGFR, PI3K, AKT isoforms and PTEN were enriched from two cell lysates using an optimized IP to MS workflow. A multiplex, targeted selected reaction monitoring (SRM)-based MS assay was developed to measure the limit of quantitation (LLOQ) of EGFR, AKT2, AKT1, PTEN, PIK3CA and PIK3R1 tryptic peptides. Multiple targets (EGFR, AKT isoforms, PTEN) were immunoprecipitated simultaneously and quantitated by targeted SRM assay.

Results and Discussion:
Immunoprecipitation using magnetic beads resulted in overall higher yield of target protein and less non-specific binding for MS applications. Enrichment of EGFR, AKT isoforms, PI3K and PTEN in two cell lysates permitted MS detection and quantitation. EGFR, AKT1, AKT2, PI3K and PTEN were quantitated in low nanogram range by LC-MRM/MS in two cell lysates and human plasma matrix. Multiple targets IP resulted in simultaneous identification and quantitation by MS.

Conclusions:
Optimized IP-MS workflow permits detection and quantitation of EGFR, PI3K, AKT and PTEN proteins and PTMs at sub to low nanogram concentrations.

P-183.00

INTEGRATED PROTEOMICS FOR CANCER STEM CELLS

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Cancer stem cells (CSCs) are considered responsible for the therapeutic resistance and recurrence of malignant tumors/cancer. To understand the molecular mechanism of CSC maintenance/differentiation, we established glioma stem cell (GSC) clones from patient tissues having the potential to differentiate into glioblastoma, and subjected to DNA microarray/ iTRAQ and 2D-DIGE based integrated proteomics.

We identified 21,857 mRNAs and 8,471 proteins which were integrated into an gene/proteomics expression analysis chart (iPEACH:PTC/JP2011/58366, Mol Cell Proteomics, 2013 12(5): 1377-94), and the upregulated 469 mRNAs/196 proteins, downregulated 114 mRNAs/ 212 proteins in the differentiating GSCs were subjected to the GO and network analyses. The identified genes/proteins were classified into the groups: upregulated groups, extracellular matrices (ECMs) (18%); membrane (10%); adhesion/cell communication (6%); and downregulated groups; intracellular (39%); cytoplasm/organelle (35%), and others. We also observed the upregulation of glioma specific proteins in the differentiating GSC, such as vimentin, VEGF, EGFR, MAPKs, KRAS, Musashi, CD44, GFAP, downregulation of neural stem cell markers, CD133, SOX2 etc, in proteomics/mRNA levels.

The induction of ECMs dramatically accelerated the GSC differentiation and proliferation, which were suppressed by integrin alphaV blocking antibody or RGD peptide. In addition, the expression of integrin alphaV and its ligand FN was prominently increased in glioblastomas developed from mouse brain GSC xenografts. The combination treatments of anti-cancer drug TMZ and RGD inhibited glioma progressions, induced their apoptosis, and lead the longer survival of mouse GSC xenograft models.

These results indicate that GSCs induce/secrete ECMs to develop microenvironments, namely □gdifferentiation niches□h that further stimulate GSC differentiation and proliferation via the RGD motif.

A combination of RGD treatment with TMZ could have the higher inhibitory potential against the glioma recurrence that may be regulated by the GSCs in the differentiation niche. Functional combination with other niche factors will be discussed. PLOS ONE, 2013 8(5):e59558

P-184.00

A NOVEL AND STRAIGHTFORWARD EXPERIMENTAL NULL STRATEGY ENABLES ACCURATE EVALUATION AND CONTROL OF FALSE-DISCOVERY OF SIGNIFICANTLY-ALTERED-PROTEINS IN LABEL-FREE QUANTITATIVE PROTEOMICS

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Introduction:

False-discovery of significantly-altered-proteins due to technical/biological variability is a prevalent problem;evaluation and control of False-Altered-protein-Discovery-Rate(FADR) is critical but represent a daunting challenge as current parametric algorithms (e.g. multiple-testing-correction), which work poorly with LC/MS-based proteomics data, largely due to the fact that some critical assumptions by the parametric algorithms are not met (e.g. sample size; distribution of p-values). Here an experimental-null(EN) strategy was developed, which experimentally obtains the null distribution, which was demonstrated to have achieved much more accurate evaluation and control of FADR in label-free quantitative experiments.

Methods:

Four sample sets, representing a wide range of proteomic matrices with biological replicates of 5-10 were used to develop and evaluate the EN strategy: All samples were analyzed by a label-free, LC/MS-based quantification. Null distributions in each set were obtained. We compared the EN strategy against prevalent statistical methods including permutation method or BH method combined with standardized t-test, LIMMA t-test and Mann-Whiteny Wilcoxon(mww) test.

Results and discussion

The EN strategy was validated with the two spiked-in sample-sets with known true-positives/negatives and true protein ratios. While EN strategy achieved highly accurate estimation, strikingly, BH with t-test, LIMMA t-test or MWW test all resulted in severe underestimation of false-positives by at least 6-fold. Furthermore, EN showed a significant better specificity-sensitivity ROC curve over all other algorithms. Evaluation with two biological sample-sets obtained from pre-clinical studies, showed BH with t-test, LIMMA t-test or MWW was over stringent and cohorts of well-known true positives were missing. By comparison, EN enabled the identification of all known true-positives.

Conclusion the EN strategy has been showed to achieve more accurate prediction of FADR in quantitative proteomics and significantly superior specificity-sensitivity and precision, over the prevalent statistical strategies. The strategy is straightforward and easy to implement, and thus affording a promising alternative to statistical methods for FADR control.

P-185.00

PROTEOMIC AND DEGRADOMIC PROFILING OF GLIOBLASTOMA MULTIFORME

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Glioblastoma multiforme (GBM), a WHO grade IV astrocytoma, is the most common and aggressive primary tumor of the brain. With median survival rate of less than 15 months, GBM accounts for more than half of all neoplasm of the brain. Standard of care to date consist of maximal resection of the affected area followed by extensive radiochemotherapy. Nevertheless, despite extensive research efforts to date, clinical prognosis remains poor. The present study looks at the differential regulation of proteins and proteolytic cleavage events in GBM tissues versus non-malignant tissues of the brain. In addition, proteomic profiles for disease progression from early stages of astrocytomas to GBM will also be presented. Brain and glioma tissues were obtained from neurosurgery after receiving patients' consent and ethical approval by the local authorities.

Directly after sampling, tissues were frozen in liquid nitrogen and stored at -80°C. Multiplexed analysis of extracted proteins from human clinically dissected tissues, along with control tissues were carried out using isotopic labeling followed by two-dimensional liquid chromatography tandem mass spectrometry (LC-MS/MS). Quantitative analyses of GBM tissues show that over 3,000 proteins were consistently identified, with approximately 1,500 proteins changed between GBM versus controls, and more than 1,000 proteins changed between GBM versus lower grade astrocytomas.

Many of which are known to be involved in the regulation of neuronal diseases, synaptic transmission as well as neurological system processes. Interestingly, a number of proteases as well as proteins that are associated with proteolytic processing were also found to be differentially regulated, with more than 2,000 N-terminal peptides were consistently identified. Taken together, our study provides a comprehensive profile of protein alterations/regulations in different disease states. Such knowledge would provide valuable information for improving the diagnosis of the disease, as well as identifying potential clinical markers for preclinical detection and surveillance of disease severity.

P-186.00

SECRETOME AND DEGRADOME PROFILING SHOWS THAT KALLIKREIN-RELATED PEPTIDASES 4, 5, 6, AND 7 INDUCE TGFB–1 SIGNALING IN OVARIAN CANCER CELLS

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Kallikrein-related peptidases, in particular KLK4, 5, 6 and 7 (4–7), often have elevated expression levels in ovarian cancer. In OV-MZ-6 ovarian cancer cells, combined expression of KLK4–7 reduces cell adhesion and increases cell invasion and resistance to paclitaxel.

The present work investigates how KLK4–7 shape the secreted proteome ("secretome") and proteolytic profile ("degradome") of ovarian cancer cells. The secretome comparison consistently identified > 900 proteins in three replicate analyses. Expression of KLK4–7 predominantly affected the abundance of proteins involved in cell-cell communication. Among others, this includes increased levels of transforming growth factor β-1 (TGFβ-1). KLK4–7 co–transfected OV-MZ-6 cells share prominent features of elevated TGFβ-1 signaling, including increased abundance of neural cell adhesion molecule L1 (L1CAM).

Augmented levels of TGFβ-1 and L1CAM upon expression of KLK4 – 7 were corroborated in vivo by an ovarian cancer xenograft model. The degradomic analysis showed that KLK4–7 expression mostly affected cleavage sites C-terminal to arginine, corresponding to the preference of kallikreins 4, 5 and 6. Putative kallikrein substrates include chemokines, such as growth differentiation factor 15 (GDF 15) and macrophage migration inhibitory factor (MIF). Proteolytic maturation of TGFβ-1 was also elevated. KLK4–7 have a pronounced, yet non-degrading impact on the secreted proteome, with a strong association between these proteases and TGFβ-1 signaling in tumor biology.

Reference:
Shahinian H, Loessner D, Biniossek ML, Kizhakkedathu JN, Clements JA, Magdolen V, Schilling O.Mol Oncol. 2014 Feb;8(1):68-82.

P-187.00

DEMONSTRATION OF ORTHOGONAL COMPLEMENTARY ENRICHMENT METHODS FOR ENHANCED PHOSPHOPEPTIDE PROFILING OF DRUG-TREATED GASTRIC CARCINOMA CELLS

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Cell Signaling Technology

Post-translational modification (PTM) of proteins, including phosphorylation, acetylation, methylation, and ubiquitination, are critical events in all aspects of cellular signaling. Antibody-based enrichments of post-translationally modified peptides combined with LCMS have proven to be powerful methods for the study of PTMs in a wide variety of cells and tissues, and in profiling various disease states.

These antibody-based methods involve complex protocols that necessitate great care to achieve optimal results and reproducibility. The AssayMap BRAVO platform allows automation of antibody-based peptide enrichments, simplifying the enrichment protocol and providing results that can be superior to manual methods.

Manual versus automated protocols have been compared with respect to number of post-translationally modified peptides identified and relative abundance of those peptides.

P-188.00

GLOBAL ANALYSIS OF THE PHOSPHOPROTEOME OF HUMAN BLASTS REVEALS PREDICTIVE PHOSPHORYLATION MARKERS FOR THE TREATMENT OF ACUTE MYELOID LEUKEMIA WITH AC220

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Acute Myeloid Leukemia (AML) results from a combination of oncogenic events that can involve multiple signal transduction pathways including mutation-induced activation of tyrosine kinases. Thus, kinase inhibitors are increasingly studied as promising targeted clinical approaches. However, only subsets of patients respond to the respective targeted therapies. Internal tandem duplication (ITD) of the receptor-type tyrosine-protein kinase FLT3 is one of the most common mutations in AML, causing constitutive activation of FLT3.

AC220 (Quizartinib®) a selective inhibitor of FLT3 was recently applied in a phase II open-label study (ACE, NCT00989261) with relapsed AML patients. The results in the first cohort of patients with AML relapsed or refractory to first-line chemotherapy showed that the FLT3-ITD mutation status correlates with response, but does not perfectly stratify the patients into responders and non-responders.

We therefore sought to identify phosphorylation events (phosphosignatures) by mass spectrometry based phosphoproteomics from patient samples that predict clinical response with high accuracy, especially in the group of FLT3-ITD-positive patients.

P-190.00

PROTEOMICS PROFILING OF THE THE INTERSTITIAL FLUID OF MURINE BREAST CANCER.

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Introduction and objectives: Neoplasms are complex tissues, constituted primarily by tumoral cells and surrounded by a rich extracellular matrix (ECM) and different types of cells, including fibroblasts, myo/epithelial, endothelial, and infiltrating immune cells among others. Besides providing a physical scaffold for the tumor, within this complex micro-environment, an important altered crosstalk occurs, which can promote and enhance the carcinogenesis. This alteration is reflected by the disturbed expression and secretion profile of proteins secreted from tumor and stromal cells (cancer secretome) which includes proteases, such as cysteine cathepsins.

Classically, the analysis of the cancer secretome using mass spectrometry-based proteomics methods has employed the cell conditioned media as an in-vitro surrogate. Whereas, the in-vivo representation, the interstitial fluid (IF) is seldom evaluated, mainly due to the difficulty in its collection and sample complexity, i.e. highly abundant proteins over-shadowing less abundant. Thus, methods that analyze the IF are needed.

Methods: An integrated method to collect the interstitial fluid of solid breast tumors, from a murine cancer model (MMTV-PyMT) of mice with or without an altered cysteine cathepsin expression, followed by chemical isotopic labeling of peptides was established. Identification and relatively quantification of the in-vivo secreted proteins was carried out using mass-spectrometry based proteomics methods.

Results and discussion: Over 1800 proteins could be identified and relatively quantified in a single proteomics experiment by this method, with around 50% being annotated as secreted/extracellular proteins or classified as non-classically secreted proteins.

Conclusions: A method to analyze the IF has been adapted, allowing a closer representation of the in-vivo secretome, providing a feasible and reproducible technique to give insights to the role specific cysteine cathepsins have in shaping the cancer secretome

P-191.00

PROTEOMIC ANALYSIS IN PATIENTS WITH HEPATOCELLULAR CARCINOMA AFTER LIVER RADIOEMBOLIZATION. PRELIMINARY RESULTS

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Background and aims: Liver radioembolization activates inflammatory pathways and induces oxidative stress and endothelial injury with partial activation of the coagulation cascade. Proteomics is a large-scale study of proteins and may allow us to identify driver proteins of these pathogenic processes.

Methods: We prospectively collected serum at different time points (baseline, 24 hours, 1 and 8 weeks after radioembolization) in 4 patients with HCC and portal vein invasion treated with radioembolization to perform differential proteomic analyses. Proteomic analysis was performed by two complementary approaches namely 2D-DIGE and a gel-free method based on isobaric labeling of peptides (iTRAQ) coupled to nanoLC-ESI-MS/MS analysis. Differential proteins were then verified by targeted MS analysis (Selective Reaction Monitoring, SRM).

Results: Fifteen and 69 differential proteins were identified by 2D-DIGE and iTRAQ analysis respectively. The regulated proteins display a time-dependent trait suggesting impairment of functional pathways including complement cascade, lipoprotein homeostasis, acute phase response and protease activity. SRM are currently being used to verify the results in the 4 patients analyzed to then select the best candidates and extend the study to more patients. Preliminary results suggest down-regulation of vitamin D BP, providing valuable information for the follow-up of patients after radioembolization.

Conclusions: Our protein panel provide a dynamic envision of the patient's response to radioembolization. Combination of unsupervised and targeted proteomics approaches arises as a powerful tool to discover novel biomarkers that may prove their usefulness in the follow-up of HCC patients after radioembolization. These results require further validation.

P-191.00
PRE-ANALYTICAL VARIABLES FROM BIOBANK SAMPLES IN MALIGNANT MELANOMA

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Introduction and objectives
The biobank is a promising infrastructure activity that establishes the research resource. A general concept of biobank is that each biobank follows its own protocols and in general implements its own quality criteria. Therefore, it is important to build standardized and highly qualified biobank. However, little is taken into account regarding pre-analytical variables. The goal of this study was to provide "proteomics communities" practical point of notice in pre-analytical variables.

Materials and Methods
Five types of blood samples were collected per patient with malignant melanoma (MM) or suspect melanomas; plasma (EDTA, citrate, heparin), serum, and whole blood using BD Vacutainer (BD, UK). Seventy ?L aliquots of each blood samples/fractions were collected into high-density tube sample format, 384 tubes (Thermo Scientific, MA). The sample processing procedure was achieved in the format using a liquid handling robot (Hamilton Microlab STAR liquid handling robotic workstation, Hamilton, Reno, NV). The total process time is within 2 hours, including the workflow from the needle into a patient to storage of 384 tubes rack at -80ºC.

Results and Discussion
To date, we have collected more than 20,000 blood sample tubes. Of these, around 1% sample tubes have hemolysis. In addition, less than 1% sample tubes have coagulation in whole blood sample and/or serum sample. After taking five types of blood samples, these phenomena were observed. Hemolysis and coagulation of blood sample depend on the individual sample tubes before aliquot. HUPO Plasma Proteome Project has demonstrated regarding specimen collection and handling. Therefore, it will be necessary to interact with the proteomics communities.

Conclusions
Southern Swedish Biobank operation is ongoing within MM patients. We have archived tens of thousands of blood samples that is linked to clinical data registered. These samples are a resource to be useful within proteomics communities.

P-192.00
STOOL PROTEOMICS REVEALS NOVEL CANDIDATE BIOMARKERS FOR COLORECTAL CANCER SCREENING

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Introduction and objectives: Early detection of colorectal cancer (CRC) and its precursor lesions is an effective approach to reduce CRC mortality rates. The fecal immunochemical test (FIT) is a non-invasive CRC screening test that detects small traces of the blood protein hemoglobin. Although beneficial in its current form, the FIT test characteristics leave room for improvement. The aim of the present study was to identify and validate novel protein biomarkers in stool that complement or outperform the current hemoglobin-based test, to improve its diagnostic accuracy.

Methods: Proteins isolated from stool from 10 subjects without any signs of colorectal neoplasia (controls) at colonoscopy and from 12 CRC patients were analyzed by GeLC-MS/MS (discovery set). Data were analyzed by comparing protein abundances, measured as spectral counts.Analysis of differential proteins was performed using the beta-binomial test. Findings were validated by mass spectrometry (Q-Exactive) in an independent series of 292 stool samples obtained from control subjects (n=109) and subjects with adenomas (n=55), advanced adenomas (n=53), or CRCs (n=75).

Results and Discussion: In total 830 human proteins were identified in the discovery set, of which 134 were significantly enriched in CRC. These included 78 proteins that were significantly more enriched in FIT-negative CRC stool samples compared to controls. Preliminary analysis of the validation set indicates that more than half of these markers are significantly more abundant in CRC samples compared to controls.

Conclusion: Proteome profiling of stool samples revealed novel candidate biomarkers to improve current CRC screening tests. More data analysis is currently ongoing to select most promising protein biomarkers for clinical assay development.

P-193.00
SEARCH AND IDENTIFICATION OF PEPTIDE BIOMARKERS OF COLORECTAL CANCER IN SERA.

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Introduction and objectives
Colorectal cancer (CRC) is a common and deadly disease in the world. The average lifetime risk to develop nonhereditary, sporadic CRC is approximately 5%. Each year more than one million people are diagnosed with CRC and about half of them die from this malignancy. Stage of the disease, is an important prognostic factor, with five year survival rates of more than 90% for localized CRC (stage I) and only about 10% for CRC that metastasized to distant organs(stage IV). The aim of the present work was a search and identification of peptide markers of CRC in sera using modern mass spectrometry techniques.

Methods
Blood sera obtained from 50 patients with CRC and 50 healthy donors (control) were used for isolation and identification of peptides. Serum samples of each analyzed groups were fractionated using magnetic beads with weak cation exchange surfaces, obtained eluates were analyzed by nanoLC-MS/MS using ABSciex TripleTOF 5600. All samples were analyzed by DDA (identification of serum peptides) and by SWATH (for label-free relative quantitative mass spectrometry analyses) approaches.

Results and Discussion
As a result of LC-MS/MS analysis of sera more than 6000 unique peptides originated from the almost 1000 unique proteins were identified. Among identified peptides 786 were unique for CRC samples, and 125 of those were originated from the proteins unidentified in the control samples. For the control group there were 1075 unique peptides, 259 of which were originated from the proteins unidentified in CRC samples.

Conclusions
We believe that the presented data set contains valuable information which will enable interested researchers to identify of new potential biomarkers for colorectal cancer.

P-194.00

PROTEOME ANALYSIS OF OVARIAN CANCER ASCITES AS A MEDIUM FOR INTERCELLULAR COMMUNICATION

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Introduction and objectives: Ovarian cancer ascites is a native medium for cancer cells that allows investigation of the secretome of cancer cells in their natural environment. On the one hand, this medium is of interest as a promising source of potential biomarkers, on the other hand, as a medium for intercellular communication. Studies of the ascites with the use of omics technologies can help understand the peculiarities of cancer cell activity in the organism and elaborate new therapeutic approaches. The aim of this study was to elucidate specific features of malignant ascites proteome.

Methods: Proteomes of ascites from ovarian cancer patients and those of portal alcoholic cirrhosis were compared. The content of several small nuclear RNAs in ascites was analyzed. To confirm the result of proteome analysis we examined the proteins exported from ovarian cancer cells in vitro. SK-OV-3 cells were grown for 24 h in serum-free medium, next vesicle-associated and free proteins were separated by differential centrifugation and profiled by mass spectrometry.

Results and discussion: Proteomic analysis allowed us to identify 1632 and 1139 proteins in ovarian cancer and cirrhosis ascites, respectively, 663 proteins were specific for malignant ascites. Functional analysis of the ascites proteome demonstrated that the major differences between cirrhosis and malignant ascites were observed for the cluster of spliceosomal proteins. To confirm this result we demonstrated that several splicing RNAs were exclusively detected in malignant ascites, where they probably exist within protein complexes. Also, this result was confirmed in vitro using ovarian cancer cell line.

Conclusions: Ascites contains various components that participate in cellular communication. Identification of spliceosomal proteins and RNAs in an extracellular medium is of particular interest, the finding suggests that they may play a role in the communication between cancer cells.

P-195.00
PROTEOMICS-BASED DISCOVERY OF BIOMARKERS FOR PAEDIATRIC ACUTE LYMPHOBLASTIC LEUKAEMIA: CHALLENGES AND OPPORTUNITIES

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Introduction and Objetives: There are important breakthroughs in the treatment of paediatric acute lymphoblastic leukaemia (ALL) since 1950, by which the prognosis of the child majority suffered from ALL has been improved. However, there are urgent needs to have disease-specific biomarkers to monitor the therapeutic efficacy and predict the patient prognosis.

Methods: The present retrospective study overviewed proteomics-based research on paediatric ALL to discuss important advances to combat cancer cells and search novel and real protein biomarkers of resistance or sensitivity to drugs which target the signalling networks.

Results and Discussion: We highlighted the importance and significance of a proper phospho-quantitative design and strategy for paediatric ALL between relapse and remission, when human body fluids from cerebrospinal, peripheral blood, or bone-marrow were applied. The present article also assessed the schedule for the analysis of body fluids from patients at different states, importance of proteomics-based tools to discover ALL-specific and sensitive biomarkers, to stimulate paediatric ALL research via proteomics to 'build' the reference map of the signalling networks from leukemic cells at relapse, and to monitor significant clinical therapies for ALL-relapse.

Conclusions: Individualized medicine is a concept which has gained popularity during the last decade. To reach such an important step in paediatric ALL -relapses-, experts in leukaemia (clinicians) and proteomics (biotechnologists) must work together, to establish homogeneous protocols, thereby improving the current data and obtaining new information. This will certainly improve and compact the relevant information we have obtained in the past, and will ultimate the efficacy/therapies via the future resulting data, as undoubtedly too little prognostic knowledge is known at paediatric ALL-relapse.

Topic

2

Regulation of cellular pathways. Role of PTMs

OP005 - CONVERGENCE OF UBIQUITYLATION AND PHOSPHORYLATION SIGNALING IN RAPAMYCIN-TREATED YEAST CELLS

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The target of rapamycin (TOR) kinase senses the availability of nutrients and coordinates cellular growth and proliferation with nutrient abundance. Inhibition of TOR mimics nutrient starvation and leads to the reorganization of many cellular processes, including autophagy, protein translation, and vesicle trafficking. TOR regulates cellular physiology by modulating phosphorylation and ubiquitylation signaling networks, however, the global scope of such regulation is not fully known.

Here, we used mass spectrometry (MS)-based proteomics approach for the parallel quantification of ubiquitylation, phosphorylation, and proteome changes in rapamycin-treated yeast cells. Our data constitutes the most detailed proteomic analysis of rapamycin-treated yeast to-date with 3,590 proteins, 8,961 phosphorylation sites, and 2,498 di-Gly modified lysines (putative ubiquitylation sites) quantified. The phosphoproteome was extensively modulated by rapamycin treatment, with more than 900 up-regulated sites one hour after rapamycin treatment. Dynamically regulated phosphoproteins were involved in diverse cellular processes, prominently including transcription, membrane organization, vesicle-mediated transport, and autophagy. Several hundred ubiquitylation sites were increased after rapamycin treatment and about half as many decreased in abundance.

We found that proteome, phosphorylation, and ubiquitylation changes converged on the Rsp5-ubiquitin ligase, Rsp5 adaptor proteins, and Rsp5 targets. Putative Rsp5 targets were biased for increased ubiquitylation, suggesting activation of Rsp5 by rapamycin. Rsp5 adaptor proteins, which recruit target proteins for Rsp5-dependent ubiquitylation, were biased for increased phosphorylation. Subcellular localization and stability of transmembrane permeases and transporters is known to be regulated by Rsp5. We found that permeases and transporters were biased for reduced ubiquitylation and reduced protein abundance. The convergence of multiple proteome-level changes on Rsp5 indicates a key role for Rsp5 in the response to rapamycin treatment.

Collectively, these data reveal new insights into the global proteome dynamics in response to rapamycin treatment and provide a first detailed view of the co-regulation of phosphorylation and ubiquitylation-dependent signaling networks by this compound.

OP006 - A NOVEL DATA-INDEPENDENT APPROACH ALLOWS AN EXTREMELY DETAILED STRUCTURAL MAP OF PROTEIN COMPONENTS OF MITOCHONDRIAL COMPLEXES AND SUPERCOMPLEXES

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Introduction and objectives

The fine-tune stoichiometry of mitochondrial respiratory chain components is essential to maintain an efficient metabolism and to minimize ROS production. However, the molecular mechanisms by which the protein components are assembled to form complexes and supercomplexes are still poorly understood.

Methods

We have developed a proteomics approach which combines Blue-Native separation of mitochondrial complexes and a newly-developed data-independent mass spectrometry scanning method (Blue-DiS) for a highly detailed structural mapping of the different respiratory chain complexes. Blue-DiS methodology represents an important improvement in sensitivity for shotgun proteomics and allows the comprehensive monitoring of selected ions with a very high dynamic range. Computational analysis of the information stored in these unbiased maps using in-house algorithms allowed us a high-throughput identification of protein components, including post-translational modifications (PTMs).

Results and Discussion

We have constructed a highly detailed map of the protein composition of OxPhos complexes and supercomplexes, giving a molecular insight on how these proteins dynamically gather together to form the mature complexes. We have also uncovered differences in protein composition from selected tissues and cell lines, suggesting that some proteins behave distinctly in a tissue-specific way.

Preliminary analysis of specific PTMs revealed that these modifications are not evenly distributed across tissues and depend on the size and nature of the supercomplexes, suggesting a post-translational regulation of complex and supercomplex formation.

Conclusions

Using our novel data-independent approach we have performed a comprehensive characterization of the protein composition and critical regulatory PTMs involved in the assembly and the function of mitochondrial complexes and supercomplexes.

OP007 - CHAFRADIC - A NOVEL MULTIFUNCTIONAL TOOL FOR PTM-SPECIFIC PROTEOMICS

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Introduction

PTM-based regulation is important for cellular homeostasis. Up to now only few PTMs are amenable to mass spectrometric analysis, mostly due to their low stoichiometry and the resulting need for enrichment. However, when considering the high number of known PTMs and their mutual impact via PTM crosstalk as well as formation of PTM codes, the field is longing for novel strategies to enrich for so far inaccessible PTMs. Here, we present a novel technique, based on a two-dimensional charged-based separation that is deduced from COFRADIC but offers new possibilities for enriching multiple PTM-peptides. Among the numerous possible new applications we here demonstrate its usability for enriching N-terminal peptides, disulfide peptides and ubiquitinated peptides with high sensitivity.

Methods

The method is based on optimized and reproducible cation exchange chromatography to separate peptides in distinct charge state bins. Each charge state fraction obtained from a first dimension separation will be specifically derivatized and subsequently separated under the same conditions. The chemical/enzymatic derivatization induces charge state shifts which allow separating the peptide class of interest (e.g. N-terminal or disulfide peptides) from the bulk of peptides within the second dimension separation.

Results

Application of ChaFRADIC for enrichment of N-terminal peptides from yeast resulted in the identification of over 1,700 non-redundant N-terminal peptides from only 50 µg of protein, 1500 of which quantified between two different samples. We furthermore used ChaFRADIC to enrich disulfide peptides, identifying >1,000 different disulfide sites in over 600 proteins in human platelets. Thus, for integrin beta-3 we could detect 21 out of 56 known disulfide peptides (~38%) from complete platelet lysate, analyzing only two fractions by LC-MS.

Conclusion

A novel HPLC-based method for enrichment of post-translational modified peptides, applied e.g. for the large-scale enrichment of disulfide peptides.

OP008 - PROTEOMIC ANALYSIS OF CELLULAR CHOLESTEROL REGULATION USING TARGETED MASS-SPECTROMETRY

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Targeted proteomic techniques, such as selected reaction monitoring (SRM) or data-independent acquisition (DIA)/SWATH-MS, produce complete datasets across many samples with accurate quantification. Therefore, targeted proteomics is ideally suited for systems biology studies. Here, we employ targeted proteomics to study cellular cholesterol regulation, a crucial biological process underlying the heritable complex traits of blood lipid levels and cardiovascular disease. For the first time, recent genome-wide association studies (GWAS) report a comprehensive list of over 150 genomic loci that encode proteins contributing to this disease. Focusing on these proteins and known cholesterol regulators, we generate a network model describing cellular cholesterol regulation.

We employ SRM and SWATH-MS to quantitatively monitor >2000 proteins in four different mammalian cell lines following application of specific perturbations. Perturbations on the key pathways of cellular cholesterol regulation are performed with drugs or siRNAs. A logic-based network model is generated using the quantitative proteomic data.

Specific changes in protein levels following the different perturbations are observed, for example the levels of enzymes in the cholesterol synthesis pathway are strongly regulated. Furthermore, we describe differential regulation in different cell lines or changes in the protein levels of proteins so far not known to be regulated. The main result is a logic network model describing the functional interactions of cellular cholesterol regulators as a logic circuit. This network model reproduces the experimental data and can be used to predict the effect of novel perturbations or as a potential tool to identify novel drug targets.

This study shows the potential of targeted proteomics in studying complex biological processes with high clinical relevance. To understand complex diseases caused by several hundred genes and environmental factors, quantitative models are needed. The presented approach can easily be adapted to other biological processes and highlights the potential of targeted proteomics for such systems biology studies.

P-196.00

BORNA DISEASE VIRUS INFECTION IMPACTS HOST PROTEOME AND HISTONE LYSINE ACETYLATION IN HUMAN OLIGODENDROGLIA CELLS

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Background. Borna disease virus (BDV), a neurotropic RNA virus that infects several animal species including humans, impacts host gene expression, but the underlying mechanism remains poorly understood. Histone lysine acetylation (Kac) can modulate gene expression; however, BDV's impact on the protein expression and histone Kac of human oligodendroglia (OL) cells remains an open question.

Methods. Through stable isotope labeling for cell culture (SILAC)-based quantitative proteomics coupled with bioinformatic analysis, the differential proteomic and histone Kac profiles of human strain BDV Hu-H1-infected OL cells were assessed vis-a-vis non-infected OL cells.

Results. Post-BDV infection, 4383 quantifiable differential proteins were identified and functionally annotated to metabolism pathways, immune response, DNA replication, DNA repair, and transcriptional regulation. Eighty-four and eleven transcription factors showed significantly increased and decreased expression post-BDV infection, respectively. Two histone acetyltransferases (GCN5 and PCAF) were significantly downregulated and four histone deacetylases (SIRT1, SIRT2, HDAC4, and HDAC7) were significantly upregulated post-BDV infection. Of the thirty quantifiable Kac sites identified post-BDV infection, fifteen Kac sites and one Kac site exhibited decreased and increased acetylation, respectively, suggesting a histone Kac-mediated mechanism underlying the observed proteomic changes.

Conclusions. Human OL cells display significant proteomic and histone Kac changes in response to BDV Hu-H1 infection.

P-197.00

PATHOPHYSIOGICAL TENSILE STRETCH CHANGES THE HUMAN CEREBRAL ENDOTHELIAL CELL PROTEOME

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Mechanical forces exerted through pathophysiogical blood pressure affect the vascular wall at both the cellular and molecular level. One mechanical force endothelial cells experience is the result of radial and circumferential stretch due to the pulsatile nature of cardiac ejection of blood. Constant excessive radial and circumferential stretch of blood vessels due to high blood pressure may cause unnecessary changes in vascular components (e.g., endothelial cells) that may, in the long term, lead to pathological conditions (e.g., atherosclerosis or aneurysm formation).

We propose that improved understanding of the consequences of cyclic radial and circumferential stretch on the proteome expression signature of human cerebral microvascular endothelial cells (HCMEC) would be useful in demonstrating early pathogenesis of vascular disease. In this study, HCMECs were grown in silicone chambers and subjected to uniaxial cyclic stretch at 5% and 20% intensity to mimic physiological and pathological radial and circumferential stretch parameters respectively for a short and longer duration (2 or 18hrs). Global proteomes were analyzed using two different strategies. The first involved label free quantification and 6-plex tandem mass tag (TMT) for wider proteome coverage. Validation of differentially expressed proteins, where possible, was performed by Western blotting. Stretch was found to produce cell morphology changes, with cells becoming elongated in a direction approximately perpendicular to the applied stretch. Preliminary results showed that the protein ICAM-1 (which is also upregulated post TNFα exposure) was upregulated with stretch in a dose-dependent manner over 18hrs duration.

These results suggest that HCMEC exposed to mechanical forces that mimic pathophysiogical stress demonstrate proteomic signatures associated with early endothelial dysfunction.

P-198.00

RELEVANCE AND REGULATION OF ARGININE METHYLATION OF THE CARDIAC VOLTAGE-GATED SODIUM CHANNEL

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Introduction and Objectives:

We have previously provided the first evidence of arginine methylation (ArgMe) within the voltage-gated ion channel superfamily, which consists of 143 proteins. R513, R526 and R680 in the alpha subunit of the cardiac voltage-gated sodium channel (Nav1.5) were found to be mono- or dimethylated in Nav1.5 purified from a stable HEK cell line (1). The functional relevance of Nav1.5 ArgMe is underscored by the fact that R526H and R680H are Nav1.5 mutations causing cardiac arrhythmia. Objectives of the current work: 1) to describe ArgMe of Nav1.5 isolated from cardiac tissue, 2) to describe regulation of ArgMe by mutations in residues adjacent to R513 leading to cardiac disease, and 3) to describe the cross-talk between Nav1.5 ArgMe and phosphorylation.

Methods:

Nav1.5 was isolated from human cardiac tissue. Methylation and phosphorylation reactions were done in vitro using PRMT3 and PKA, respectively, and synthetic peptides. Reactions were monitored by MALDI-TOF.

Results and Discussion:

Nav1.5 isolated from human cardiac tissue was mono and dimethylated (at least) at R526. The mutation T512I, described in a patient with cardiac conduction disease, significantly reduced rate of R513 methylation by PRMT3. The mutation G514C, which disrupts the RG methylation motif and has been described in a patient with cardiac conduction disease, blocked R513 methylation by PRMT3. Phosphorylation of S516 completely inhibited R513 methylation by PRMT3. Methylation of R513 reduced rate of S516 phosphorylation by PKA. Overall, our results suggest a very sensitive regulation of Nav1.5 ArgMe and highlight the pathophysiological relevance of Nav1.5 ArgMe.

Conclusions:

Our work continues to highpoint the relevance of ArgMe outside the histone paradigm, provides the first insights on how ArgMe of voltage-gated ion channels is regulated, and opens the door to pharmaceutical intervention to balance ArgMe-phosphorylation equilibria in cardiopathological states.

P-199.00

IDENTIFICATION OF NEW MECHANISMS OF CELLULAR RESPONSE TO ANTICANCER THERAPY BY TRACKING CHANGES IN POST-TRANSLATIONAL MODIFICATIONS BY UBIQUITIN AND UBIQUITIN-LIKE PROTEINS.

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Introduction and objectives:

Pancreatic Ductal Adenocarcinoma (PDAC) is a very aggressive malignancy characterized by excessive resistance to all known anticancer therapies, a still largely elusive phenomenon. In order to identify original mechanisms, we have explored the role of post-translational modifications (PTMs) mediated by members of the ubiquitin family. Though alterations of these pathways have been reported in different cancers, no methodical search for these kinds of anomalies has been performed so far. Methods: We have studied the Ubiquitin, Nedd8, and SUMO1 specific proteomes of a pancreatic cancer cell line (MiaPaCa-2) and identified variations induced by Gemcitabine, the standard PDAC's chemotherapeutic drug.

Results and Discussion

PTMs profiles of MiaPaCa-2 cells contained both known major substrates of all three modifiers as well as original ones. We identified Gemcitabine-induced changes in Ubiquitin, Nedd8 and SUMO1 specific proteomes. Gemcitabine treatment altered the PTM profile of proteins involved in various biological functions, some known cancer associated genes, many potentially cancer associated genes, and several cancer signaling networks, including canonical and non canonical WNT and PI3K/Akt/MTOR pathways. Some of these altered PTMs formed groups of functionally and physically associated proteins. Importantly, we could validate the Gemcitabine-induced PTMs variations of relevant candidates and we could demonstrate the biological significance of such altered PTMs by studying in detail the sumoylation of SNIP1, one of these new targets.

Conclusions

This study will help to better understand mechanisms of PDAC resistance to gemcitabine

P-200.00

OMICS PROFILING OF PROTEIN AND METABOLITE CHANGES DURING AGEING OF *CASPASE-2* DEFICIENT MICE

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Caspase-2 is an important proteolytic enzyme implicated to function in both apoptotic and non-apoptotic signalling pathways. However, the mechanisms of its functions remain poorly understood. Using $Casp2^{-/-}$ mice we have demonstrated roles for this enzyme in antioxidantdefense, the DNA damage response, tumour suppressor and ageing. Importantly, $Casp2^{-/-}$ mice display several premature ageing-related phenotypes.

To determine the mechanism of caspase-2 function in these processes we have used a combination ofomics approaches. Quantitative proteomics involving iTRAQ labelling was used to analyse livers from young (6-9 weeks) and aged (18-24 month) WT and $casp2^{-/-}$ mice. Livers were also analysed by N-terminomics involving isotopic dimethyl labelling of terminal amine residues of proteins to identify *in vivo* caspase-2 substrates. In addition, both liver tissue and serum from these mice were subjected to metabolomics analysis. In total, 1510 high confidence liver proteins were identified of which, 170 (11%) were found to be differentially expressed in $Casp2^{-/-}$ mice.

Pathway analysis of these proteins revealed metabolic processes and oxidative phosphorylation to be the most enriched in $Casp2^{-/-}$ livers. Some changes in the enriched metabolic pathways were reflected by coinciding changes at the metabolite level. Interestingly, during ageing of WT mice, the expression of 549 (36%) proteins were altered while during ageing of $Casp2^{-/-}$ mice the expression of only 201 proteins (> 60% less) were found to be differentially expressed. This suggests that caspase-2 or its substrates may have important roles in regulating age-related homeostatic reprogramming. Results of our studies will be presented at this conference.

P-201.00

IN GEL DERIVATIZATION OF HISTONE H3 FOR QUANTITATIVE ANALYSIS OF HISTONE MODIFICATIONS

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Histone methylation is one of the most important part of epigenetics, and have emerged as a critical player in the regulation of gene expression, cell cycle, gene stability, and nuclear architecture. Mass spectrometry (MS) is a powerful tool to identify and quantify PTMs on histone.

As there are many hydrophilic lysine and arginine residues on the N-terminal of histone H3, chemical derivatization of histones with propionic anhydride, deuterated acetic anhydride and N-hydroxysuccinimide ester followed by trypsin digestion have been widely used to generate compatible peptides for LC-MS analysis. However, those reactions were only confirmed and applied in solution, while some biological sample can only be separated by SDS-PAGE, and in-gel digestion should be conducted for those sample in gel. However, none of those derivatization methods have been applied in in-gel digestion. Here, we verified N-Propionylation of Histone H3 by N-hydroxysuccinimide ester in gel and demonstrated that this method can achieve comparable specificity and efficiency for histones derivatization in gel with highly reproducibility.

With this derivatization method, we succeeded to quantitatively analysis the histone PTMs in wild type and ¦ÂE treated Arabidopsis thaliana, which reveals that ¦ÂE may affect the function of methyltransferase in Arabidopsis thaliana.

P-202.00

MRM-BASED QUANTIFICATION OF ACETYLATION METABOLISM ENZYMES

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As a key posttranslational modification, protein acetylation plays critical roles in regulating/coordinating cell metabolism. Acetylation is a prevalent modification in enzymes. Zhao et al. have identified thousands of acetylation proteins during glycolysis, gluconeogenesis, the tricarboxylic acid (TCA) cycle, the urea cycle, fatty acid metabolism, and glycogen metabolism in human liver tissue.

Protein acetylation modification is sub-stoichiometric amounts, therefore extracting biologically meaningful information from these acetylation sites requires an adaptable, sensitive, specific and robust method for their quantification. MRM-MS is a targeted quantitative technology commonly performed on QQQ-MS that generates unique fragment ions associated with their corresponding precursor ions that can be quantified in a very complex matrix, so our research focus on determining acetylation stoichiometry of some important metabolism enzymes using MRM-MS. Firstly, we choose appropriate peptides that can be used to absolutely quantify acetylation by MRM based on discovery experiments. Secondly, we synthesize peptides with incorporated stable isotopes (13 C, 15 N, etc.) as internal standards to mimic native peptides (AQUA) derived from proteolysis and develop method. And furthermore, we can use MRM-MS to quantify these affinity-purified acetylated peptide under different physiology and pathophysiology conditions.

The development of this quantitative workflow is a pivotal step for advancing our knowledge and understanding of the regulatory effects of protein acetylation in physiology and pathophysiology.

P-203.00

THE INFLUENCE OF SIALIC ACID MODIFICATION OF SURFACE PROTEINS ON INTRACELLULAR SIGNALLING

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Introduction

Post-translational modifications (PTMs) of proteins refer to the addition of chemical modifying groups to proteins after their synthesis. Glycosylation is the most complex and ubiquitous type of PTMs found in nature, due to the huge diversity of the attached sugars. Recently, some PTMs have been shown to have a significant influence on other PTMs, which in turn can alter the function or activity of proteins. Phosphorylation, O-GlcNAcylation and acetylation have been proposed to interplay upon regulation of signalling pathways, however very little is known about the effect of manipulating sialic acids on surface glycoproteins. The aim of this study was to investigate the alteration of sialylation on surface proteins after short stimulation of the epidermal growth factor receptor in HeLa cells.

Method

Membrane proteins from HeLa cells were enriched by NaCO2 treatment and ultracentrifugation after EGF stimulation for 0, 2, 5 and 10 min. The changes at the N-linked sialylated glycopeptides upon stimulation were assessed using iTRAQ. After trypsination and dephosphorylation, sialylated glycopeptides were enriched using TiO2, deglycosylated using PNGaseF, fractionated using HILIC and subsequently analysed using an Orbitrap Fusion. For the analysis of intact sialylated glycopeptides an in-house developed program (GPMAW) was used together with a novel strategy for analysis of intact sialylated glycopeptides (peptide-glycan mass matching (PGMM)).

Results and discussion.

Short time stimulated HeLa cells revealed a significant site-specific desialylation of glycans on proteins like Clusterin, Intercellular adhesion molecule 1 and Thrombospondin-1 and several proteins with EGF domains. In addition, we observed increased sialylation on glycoproteins on the surface of HeLa cells after short time EGF stimulation suggesting a substantial modulation of sialic acid on the surface of HeLa cells after stimulation.

Conclusions

Stimulation of cells not only result in alteration of phosphorylation but also initially of remodulation of surface sialylation which effect the downstream signalling pathway.

P-204.00

QUANTITATIVE PROTEOMICS FOR STUDYING HUMAN STEM CELLS DIFFERENTIATION

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Mesenchymal Stem Cells (MSC) have shown the ability to differentiate to several cell types such as osteoblasts and adipocytes depending on the stimuli they receive. As the earliest molecular mechanisms that lead to the osteoblastic commitment remains unclear and considering the fact that phosphorylation is important modification regulating cellular processes, we studied the dynamics of both phosphorylation sites and proteins abundances during the first 24 hours of differentiation.

In order to achieve our objectives, we employed mass spectrometry-based quantitative proteomic/phosphoproteomics setup, using SILAC as isotopic label (Ong et al. 2002). hMSC were differentially encoded by 'double-triple' SILAC (Rigbolt et al. 2011) and stimulated into osteoblastic differentiation (Kratchmarova et al. 2005). Two sets of such 'double-triple' SILAC labeled cells were designed to cover six experimental situations: two controls (time 0), 30 minutes, 1h, 6h and 24h after stimulation. For the phosphoproteomics setup, phosphopeptide enrichment was performed using metal affinity chromatography. All samples were analyzed by LC-MS/MS on LTQ-Orbitrap Velos and Q-Exactive hybrid mass spectrometers.

Three datasets belonging to three biological replicas were analyzed together using MaxQuant 1.3.0.5. We identified 15133 unique phosphorylation sites and 6513 proteins with an FDR smaller than 1%. Dynamics during the differentiation were analyzed using hierarchical clustering to group sites and proteins depending on their quantitation profile in the course of differentiation. NetworKIN and Metacore bioinformatics platforms were used to predict possible kinases and transcription factors responsible for the observed dynamic changes of phosphorylation sites and protein abundances, respectively.

Combining these methods, we were able to extract molecular mechanisms that might play important roles regulating the initial stages of osteoblast differentiation, nonetheless further studies should be performed to consolidate these findings.

P-205.00
IDENTIFICATION OF N-TERMINALLY ACETYLATED KINETOCHORE PROTEINS USING HIGH RESOLUTION MASS SPECTROMETRY

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Introduction:
During mitosis a series of events leads to the formation of two genetically identical daughter cells. At the center of this process stands the kinetochore, a large protein assembly, mediating and regulating chromosome segregation. Recently, it has been suggested that N-terminal acetylation, a co-translational modification, is involved in complex formation and protein turnover, two important features for the proper functioning of the kinetochore. The final aim of this study is to establish if N-terminal acetylation of kinetochore proteins is associated to cell cycle progression, particularly during mitosis.

Material and Methods:
We developed an efficient procedure to enrich protein extracts for N-terminal peptides of proteins for mass spectrometric analysis. N-termini of proteins and lysines are modified by dimethylation and after tryptic digest, newly generated N-termini are sequestered by active NHS beads, leaving modified N-termini in the supernatant. We measured lysates and enriched fractions from mitotic HeLa cells with an Q-Exactive massspectrometer.

Results and Discussion:
We were able to identify the N-termini of 3313 proteins in mitosis, 2385 of them were acetylated, suggesting a lower overall acetylation rate in mitosis compared to steady-state data sets. We performed as well an extensive statistical analysis of the initial amino acid sequence, finding a good overlap between the established knowledge about stabilizing and destabilizing residues. In addition, we were able to identify 30 kinetochore proteins, 28 of them acetylated and two unmodified. We also performed a quantitative proteomic analysis using stable isotope labeling of amino acids in cell culture. Protein extracts from cells just before the onset of mitosis and in metaphase were compared and results suggest that N-terminal acetylation might not be as static as it was thought.

Conclusions:
Our data provide the methodical basis to study N-terminal acetylation and gives a first evidence that N-terminal acetylation might be regulated during cell cycle.

P-206.00

GLYCOPROTEOMIC STUDY IN RAT SERUM USING ALBUVOID-A SERUM REDUCTION TOOL

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Glycosylation status in serum is indicative to metabolic state of the body and thus it has great potential as biomarker for diseases such as cancer and diabetes and for monitoring drug treatments.

Serum reduction is a critical step in many proteomic studies of serum, including glycol-proteomics. Here, we evaluate a method that specifically depletes albumin by avoidance strategy - called AlbuVoid™, which consequently enriches for the low abundance serum protein content. Such a strategy compares favorably to high abundance immuno-depletion methods judging from the number of glycolsolation sites identified, reproducibility, cost effectiveness and its work flow design. After serum depletion and trypsin digestion, the samples are labeled with isobaric tags (TMT) prior to glycol-peptide enrichment through lectin beads (ConA).

Using this workflow, more than 500 glyco-peptides (representing more than 280 glycosylation sites) were routinely identified and quantified. For larger number of samples, label free method can potentially be used with further testing and optimization. This method is not limited to rats, but can be readily applied to other species.

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Topic 2: Regulation of cellular pathways. Role of PTMs

P-208.00

IMMOBILIZATION OF ANTIBODIES ON FE3O4@POLYDOPAMINE CORE¨CSHELL MICROSPHERES FOR SELECTIVE ENRICHMENT OF LYSINE-ACETYLATED PROTEINS AND PEPTIDES

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Protein lysine acetylation is a dynamic and reversible post-translational modification and plays an important role in life processes. However, the low amounts of the acetylated peptides or proteins could hardly be detected before enrichment.

In this study, for the first time, antibody-immobilized Fe3O¬4@polydopamine core¨Cshell microspheres were developed for selective enrichment of acetylated proteins and peptides. covalent strategy based on schiff base reaction and covalent-orientated strategy through the ¡°crystallizable fragment (Fc)¡± receptor protein A were both used in the process of antibody immobilization. At first, standard proteins composed of acetylated bovine serum albumin, myoglobin, ¦Á-casein and ovalbumin were used as model proteins to compare and verify the enrichment efficiency of these two strategies.

Then, the synthesized peptide was employed to confirm the selective enrichment behavior of the covalent-orientated antibody immobilization microspheres .The results turned out that covalent-orientated antibody immobilization strategy performed better than covalent strategy .Moreover, the covalent-orientated antibody immobilization microspheres were successfully applied to analyze mouse liver tissue proteins.

P-209.00

UNCOVERING SMALL UBIQUITIN-LIKE MODIFIER SIGNALING NETWORKS

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Small Ubiquitin-like Modifiers (SUMOs) are essential for eukaryotic viability and regulate large numbers of target proteins. We are interested in systems-wide understanding of dynamic SUMO signal transduction, employing proteomics approaches (Vertegaal 2011). Our projects are revealing hundreds of SUMO target proteins, demonstrating that SUMOylation plays a role in all nuclear processes, including transcription, chromatin remodelling, pre-mRNA splicing, DNA repair and ribosome assembly. Dynamic studies include alterations in SUMOylation during cell cycle progression (Schimmel et al. 2014) and changes in SUMOylation in response to DNA damage (Vyas et al. 2013).

Phosphorylation, acetylation and ubiquitination can be studied by mass spectrometry in a site-specific manner. This is still challenging for SUMOylation due to the low stoichiometry of SUMOylation, potent SUMO proteases and particularly due to large C-terminal tryptic SUMO fragments. Currently, modest numbers of SUMOylation sites have been identified, employing a SUMO mutant that includes an arginine at its C-terminal part at a position that corresponds to the location of an arginine in the C-terminal part of SMT3, yeast SUMO (Schimmel et al. 2014, Matic et al. 2010). Despite identifying relatively small numbers of SUMO acceptor sites, these studies have provided insight in SUMOylation consensus motifs (Matic et al. 2010; Schimmel et al. 2014).

We aim to improve the site-specific aspect of our work. Since a significant percentage of SUMO acceptor lysines are not situated in SUMOylation consensus motifs, identifying SUMO acceptor lysines is vital to generate SUMOylation-deficient mutants for subsequent functional analysis.

P-210.00

DEFINING THE INTERACTOME OF THE TUMOR SUPPRESSOR CYLD AND ITS ROLE IN EGF SIGNALING

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Introduction and Objectives

Epidermal growth factor (EGF) receptor (EGFR) plays a key role in the regulation of cell proliferation, differentiation, survival and migration, being thus pivotal for a correct cell physiology. Consequently, EGFR pathway is subjected to a very tight regulation that, when aberrant, explains the origin of several human diseases. Post-translational protein modifications are crucial in the control of EGFR signal transduction and, specifically, ubiquitination has arisen as one of the essential modifications to regulate this pathway. It is therefore critical to keep a balanced function of the enzymes responsible for both the attachment and removal of ubiquitin moieties from effector proteins involved in the EGF-induced response. The engagement of Cylindromatosis tumor suppressor (CYLD) deubiquitinase in EGFR pathway has been very recently documented. However, its role in the signaling events triggered by EGF remains obscure. Considering the relevance of CYLD in other central signaling mechanisms, namely the TNF-induced NFkB pathway, we aim to unveil the role of CYLD in EGF-triggered networks.

Methods

In order to decipher the implication of CYLD in the signaling cascades initiated by EGF, we stably knocked down the expression of the deubiquitinase and combined protein enrichment methods with SILAC-MS in non-stimulated and EGF-stimulated control and KO cells.

Results and Discussion

Our analysis allowed the detection of CYLD partners, together with the quantification of the tyrosine-phosphorylation status of over 600 proteins upon EGF stimulation in an unbiased manner. Among the last, a significant set of differentially responding proteins to EGF in KO with respect to control cells are well known effectors of the EGFR pathway, responsible for signal transduction, receptor modification, internalization and trafficking.

Conclusion

Our results demonstrate that the downregulation of CYLD deeply affects the cellular response to EGF and help to a better understanding of the function of this deubiquitinase in signal transduction mechanisms.

P-211.00

BOTTON-UP AND MIDDLE-DOWN APPROACHES FOR COMPREHENSIVE ANALYSIS OF CHROMATIN-BINDING PROTEINS AFTER TREATMENT WITH SODIUM BUTYRATE.

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Histone deacetilase inhibitors have emerged as promising targets in cancer therapeutics. Inhibition of histone deacetilase leads to increased acetylation and inhibition of cell proliferation in cancer cells. Changes in histone acetylation remodel chromatin structure, which should be concurrent to changes in chromatin remodeling proteins. In this work, we aimed to quantify PTMs on histones to correlate them to changes in chromatin-binding proteins amounts on acid nuclear extracts of HeLa cells untreated (control) and treated with sodium butyrate (SB, a histone deacetilase inhibitor.). Commercially available acid nuclear extract from HeLa cells was digested in-solution with trypsin and ArgC. Samples were submitted to direct shotgun LC-MS/MS proteomics analysis in triplicates in an Orbitrap Velos using a 180 min gradient.

Identification and protein quantification were performed in Xcalibur and Proteome Discover, respectively (Thermo). We identified 1771 proteins from nuclear extract after trypsin digestion and 898 after ArgC digestion in control cells. In sodium butyrate treated cells 1297 proteins were identified after trypsin digestion and 704 after ArgC digestion. Of the proteins identified after ArgC digestion, 65% and 66% overlapped with trypsin data set in control and SB treatment, and the ArgC data added 10% and 15% of novel identifications in control and SB treatment, respectively. ArgC digests were the main data source for identification of histones. Several chromatin remodeling proteins were found including HDAC, RBBP4, RBBP7, MTA, Lysine-specific histone demethylase 1, REST corepressor 1, zinc finger containing proteins and DNA helicases.

As expected, overall acetylation on histones increased after sodium butyrate treatment. Additionally, there were changes in the amounts of chromatin binding proteins in sodium butyrate treated cells. We propose that digestion with trypsin and ArgC can be used to identify and quantify PTMs not only from histones but also for a wide range of chromatin binding proteins.

P-212.00

IDENTIFICATION OF THE MYOFIBRILLAR Z-DISC AS A NODAL POINT IN SKELETAL MYOCYTE SIGNALING BY LARGE-SCALE PHOSPHOPROTEOMICS

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The myofibrillar Z-disc is an essential, structure-bearing component of muscle fibers which directly interacts with thin filaments, intermediate filaments and titin. While originally considered a passive structural unit, its role in signal transduction has become apparent. To improve our understanding of signaling processes in and out of the Z-disc, we delineated the phosphorylation landscape of cultured skeletal myotubes and studied the role of Z-disc proteins in hypertrophic signaling processes. To characterize kinase-mediated phosphorylation events in contracting C2C12 cells, we established a large-scale phosphoproteomics workflow employing LC-MS combined with SCX chromatography and TiO2 affinity enrichment of phosphopeptides. Following up the results of our basal phosphoproteome study, we analyzed the PI(3)K/Akt dependent signaling pathway in a SILAC approach using CID, MSA and HCD fragmentation on an Orbitrap Elite system.

We delineated the phosphorylation landscape of skeletal myocytes comprising more than 10.000 phosphosites. Remarkably, we show that the vast majority of Z disc-specific and Z disc-associated proteins are phosphoproteins accounting for more than 60% of all localized sarcomeric phosphorylation sites. We identified Filamin C (FLNC) and its interaction partners as a group of highly phosphorylated proteins indicating a key role for these constituents in Z-disc signaling. In total, we found 12 distinct FLNC phosphosites including several potential PKC and Akt motifs. Using in vitro kinase assays, we identified murine FLNC Ser-2625 as novel PKCalpha target. Pathway analysis showed that the majority of myofibrillar proteins involved in Z disc assembly and maintenance are targets downstream of PI(3)K/Akt. In our work, we continued to dissect the IGF-1-activated PI(3)K/Akt pathway leading to skeletal myotube hyperthrophy using SILAC and kinase signaling pathway inhibitors.

To conclude, we characterized the myofibrillar Z-disc as a nodal point of skeletal myocyte signaling. We propose that FLNC acts as a signaling hub whose localization and protein interactions are controlled by cytosolic kinases.

P-213.00
INTEGRATIVE BIOINFORMATICS FOR PROTEIN PTM NETWORK DISCOVERY: ANALYSIS OF A CANCER DRIVER GENE

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Introduction and Objectives
While post-translational modifications (PTMs) play a pivotal role in numerous biological processes by modulating regulation of protein function, critical gaps remain in the current research framework for studying PTMs. Most of the current PTM resources do not connect modified proteins with their modifying enzymes; our understanding of phosphorylation events in signaling networks is fragmented, largely due to the lag in elucidating kinase-substrate interactions. To support integrative understanding of PTMs in systems biology context, we have developed the iPTMnet (http://proteininformationresource.org/iPTMnet), with the initial focus on phosphorylation.

Methods
We employed a systematic approach to mine PTM databases and used the RLIMS-P and eFIP text mining tools to extract kinase-substrate information and their functional impact and biological context from scientific literature. We adopted the Protein Ontology (PRO) to provide a formal representation for the various PTM forms and complexes. As a scientific use case, we have built a beta-catenin knowledge map, a multi-relation network that captures PTM enzyme-substrate relationships, protein-protein interactions, transcription factors and targets, as well as cancer driver mutations to explore the underlying mechanisms by which beta-catenin may be involved in a diverse cancer types.

Results and Discussion
 From the beta-catenin knowledge map, we have identified novel roles for several beta-catenin PTM sites based on their mutation frequency in cancer cells overall and their co-occurrence patterns with other mutations in individual cancer types. We also identified candidate kinases that may mediate the positive effect of CDK5 on beta-catenin co-activator activity.

Conclusions
Our integrative systems approach, which combines data mining, text mining and ontological knowledge representation to capture rich information about proteins and PTMs, will be generally applicable to build PTM networks and knowledge maps that provide new insights into other proteins and their involvement in biological processes.

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Topic 2: Regulation of cellular pathways. Role of PTMs

P-214.00

THE ROLES OF HAUSP/USP7 DEUBIQUITINATING ENZYME REGULATING DNA DAMAGE RESPONSE

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Ubiquitin specific protease HAUSP/USP7 regulates the dynamics of the p53 and Mdm2 network in response to DNA damage by deubiquitinating both p53 and its E3 ubiquitin ligase, Mdm2. Its concerted action increases the level of functional p53 by preventing proteasome-dependent degradation of p53. However, the protein substrates that are targeted by HAUSP to mediate DNA damage responses in the context of the HAUSP-p53-Mdm2 complex are not fully identified.

Therefore, to explore the possible intracellular molecular events underlining DNA damage responses, two-dimensional electrophoresis (2-DE) and other proteomics-based approaches in HAUSP-overexpressed human cancer cells were performed. These results revealed 35 spots from MALDI-TOF/TOF analysis. Interestingly, NUL, one of 35 proteins, was strongly bound with HAUSP through its N- and C-terminal regions and these two partial peptides were deubiquitinated by HAUSP. Further, NUL exists as a component of the HAUSP-p53-Mdm2 complex, and both Mdm2 and p53 are required for the interaction between HAUSP and NUL.

Importantly, the HAUSP-NUL interaction was increased upon ionizing irradiation significantly, leading to NUL stabilization. Taken together, this study reveals a new component of the HAUSP-p53-Mdm2 complex that governs dynamic cellular responses to DNA damage.

P-215.00

SIRT5 DESUCCINYLATES MITOCHONDRIAL ISOCITRATE DEHYDROGENASE 2 AND REGULATES TUMORGENESIS

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Lysine succinylation is a novel post-translational modification. Diverse metabolic enzymes located in mitochondria can be succinylated. It has been well-known that SIRT5 is a lysine deacetylase on the basis of sequence similarity. But recent progress has shown that SIRT5 is a NAD-dependent protein lysine demalonylase and desuccinylase. SIRT5 can remove the succinyl moieties from target lysines, which may regulate the biological activity of the target proteins.

Dysfunction of acetylation process is often associated with several diseases, especially cancer. It is unknown whether SIRT5 is implicated in cancer pathogenesis. To establish the relationship between SIRT5 expression and tumorigenesis, we examined its expression level in a number of human cancer cell lines, including cervical cancer, gastric cancer, breast cancer, colorectal cancer and hepatocellular carcinoma. Human cancer cell lines express varying levels of SIRT5 protein, ranging from clearly detectable to almost undetectable. In human hepatocellular carcinoma cell line MHCC97H and HCCLM3, both of which exhibit a rather lower SIRT5 expression level, SIRT5 overexpression in the two cell lines can inhibit cell proliferation and promote cell apoptosis. Therefore, our findings indicate possible important link between SIRT5 and tumorgenesis.

Furthermore, overexpression of Sirt5 and/or IDH2 (isocitrate dehydrogenase 2) in cultured cells show that SIRT5 interacts with IDH2, a key enzyme in the TCA cycle, resulting in change of IDH2 activity. In our study, we found SIRT5 has a interaction with IDH2, which indicates that SIRT5 may be involved in regulating cell metabolism and energy production by regulating this kind of reversible modification, and then has an important function in tumorgenesis.

P-216.00

TARGETED LC-MS EXPERIMENTS FOR THE ACCURATE MAPPING OF ADP-RIBOSYLATION SITES

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Introduction and Objectives

Adenosine diphosphate (ADP)-ribosylation at arginine residues is a reversible modification which plays an important role in inter-and intracellular signaling, transcription, DNA repair, apoptosis and necrosis. However, due to the complexity of the modification and the fragmentation pattern in MS/MS experiments the identification of ADP-ribosylated peptides is difficult. To determine ADP-ribosylation sites in a protein containing several adjacent arginine residues, MS3 experiments were performed which utilized both CID and HCD spectra analyzed in the Orbitrap. The use of multiple enzymes and high resolution MS/MS data greatly assisted in mapping the ADP-ribosylation site.

Methods

Inclusion lists were used to perform MS2 scan only on the ADP-ribose containing peptides. After the CID scan two MS3 scans (CID and HCD), on the remaining ornithine containing peptide were performed. All MS2 and MS3 scans were acquired in the Orbitrap to provide accurate and high resolution MS/MS spectra.

Results and Discussion

Using this technique the site of ADP-ribosylation on the C-terminus of the target protein, which contains four arginine residues in close proximity, could be mapped. The use of two enzymes; chymotrypsin and Glu-C resulted in peptides of different lengths further validating the site of ADP-ribosylation.

Conclusion

Utilizing different fragmentation techniques (CID and HCD) and enzymes (e.g. chymotrypsin and Glu-C) the accurate mapping of ADP-ribosylation sites can be achieved. The use of multiple enzymes and an MS3 fragmentation approach is an attractive alternative when ECD/ETD fragmentation experiments are not possible.

P-217.00

ELUCIDATING THE ROLES OF LYSINE METHYLTRANSFERASES BASED ON THEIR NON-HISTONE PROTEIN TARGETS USING A NOVEL TECHNIQUE

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Introduction and Objectives: Lysine methylation is a post-translational modification best known for its occurrence on histones where it regulates transcription through compaction of heterochromatin, and the activation and repression of genes in euchromatin. It is also implicated in cell cycle regulation and development. Its complexity is further amplified by its multiplicity: monomethylation, dimethylation and trimethylation at the epsilon amino group of the lysine residue. These differing amounts of methylation correlate with different functions and locations in the genome. The discovery of non-histone targets of methylation has sparked interest in the other processes, such as development, which is affected by methylation. Our research focuses on describing the role of lysine methyltransferases based on their non-histone protein targets using a novel enrichment technique.

Methods: We overexpressed lysine methyltransferases SMYD1, SMYD2, SMYD3, SMYD4, SMYD5, SET7/9 and G9a in the human embryonic kidney (HEK) 293 cell line. The cellular protein was tryptically digested and the methylated peptides were enriched using a novel technique called Protein Methylation Enrichment by Neutralizing Amine through Derivatization (PROMENADE). The enriched peptides were analysed by electrospray ionization liquid chromatography mass spectroscopy (ESI LC-MS/MS).

Results and Discussion: 111 unique lysine methylation sites have been found using this approach. This method identified known methylation targets such as dimethylation on histone H3 lysine 27 and has also uncovered some novel targets such as trimethylation on lysine 758 of semaphorin-3C. The methylation of semaphorin-3C, a protein responsible for cardiac development, was found in the SMYD1 and SMYD2 transfected samples suggesting a role for these methyltransferases in semaphorin-3C modification.

Conclusions: Using this approach we have identified novel non-histone targets of methylation. Upon validation and pathway analyses, the biological role(s) of these methyltransferases and their targets will be investigated.

P-218.00

DECIPHERING THE CLEAVAGE SITE SPECIFICITY OF THE SHEDDASES ADAM10 AND ADAM17

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Introduction and Objectives

Various transmembrane proteins (e.g. APP, pro-TNF-alpha) are released from the cell surface by the ADAM (A Disintegrin And Metalloproteinase) family of transmembrane proteases. Two members of this family, ADAM10 and ADAM17, are known to be dysregulated in many diseases and hence are potential targets for the development of novel drug therapies. As the proteases have specific as well as common substrates, we aimed to investigate the cleavage site specificity of recombinant ADAM10 and ADAM17 using a modified version of the PICS approach, Q-PICS (Quantitative Proteomics for the Identification of Cleavage Sites).

Methods

Two peptide libraries with blocked primary amines were prepared from a yeast proteome. Six technical replicates of each of the two libraries were incubated with the pro- and catalytic domain of either murine ADAM10 or ADAM17, followed by TMT-Sixplex labeling of newly formed N-termini. Samples were analyzed by LC-MALDI MS and LC-ESI MS.

Results and Discussion

Overall we identified 218 and 365 cleavage sites for ADAM10 and ADAM17, respectively. Of these, 109 cleavage sites were shared by both proteases and mainly exhibited leucine in P1'. Regarding the specific cleavage sites, ADAM10 showed a tendency to cleave N-terminal of aromatic amino acids, whereas ADAM17 preferentially hydrolyzed peptide bonds before valine. At the P1 site both sheddases preferred proline, alanine as well as basic residues. Two complementary technologies of mass spectrometry increased the amount of cleavage sites identified by up to 50% compared to LC-ESI MS alone making the results more reliable. Quantitative data of the TMT labeling strategy confirmed the reproducibility of the proteolytic events.

Conclusion

Using Q-PICS we were able to determine the cleavage site specificities of two important sheddases, which delivers the base for further studies, i.e. the design of specific peptide inhibitors or the identification of substrates and cleavage sites in vivo.

P-219.00

ACETYLATION,¡AINSTEAD OF UBIQUITINATION, MEDIATES PROTEASOMAL DEGRADATION OF CORE HISTONES DURING SPERMATOGENESIS AND DNA REPAIR

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Histone acetylation plays critical roles in chromatin remodeling, DNA repair, and epigenetic regulation of gene expression, but the underlying mechanisms are unclear. Proteasomes usually catalyze ATP- and polyubiquitin-dependent proteolysis.

Here we show that the proteasomes containing the activator PA200 catalyze the polyubiquitin-independent degradation of the core histones. Most proteasomes in mammalian testes (¡§spermatoproteasomes¡¨) contain a spermatid/sperm-specific $f\tilde{N}$-subunit $f\tilde{N}$4s/PSMA8 and/or the catalytic $f\grave{O}$-subunits of immunoproteasomes in addition to PA200. Deletion of PA200 in mice abolishes acetylation-dependent degradation of somatic core histones during DNA double-strand breaks, and delays core histone disappearance in elongated spermatids. Purified PA200 greatly promotes ATP-independent proteasomal degradation of the acetylated core histones, but not polyubiquitinated proteins.

Furthermore, acetylation on histones is required for their binding to the bromodomain-like regions in PA200 and its yeast ortholog, Blm10. Thus, PA200/Blm10 specifically targets the core histones for acetylation-mediated degradation by proteasomes, providing mechanisms by which acetylation regulates histone degradation, DNA repair, and spermatogenesis.

P-220.00

A SECRETED PROTEIN FROM M. TUBERCULOSIS SUPPRESSES NF-ÊB SIGNALING PATHWAY AND PROMOTES INTRACELLULAR SURVIVAL OF MYCOBACTERIA

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NF-ÊB signaling pathway is critical for the regulation of host inflammation. Induction of pro-inflammatory cytokines is beneficial to the host defense against invading pathogenic bacteria.

Consequently, intracellular pathogens such as M. tuberculosis (Mtb) deploy numerous countermeasures against host inflammation to promote their survival within macrophages. In this study, we sought to identify secreted proteins from Mtb which can modulate host NF-ÊB signaling pathway and intracellular survival of mycobacteria. Using luciferase reporter assay, we identified a secreted protein from Mtb (which is referred to as Mubp1) which can block NF-ÊB signaling. Quantitative PCR analysis and enzyme-linked immunosorbent assay showed specific inhibition of pro-inflammatory cytokine production by Mubp1 in macrophages.

Furthermore, results from macrophage infection and mice infection experiments showed that the absence of Mubp1 leads to a decrease in bacterial load. Collectively, Mubp1 promotes intracellular survival of mycobacteria by suppressing host inflammation through inhibition of NF-ÊB signaling pathway.

P-221.00

QUANTITATIVE PHOSPHORYLATION ANALYSIS OF HOST RESPONSES TO DENGUE VIRUS INFECTION

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Introduction and Objectives

Dengue virus (DENV) has four serotypes and infection with one serotype provides no protection from infection with the others, so secondary/sequential infections with more than one serotype are common. The increased severity in secondary infections is highly associated with antibody dependent enhancement (ADE) leading to increased virus replication. To understand the role of protein phosphorylation in host-DENV responses, we performed a comparative analysis of host cell phosphoproteome in response to DENV infection (primary) and infection under ADE (secondary).

Method

Human cell line K562, DENV-2 and DENV E protein antibody (4G2) were used. Three conditions were established: (1) Mock, (2) DENV-2 infection and (3) DENV-2 infection under ADE. Stable isotope dimethyl labeling was used for quantitation. Phosphopeptides were affinity-enriched by TiO2, fractionated by HILIC and analyzed by nano-HPLC-MS/MS (Orbitrap XL). Data was searched against swiss-prot database using MaxQuant.

Results and Discussion

In total, 2930/2482 phosphopeptides from 1410/1266 proteins were identified/quantified in three conditions. Phosphorylation levels of quantified phosphopeptides were clustered into eight groups using Perseus program, illustrating various patterns of phosphorylation regulation in response to DENV infection. A total of 317 phosphopeptides showed significant regulation in secondary infection compared to primary infection, among which 50% showed up/down regulation in primary infection but were re-stored in secondary infection; 10% showed up/down regulation in primary infection and were further regulated in the same trend in secondary infection; the rest exhibited no significant regulation in primary infection, however were up/down regulated in secondary infection. These altered phosphoproteins are related to many important cellular signaling pathways. This phosphoproteomic study provides new clues for functional studies, and may lead to better understanding of the pathogenic mechanisms in host responses to DENV infection via protein phosphorylation.

Conclusions

This study provided evidence indicating the critical role of protein phosphorylation involving in pathogenesis of DENV infection.

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Topic 2: Regulation of cellular pathways. Role of PTMs

P-222.00

COMPARABLE PATHWAY ANALYSIS OF IGA NEPHRITIS GLOMERULI WITH NORMAL ONE BASED ON SWATH APPROACH

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IgA nephropathy (IgAN) is considered as the most common cause of primary glomerulonephritis. Up to 50 percent of these patients undergoing progressive disease ultimately leading to end-stage renal disease. High throughput proteomics technologies have been applied to investigate the underlying mechanisms of diseases. SWATH approach was established recently as a platform for targeted proteomics in IMSB, ETHZ Zurich.

Quantitative information of identified proteins from SWATH approach makes the comparable pathway analysis of IgAN glomerulus as best method as identify disease-associations for such disease.

From fixed and paraffin embedded (FFPE) human kidney biopsy specimens of IgAN, glomerular sections were collected by laser microdissection (LMD). Peptides were extracted from the glomerular sections by using the OSDD method and purified by C18 tip. A data independent MS acquisition method (SWATH) was used to collect information of all peptides and their fragments contained in the samples and a targeted data analysis strategy using the OpenSWATH software tool was used to analyze the SWATH data sets. Compare to the normal kidney glomerulus, the proteins with over 2 times changing on the expression intensity was recognized as targets of pathway analysis. Pathway analysis was done and confirmed by using IPA™ and IPAD platforms.

Within total of 3450 identified proteins by SWATH approach, there are around 200 proteins were figured out with over 2 times changed in IgAN glomerulus than normal one. Localization of 25 proteins were plasma membrane, 94 proteins were located as cytoplasm, and 5 complement component family proteins were increased. Comparable pathway analysis based on SWATH approach, could be considered as a advantaged way to get information of some disease, due to both of quantitative and qualitative results were provided in targeted analysis. This study shows a challenging to derive biological insight and identify disease-associations and possibility of help to improve treatment.

Abstracts Book - HUPO 2014

Topic 2: Regulation of cellular pathways. Role of PTMs

P-223.00

PROTEOME ANALYSIS OF ENRICHED CARDIAC PROGENITOR AND DOPAMINERGIC NEURONS DERIVED FROM HUMAN EMBRYONIC STEM CELLS REVEALS NOVEL PATHWAYS AND MARKERS

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Human embryonic stem cells (hESCs) are pluripotent cell lines with the potential to form any human cell type. Gene expression analyses of hESCs and their differentiated cells will help to uncover or further define signaling pathways and molecular mechanisms involved in the maintenance of self-renewal and pluripotency. hESC derived cardiac progenitor cells (CPC) and dopaminergic progenitor cells (DPC) are invaluable sources for drug-screening, disease modelling, and cell therapy.

However, the major problem with the use of hESC derived cells is the inefficiency of the differentiation protocols and the lack of a specific surface marker that can be used for FACS and MACS based purification. Therefore, the purification and extraction of specific ESC-derived cell types and the consistency and reproducibility of sample generation are thus considered important issues. Here we used a proteomics-based approach to identify surface markers in a purified population of CPCs and DPCs generated from hESCs. For CPCs enrichment, we used a sequence encoding hygromycin resistance gene under the control of islet-1 (isl1) promoter. For DPCs enrichment, the GFP reported gene introduced to coding sequence of LMX1A locus and progenitors were purified by sorting the positive cells. Subsequently, enriched cells were analyzed by flow cytometry, immunostaining, western blot and real-time PCR to confirm enrichment of two cell types. Then, we applied a non-labeling quantitative shotgun proteomics to analyze enriched cells. In enriched DPCs, axonal development and export proteins and glutamate catabolic proteins overrepresented compared to control cells. Furthermore, new cell specific markers for DPCs were identified.

Proteome analysis of CPCs also showed that cell fate involves isl-1 and Notch signaling pathway that may be leveraged for regenerative approaches. Our results showed that a quantitative shotgun proteomics analysis of enriched hESC derived cells provided a unique approach for novel cell surface markers and pathways.

Abstracts Book - HUPO 2014

Topic 2: Regulation of cellular pathways. Role of PTMs

P-224.00

A PROTEOMIC ANALYSIS OF P27(KIP1)-BINDING PROTEINS REVEALS A ROLE OF THIS PROTEIN ON THE REGULATION OF TRANSCRIPTION THROUGH ITS INTERACTION WITH RNA POLYMERASE II

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The proteome quest
to understand
biology and disease

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Topic 3

Microvesicle and organelle proteomics

OP013 - HEPATIC EXOSOMES IN METABOLISM AND DISEASE

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Hepatic function is essential for homeostasis of the organism and outcome of different endogenous and external estresses greatly depends on the integrated response ejected by this organ. Liver injury ranging from mild infection to life-threatening liver failure is a serious worldwide health issue and a major goal in liver pathology is the identification of molecular markers for its early detection, i.e. before clinical manifestations are produced.

Our group is studying the physiological role of extracellular vesicles in the hepatic function in normal and pathological conditions to identify novel low-invasive markers for liver injury. Our group demonstrated that hepatocytes are able to secrete exosome-like vesicles enriched in metabolic enzymes. We are currently achieving a thorough qualitative and quantitative analysis by transcriptomics, proteomics and metabolomics of hepatocyte-derived extracellular vesicles challenged to different model toxins as well as the effect that these vesicles have on blood homeostasis.

We have detected a significant number of RNAs and proteins in these vesicles that are altered by the liver toxins. Our work provides a repertoire of low invasive candidate markers for liver damage. In addition, we have detected a number of metabolites that are enriched in hepatocytes-released vesicles that support a physiological role of these vesicles in several cellular pathways.

OP014 - PROTEOME-WIDE PROFILING OF SERUM EXOSOMES FOR IDENTIFICATION OF SCIRRHOUS GASTRIC CANCER BIOMARKERS

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Introduction and objectives

Recently biological significance and clinical utility of exosomes have been extensively discussed. In the field of cancer diagnosis, exosomes are fascinating targets for biomarker discovery due to their molecular characteristics. Indeed a set of molecules expressed in original tumor cells can be detectable from exosomes in blood circulation. However, despite enormous interest in exosomes, difficulties in exosome isolation from biological fluids have significantly hindered effective discovery of biomarker components.

Methods

In the present study, we developed Second-Exo method (size exclusion chromatography on drip column for exosome isolation) allowing rapid collection of highly-pure exosomes in flow-through fraction by simple gravity drip. To identify early detection biomarkers for scirrhous gastric cancer, we employed Second-Exo method to perform quantitative proteome profiling of serum exosomes from 58 individuals (10 normal controls, 17 early-stage gastric cancer patients, 17 advanced-stage gastric cancer patients, and 14 scirrhous gastric cancer patients). After statistical selection of biomarker candidates on the Expressionist proteome server system, exosome sandwich ELISA was next established for the validation experiments with independent sample set.

Results and Discussion

From LC/MS/MS analysis, CEACAM5 (CEA) was found on gastric cancer patients□f serum exosomes, as well as 6 tetraspanin family proteins were identified as exosome markers. On the Expressionist server, 123,094 peptides were quantified and subjected to statistical selection of biomarkers, resulting in identification of 162 peptides which showed scirrhous gastric cancer specific expression patterns ($p < 0.001$, Student□fs t-test). Among them, an exosomal surface antigen was finally confirmed as potential scirrhous gastric cancer biomarker by exosome sandwich ELISA using 200 serum samples from BioBank Japan.

Conclusions

Thus Second-Exo method (a kit is now commercially available from GL Science company) can provide the best way for high-throughput exosomal biomarker screening studies using hundreds of clinical specimens.

OP015 - GLYCOPROTEINS ON THE SURFACE OF MDSC EXOSOMES

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We have shown that exosomes shed by myeloid-derived suppressor cells (MDSC) are abundant in the tumor microenvironment and suppress the immune system in tumor-bearing mice by communicating biological activity to both parental cells and macrophages (Burke et al 2014).

Glycoproteins on the surface of exosomes are thought to be involved both in recognizing target cells and in transferring molecular signals. Consequently we have extended a proteomic strategy developed to characterize cell surface glycoproteins to identify glycoproteins on the exosome surface. Because these exosomes have a chemotactic effect on their parental cells, we have also characterized MDSC cell surface proteins. We have used a variation (Weekes et al, 2010) of the strategy proposed by Zhang et al (2003) in which sugar moieties are oxidized with periodate and the resulting carbonyl groups are alkylated by amino-oxy-biotin. The derivatized glycoproteins are enriched on a streptavidin column, sequentially digested on-column with trypsin and PNGase, and the respective peptides are recovered for analysis by LC-MS/MS.

Currently 74% of 650 proteins enriched from MDSC using amino-oxy-biotin (5 biological replicates) and 64% of 1100 proteins enriched from exosomes (5 biological replicates) are classified as cell surface. We conclude that this enrichment strategy can be applied successfully to the smaller exosomes. Based on the UniProtKnowledgeBase (February 2014), more than 44% of the MDSC cell surface glycoproteins function to bind proteins, while approximately 54% of the exosome surface proteins have this function. Currently thirty-seven CD proteins are identified on the surface of the parental cells, and eight on the exosomes. A number of binding partners are recognized between the cell and exosome surfaces. Cell surface glycoproteins shared by the parental cells and shed exosomes include annexins and the proinflammatory molecules S100 A8 and S100 A9.

OP016 - CLINICAL AND TECHNICAL POTENTIALS AND PROSPECTS OF THE EV ARRAY

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The Extracellular Vesicle (EV) Array (Jørgensen et al., 2013, JEV) facilitate the ability to detect and profile extracellular vesicles for the presence of multiple surface exposed antigens simultaneously. The EV Array uses only small amounts (1 - 100µL) of starting material and is run in a high-throughput manner. The possibilities of the technology have been tested in various technical and clinical correlations.

Most interestingly, the analysis of plasma samples from lung-cancer patients, lung-infected patients and healthy controls revealed a pronounced diagnostic potential of the EV Array.

Other applications tested by the EV Array: (i) investigating whether the maturation stage of dendritic cells was reflected in the exosomes released from these cells and if the exosomal profile differed between dendritic cells grown in monocultures and dendritic cells grown in co-cultures with T cells, (ii) the influence of hypoxic conditions on cancer cell lines were investigated in relation to the number and phenotypes of the exosomes produced by these cells, and (iii) a phenotypic characterization of leukocyte-derived exosomes was performed to test whether the profile of these exosomes reflects the distribution of the leukocyte subpopulations present in blood and to generate an exosomal profile for normal healthy individuals. Taken together these studies illustrate the numerous applications of the EV array.

P-225.00

A POST-TRANSLATIONAL MODIFICATION PROTEOMICS STUDY OF INS CELL MITOCHONDRIA

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Introduction and objectives

Mitochondria oxidize fuel for the generation of energy in the form of ATP. Mitochondria are involved in metabolic and physiological signaling tasks such as cell cycle, cellular differentiation and cell growth. Mitochondrial dysfunctions or mutations in the mitochondrial genome have been associated with a variety of diseases and phenotypes including type 2 Diabetes.

We have implemented quantitative proteomic approaches to study the phosphoproteome and acetylome of the mitochondria of INS cells and decipher signaling events related to specific stimuli.

Methods

INS cells and isolated mitochondria from INS cells were lysed with RIPA buffer. Proteins were precipitated with methanol/chloroform. After protein digestion with trypsin, TMT isobaric tagging was used to label the differential extracts. Samples were either enriched for TMT-labeled phosphorylated peptides with TiO_2 magnetic beads or TMT-labeled acetylated peptides with acetyl-lysine antibodies. Samples were analyzed in triplicate with RP-LC MS/MS.

Results and Discussion

About 1000 proteins were identified and quantified in the mitochondria extracts with a high number of specific identities based on organelle enrichment with GO annotation. In parallel, analyses of the whole INS cells provided a significant portion of the mitochondrial proteome. Several phosphorylated and acetylated sites were specifically identified in the mitochondrial proteins. While some of those PTM sites have been already described, others have not been reported previously. Quantitative analysis of the regulation of the pinpointed PTM sites will be performed thanks to the isobaric tagging technology.

Conclusions

Two quantitative proteomic workflows have been optimized to specifically analyze phosphorylated peptides and lysine-acetylated peptides from the mitochondria. These approaches have been applied to study the regulation of PTM in the INS Cell mitochondria under specific stimulation. Analysis of the data is expected to provide relevant insights of the signaling events occurring in the mitochondria.

P-226.00

NUCLEAR PROTEOMICS REVEALS THE LINK BETWEEN MINICHROMOSOME MAINTENANCE COMPLEX AND GLUCOSE-INDUCED PROLIFERATION OF RAT PANCREATIC INS-1E B-CELLS

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Proper functioning of pancreatic β-cells is a central point for glucose homeostasis control, and therefore a main problem regarding type 2 diabetes onset and evolution. The ability of β-cells to proliferate upon certain stimuli, such as elevated glucose concentration, is an essential property to overpass a major problem of the pathology: the decrease of pancreatic β-cell mass leading to a lack of insulin production.

However, high glucose concentrations are also a critical inducer of β-cell dysfunction, when proliferation become unable to overcome increased insulin demand. The control of β-cell proliferation represents a key target for the development of new therapeutic molecules for type 2 diabetes treatment. To get new insights into β-cell replication, we investigated the modulation of nuclear proteins of INS-1E cells submitted to medium and high glucose concentrations for 24h. The SILAC approach identified 24 nuclear proteins whose expressions were modified by chronic high glucose concentration.

A wide Downstream Effects Analysis assigned the vast majority of the differentially expressed proteins to proliferation and cell cycle. Interestingly, our work identified the MiniChromosome Maintenance complex as a target of high glucose concentration, linking for the first time the increase of expression of its 6 components to glucose-induced stimulation in β-cells.

P-227.00

LABEL-FREE QUANTITATIVE MASS SPECTROMETRIC ANALYSIS OF COMPLEX PROTEOME ALTERATIONS IN THE AGING MUNICH WISTAR FRÖMTER RAT BRUSH BORDER MEMBRANE

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While the clinical relevance of albuminuria is well documented, the significance of renal barrier components remains of considerable interest. Recent studies describe a role for renal proximal tubules (PT) in albumin reabsorption and reclamation as an essential component of the urinary barrier to albuminuria.

Understanding the mechanism and extent of reabsorption of filtered proteins by the PT cells requires a better understanding of their brush border membranes (BBM). In this study, we quantified differential protein expression in BBMs from the Munich Wistar Frömter (MWF) model of albuminuria. BBMs proteins from MWF males (7 wks and 32 wks , n=3) were trypsinized and peptides subjected to LC-MS/MS analysis using Orbitrap Velos Pro high resolution MS.

Protein identification, quantitation, and statistical analysis was conducted using the IdentiQuantXL™ platform and bioinformatics via Ingenuity Pathway Analysis. 953 unique BBM proteins, splice variants or isoforms were identified, quantified, and compared. 94 proteins detected in both groups were differentially expressed (p

P-228.00
IN DEPTH PROFILING OF PROTEINS IN EXOSOMES RELEASED FROM SCIRRHOUS GASTRIC CANCER CELL

Satoshi Muraoka[1], Ayako Sato[1], Hideaki Kume[1], kazunari Hashiguchi[1], Jun Adachi[1], Yuuki Hashimoto[1], Kazuna Fukamizu[1], Takeshi Tomonaga[1]
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Exosomes are released from various cells into the extracellular space. They are small particle membrane proteins with a diameter 40-100nm and contain mRNAs, microRNAs, and proteins that could be transferred to target cells causing epigenetic or biologic changes. Exosomes are in many biological fluids such as blood, urine, seminal fluid, saliva, tear fluid, breast milk, and amniotic fluid and in vitro culture of cell line. They have a potential source of biomarker for diagnosis and prognosis. Scirrhous gastric cancer is unique among gastric cancer.

The prognosis of patients with scirrhous gastric cancer is extremely poor and the 5-year survival rate is significantly lower than patients with other types of gastric cancer. In this study, we report proteomic profiling of exosome released from scirrhous gastric cancer cell lines to identify novel biomarkers of scirrhous gastric cancer. Exosomes were isolated from culture medium of scirrhous, non-scirrhous gastric cancer, and normal gastric cell lines and profile of their exosomal proteins was analyzed by a quantitative proteomics approach using iTRAQ labeling. Some proteins were differentially expressed in scirrhous gastric cancer cell lines as compared with the other cell lines.

Our results indicate that exosomes derived from scirrhous gastric cancer have unique protein expression signatures compare to exosome from non-scirrhous gastric cancer or normal gastric cell. These results provide not only novel diagnostic biomarkers or therapeutic targets of scirrhous gastric cancer but will aid in understanding the molecular biology of the disease.

P-229.00

ISOLATION AND PROTEOMIC ANALYSIS OF EXTRACELLULAR MEMBRANE VESICLES OF STREPTOCOCCUS PNEUMONIAE

Chi Won Choi[1], Edmond Changkyun Park[1], Sung Ho Yun[1], Youn Hee Hong[1], Yeol Gyun Lee[1], Sang-Yeop Lee[1], Gun-Hwa Kim[1], Seung Il Kim[1]
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Objectives: Bacteria constitutively release extracellular membrane vesicles (MVs) to communicate with the environment in natural conditions. MVs are spherical structures that contain various native bacterial components, which are delivered to the environment where they fulfill various roles. A wide range of Gram-negative bacteria secrete MVs, which are known as outer membrane vesicles (OMVs). However, the extracellular MVs from Gram-positive bacteria have been studied much less than OMVs from Gram-negative bacteria.

Methods: Here, we report the exsitance of extracellular MVs of Gram-positive bacteria Streptococcus pneumonia BAA-255. We isolated MVs and examined their proteomic and clinical properties.

Results: Proteomic analysis reveled that a total of 104 protein components are included in S. pneumoniae derived MVs. Among them, extracellular proteins and membrane proteins take major portion of the identified proteins. In addition, S. pneunoniae MVs can induce immunity to bacterial infection in mice without cytotoxic effect.

Conclusion: These findings suggest that there may be a specific sorting mechanism for vesicular proteins and MVs of Gram-positive bacteria can be used for acellular vaccine

P-230.00

PROTEOMIC ANALYSIS OF IMMUNOAFFINITY-ISOLATED URINARY EXOSOMES

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Proteomic analysis of immunoaffinity-isolated urinary exosomes Siri Hildonen, Trine Grønhaug Halvorsen, Lèon ReubsaetDepartment of Pharmaceutical Chemistry, School of Pharmacy, University of Oslo, Oslo, Norway. Exosomes are 20-100 nm vesicles of endocytic origin that are secreted by most cell types in culture. Exosomes are vehicles for transport and delivery of various molecules such as proteins, nucleic acids and lipids. Exosomes have been found in various types of body fluids, such as blood, urine, saliva, cerebrospinal fluid, breast milk, etc.

Research on exosomes has intensified after these small vesicles were found to be not only involved in normal physiological processes but that they are also important mediators in pathophysiological conditions.

Exosomes is composed of a common set of membrane and cytosolic molecules. In addition they contain unique proteins linked to cell type–associated functions and as so they are considered rich reservoirs of disease biomarkers. One main advantage of targeting exosomes for biomarker discovery is that they can be collected minimally invasive, such as those from the urine or from the blood. There are different ways to isolate exosomes from body fluids: by ultracentrifugation, by ultrafiltration or by immunoaffinity methods.

Current diagnostic, therapeutic, and prognostic tools for kidney related diseases are limited and imperfect. We have developed a method to isolate exosomes and to identify exosomal proteins that is to be used in discovery of more specific biomarkers of kidney related diseases or pathogenesis. In short an immunoaffinity method was developed to isolate exosomes from urine by the use of magnetic bead coupled antibodies recognizing exosomal marker proteins.

The isolation of exosomes was confirmed by immunofluorescence microscopy. Several proteins were identified by a bottom-up proteomic analysis of the isolated exosomes.

P-231.00

MILK FAT GLOBULE PROTEOMICS FOR UNDERSTANDING THE CONTRIBUTION OF EPITHELIAL CELLS IN INNATE IMMUNITY OF THE MAMMARY GLAND

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Introduction and objectives
Milk fat globules (MFGs) are extracellular microvesicles released by mammary epithelial cells (MECs) to enable fat secretion and dispersion in milk. MFGs are structured as fat droplets surrounded by a tripartite membrane derived by the secreting cell, and carry various amounts of cytoplasmic contents with them. Here, we exploited MFGs as a tool for discovering how the MEC proteome changes following bacterial infection.

Methods
MFG protein (MFGP) profiles were evaluated in sheep suffering bacterial mastitis caused by two different bacterial pathogens: Mycoplasma agalactiae and Streptococcus uberis. MFGPs were isolated from milk by centrifugation and washing of the fat ring, followed by protein extraction by fat crystallization and treatment with detergents. Then, MFGPs were evaluated by 2D-DIGE/MS and by SDS-PAGE followed by tandem mass spectrometry and label-free quantification. Differential proteins were evaluated by Ingenuity Pathway Analysis. The validity of the findings obtained by MFG proteomics was then assessed by means of immunohistochemistry and confocal immunomicroscopy of affected tissues.

Results and Discussion
Proteomics of MFGs released from MECs during a bacterial infection enabled the detection of several proteins involved in inflammation, chemotaxis of immune cells, and antimicrobial defense, including cathelicidins and calprotectin (S100A8/S100A9). Immunohistochemistry and confocal immunomicroscopy of mammary tissues confirmed the ability of MECs to produce and release antimicrobial and immune defense proteins, confirming their contribution to the innate immune response to pathogens in the mammary gland.

Conclusion
The MFG vesicle model proved successful for probing the MEC proteome during infection in vivo, for gathering precious information on the contribution of MECs to the immune response of the mammary gland, and for identifying novel markers for diagnosis of mammary infections. In addition, the results obtained in this work can also provide useful insights on the contribution of epithelial cells to the innate immune response in other secretory epithelia.

P-232.00

PROTEOMIC ANALYSES OF EXTRACELLULAR VESICLES FROM THE PARASITIC TREMATODES FASCIOLA HEPATICA AND DICROCOELIUM DENDRITICUM

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Introduction and objectives: Helminthiases are considered the most neglected diseases, with a third of the human population affected by at least one species of parasitic helminths. The trematode species Fasciola hepatica (liver fluke) has a high impact on human health, and together with the related species D. dendriticum, causes important economic losses in the veterinary sector. Studies on these parasites' secretome revealed the presence of extracellular vesicles (EVs) in both helminths. With the aim of identifying potential biomarkers for these diseases, we have characterized and compared the proteins in EVs from F. hepatica and D. dendriticum.

Methods: Isolation and characterization of parasites EVs were carried out using differential ultracentrifugation coupled to ultrafiltration, followed by transmission electronic microscopy (TEM) analysis to confirm their size and morphology. Vesicles were lysed in water and total protein content was analyzed by LC-MS/MS. Protein identifications were done by MASCOT and ProteinPilot analyses. MASCOT searches were performed against the NCBI "other metazoan" data set, and against the transcriptome data set of the related species, E. caproni.

Results and discussion: 222 and 84 proteins were identified in F. hepatica and D. dendriticum EVs, respectively. These proteins constitute more than 50% of the molecules detected in the secretome of these parasites. Qualitative and quantitative differences were detected in the proteins identified in EVs obtained from both trematode species. While in F. hepatica the most abundant group of proteins were proteases (cathepsins), in D. dendriticum the most prominent group corresponded to metabolic enzymes. The existence of EVs in these helminths explains the secretion of atypical proteins, with no transmembrane domains, GPI-anchors and/or signal peptides. Conclusions: EVs constitute a major mechanism for secreting proteins in parasitic trematodes. EVs carry a common group of proteins, but also specific proteins that could constitute potential biomarkers for control of helminthiases.

P-233.00
QUANTITATIVE PROTEOMIC ANALYSIS OF MICROPARTICLES IN HYPERTENSIVE PATIENTS WITH ELEVATED CARDIO-RENAL RISK

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Introduction and objectives:

An extensive group of hypertensive patients treated by means of renin-angiotensin-aldosterone system (RAAS) blockage refers sustained and de novo albuminuria, which in turn denotes therapeutic inefficiency characterized by increased cardio-renal risk. Adequate prognostic biomarkers are mandatory to predict therapeutic response to RAAS blockage, in order to minimize damage of target organs. Microparticles are 0.1 nm to 1 μm vesicles released by activated or apoptotic cells, which have been associated with thrombosis and endothelial dysfunction. The aim of this study was to analyze the proteome of blood circulating microparticles from albuminuric patients with respect to patients with arterial hypertension without albuminuria, in the search for novel easy-accessible biomarkers with prognostic value.

Methods

Patients with arterial hypertension were recruited into three groups of study: normoalbuminuric, de novo microalbuminuric (less than 3 years), and sustained microalbuminuric. Circulating microparticles were isolated from blood plasma by ultracentrifugation. Protein content was solubilized, digested with trypsin, and the peptides were labeled with 8-plex iTRAQ reagents. The iTRAQ mixtures were analyzed in an LTQ-Orbitrap XL ETD. Quantification was carried out with QuiXoT software. Functional classification of identified proteins and categorical enrichment analysis was performed using DAVID Bioinformatic Resources 6.7 (NIH).

Results and discussion

More than 1000 proteins from circulating microparticles of hypertensive patients were identified. An important subset of proteins involved in metabolism, adhesion and apoptosis were found. Significantly abundant proteins found in microalbuminuric patients allowed to efficiently classify patients due to their cardio-renal risk.

Conclusions

The proteome of circulating microparticles is a novel easy-accessible source for prognostic biomarkers of therapeutic response in hypertensive patients treated with RAAS blockage.

P-234.00

PROTEOMIC STUDY OF BLOOD-DERIVED MICROPARTICLES IN MULTIPLE SCLEROSIS

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Introduction and objectives Microparticles (MP) are produced by a variety of cells, spontaneously or in response to different stimuli, and play important roles in cell-cell communication. Proteins specific to the original cell type are incorporated in the MP, making MP composition reflect physiological changes and pathological conditions. MP are suitable targets for biomedical research, as they can be readily sampled from blood, in which they are very stable. Blood-derived MP have been found to associate with several inflammatory diseases and, interestingly, with some central nervous system (CNS) pathologies. Here, we present a proteomic analysis of blood-derived MP from patients affected by multiple sclerosis, a pathology in which MP release from endothelial cells into the circulation might be favored by blood-brain barrier damage.

Methods Blood-derived MP have been purified by molecular exclusion techniques from patients affected by relapsing remitting multiple sclerosis in the active- or remitting-phase of the disease or healthy donors. MP protein content has been analyzed by LC-MS/MS and classified according to Gene Ontology.

Results and Discussion The effectiveness of the purification procedure has been validated by transmission electron microscopy of the purified microparticles and by showing that the isolated material is enriched in membrane-bound and cytoplasmic vesicle proteins. Several proteins identified only in active-phase patients are typical of the CNS, indicating this origin for MP production and probably reflecting local damage. Moreover, a significant enrichment in proteins involved in biological processes strictly related to multiple sclerosis, such as synaptic transmission, cell death, and immune response, characterizes the purified MP.

Conclusions Our analysis, for the first time, has demonstrated the presence of molecular signatures of CNS damage in blood-derived MP, which provide a new perspective on the pathogenetic mechanisms of multiple sclerosis and represent potentially valuable biomarker sources.

P-235.00

A TRANSFER LEARNING FRAMEWORK FOR ORGANELLE PROTEOMICS DATA

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Introduction and Objectives

Organelle proteomics is the systematic study of proteins and their assignment to organelles, and is a field of rapidly growing importance. The knowledge of subcellular localisation of proteins is extremely desirable to biologists, as it can assist elucidation of a protein's role within the cell, as proteins are spatially organised according to their function and specificity of their molecular interactions. Experimental organelle proteomics requires sophisticated experimental designs and data analyses in order to obtain accurate datasets, and to collectively infer the localisation of thousands of proteins [1]. Here, we use freely available data from repositories to improve upon the classification of experimental, condition and organism-specific protein subcellular localistation predictions.

Methods

We use state-of-the-art machine learning in a transfer learning framework [2] to simultaneously exploit several sources of information available with which to assign a protein to its subcellular compartment. These sources encompass our main (primary) data produced from experimental high-throughput mass spectrometry (MS)-based methods and in silico (auxiliary) data such as sequence-based predictions and Gene Ontology information.

Results and Discussion

We find that integrating data, from a second more plentiful auxiliary data source, directly in to classifier training and classifier creation results in the assignment of proteins to organelles with high generalisation accuracy. Furthermore, we show our hybrid, data fusion approach outperforms a single classifier trained on each single data source alone.

Conclusion

A new transfer learning framework for predicting protein localisation to unify multiple sources of information to improve experimental proteomics data is proposed. This novel machine learning methodology forms part of the Bioconductor pRoloc [3] suite of computational methods available for organelle proteomics data analysis.

P-236.00

FRACTIONATION PROFILING: A FAST AND VERSATILE APPROACH FOR MAPPING VESICLE PROTEOMES AND PROTEIN-PROTEIN INTERACTIONS.

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Introduction and objectives

Clathrin-coated vesicles mediate protein transport between the trans-Golgi network and endosomes, and also facilitate plasma membrane endocytosis. Their function is critically important in all eukaryotes. Defects in clathrin-dependent trafficking are associated with severe developmental phenotypes in multicellular organisms; similarly, parasitic trypanosomes, the causative agents of sleeping sickness, are not viable without clathrin-mediated endocytosis. Despite recent technical advances, the proteomic characterization of transport vesicles has remained extremely challenging, due to their transient nature and low abundance.

Methods

We have developed 'fractionation profiling', a method for rapid proteomic analysis of membrane vesicles and protein particles. The approach combines SILAC quantitative proteomics with subcellular fractionation, to generate signature protein abundance distribution profiles. Profiles are grouped through cluster analysis, to identify proteins with shared fractionation behavior.

Results and Discussion

To validate the method, we first profiled >3,500 proteins from HeLa cells, and identified known clathrin-coated vesicle proteins with >90% accuracy. We then successfully applied the method to a variety of less well-characterized systems, including human neuronal SH-SY5Y cells, the murine dendritic cell line MutuDC, drosophila S2 cells, and trypanosomes. We report here the first clathrin-coated vesicle proteomes from these cell types. In addition, the cluster analysis extends to all profiled proteins, and thus identifies a diverse range of known and novel cytosolic and membrane-associated protein complexes. We show that it also allows the detailed compositional characterization of complexes, including the delineation of sub-complexes and subunit stoichiometry. Our predictions are presented in an interactive database. Although we have focused our analysis primarily on clathrin-coated vesicles, the method will be suitable for many other types of vesicles.

Conclusions

Fractionation profiling is a universal tool for defining vesicle proteomes from any cell type, but also for the complementary fast generation of protein interaction maps.

P-237.00

SUBCELLULAR PROTEOMICS OF HUMAN EMBRYONIC STEM CELLS

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Introduction and objectives

Since the first human embryonic stem cell (hESC) line derivation in about 15 years ago, these eminent cells provided exquisite source for studding cell differentiation to human embryonic development. As long as one of the ultimate goals of hESCs applications is cell therapy, more detailed analysis is so critical and indispensable. Since the function of a protein is strongly associated with its localization in cell, a complete and accurate picture of the proteome of ESCs cannot be achieved without knowing the subcellular locations of proteins.

Methods

hESC line H9 has been grown on mouse embryonic fibroblast (MEF) and the quality of harvested cells were assessed through various techniques such as flow cytometry, RT-PCR and karyotype analysis. Freshly harvested cells have been fractionated on discontinuous sucrose gradient into six subcellular fractions (Cytoplasm, nucleus, mitochondria, plasma membrane, light and heavy microsomes). Western blotting for organellar marker proteins has been used to confirm the efficiency of subcellular fractionation. All the fractions were subjected to gel-assisted digestion following by mass spectrometry analysis by Triple TOF Mass spectrometry and label free quantitation by IDEAL-Q.

Results and Discussion

Gene antology analysis and prediction of transmembrane helix and subcellular localization of proteins through various bioinformatic tools alongside western blotting confirmed the efficiency of fractionation. Clustering of all quantified proteins in fractions provided insight into tracking signaling proteins that shuttle between different compartments and some of signaling pathways are highlighted such as calcium signaling.

Conclusions

Subcellular fractionation of hESCs provided greater proteome coverage compared to whole cellular lysate and also a greater depth of signaling protein analysis. Our report is the most comprehensive subcellular proteomic analysis in hESC which provide powerful data for further detailed studies.

P-238.00

CENTRAL COMPONENTS OF THE PEROXISOMAL MATRIX PROTEIN IMPORT MACHINERY ARE SUBSTRATES OF CYTOSOLIC KINASES

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Peroxisomes are ubiquitous, single membrane-bound organelles with vital physiological and metabolic functions in eukaryotic cells. Defects in their biogenesis and metabolism are associated with severe, often lethal human disorders such as the Zellweger Syndrome. Since peroxisomes do not contain DNA, matrix proteins synthesized on cytosolic ribosomes need to be post-translationally imported across the peroxisomal membrane. This process is mediated by a large membrane-spanning multi-protein complex, the peroxisomal importomer. Pex14p and Pex5p have recently been demonstrated to be involved in the formation of a large and highly dynamic pore enabling the translocation of peroxisomal proteins into the organelle in Saccharomyces cerevisiae. However, information about the dynamic regulation of this central gateway to peroxisomes is still lacking.

We used SILAC combined with affinity purification-MS to identify transient interaction partners of Pex14p in S. cerevisiae. Furthermore, endogenous Pex14p and Pex5p were affinity-purified and subjected to comprehensive phosphoproteomics analyses using different proteolytic enzymes and high resolution LC/MS. To identify the kinases mediating distinct phosphorylation events in these proteins, we developed a site-specific MS-based in vitro kinase assay using non-radiolabeled ATP. Results were confirmed by radioactive kinase assays using wild-type proteins and phosphosite mutants followed by in vivo functional analyses.

We identified Hrr25p, a Ser/Thr kinase of the casein kinase 1 family, as transient interaction partner of the Pex14p complex indicating the phosphorylation of components of the peroxisomal importomer. Comprehensive phosphorylation analyses of endogenous Pex14p and Pex5p resulted in the identification of a battery of distinct phosphorylation sites. Using in vitro kinase assays, we revealed that both proteins are substrates of numerous cytosolic kinases. Our ongoing in vivo functional analyses suggest that reversible phosphorylation events of the components of the peroxisomal translocation pore affect import of matrix proteins into the organelle. We thus propose that cytosolic kinases regulate protein import into peroxisomes.

P-239.00

URINARY EXOSOMES REFLECT PROTEOMIC CHANGES AT TISSUE LEVEL. A NOVEL TOOL FOR NON-INVASIVE DETECTION OF KIDNEY INJURY IN DIABETIC NEPHROPATHY.

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Introduction And Objectives:

Diabetic nephropathy (DN) is a progressive kidney disease and the most frequent cause of end-stage renal disease. The early stages are asymptomatic. Thus, a better understanding of its pathogenesis and non-invasive monitoring in substitution of renal biopsy is required for earlier diagnosis. Exosomes are urinary secreted vesicles considered a mechanism of non-classical secretion of proteins. The aim was to investigate if proteome alterations in response to DN occuring in kidney tissue could be monitored in an accessible fluid as urine and to investigate a role for exosomes in this context.

Methods:

Ten 5-week-old male Wistar-Kyoto rats were used. Diabetes was induced in 5 rats by a single intraperitoneal injection of streptozotocin (STZ). Eight weeks later, 24h urine was collected and kidney tissue was extracted. Urinary exosomes were isolated by ultracentrifugation and albumin depleted. Tissue proteome from control and DN rats was compared by DIGE and varied proteins were confirmed by WB and IHQ. Proteins varied in tissue were investigated in urine and exosomes by SRM-LC-MS/MS. Human kidney tissues from 11 individuals were collected (four control and seven kidney diseased).

Results And Discussion:

Twelve tissue proteins were significantly different. The strongest variation (more than 4-fold decreased) was confirmed in human kidney tissue. This protein was not detected in urine but it was detected in urinary exosomes only from control rats, following the same trend observed in tissue. SRM-LC-MS/MS in urinary exosomes confirmed this protein downregulation in DN rat. Urinary exosomes isolated from DN patients also showed a clear decrease for this protein in comparison to exosomes from healthy individuals.

Conclusions:

Urinary exosomes reflect changes occurring at tissue level during kidney disease. Proteome changes shown here can be used to monitor kidney injury directly in urine, overcoming one of the limitations for early diagnosis of DN.

P-240.00
PROTEOMIC PROFILING OF EXOSOMES FROM CEREBROSPINAL FLUID

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Introduction: Exosomes are microvesicles that are released from cells into the extracellular space. Since they are present in biological fluids such as blood and cerebrospinal fluid (CSF), they gained attention in recent years as carriers of disease biomarkers. Proteomic biomarkers in CSF exosomes are of great interest for the diagnosis of neurodegenerative diseases, where no reliable biomarker has been described yet. To date, there is little information about the proteome of CSF exosomes and in-depth proteomic studies are urgently needed.

Objective: Proteomic profiling of CSF exosomes to identify biomarker candidates. Methods: Lumbar CSF samples of patients without signs of neurodegeneration and with normal CSF diagnosis were pooled and exosomes were isolated by differential centrifugation. Purified exosomes and untreated CSF were reduced and alkylated with TCEP and iodacetamide and digested using trypsin/LysC over night at 37°C. The digested samples were separated on a 0.075x500mm C18 column with an Ultimate 3000 RSLCnano system and were analyzed by data-dependent acquisition (Top12) using a Thermo Q Exactive mass spectrometer.

Results and Discussion: In the exosome preparation, we identified a total of 19884 peptides from 2423 proteins (FDR 0.01). To the best of our knowledge, this is the highest number of proteins identified in CSF exosomes in a single study to date. The list of proteins included several exosomal marker proteins such as flotillin-1, CD63, ALIX and TSG101. These marker proteins could not be identified in untreated CSF showing the successful enrichment of exosomes. In the untreated CSF sample, we identified 11503 peptides from 1694 proteins. By quantitative comparison of exosomal preparations and untreated CSF, we were able to clearly identify exosomal enriched proteins and distinguish contaminating proteins.

Conclusion: Our study is a further step in the elucidation of the exosomal CSF proteome and enables the identification of interesting biomarker candidates for further validation.

P-241.00

PROTEOMIC IDENTIFICATION OF MICROVESICLE BIOMARKERS IN ST-ELEVATION MYOCARDIAL INFARCTION

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Introduction and objectives: Membrane microvesicles (MVs) are released from activated cells, most notably platelets, into the circulation. They represent an important mode of intercellular communication and their number is increased in patients with acute coronary syndromes. We present here a differential proteomic analysis of plasma MVs from ST-elevation myocardial infarction (STEMI) patients and stable coronary artery disease (SCAD) controls. The objective was the identification of MVs biomarkers/drug targets that could be relevant for the pathogenesis of the acute event.

Methods: Plasma MVs were isolated by an ultracentrifugation-based procedure, and characterized by FACS and electron microscopy. Subpopulations distribution was confirmed by dynamic light scattering. Proteome analysis was based on 2D-DIGE, and mass spectrometry. Validations were by western blotting in an independent cohort of patients and healthy individuals. Ingenuity Pathway Analysis (IPA) was used to predict possible interactions between proteins and their relation with disease. Results and discussion: We detected 117 differentially regulated protein features between STEMI and SCAD MVs (fold change$\geq$2; p

Conclusions: In conclusion, we provide a unique panel of proteins that vary between plasma MVs from STEMI and SCAD patients and that might constitute a promising source of biomarkers/drug targets for myocardial infarction. We believe our study provides novel information on the critical role microvesicles play in the pathophysiological events underlying an acute myocardial infarction.

P-242.00

PHENOTYPIC CHARACTERIZATION OF EXOSOMES PRESENT IN BLOOD BY EXTRACELLULAR VESICLE (EV) ARRAY – IS THE EXOSOME PROFILE A REFLECTION OF THE DISTRIBUTION OF THE LEUKOCYTE SUBPOPULATIONS IN BLOOD?

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Introduction: It is generally accepted that exosomes play an important role in the communication between cells. Studies have shown that the quantity and molecular composition of exosomes shed from various cell types differs considerably. It is therefore expected that blood contains a wide range of exosomes with different phenotypes, reflecting the phenotype of the cells that produced them. Consequently, it was investigated whether exosomes present in blood reflect the specific subtype composition of the leukocytes in blood from healthy individuals.

Methods: Blood samples from 10 healthy individuals were analyzed by flow cytometry for the subtype composition using antibodies specific for T cells, B cells, NK cells, monocytes and granulocytes. The expression of the tetraspanin exosomal markers CD9, CD63 and CD81 was also determined for each subtype. From the same blood samples, plasma was extracted and used for the phenotypic investigation of the exosomes present in whole blood using the EV Array (Jørgensen et al., 2013, JEV). The EV Array is based on the antibody capture of exosomes by a panel of surface markers with subsequent detection of the captured exosomes by biotin-labeled anti-tetraspanin antibodies. The panel included the subtype-specific surface markers and together with the exosome specific markers a total of 28 markers were analyzed.

Results: The EV Array demonstrated only low intensities for the majority of subtype-specific markers, while few surface markers, e.g. CD8 and CD14, appeared at higher intensities. In addition, the results indicated that the 3 general exosomal markers, CD9, CD63 and CD81, were differentially expressed on the different leukocyte subpopulations.

Conclusion/Summary: Based upon a panel of 12 different immune markers, the results indicate that there is no direct correlation between the amount and phenotype of the exosomes and the corresponding subtype composition of leukocytes in blood.

P-243.00

COMPARISON OF SUPER-SILAC AND SWATH-MS TECHNIQUES: APPLICATION FOR QUANTIFYING THE MOUSE SYNAPTIC MITOCHONDRIAL PROTEOME.

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Stable-isotope labeling by amino acids in cell culture (SILAC) is a key method for quantitative proteomics. Expansion of SILAC for tissue proteome quantification using a mix of multiple SILAC-labeled cell lines as internal standards (super-SILAC) has allowed for biological applications of this technique (1,2). While exhibiting excellent reproducibility and accuracy, the super-SILAC method may prove cost-prohibitive when performing a high number of samples; therefore, we chose to compare the performance of super-SILAC with that of a label-free method, SWATH-MS (3).

We used our previously developed super-SILAC mix for quantitative mass spectrometry-based proteomics of mouse brain mitochondria (4) to quantify the proteome differences between synaptic mitochondria isolated from 1 and 6 week old mice (n = 4). In order to evaluate the reproducibility of quantification achievable by SWATH-MS compared to super-SILAC, and the correlation between the two methods, we generated a mitochondrial reference spectral library and subjected the same experimental samples used for super-SILAC to cyclic data-independent acquisition of mass spectra using 25-Da swaths in a similar manner to the previously established method (3,5). In addition to assessing the above metrics, the biological functions of the proteins with significant expression changes between 1 and 6 weeks of age were investigated using bioinformatic approaches and altered protein expression levels were examined orthogonally with Western blot analysis.

Our comparison of the super-SILAC- and SWATH-MS-derived quantitative values obtained from the same synaptic mitochondria samples revealed significant correlation between the two and advantages of each technique. These results and our other recent findings (6) suggest that label-free SWATH-MS has great utility for analysis of in vivo derived samples.

P-244.00

THE AGING MOUSE BRAIN SYNAPTIC MITOCHONDRIAL PROTEOME: PRESERVATION OF FUNCTION THROUGH HORMESIS.

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Increasing evidence suggests mitochondrial dysfunction contributes to aging and several forms of neurodegeneration. To prevent the accumulation of dysfunctional mitochondria, the cell has adopted several mechanisms for controlling mitochondrial quality. Mitochondria are enriched at synapses, this is important for local energy production and calcium regulation which supports synaptic transmission. Due to the high metabolic demands of neuronal synapses this pool of mitochondria is suspected to encounter more oxidative stress and accumulate more age-associated damage.

Therefore, we set out to determine if synaptic mitochondrial dysfunction and loss of integrity results from age-related alterations in protein expression. We developed a super-SILAC mix for quantitative mass spectrometry-based proteomics of mouse brain mitochondria and accurately quantified the proteome differences between synaptic mitochondria isolated from sequential age-matched mice (5, 12, and 24 months of age). Altered protein expression levels were verified orthogonally with Western blot and focused on those enriched in pathways relevant to aging including mitochondrial bioenergetics, transport, oxidative stress response and mitophagy. Functional confirmation of altered mitochondrial bioenergetics was conducted using the Seahorse XF24 machine.

During the process of aging we find that dynamic proteomic alterations occur in synaptic mitochondria. Despite direct (mitochondrial DNA deletions) and indirect (increased antioxidant protein levels) signs of mitochondrial damage in the aged mice, there was an overall maintenance of mitochondrial function. Aging is not necessarily pathogenic, and in healthy aging, organs, cells and subcellular organelles can respond to gradual age-associated stress, such that adaptive changes to these insults can lead to maintained or even improved outcomes, a concept known as hormesis. The proteomic changes observed likely allow the cell to adapt to functional requirements and counteract age-associated cellular stress and damage enabling maintenance of mitochondrial energetics at the synapse. Therefore the synaptic mitochondrial proteomic changes that occur with aging correlate with preservation of synaptic mitochondrial function.

P-245.00

PROTEOMIC STUDY OF EXTRACELLULAR VESICLES OF HUMAN MACROPHAGES IN RESPONSE TO CANDIDA ALBICANS INFECTION

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Introduction

Candida albicans is an opportunistic pathogen causing symptomatic infections especially in patients with compromised immune functions. Macrophages are phagocytic cells that play an essential role in the primary response to pathogens. During the interaction with the pathogen, immune cells actively communicate with each other by secreting proteins that can be secreted through different mechanisms including classical and non-classical mechanisms. The non-classical secretion includes diverse types of extracellular vesicles (EVs) that differ in size or origin such as exosomes. These exosomes have a potential role mediating their response to the infections. In the aim of understanding the effect of the macrophage EVs, we present a comparative proteome profile of C. albicans infected human macrophages compared with the control macrophages.

Methods

Extracellular vesicles were isolated from culture supernatants as previously described. Three biological replicates of THP1 control macrophages and after 3h of co-incubation with C. albicans (1:1) were obtained in order to perform a quantitative analysis with iTRAQ labeling combined with a bioinformatic analysis.

Results and Discussion

We set up a C. albicans-THP1 macrophages interaction model showing and increase in pro-inflamatory cytokines along different times of interaction. Proteomic analysis of extracellular vesicles of THP1 macrophages in response to infection with C. albicans allow us to detect an increase in the secretion of EVs in response to the yeast, identifying a high number of proteins related to the response of the immune cells to fungal infection. We are performing a quantitative proteome analysis with iTRAQ to allow us to quantify the changes in the components of macrophage EVs in response to C. albicans infection.

Conclusion

The proposed model is suitable for analysis of the human macrophages response to C. albicans and could be also useful for the study of the macrophage response against other microorganisms.

P-246.00
THE SUBCELLULAR PROTEIN ATLAS

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Compartmentalization of biological processes is a fundamental principle of eukaryotic cells that gathers the molecules needed for a specific function and enables multiple processes to occur in parallel. Despite a great deal of research, basic questions about the spatial organization of many proteins and biological processes remain unanswered. The Subcellular Protein Atlas aims to systematically localize the human proteome using an antibody-based approach as part of the Human Protein Atlas project. In total, over 10.000 human proteins have been localized. In late 2013, the Human Protein Atlas database was relaunched with a new design and stricter validation criteria incuding the following techniques. Gene silencing using siRNA for validation of antibody binding and protein localization (1), fluorescently tagged proteins (FP) as a complementary technique (2) and paired antibodies targeting the same protein. Also, the panel of cell lines has been expanded and RNA-seq is used to cherry pick thr best cell lines for each protein (3).

Interestingly our results indicate that as much as 50% of all proteins localize to multiple compartments, almost 50% show variation in localization between cell lines and approximately 5-10% show cell cycle dependent expression. Defects in compartment organisation also underlie the cause of many forms of human disease, for instance cancer. In order to further investgate the implicacations of this we used a cell line model for malignant transformation to identify proteins with an altered expression profile upon cell transformation.

Here we discuss the importance of spatial proteomics and present the content and results of the Subcellular Protein Atlas as well as the path to a complete coverage of the human proteome.

P-247.00

PROTEOMIC CHARACTERIZATION OF DETERGENT-RESISTANT PLASMA MEMBRANE MICRODOMAINS: SIMILARITIES AND DISSIMILARITIES BETWEEN PLANT AND OOMYCETE

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The plasma membrane (PM) is considered as one of the most interactive and dynamic membrane structures of the cell. It is involved in many biological processes such as metabolite and ion transport, endocytosis, defense against pathogens, cell differentiation and proliferation, gaseous exchanges, etc. Some of these dynamic processes occur in specific domains in the PM. These microdomains enriched in sphingolipids and sterols, are resistant to certain concentrations of detergents.

The aim of this work was to determine the composition and main functions of such detergent-resistant microdomains (DRMs) in poplar (Populus trichocarpa) and a pathogenic oomycete Saprolegnia parasitica, through a comprehensive quantitative proteomic analysis using gel-based and solution (iTRAQ) approaches. The characterization of DRMs is important to understand the mode of interaction of specific proteins with sterols and sphingolipids and to gain insight into the protein composition and biological activity of subdomains from the PM. In this study, compared to PM, many proteins related to cell wall biosynthesis, stress responses, signaling processes, molecular and ion transport were found to be significantly enriched in DRM from each species. An important proportion of the most enriched proteins in DRM corresponded to $(1{\rightarrow}3)$-β-glucan synthases, indicating that the isolated microdomains are the site for callose biosynthesis and regulation.

Furthermore, the predicted specific structural features of the oomycete glucan synthases is compared to the properties of the corresponding enzymes from higher plants. The data will be presented and discussed in relation with these key biological processes in plant and oomycete model systems through comparative integrated model connecting all DRM-enriched proteins.

P-248.00

PROTEOMIC ANALYSIS OF CEREBROSPINAL FLUID EXTRACELLULAR VESICLES: A COMPREHENSIVE DATASET

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Introduction and objectives: Extracellular vesicles (EVs) are present in human cerebrospinal fluid (CSF), yet little is known about their protein composition. Here we provide an in-depth analysis of the proteome of CSF EVs by high resolution tandem mass spectrometry (MS/MS) in conjunction with bioinformatics. Our main aim was to build a dataset of proteins present in CSF EVs which may provide the basis for biomarker studies in neurological diseases.

Methods: Two independent pools of CSF (6 mL each) were constructed for proteomic analysis. EV isolation was performed using ultracentrifugation, collecting vesicles precipitating at 20,000xg (P20) and at 100,000xg (P100). EVs proteins were separated with SDS-PAGE and digested with trypsin. Extracted peptides were analyzed using a QExactive mass spectrometer, and raw data were processed with MaxQuant. Spectral counts were used for relative protein quantification of P20 and P100 fractions. Electron microscopy was performed to study EV size.

Results and discussion: EVs in the P100 fraction presented an average diameter of 48.8 ± 3.2 nm, consistent with that of exosomes. Interestingly, EVs with different size were present in the P20 fraction. A total of 1315 proteins were identified in EVs isolated from the two CSF pools, including 230 novel EV proteins. The proteome of CSF EVs was enriched in exosomal markers such as alix and syntenin-1, heat shock proteins and tetraspanins and contained a high proportion of brain-derived proteins (n=373). Notably, several known biomarkers for neurodegenerative diseases such as the amyloid precursor protein, the prion protein and DJ-1 were identified in the EV fractions, with a differential enrichment in the P20 or P100 fractions.

Conclusions: Our dataset represents the first comprehensive inventory of the EV proteome in CSF, underscoring the biomarker potential of these sub-fractions. Further comparative studies on CSF EVs isolated from patients diagnosed with neurological disorders are warranted.

Proteogenomics

OP009 - A NOVEL UNPRECEDENTEDLY COMPREHENSIVE PROTEIN IDENTIFICATION STRATEGY, MASS SPECTROMETRY AND RIBOSOME PROFILING: THE PERFECT MATCH.

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Introduction

The recently developed ribosomes profiling (RIBO-seq) technique provides genome-wide information about protein synthesis with sub-codon precision by monitoring mRNA that enters the translation machinery, while highly sensitive mass spectrometry provides information about the protein abundance of a sample. We present a proteogenomic pipeline merging these two techniques.

Methods

To investigate the impact of using RIBO-seq predicted translation sequences as a search database we used matching RIBO-seq, gel-free shotgun and N-terminal COFRADIC proteomic data from mouse embryonic stem cells (mESC) and human colorectal cancer cells (HCT116). Both cycloheximide (CHX) and lactimidomycin (LTM) treated cell line samples were analyzed, respectively resulting in an overall CDS and accumulated translation initiation site signal. We implemented our proteogenomic pipeline in the GalaxyP environment and compared the protein/peptide/PSM identification results with a regular search against SwissProt using the SearchGUI/PeptideShaker toolkit.

Results/Discussion

After pioneering work, we recently developed a new integration pipeline making full advantage of all the information present in the RIBO-seq data. An optimized translation start site prediction algorithm in combination with the compiled complete map of synthesized proteins - optionally also including non-synonymous sequence polymorphism info - results in a custom database of translation products. Preliminary analysis on the mESC data demonstrate that searches against this custom database outperform searches against SwissProt (an increase of 6,9 and 5,5% on respectively the peptide/PSM level) for the shotgun experiment. The N-terminal COFRADIC analysis results in the identification of N-terminally truncated/extended protein forms, next to translated uORFs. Notably, the characterization of these new translation products revealed the use of multiple near-cognate (non-AUG) start codons.

Conclusion

As deep sequencing techniques are becoming more standard and less expensive, we anticipate that these custom-tailored DB's will become indispensable in MS-based proteomics.

This proteogenomic pipeline is implemented in GalaxyP, allowing non-bioinformaticians to create these custom databases and perform subsequent searches.

OP010 - PERSONALISED PROTEOMICS BY MEANS OF INDIVIDUALISED PROTEIN MICROARRAYS

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Introduction and objectives
Genome sequencing of individuals will soon be applied routinely in clinical settings. Despite this remarkable progress, however, insights into disease biochemistry frequently remain preliminary. Much disease-relevant regulation occurs through control of protein expression and isoform formation. We are taking advantage of sequence information from individual patients for a directed characterisation of protein isoforms (mutations and splice variations), utilising a newly developed technique of producing personalised protein microarrays.

Methods
The genes represented in a tissue's total RNA are copied onto a microarray by on-chip PCR amplification, using gene-specific primer pairs that are attached to the surface at distinct locations. The arrayed DNA copies then act as templates for in situ cell-free protein expression, yielding microarrays that present the protein content of a particular tissue of an individual person. This allows analysing personal variations in protein interactions with other proteins, nucleic acids and small chemical compounds.

Results and discussion
Typical results from such studies in human and Trypanosoma brucei will be shown that document the power of the assay format and the different analysis types that can be performed. Disease-relevant variations were found, with an emphasis on cancer and sleeping sickness, that could be confirmed by in vitro and in vivo assays. Another application is the definition of disease-relevant variations of the immune-response of individual patients.

Conclusions
Our objectives are the detection of disease-related protein variations, characterisation of functional differences, and a targeted identification of therapeutically relevant compounds. For future therapy, knowledge of protein isoforms and their combinations in individual patients will be critical for therapeutic approaches that target disease-relevant protein conformations, leaving the molecules in healthy tissues unaffected. The personalised array is complemented by a set of antibody pairs that recognise protein isoforms that a different between healthy and tumour tissues.

OP011 - A FAST DECOY-FREE APPROACH FOR RELIABLE IDENTIFICATON OF PSMS

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Traditionally, in mass spectrometry-based proteomics, target-decoy approaches are used to establish the false discovery rate (FDR), the ratio of incorrect to correct Peptide-to-Spectrum Matches (PSMs) at a certain cutoff.

In these approaches, the experimental spectra are also searched against nonsense, 'decoy' databases (often created by reversing or shuffling the sequences in the original database), and the number of hits in such a database is taken to be an estimate of the number of false hits obtained by searching the original database, so FDR = #decoys / #originals. However, this approach is not always optimal, especially for Proteogenomics where whole genomes are searched and the vast increase of high-scoring false hits makes it extremely difficult to separate true hits from false ones, and consequently to estimate an FDR. Additionally, popular and reliable software tools, such as Percolator, need to be retrained each time for each dataset, a process that consumes impractically large amounts of computing time in proteogenomics due to the combined size of the original and the decoy database.

In this work, we therefore developed a new FDR estimation method that no longer relies on the traditional 'decoy' databases. Instead, we trained a binary classifier once on a collection of heterogeneous data to classify correct and incorrect PSMs. We used Mascot ranks to label correct and incorrect PSMs. We built different models according to the rank used to model incorrect PSMS. All models perform quite well, and interestingly, the model trained on rank1 hits and decoy hits shows the lowest performance when tested on datasets in which ranks lower than rank1 are used to model incorrect hits, indicating that decoys are not very good at modeling incorrect PSMs. We thus present a fast method to confidently classify PSMs, which does not require retraining for each data set.

OP012 - AN INTEGRATED PROTEOGENOMIC STRATEGY FOR GENOME ANNOTATION AND GLOBAL POST-TRANSLATIONAL MODIFICATIONS DISCOVERY IN THE MODEL CYANOBACTERIUM SYNECHOCOCCUS SP. STRAIN PCC 7002

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Introduction and objectives

Cyanobacteria are a highly diverse group of photosynthetic prokaryotes and play crucial roles in global carbon and nitrogen cycles. Synechococcus sp. PCC 7002 (hereafter Synechococcus) is a model cyanobacterium and has been extensively used for studies concerned with photosynthesis and biofuels development. In spite of the biotechnological and basic science relevance of Synechococcus there has been no comprehensive analysis undertaken to provide experimental support of its in silico-based genome annotation to facilitate systems level analysis. To gain fundamental insights into this model cyanobacterium, we developed an integrated workflow for the genome annotation and whole proteome analysis of post-translational modifications (PTMs) in Synechococcus.

Methods

Protein extracts were prepared from Synechococcus cultures, subjected to Glu-C and Trypsin digestion, producing peptide mixture. The mixture is analysed by means of nanoLC¨CMS/MS with a LTQ-Orbitrap Elite mass spectrometer. MS/MS peptide spectra searched against specific organism genome sequence validating and correcting genomic annotations, as well as identifying novel protein-coding genes and diverse PTMs.

Results and Discussion

In all, we validated 2938 of the 3186 predicted protein-coding genes ($> 92\%$), identified 118 novel protein coding genes and corrected 38 predicted gene models in the Synechococcus genome. In particular, without specific enrichment strategies, we have revealed a large repertoire of post-translational modifications (PTMs) using the same proteomic datasets and provided a holistic view of PTM events in Synechococcus. The identified PTM events constitute an important advance in understanding the physiological functions underlying these PTMs and facilitate the elucidation of complex cellular machinery in the specific context of Synechococcus and cyanobacteria in general.

Conclusions

As the strategy is relatively fast and applicable to any sequenced prokaryotic organism we expect that it will become an integral part of ongoing genome sequencing and annotation efforts.

P-249.00

QUANTITATIVE AND QUALITATIVE PROTEOME CHARACTERISTICS EXTRACTED FROM IN-DEPTH INTEGRATED GENOMICS AND PROTEOMICS ANALYSIS

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Introduction:
Quantitative and qualitative protein characteristics are regulated at genomic, transcriptomic, and posttranscriptional levels. Here, we integrated in-depth transcriptome and proteome analyses of liver tissues from two rat strains to unravel the interactions within and between these layers.

Results:
We obtained peptide evidence for 26,463 rat liver proteins. We validated 1,195 gene predictions, 83 splice events, 126 proteins with non-synonymous variants, and 20 isoforms with non-synonymous RNA editing. Quantitative RNA sequencing and proteomics data correlate highly between strains but poorly among each other, indicating extensive nongenetic regulation. We therefore propose a protein-specific "conversion factor" between mRNA levels and protein levels. Our multilevel analysis identified a genomic variant in the promoter of the most differentially expressed gene Cyp17a1, a previously reported top hit in genome-wide association studies for human hypertension, as a potential contributor to the hypertension phenotype in SHR rats.

Conclusion:
These results demonstrate the power of and need for integrative analysis for understanding genetic control of molecular dynamics and phenotypic diversity in a system-wide manner.

P-250.00

NEXTSEARCH: DISCOVERY OF NOVEL TRANSCRIPTS USING NUCLEOTIDE-BASED SPLICE GRAPHS

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Proteogenomics has recently emerged as a new research field that combines knowledge and findings from proteomics and genomics. One common practice is to identify peptides from mass spectrometry data and use such proteomic findings to improve gene annotations.

The previous methods often involve the construction of protein databases consisting of aminP-acid sequences that may well come from a 6 frame translation of whole genome sequences, or translated splice graphs built from next-generation sequencing results.

Representation of protein sequences in terms of amino acids makes it difficult to add alternative splice and/or junction variation events because they may result in frame shifts during translation, necessitating additional translation for each event. In our newly proposed method NEXTSearch (Nucleotide Exon Graph Transcript Search), a splice graph remains as nucleotide sequences. Therefore, it is much easier to add hypothetical novel events such as exon skipping and junction variation than previous methods, achieved by simple insertion of new nodes and/or edges to the splice graph.

Representing protein sequence database as a graph structure consisting of nucleotide sequences requires a proteomics search tool that can conduct searches against nucleotide sequences instead of amino acid sequences. We have modified MODa so that it can traverse a nucleotide splice graph while it interprets tandem mass spectra.

NEXTSearch constructs a compact splice graph by using all transcripts of the Ensembl database and adding hypothetical alternative splice and junction variation events. Then, peptides are identified by applying the modified version of MODa against the nucleotide sequences in the splice graph form. The identified peptides are mapped back to the splice graph in order to confirm any putative transcript events included in the peptides. It is also possible to visualize identified peptides in the UCSC Genome Browser, by converting these peptides into a general feature format.

P-251.00

DISCOVERY OF NOVEL ALTERNATIVE SPLICE VARIANTS IN HUMAN HEPATOCELLULAR CARCINOMA CELLS

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Alternative splicing allows a single gene to produce several protein products and widely expands proteome diversity. Alternative splicing can affect expression, subcellular distribution, and functional activities of a gene product, which is recognized increasingly as a player in cancer development.

We applied a proteogenomics strategy to discover novel alternative splice variants in human hepatocellular carcinoma (HCC) cells. A large data set of mass spectra derived from HCC cells were generate by LTQ-Orbitrap. A searchable database with all possible encoding exon junction was build in house for the discovery of novel exon splicing events. Three search engines were used in database search. 478 novel splicing events was identified by X!tandem, using the home made exon-junction database. Among these 478 novel splicing events, 65 events were re-identified by SEQUSET and 27 events were re-identified by MAXQUANT.

SRM was employed to validate these identified novel splicing events. In all, 14 novel splicing events were confirmed with SRM.

P-252.00

CAPER 3.0: A SCALABLE CLOUD-BASED PIPELINE FOR THE DATA-INTENSIVE ANALYSIS OF PROTEOMIC DATASETS

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Introduction and Objectives£ºOne of the most important goals of Human Proteome Project(HPP) is to identify and characterize at least one protein product and as many post translation modifications, single amino acid polymorphisms and alternative splice variants as possible from human protein-coding genes [J. Proteome Res. 2014; 13:15−20]. Rapid development of high-throughput technologies, such as mass spectrometry and next generation sequencing) ,enables us to perform these detections in a proteogenomic way. [J. Proteome Res. 2014; 13:1−4]. However, proteogenomic approaches often need high-volume computation, with a total of about 100 CPU*hrs for a typical bacterial genome [Bioinformatics 2014; btu051], let alone human genome. In the past two years, we developed CAPER [J Proteome Res. 2013; 12:179-86] and updated it [J. Proteome Res. 2014; 13:99−106] by presenting a powerful toolbox and integrating a configurable workflow system to facilitate the bioinformatic analysis of the heterogeneous datasets from HPP,. However, the previous CAPER system can not tackle the above data-intensive problems.

Results£ºIn CAPER 3.0, we integrated a data-intensive pipline based on Hadoop and the Amazon Cloud Services. The pipline is designed to perform parallel database searching, process RNA-Seq data, map the identified peptides back to the genome and present the results by the CAPER server. Results and Discussion It offers bioinformatic solutions to problems such as discovery of novel gene based on six-frame translation, detection of variant peptides based on dbSNP/COSMIC or custom RNA-Seq datasets and identification of gene isoforms. A local running package is provided to download, with which users could easily launch an arbitrarily sized cluster and deploy a CAPER 3.0 computing environment in the cloud.

Conclusions£ºThe new features of CAPER 3.0 allow users to conduct large-scale protegenomic analysis without maintaining expensive local server clusters. These updates will offer a potential solution to the big data computational problems in HPP.

P-253.00

REVISITING THE IDENTIFICATION OF CANONICAL SPLICE ISOFORMS THROUGH INTEGRATION OF FUNCTIONAL GENOMICS AND PROTEOMICS EVIDENCE

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Introduction and objectives. Canonical isoforms in different databases have been defined as the most prevalent, most conserved, most expressed, longest, or the one with the clearest description of domains or post-translational modification. In this study, we revisited these different definitions of canonical isoforms based on functional genomics and proteomics evidence, focusing on the mouse data.

Methods. We calculated an average functional relationship (AFR) score for each isoform of the gene using a novel functional relationship network-based approach for the mouse [Li, H.-D., et al., bioRxiv, doi: 10.1101/001719 2013]. We defined the highest connected isoform (HCI) as the one with the highest AFR score. The remaining are the non-highest connected isoforms (NCIs) of multi-isoform genes. We then investigated the expression signature of HCIs and NCIs at the transcript and protein levels using RNA-seq and mass spectrometry proteomic data, respectively.

Results and discussion. We showed that 46% of these HCIs are not the longest transcripts. In addition, this approach revealed many genes that have more than one highly connected isoforms. Averaged across 175 RNA-seq datasets covering diverse tissues and conditions, 65% of the HCIs show higher expression levels than NCIs at the transcript level. At the protein level, these HCIs highly overlap with the expressed splice variants, based on proteomic data from eight different normal tissues of the mouse.

Conclusions. Our results suggested that a more confident definition of canonical isoforms can be made through integration of multiple lines of evidence, including highest connected isoforms defined by biological processes and pathways, expression prevalence at the transcript level, and relative or absolute abundance at the protein level. This integrative proteogenomics approach can successfully identify isoforms that are responsible for the canonical functions of genes. This scheme is being extended to the functional networks of the human.

P-254.00

HGNC GENE FAMILY RESOURCES

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HUGO Gene Nomenclaure Committee (HGNC)

The HUGO Gene Nomenclature Committee (HGNC) has assigned unique approved symbols and names to over 38,000 human loci to date. Over 19,000 of these are protein-coding genes, but we also name pseudogenes, phenotypic loci, genomic features and non-coding RNAs. Our website, genenames.org, is a searchable repository of HGNC approved nomenclature and associated resources.

Each locus has an individual "symbol report" which can include links to genomic, proteomic and phenotypic information. Approved gene symbols are based on names describing structure, function or homology, where possible. HGNC also create web pages for specific groups of genes; these "gene families" are mostly grouped together by homology and/or function but sometimes by other shared information such as structure or genomic location. Many of these families have specialist advisors who are experts in that particular area of biology.

Recently we have greatly expanded the scope of these pages, including a hierarchical structure for all the constituent subclasses for some large families such as the G-protein-coupled receptors and zinc finger families. We also now provide links from our individual symbol reports to our improved gene family resources. If you know of a gene family that you think we should include or update, please contact us via hgnc@genenames.org or talk to us during this meeting.

P-255.00
PROTEOMIC ANALYSIS IN PTERYGIUM

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Introduction and objectives: Pterygium is characterized by abnormal fibrovascular corneaconjunctival tissue. In spite of a number of investigations to elucidate the pathogenesis of this ocular surface disease, it still remains poorly understood. The most widely accepted triggering factor involves ultraviolet (UV) radiation and resultant oxidative stress. In this study, protein biomarkers that are differentially expressed in pterygium compared to healthy conjunctiva were identified through a proteomic approach.

Methods: We analyzed proteins of pterygial and healthy conjunctival tissues obtained from 24 patients undergoing pterygium excision. Total proteins of pterygia and healthy conjunctiva were separated by one-dimensional electrophoresis, and protein bands of interest were excised and subjected to LC-MS/MS analysis. A web-based gene ontology program, DAVID, was used to classify 230 proteins that were differentially expressed in pterygial tissues. Western blot and immunohistochemistry were conducted for further validation.

Results and Discussion: Three proteins, aldehyde dehydrogenase, dimeric NADP-preferring (ALDH3A1), protein disulfide-isomerase A3 (PDIA3) and peroxiredoxin-2 (PRDX2), were significantly overexpressed in pterygium and further overexpressed in recurrent pterygium. Result of western blot and immunohistochemistry confirmed the substantial overexpression of these three proteins in pterygium compared to healthy conjunctiva and these proteins were preferentially detected in the basal epithelial layer of pterygium.

Conclusions: We assumed that UV exposure and resultant oxidative stress lead to increased ALDH3A1, PDIA3 and PRDX2 levels, providing resistance to UV-induced apoptosis and a hyperproliferative state. Further studies are required to establish the functional roles of these proteins in pterygium formation.

P-256.00

THE FUNCTIONAL ROLE OF INFLAMMATORY BIOMARKER IN RECURRENT PREGNANCY LOSS

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Recurrent pregnancy loss (RPL) is defined as at least three pregnancy losses in series after 20-28 weeks of gestation. According to our previous proteomic study, ITI was weakly expressed at a molecular weight of 120 kDa, but was highly expressed at a modified molecular weight of 36 kDa in RPL patients. Here, the aim is to identify the role of two different fragments in RPL, and our findings suggest that it may be considered as a cause of pregnancy loss.

Immunoprecipitation assay demonstrates ITI full length form bound with KLK. We performed enzyme-linked immunosorbent assays (ELISA) and cytokine meta-analysis. Expression of KLK resulted in stabilization of ITI in a dose-dependent manner. The cytokines which were secreted from Th1 cells were higher in 36 kDa of ITI transfected culture medium, while Th2 and Th17-related cytokines were shown upregulated in the full-length of ITI-transfected culture medium.

These results were consistent with the data that showed in serum samples. Taken together, the full-length of ITI plays a protective role during the maintenance of pregnancy, but a cleaved form of ITI might be a crucial inflammatory factor which leads to RPL.

P-257.00

SPLICE ISOFORMS IN TRIPLE-NEGATIVE BREAST CANCERS

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The goal of this study is to analyze the specific splice isoforms expressed in triple-negative breast cancers (TNBC). Triple-negative breast cancers comprise a heterogeneous group of cancers; the subtypes have conflicting prognosis. The original mass-spectrometric raw files from 126 human TNBC samples were downloaded from the EMBL_EBI PRIDE Archive.

The samples were derived from patients with good and poor prognoses. Using our analysis pipeline, we are in the process of identifying known splice isoforms and novel peptides from these samples. Our goal is to compare the different isoforms expressed in these two groups. Since the specific functions of the splice isoforms are not yet known, we use computational tools including sequence comparisons, structure, function, and interaction predictions to annotate these proteins.

This project is part of the C-HPP Chromosome 17 work on breast cancers and the continuation of a stream of studies on differential expression of splice isoforms of key proteins in pathways important to subtypes of human breast cancers [Menon et al, 2011, 10:5503-11; Liu, S, et al. JPR 2013, 12:45-57; Menon, R, et al, JPR 2014, 13:212-27; Omenn, GS, et al, JPROT 2014; May 5. pii: S1874-3919(14)00179-1].

Topic

5

Proteomics of obesity and related metabolic liver disorders. Metabolomics

OP031 - OMICS IN NUTRITION AND ITS PERSPECTIVES FOR PEDIATRICS RESEARCH AND HEALTHCARE

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Introduction and objectives

Proteomics and Metabonomics in nutrition are established platforms for discovery and first-stage validation of biomarkers, and studying interactions between genetics, environment, and health. High protein intake observed with infant formula feeding, in excess of metabolic requirements, may predispose to an increased obesity risk in later life. The present study investigated the effect of low or high protein intake on the metabolism of term infants in the first year of life.

Methods

We assessed the impact of breast- and formula-feeding (differing in protein content) on the metabolism of term infants from overweight and obese mothers. From birth to 3 months of age, infants received exclusively either breast- or starter formula-feeding, and until 6 months exclusively either a formula designed for this study, or breast-feeding. From 6 to 12 months, infants received complementary weaning food. Metabonomics was conducted on the infants' urine and stool samples collected at the age of 3, 6 and 12 months.

Results and Discussion

Infants receiving any of the administered formula had higher fecal levels of short-chain fatty acids and amino acids. Urine metabonomics revealed a relationship between bacterial processing of dietary proteins and host protein metabolism stimulated with increasing protein content in the formula. Moreover, formula fed infants were metabolically different from breast-fed infants, at the level of lipid and energy metabolism. A dose dependent metabolic response was observed with the protein content in formula-fed infants, who differed metabolically from breast-fed infants.

Conclusions

Non-invasive application of metabolomics to urine and stool enables the monitoring of the metabolic response and nutritional requirements of infants receiving different types of feeding during the first year of life. We expect the development of molecular phenotyping and system biology in neonatology to deliver mechanistic hypotheses that could be targeted with personalized nutritional intervention for preventive healthcare in early life.

OP032 - CHARACTERIZATION OF BLOOD PLASMA METABOLOME OF HEALTHY MEN BY MASS SPECTROMETRY APPROACH

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Nowadays, scientists' efforts are directed to apply omics techniques to create new tests for the diagnostics of diseases, to assess risk of their development and to predict the response of the patient's organism on the treatment. However, omics technologies didn't reach yet a stage of clinical application. In particular it concerns omics- tests based on metabolomics, the youngest omics science, when a comprehensive set of low-molecular substances of biological object can be measured in a single run. In order to implement such analysis in clinic, it is necessary to define reference ranges (i.e. norm) for blood plasma metabolites on the basis of the metabolomic analysis of blood plasma samples collected from the healthy volunteers.

In our study, blood plasma samples were collected from 50 volunteers (21-34 years old) selected after advanced medical examination with health standards typical of cosmonauts. The state of health was confirmed by the results of biochemical, hematologic and immunological tests for each sample. After the removal of blood plasma proteins with methanol, the remaining plasma metabolite fractions were analyzed directly using electrospray hybrid quadrupole time-of-flight mass spectrometer (maXis Impact, Bruker Daltonics Gmbh) in the mode of detection of both positive and negative ion charge. Mass spectra were obtained with resolution up to 1-3 ppm using of losartan (Mr=411.95) as internal standard.

On the average 4000 metabolite's ions were detected in each blood plasma sample. For intensity data of metabolites mean values, standard deviations, CV, ranges (min and max) were defined. The most abundant metabolites were identified using an accurate mass tag and an isotope pattern method. The obtained data for blood plasma metabolites (the metabolomic passport of blood plasma of healthy men) allows detecting individual differences in patients metabolome induced by diet, evaluating age-related changes, detecting xenobiotics in the blood of patients.

OP033 - TOWARDS SPATIAL METABOLOMICS: DATABASE DRIVEN METABOLIC ANNOTATION FOR IMAGING MASS SPECTROMETRY

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Introduction

Imaging Mass Spectrometry (imaging MS) can capture spatial localization of various molecules including proteins, peptides, and metabolites. Imaging of metabolites is essential to understand the function of a protein, by screening for metabolites that accumulate after protein modification, for example, after enzyme inhibition. The recently emerged high-resolution imaging MS is a promising technique for spatial metabolomics. However, molecular annotation of large datasets delivered by this technique is still a major challenge. We present a novel database-driven and high-throughput approach to generate hypotheses on metabolites represented in high-resolution imaging MS data.

Methods

High-resolution MALDI-FTICR imaging MS data of rat brain sections was acquired on a 12T solarix XR (Bruker Daltonics). Rather than trying to identify molecular species of millions of individual peaks, we restricted each imaging dataset to signals potentially corresponding to molecules from metabolic databases (HMDB, KEGG, LIPID MAPS, ChEMBL). This substantially reduced the search space to metabolites that are plausibly within the sample. Sum formulas of all metabolites were considered and corresponding ion images were generated. For each sum formula, a presence score was calculated which integrates various spatial and spectral characteristics. Finally, hypothetic annotations the highest ranked sum formulas to metabolites could be propagated.

Results

Hundreds of sum formulas were detected as corresponding to metabolites present in the tissue sections. Comparison of results for different biological and technical replicates confirmed the robustness of the approach. The evaluation was performed using both the software for sum formula prediction (SmartFormula, Bruker Daltonics) and MS/MS identification and confirmed the potential of our approach to provide relevant hypotheses on metabolites present in a tissue section.

Conclusions

Our novel database-driven approach to spatial metabolomics can perform hypothetical metabolic annotation for high-resolution imaging MS and propose hundreds of metabolites present in the tissue sections.

OP034 - PLASMA PROFILING REVEALS NOVEL BIOMARKERS FOR DRUG-INDUCED LIVER INJURY IN HUMANS

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Drug-induced liver injury (DILI) is a major concern in drug development where it is the single leading cause for termination of drug development and safety-related withdrawal of approved drugs from the market. ALT is the current standard biomarker, but there is a need for more specific, sensitive and predictive markers.

In this study, we aimed at finding novel DILI biomarkers by analysing in total 915 serum and plasma samples from 208 individuals across three independent cohorts with antibody suspension bead arrays.

Initially, almost 5000 antibodies from the Human Protein Atlas were used to profile baseline serum samples from an acetaminophen study in healthy volunteers (HV APAP). Hits from this approach together with other proteins interesting in the context of liver toxicology were used to compose a targeted DILI antibody array of 300 antibodies targeting 260 proteins. This array was used to screen the complete HV APAP set (n = 355), plasma from an HIV/tuberculosis treatment study (n = 472) and serum from a confirmatory cohort (n = 88).

By the application of highly multiplexed antibody suspension bead arrays, we have identified two proteins (Marker 1 and 2), which were elevated in cases compared to controls in all three clinical cohorts. In both longitudinal screening cohorts, Marker 1 was elevated already in baseline samples, indicating a potential predictive use. Marker 2 showed significant elevation after treatment initiation. Interestingly, this marker seemed to respond more rapidly compared to ALT in a number of individuals. Both markers could be verified in the third confirmatory cohort.

In summary, two candidate biomarkers were identified to be elevated in DILI cases on a variety of drug treatments. Additional clinical studies with other hepatotoxic drugs will be needed to replicate and further validate the results of this study.

P-258.00

A COMPREHENSIVE AND SCALABLE AUTOMATED WORKFLOW FOR HUMAN PLASMA PROTEOMICS AND ITS APPLICATION TO THE DIOGENES CLINICAL STUDY

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Introduction and objectives

Blood is the body fluid most used in routine clinical practice. Protein biomarker discovery in a high number of samples results in increased significance of the candidates before further validation. We have developed and validated an automated workflow and applied it to the proteomic analysis of more than 1000 human plasma samples from the human dietary intervention study "DiOGenes" to discover predictive biomarkers of weight maintenance.

Methods

Human plasma samples were obtained from the DiOGenes study (http://www.diogenes-eu.org/).[1] Fourteen abundant plasma proteins were removed with multiple affinity columns and HPLC systems. Buffer exchange was performed with SPE. Reduction, alkylation, digestion with trypsin, and labeling with isobaric tags were carried out on a liquid handler. Two SPE steps for sample purification completed the sample preparation. Samples were analyzed with RP-LC MS/MS.

Results and Discussion

We first optimized the analysis of human plasma samples in both small pre-clinical and large clinical studies. Several iterative evaluation and validation steps were performed. In particular, proteome coverage, recovery, reproducibility, and quantitative trueness and precision were assessed at every stage of the sample preparation and analysis. The developed robotic platform was shown to provide better quantitative trueness and precision than manual operation. An average coefficient of variation of 8% was obtained on the protein quantitative values for 96 identical samples prepared with the automated workflow. At an FDR of 5%, protein ratios below 0.769 and above 1.3 represented true quantitative differences.

The automated proteomic platform was then applied to the DiOGenes study. Plasma samples from 500 donors collected at baseline and at the end of the nutritional intervention were analyzed within 15 weeks.

Conclusions

A robust proteomic platform to analyze plasma samples with appropriate throughput was developed and applied to a large-scale human clinical study.

P-259.00

MATURE ADIPOCYTE PROTEOME REVEALS ALTERED METABOLIC AND REGULATORY ACTIVITY BETWEEN LEAN, OVERWEIGHT AND OBESE: USING PROTEOMIC APPROACH AND NETWORK ANALYSIS.

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Introduction: The human body maintains itself in dynamic equilibrium adapting to environmental changes. Over-nutrition and decreased physical activity, dramatically increased prevalence of overweight and obesity. The normal metabolic function of adipose tissue becomes overburdened leading to increased risk of chronic metabolic diseases. We undertook to study the proteome from lean (control), overweight (OW) and obese (OB) subjects to gain insight into how changes with caloric excess affect protein expression and metabolic pathways in adipose tissue, using 2D-DIGE and MALDI-TOF.

Materials and Methods: Subcutaneous abdominal adipose tissue was obtained from 22 age matched healthy female patients undergoing elective liposuction and divided into 3 groups according to their BMI : Lean (N=7, BMI 23.3±0.4), Overweight (N=8, BMI 27.9±0.6) and Obese (N=7, BMI 44.8±3.8). Mature adipocytes were harvested from adipose tissue and the total proteins isolated and separated by 2D-DIGE. Differentially abundant proteins between the groups were identified by MALDI-TOF and entered into Ingenuity Pathway Analysis (IPA).

Results: Protein spots showing significant changes in abundances between lean, overweight and obese (Progenesis statistical software (ANOVA-test p1.5) were selected for further analysis.
Compared to lean, proteins up-regulated in OW and OB were 36 and 46; down-regulated were 9 and 43 respectively. The OB additionally had decreased abundances in proteins involved in lipid and carbohydrate metabolism. IPA identified pathways relating to cell to cell signaling interaction in OW, and lipid metabolism, small molecule biochemistry and cancer in OB with highest score.

Conclusion: Our results show that mature adipocytes from OW subjects increasingly express proteins supporting expansion of adipose tissue, whereas in OB they participate in defense mechanisms against excessive fat accumulation, repressing both lipogenesis and lipolysis. The study of differentially abundant or lowered proteins between the groups provides a clearer picture about involvement of different pathways at different levels of calorie excess.

P-260.00

HEPATIC SILAC PROTEOMIC ANALYSIS OF THE PANDER TRANSGENIC MOUSE REVEALS NOVEL LIPOGENIC PATHWAYS

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PANcreatic DERived factor (PANDER) is a member of a superfamily of FAM3 proteins that are uniquely structured and strongly expressed from the endocrine pancreas and co-secreted with insulin. Animal models have indicated that PANDER can induce a selective hepatic insulin resistant (SHIR) phenotype whereby insulin signaling is blunted yet lipogenesis is increased.

The complexity of the biological networks involved with this process warranted the employment of quantitative MS based proteomic analysis using Stable Isotope Labeling by Amino Acids in Cell Culture (SILAC) to identify the global proteome differences between the PANDER transgenic (TG) overexpressing murine model to matched wild-type mice under three metabolic states (fasted, fed and insulin stimulated). The "spike-in" process was employed by equal addition of lysate obtained from livers of heavy L-Lysine (13C6, 97%) fed mice to the mice liver protein lysate (PANTG and WT) for relative quantitative analysis. Upon acquisition of the dataset by use of liquid chromatography tandem mass spectrometry (LC MS/MS, LTQ Orbitrap), geometric means and Uniprot Protein identification numbers were uploaded to Ingenuity Pathway Analysis (IPA) to reveal the effect of PANDER on hepatic signaling. IPA identified lipid metabolism and fatty acid synthesis as top cellular functions differentially altered in all metabolic states. Several molecules with a role in lipid metabolism were identified and include FASN, ApoA1, ApoA4, SCD1, CD36, CYP7A1 and ACC. Furthermore, central to the differentially expressed proteins was the revealed activation of the liver X receptor (LXR) pathway.

In summary, our SILAC proteomic approach has elucidated numerous previously unidentified PANDER induced molecules and pathways resulting in increased hepatic lipogenesis. In addition, we have demonstrated strong utility of this approach in comprehensively phenotyping animal models of hepatic insulin resistance. Taken together, PANDER strongly impacts hepatic lipid metabolism and may induce a SHIR phenotype via the LXR pathway.

P-261.00

INVESTIGATION OF ADIPOCYTE PROTEOME DURING THE DIFFERENTIATION OF BROWN PREADIPOCYTES

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Brown adipocytes oxidize fatty acids to produce heat in response to cold or caloric overfeeding. The motivation and function of the development of brown fat may thus counteract obesity, though this remains uncertain.

We investigated the brown adipocyte proteome by two-dimensional gel electrophoresis followed by mass spectrometry. Comparative analyses of proteins focused on total protein spots to filter differentially expressed proteins during the differentiation of mouse primary brown preadipocytes. A Western blot analysis was performed to verify the target proteins. The results indicated that 10 protein spots were differentially expressed with significant changes, including the three up-regulated proteins of prohibitin, hypoxanthine–guanine phosphoribosyltransferase, and enoyl-CoA hydratase protein; the 5 down-regulated proteins of triosephosphate isomerase, elongation factor 2, á-tropomyosin slow, endophilin-B1, and cofilin-1 (CFL1); and the two unequivocally expressed proteins of peroxiredoxin-1 and collagen á-1(i) chain precursor.

We found that during brown adipogenesis, CFL1 has an inhibitory effect on brown adipocyte differentiation. The overexpression of CFL1 inhibited the brown fat deposition and repressed the brown marker genes UCP1, PRDM16, PGC-1á and PPARã via actin dynamics and polymerization. These observations may be novel findings that bring new insight into the detailed mechanisms of brown adipogenesis and identify possible therapeutic targets for anti-obesity.

P-262.00

A STUDY OF TWO SEQUENTIAL MEDIA FOR IVF

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Culture media for the support of human embryos after in vitro fertilisation (IVF) and before embryo transfer has been a focus of considerable interest over the past decade. These sequential media were formulated according to the changing physiology and metabolic requirements of the human embryo and led to improvements in human IVF outcomes. In this study, we performed proteomic analysis of the two kinds of commercial media (Sydney IVF medium and VitroLife G medium).

Human serum albumin is the absolutely abundant protein in the medium sample, so we choose multi-filter cut-off workflow for sample preparation and albumin removal. Firstly, Amicon 30kD cutoff spin column is applied to separate one medium sample into 3 fractions: Filtered, Concentrated, Eluted. Then, every fraction is reduced, alkylated and digested with Lys-C or Glu-C. Secondly, each fraction is filtered with 10kD cutoff spin column like in the first step, and these fractions were collected and labeled: Filtered, Concentrated, Eluted. Altogether nine fractions of each sample are collected, and digested with trypsin. Finally, peptides are analyzed by nanoHPLC–MS/MS. It is interesting that, beside the human serum albumin, other proteins involved in embryonic development are detected in the samples except. For example, there are 30 identified proteins in Sydney Cleavage medium, which are mostly plasma proteins.

Because albumin is an important transport protein, the presence of other plasma proteins could be explained with the "contamination" during the HSA production. However, it is still unknown which of these unpredicted plasma proteins have an effect on IVF, but it is an important clue for the next analysis of the embryonal secretome during IVF procedure.

P-263.00

INTAKE OF ISOFLAVONES ATTENUATES THE DEVELOPMENT OF METABOLIC SYNDROME ASSOCIATED WITH CONSUMPTION OF A WESTERN-STYLE DIET IN C57BL/6J MICE

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Consumption of a Western-style diet (WD), characterized by high intake of high-fat dairy products and sweet items, can contribute to obesity and metabolic syndrome, and increases the risk of type 2 diabetes and non-alcoholic fatty liver disease. Recent studies have suggested that isoflavones have beneficial effects on metabolic syndrome-related disorders, but the underlying mechanisms are unclear.

Here we investigated the mechanism of the effect of isoflavones on metabolic syndrome component traits. C57BL/6J mice were fed for 8 weeks on AIN93G (control) or a high-fat/cholesterol/sucrose WD, both with or without 0.05% isoflavones. The increase in ALT, glucose, liver fat accumulation and triglyceride in liver of mice fed with the WD were all improved by isoflavones treatment. The expressions of hepatic genes related to steatosis and myofibroblasts, such as acetyl-coA carboxylase and alpha-smooth muscle actin, were also normalized by isoflavones. Further, isoflavones supplementation showed a tendency to improvement in the increases in oxidative stress (4-hydroxy-2-nonenal-modified proteins) and glycation (methylglyoxal-modified proteins) in WD-fed mice.

In mice fed with a WD, intake of isoflavones reduces liver fat accumulation and improves systemic parameters related to metabolic syndrome, probably mainly through decreasing oxidative stress and reducing the expression in the liver of genes related to steatosis.

P-264.00

DIAGNOSING IMPAIRED GLUCOSE TOLERANCE USING DIRECT MASS SPECTROMETRY OF BLOOD PLASMA METABOLITES

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Currently, the glucose tolerance test (GTT) is the primary test and 'gold standard' for a diagnosis of impaired glucose tolerance (IGT). In addition, the GTT represents an important tool for identifying a prediabetic state and cardiovascular risk factors. However, this test has low reproducibility, is time consuming (takes 2 h), and some people may get sugar shock during test. Therefore, a more rapid and reproducible test for diagnosing IGT is needed.

The goal of our study was to evaluate the capacity for mass spectrometry of blood plasma to diagnose IGT. To this end, blood plasma samples from control subjects (n = 30) and patients with IGT (n = 20) were treated with methanol and low molecular weight fraction were then analyzed by direct mass spectrometry. A total of 51 metabolite ions strongly associated with IGT were detected. The area under a receiver operating characteristic (ROC) curve (AUC) for diagnosing IGT that was based on an analysis of these metabolites was 0.93 (accuracy 90%, specificity 90%, and sensitivity 90%). The associated reproducibility was 85%. The metabolites identified (among them fatty acids, amides of fatty acids, butanediol, phosphoglycolic acid, p-cresol sulfate, ornithine, and phosphatidylcholine) were consistent with risk factors previously associated with the development of diabetes.

Thus, direct mass spectrometry of blood plasma metabolites represents a rapid, single-step, and reproducible method for the analysis of metabolites. Moreover, this method has the potential to serve as a prototype for clinical analyses that could replace the currently used glucose tolerance test with a more patient-friendly assay.

P-265.00

A PROTEOMIC APPROACH TO IDENTIFY BIOMARKERS OF FOOD CRAVING AND RESPONSE TO BARIATRIC SURGERY IN MORBID OBESITY

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INTRODUCTION: Obesity results from a combination of causes which could differentially contribute to the disease and treatment outcomes in any given patient. Food addiction has been proposed to be one of these causes, being craving one of its main correlates. This work tries to identify protein biomarkers that can help to follow the evolution of morbid obese patients after bariatric surgery and can contribute to define different endophenotypes of obesity related to food addiction.

METHODS: We have compared the serum proteome of 22 obese patients (BMI >40 kg/m2), before and 1 year after bariatric surgery, and their corresponding 22 normoweight matching controls. We also compared the serum proteome of obese patients with high and low food craving according to the FCQT-PC questionnaire. Serum samples were first depleted of the 12 most abundant serum proteins. Then protein expression was analysed by 2-D electrophoresis. We used 17 cm, 3-10 nonlinear pH range IPG strips for the first dimension; 180x200x1 mm, 12 % polyacrilamide gels for the second dimension; silver nitrate for protein staining and PDQuest software for spot detection, matching and statistical analysis. We selected proteins showing at least a 2 fold significant change (p

RESULTS: When comparing the serum proteome of obese patients before bariatric surgery with their normoweight matching controls we found significant differences in the expression levels of 19 proteins, 15 of these changes were reversed 1 year after bariatric surgery. After comparing the proteome of patients with high and low food craving we only detected significant changes in the expression of two proteins.

CONCLUSIONS: The proteins identified in this study could be good biomarkers of response to bariatric surgery as well as biomarkers of food addiction in obese patients.

P-266.00

QUANTITATIVE STUDY OF HUMAN ADIPOSE TISSUE SECRETION BY CILAIR SHOWS LOCATION-SPECIFIC DIFFERENCES BETWEEN VISCERAL AND SUBCUTANEOUS FAT DEPOTS

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Introduction

Protein secretion studies of the differentiated adipose tissue depots in the organism are considered a fundamental step to understand the energy regulation mechanisms taking place in this tissue; and essential to elucidate the relationship between the amount of specific depots, like visceral fat, and the development of several metabolic diseases such as type 2 diabetes.

In this study we use CILAIR technology (comparison of isotope-labelled amino acid incorporation rates) for the quantification of visceral (VAT) and subcutaneous adipose depots (SAT) secreted proteins including the identification of different secreted patterns among them.

Methods

The experiments were performed 4 times by incubating VAT and SAT explants obtained from obese patients after bariatric surgery. After 26.5h in free Lys and Arg culture medium, in order to reduce at minimum the amount of [12C6]Lys and [12C6]Arg, the explants were incubated 72h in culture medium containing [13C6,14N2]Lys and [13C6,14N2]Arg. The sample was divided in 2 fractions to perform 2 different trypsin digestion methods: in-gel and in-solution. The resulting peptides were separated and identified by liquid chromatography - mass spectrometry LC-MS/MS (Nano-HPLC eksigen-ABSciex and MALDI-TOF/TOF-ABSciex). The quantification was performed using the software Protein Pilot (ABSciex) that detects the heavy/light peak pairs and calculates the heavy/light ratios based on the peak areas.

Results and Conclusions.

In this work we identified know differentially secreted proteins such as adiponectin, interleukin-6 or gelsolin; as well as new proteins never associated to adipose tissue. These results attribute different endocrine roles to the most important adipose tissue depots in the organism, showing specific secretion patterns between TAV and TAS. On the other hand, the CILAIR technology probes to be a powerful tool in quantitative studies and a good alternative to SILAC approaches in secretion studies of tissue explants due to its specificity, sensitivity and reproducibility obtained in our adipose tissue secretome analysis.

P-267.00

EVALUATION OF INTRACELLULAR TRACE ELEMENTS, MRNA EXPRESSION AND PROTEIN ABUNDANCES IN HUMAN LIVER

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Micronutrients play an important role in human metabolism and either a deficiency or an excess of micronutrients might have adverse effects and lead to a disease state. At least ten trace elements are essential in humans (Mn, Fe, Co, Ni, Cu, Zn, Se, Mo, I, Cr), and their physiological and cellular concentrations are kept in homeostasis by a number of membrane transport proteins and metal-binding proteins. In this study, we evaluated intracellular trace elements, mRNA expression and protein abundances in human liver.

To achieve this purpose, we performed quantitative targeted proteomics to assess the abundance of proteins related to micronutrient homeostasis in human liver samples, and correlated them with intracellular metal concentrations. The study was complemented with the measurement of expression levels of genes related to the homeostasis of trace elements by quantitative real-time PCR, and almost 200 polymorphisms (SNPs) within and around these genes were genotyped in the same samples. These different datasets were correlated with the intracellular concentration of micronutrients.

We have identified several protein and genes clusters of metal-binding proteins showing abundances that correlated with the measure of intracellular metal concentrations, and in addition, we were able to identify several polymorphisms that modulated the mRNA expression of some proteins involved in the regulation of the micronutrient homeostasis. This dataset provides a complete quantitation study of mRNA expression levels, genetic polymorphisms and proteins related to intracellular metal homeostasis, which is essential to better understand the interconnection between the regulation of mRNA expression levels, protein abundances and intracellular metal abundances.

P-268.00

COMPREHENSIVE PROTEOMIC ANALYSIS OF 3D HUMAN LIVER SPHEROIDS FOR DRUG TOXICITY INVESTIGATION

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Drug-induced hepatotoxicity is currently one of the main reason for market withdrawal of drugs and exclusion of drugs in clinical phases. Better models than in vitro 2D cells and animal subjects are needed for testing new drugs before entering human clinical trials. Recently, 3D cell spheroids have shown to be suitable for testing chronic exposure toxicity compared to 2D cells, due to their longer lifespans and greater stability. Besides, their 3D architecture display more organ-like function than conventional monolayer cell cultures.

To demonstrate the relevance of the spheroids for investigating the toxicity of drug compounds at the proteome level, we applied label-free quantitative shotgun proteomics for the generation of protein abundance profiles from 3D liver spheroids treated with different concentrations of acetaminophen.

Proteins were extracted from acetaminophen-treated and non-treated liver spheroids using the high-intensity focused ultrasound in biochemical triplicates. After the cell lysis, protein extracts were subjected to filter aided sample preparation (FASP) for protein digestion. The peptides were analyzed on an Orbitrap Fusion instrument in data dependent mode. MS/MS spectra were searched using Mascot and validated using Scaffold.

We demonstrated that very little starting cell material (i.e 12 liver spheroids) were required to map out a large fraction of the proteome (i.e 3000 proteins) in a single MS injection. Furthermore, high reproducibility in protein abundances was obtained between biochemical replicates and across all tested acetaminophen concentrations. Thus we generated quantitative profiles for several thousands of proteins in response to acetaminophen treatment, revealing hundreds of proteins affected by that drug. Several protein pathways were identified, providing a better understanding in the mechanism of drug toxicity.

In conclusion, the results demonstrated that proteomic analysis of 3D human liver spheroids are very suitable for drug toxicity investigation and can be applied to other 3D tissue models.

P-269.00

SERUM PROTEOMIC PROFILE OF MEXICAN PATIENTS WITH "DIABESITY"

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Mexico has been subjected of an epidemiological transition from malnutrition to a country dominated by obesity, diabetes and other nutrition-related non-communicable diseases. This transition is driven by rapid urbanization, nutrition transition, and increasingly sedentary lifestyles. In Mexico, since 2000 diabetes has been the primary cause of death among women and men followed by coronary heart diseases. Recent advances in genome-wide association studies have contributed substantially to our understanding of diabetes pathophysiology, but those studies are insufficient to explain ethnic differences in "diabesity" risk.

Proteomics is a powerful tool that will help to understand the association between the obesity state and diabetes related with the ethnic responses. For this, the aim of the present study was to analyze the serum proteomic profile of Mexican Diabetics patients depending on their nutritional state. Groups were defined according to the Body Mass Index of each patient into five categories: Normal weight, Overweight, Obese class I, Obese class II/III and Control. Blood samples from all groups were collected under informed consent. Serum proteins were depleted using MARS14 columns. Abundant and non-abundant proteins were collected for DIGE labeling with Cy-fluorescent dyes and 50 μg labeled protein per dye/gel strip (24 cm pH 4-7 linear gradient strips) were separated by IEF. Second dimension was carried out in an Ettan DALT Six separation unit. Gels were scanned and DIGE images were analyzed with DeCyder 2D differential analysis software (v7.2).

Differentially accumulated protein spots were cut from preparative gels generated using unlabeled protein and stained with Coomassie Blue. Spots were digested with trypsin and LC-MS/MS analysis was performed (nano-UPLC SYNAPT-HDMS). Spots have been identified by using MASCOT search engine tool and will be integrated to the Human Diabetes Proteome Project datasets.

P-270.00

DISSECTION OF METABOLIC PATHWAYS IN THE DBDB MOUSE MODEL BY INTEGRATIVE PROTEOME AND ACETYLOME ANALYSIS

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Introduction

Alterations of protein modification in Diabetes mellitus type II have been implicated in modulating immunological and metabolical pathways and therefore have a crucial role in maintaining energy homeostasis. It has been shown that reversible lysine acetylation represents a prominent regulatory mechanism for mainly all metabolic pathways. In this study, the diabetes mouse model DbDb (Diabetes/Diabetes) was used to analyse protein abundances and acetylation levels in liver tissue under steady state conditions. This mouse model has a mutation in the leptin receptor and shows a severe type 2 diabetes phenotype. Several pathways, including fatty acid and carbohydrate metabolism, as well as insulin signalling are affected. The identification of protein changes and acetylated peptides will help to better understand the pathogenesis of diabetes mellitus type 2.

Methods

For the proteome analysis proteins were fractionated using SDS-PAGE and digested in-gel using LysC. Lysine-acetylated peptides were immunoprecipitated using anti-acetyllysine antibody. Quantification was performed with the 13C-Lys6 labeled liver tissue from the SILAC mouse and mass spectrometric analysis was performed with a QExactive mass spectrometer.

Results and Discussion

We identified more than 8500 liver proteins, including 407 significant regulated proteins. Besides significant changes of mitochondrial proteins we also found downregulated proteins in peroxisomal and nuclear compartments. Moreover, we quantified over 1604 lysine acetylation sites in and we observed a fraction of 33 regulated acetyl-lysine sites on 20 proteins. Our analysis confirmed altered acetylation levels of mitochondrial proteins, including Atp5b, Hmgcs2, and CPS1. In addition, we found several novel sites on nuclear protein and signalling molecules.

Conclusions

Taken together, our data demonstrate clear changes on protein and acetylation level on several metabolic and signalling proteins, reflecting diabetes-associated regulations. Our dataset will provide a versatile resource to connect protein abundances, site-specific acetylation sites, and metabolic pathways under insulin resistance conditions.

P-271.00

PROTEOMIC ANALYSIS OF SERUM INDICATE FIBRINOGEN AS POTENTIAL BIOMARKER IN EARLY DIAGNOSIS OF HEPATOLENTICULAR DEGENERATION IN PEDIATRIC AND ADULT SUBJECTS

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Introduction: Autosomal recessive defects in ATPase gene (ATP7B), known as Wilson's disease (WD), leading to increased accumulation of copper in liver, cornea and basal ganglia and cause liver cirrhosis or variable neuropsychological manifestations. Use of proteomic tools to support the early diagnosis of WD (hepatolenticular degeneration) in humans are still challenging issue.

Methods: Proteome profiles of WD subject's serum were separated by 2-D gel electrophoresis and identified by peptide mass fingerprinting (MALDI-MS). Western blotting and immunohistochemistry were used to validate the protein expression in situ. Dot-blot was used to prove the high-level expression of fibrinogen in other homozygous and heterozygous WD subjects compared to age mathed healthy controls (HC) and patients with blood stasis indicating cardiovascular abnormalities (BSC). The 2-DE results were verified by the partial N-terminal amino acid residues sequencing and protein database searching. Results/Disscussion: Total 31 protein spots with difference between WD and HC subjects were counted and identified. Specifically, 22 protein spots were found in more abundant quantity in the serum from the WD subjects. Thus, 4 protein spots were covering fibrinogen-γ family, 2 spots - fibrinogen-α group and single protein spot was classified as the fibrinogen-β chain. The Apo-family protein spots (A1, clusterin, E) and acute phase proteins (α2-macroglobulin and haptoglobin-α chain) have been showed. Proteins related with etiology of WD as antithrombin, complement C3, serotransferrin and other binding proteins were confirmed.

Conclusions: The liver cirrhosis and oxidative stress caused by WD may account for the abnormal increase of fibrinogen (even more than observed in BSC subjects) and further studies are needed to clarify whether fibrinogen expression may be a useful biomarker in the early diagnosis of WD.

P-272.00

SUBTRACTIVE PROTEOMIC ANALYSIS ALLOWS DEFINITION OF LIPID DROPLET ASSOCIATED PROTEIN (LDP) AND UNRAVELS DIFFERENTIAL LIVER LDP PROFILING IN FATTY LIVER DISEASE

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Eukaryotic cells store neutral lipids in cytoplasmic lipid droplets enclosed in a monolayer of phospholipids and associated proteins. Growing evidence has demonstrated that LD associated proteins play important roles in the pathogenesis and development of fatty liver disease, which features abnormal LD accumulation in hepatocytes.

However, it is remarkably unclear about liver LDP profiles and their alterations in fatty liver diseases. In this study, we compared liver proteome and lipid droplet-associated sub-proteome to identify LDP, and to quantify their changes in a diet-induced-obesity (DIO) fatty liver. Among 5000 quantified proteins, 101 of which were enriched greater than 10 folds in LD. Differential profiling of LDP proteome demonstrated altered expression profiles in whole proteome and LDP sub-proteome in DIO mice fatty liver. The function of altered LDP candidates were further validated in vivo with adenovirus-mediated gene silencing.

S100 Knockdown accelerates liver steatosis induced by high fat diet. This study has identified LDP sub-proteome of liver for the first time and suggested the potential targets for fatty liver disease therapy.

P-273.00

HIGH FAT DIET INDUCED ISOFORM CHANGES OF THE PARKINSON'S DISEASE PROTEIN DJ-1

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Introduction and objectives

Recently, an altered redox balance has been suggested to be causative for the development of major diseases like type 2 diabetes, cancer and neurodegerenation. The protein DJ-1 has been implicated in all these types of diseases and identified by us to be posttranslational modified in mouse hypothalamus tissue upon high-fat diet resulting in a shift o more acidic isoforms. These isoforms can be liked to DJ-1's redox state and therefore, we propose DJ-1 as sensitive sensor for the cellular redox state. We developed a capillary isoelectric focusing immunoassay to characterize the redox state of DJ-1 in different brain regions and tissues in a mouse model of obesity.

Methods

We used capillary isoelectirc focusing immunoassay as well as mass spectrometry to analyse Dj-1 isoform in several mouse tissues and after different feeding times of high-fat and control diet.

Results and Discussion

Large scale validation of DJ-1 isoforms in individual samples and tissues confirmed a shift in the pattern of DJ-1 isoforms towards more acidic isoforms in several brain and peripheral tissues after feeding a high-fat diet for 10 days. The effect was less pronounced after prolonged feeding times. Furthermore, the extent of the high-fat diet-induced shifts correlated with the metabolic rate of the respective tissue: Metabolically active tissues (muscle > brain) showed a more pronounced shift compared to tissues exhibiting lower metabolic activity (liver > adipose tissue).

Conclusions

DJ-1 is a common physiological sensor involved in both nutrition-induced effects and neurodegenerative disease states.

P-274.00

GAINING INSIGHT INTO OBESITY AND RELATED PATHOLOGIES BY HIGH-THROUGHPUT PROTEOMIC ANALYSIS OF HUMAN ADIPOSE TISSUE

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Introduction
While transcriptomic studies on human obesity are well documented, owing to technical hurdles little is known about the adipose tissue proteome. The few proteomic studies reported so far are focused on a variety of experimental models of obesity and cultured adipocytes, and most of them have resorted to two-dimensional electrophoresis (2-DE) for protein separation, which severely restricts the fraction of the proteome amenable to quantitative analysis.

Objectives
We aim to investigate the differential expression of proteins using stable isotope labeling (SIL) and high-throughput liquid chromatography/mass spectrometry (LC-MS) analysis of adipose tissue and adipocytes from healthy obese subjects and obese subjects with severe co-morbidities. The effects of gender and age on the proteome will be also assessed.

Methods
Proteins will be extracted from whole fat biopsies collected from morbid obese patients who have been submitted for gastric bypass surgical procedures. The iTRAQ methodology will be used for peptide SIL, and the tagged peptides will be separated with high resolution and mass analyzed based on high-throughput LC-MS.

Results and Discussion
Based on LC-MS, we have recently widened the proteome coverage in human whole adipose tissue and adipocyte samples, where nearly four thousand proteins were reliably identified. We have also compared protein abundance changes in different fat depots from a number of individuals, finding that high throughput LC-MS provides a much more robust analytical platform as compared to 2D-based methods. As soon as the necessary number of fat samples has been collected, we will carry out the differential protein expression study comparing healthy obese and obese individuals with severe co-morbidity.

Conclusions
The combination of SIL and high-throughput LC-MS proteomic approaches in obesity will improve our knowledge of human adipose tissue, thus contributing to understand the molecular mechanisms of the disease and, in particular, the role of omental fat in abdominal obesity-associated co-morbidities

P-275.00

A LABEL-FREE PROTEOME WIDE BINDING STUDY TO REVEAL FUNCTIONALLY RELEVANT SNP VARIANT DNA BINDERS

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Genome-wide association studies identified numerous risk loci for various diseases. Recent large scale next generation sequencing approaches based open chromatin and novel bioinformatics address a major challenge of human genetics, the identification of cis-regulatory variants. However, to further enhance our understanding of gene regulation mediated by cis-regulatory variants, it is essential to delineate the precise molecular mechanism underlying cis-regulatory variants and disease risk. Here, we introduce a label-free, quantitative DNA protein interaction approach which enables simple identification of allele-specific binding proteins at cis-regulatory variants.

We analyzed allele-specific differential protein binding at two predicted complex and two non-complex SNPs at the PPARG locus conferring risk for development of type 2 diabetes and found overall increased protein binding at the predicted complex regions confirming the recently developed integrated approach to identify causal variants (Claussnitzer et al., Cell 2014). Among the differential binders, we identified allele-specific binding of the transcription factor YY1 at the rs7647481 A-allele. Using siRNA we demonstrated YY1 to be implicated in regulation of both, PPARG1 and PPARG2, in cells heterozygous for the PPARG risk allele and showed cis-regulatory activity of the variants in several cell types by reporter gene assay. Thus, a framework combining bioinformatics and public data analysis with quantitative DNA protein interaction approach enables identification of cis-regulatory causal variants and moreover guide the way to delineate the mechanisms underlying genetic associations.

We have confirmed feasibility of this label-free proteome DNA binding approach in two additional complex diseases, for restless legs syndrome (RLS) where we could identify binding of Creb1 to intronic region of Meis1 regulating its enhancer function in the developing brain (Spieler et al., Genome Research 2014) and for the RAD50 locus associated to atopic diseases, where we identified differential bind of Smad3 and SP1 that impacted transcriptional activity (Kretschmer et al., Allergy 2014).

P-276.00

ADVANCED GLYCATION END-PRODUCTS AS PROSPECTIVE DIABETIC PEPTIDE-BIOMARKERS

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Glycation refers to the non-enzymatic reaction of aldoses and ketoses with protein amino groups yielding Amadori and Heyns compounds, respectively. Consecutive (oxidation) reactions yield reactive deoxysone intermediates, which can modify lysine and arginine residues to form relatively stable advanced glycation end-products (AGEs). AGEs have been linked to pathological disorders like type 2 diabetes mellitus (T2DM). Unfortunately, most established analyses target AGE-modified amino acids obtained by chemical or enzymatic hydrolysis of tissue proteins. As this method does not allow identifying proteins and modification sites, new proteomic approaches relying on characteristic MS/MS patterns of modified peptides are required. Thereby, protein modification patterns can be characterized and correlated to specific diseases to identify new biomarkers.

Thus, model peptides Ac-AK*ASASFL-NH2 containing a glycerinylated, acetylated, or formylated lysine in position 2 were synthesized and tandem-mass spectrometrically characterized. Tryptic digests of T2DM plasma were analyzed by nanoUPLC-ESI-LIT-Orbitrap-DDA-MS using gas phase fractionation. AGE-containing peptides were identified by database search and relatively quantified by a label-free approach (nanoUPLC-ESI-Orbitrap-MS) in normoglycemic lean and obese individuals and T2DM patients under good and poor glycemic control.

Tandem mass spectra of amide-AGE-modified peptides displayed complete b-ion series and no modification-related neutral losses, which facilitated subsequent analyses of plasma enzymatic digests and automatic database searches. Thus, two glycerinylated, ten formylated, and seven acetylated lysine residues were identified in two, six, and four proteins, respectively. One acetylated and one formylated peptide belonging to different proteins were found at higher levels in T2DM patients with poor glycemic control than in diabetic patients with good glycemic control or non-diabetic individuals.

The result that only 2 out of 19 identified AGE-peptides responded to the disease-status emphasizes the protein- and site-specificity of glycoxidative modifications. In conclusion, the strategy of searching protein- and site-specific biomarkers appears very promising for identifying disease-specific biomarkers linked directly to disease-related processes.

P-277.00

MS BASED APPROACHES FOR STUDYING THE PHARMACOKINETICS OF AN INNOVATIVE LIVER TARGETED COPPER CHELATOR IN ATP7B -/ MICE USED AS A MODEL FOR STUDIES OF WILSON'S DISEASE

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The Wilson's disease is a genetic orphan disease (~ 1000 cases in France) which results in a hepatic copper overload due to the deficiency of the protein responsible for the removal of excess copper from the body (ATP7B). The copper toxicity induces not only liver damage, but also neurological and psychiatric disorders.

This disease cannot be cured and it is lethal if not treated. Current treatments aim at lowering dietary copper absorption, either by chelating copper anywhere the drugs can go or by sequestering dietary copper in the intestine. Current drugs are not always satisfying and it is therefore of major interest to develop new molecules which can be able to efficiently and selectively binds copper. We have developed new bifunctional molecules, functionalized with N-acetylgalactosamine sugar known to target asialoglycoprotein receptors, able to both efficiently complex Cu(I) and target hepatocytes.

LCSRM method has been developed, for the detection and quantification of these metabolites from biological samples, to achieve optimal drug dosage. The in vivo data predict an acceptable pharmacokinetic profile in mice and along with the desired efficacy in preclinical efficacy models making the compound an attractive candidate for further development.

P-278.00

ONE-WEEK PALMITATE TREATMENT MODIFIES HUMAN ISLET INSULIN SORTING AND PROCESSING

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Juvenile obesity has doubled in children during the last 30 years, and brings up a wide range of associated health problems: increased risks of cardiovascular diseases, early development of type 2 diabetes and premature morbidity and mortality. Childhood obesity is also known to be associated with high levels of circulating insulin, which even occur before the apparition of insulin resistance. Hyperinsulinemia induces expanded lipid storage in insulin sensitive tissues and insulin resistance, worsening obesity and its associated complications.

Based on the hypothesis that insulin hypersecretion by pancreatic islets promotes the development of obesity and T2D in children, we evaluated the mechanisms associated with palmitate-induced insulin hypersecretion on a human islet model.
Human islets were cultivated for 2 and 7 days in presence of 0.5M of palmitate. Glucose stimulated insulin secretion followed by perifusion allowed detecting insulin hypersecretion after 2 days of palmitate treatment. Quantitative proteomics using isobaric labeling (TMT-6plex) was applied to these islets. It highlighted 10 proteins that were significantly regulated at both time points.

Pathway analysis assigned the vast majority of these proteins to insulin sorting and processing, showing the deleterious effect of palmitate on these crucial ß-cell mechanisms. A deep functional analysis of these results will allow to better understand the palmitate-related dysfunction in human beta-cells, a key issue to apprehend T2D onset in obese children.

P-279.00

MYOCARDIAL PROTEOME DURING METABOLIC SYNDROME AND TYPE 2 DIABETES IN ZUCKER DIABETIC FATTY RATS

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Introduction
Metabolic syndrome and Type 2 Diabetes Mellitus (T2DM) are well known contributing factors for developing heart disease. Approximately 5 % of the world population is suffering from T2DM which will lead to high prevalence of cardiovascular disease (CVD) in the future, and have a huge impact on society's health and economy.

Objective
It is important to elucidate the molecular relationship between metabolic diseases and CVDs to better understand the biochemical links between diabetes and CVD. The present project will therefore apply large scale protein analysis of the rat heart proteome to map protein perturbations linked to CVD and diabetes.

Materials and methods
Male Zucker diabetic fatty (ZDF) rats (homozygote (fa/fa)) and their age-matched lean controls (fa/+) were studied at ages 6, 12 and 24 weeks corresponding to a pre-diabetic state, onset of and late type 2 diabetes (n=3 in each group). The ZDF rat hearts were removed and the tissue proteins subjected to separation by SDS-PAGE, in-gel digestion and C18-purification before peptide analysis by nanoLC-MS/MS (LTQ Orbitrap, Thermo).

Results and discussion
Approximately 50 proteins were found significantly altered at the three stages 6, 12 and 24 weeks. The results show a clear reprogramming of metabolism as the disease progresses. Over time, proteins of fatty acid oxidation (FAO) increased whereas amino acid metabolism and respiratory chain protein levels decreased. Results on FAO are in accordance with prior findings within the area of T2DM proteomics. Polymerase 1 and transcript release factor (PTRF) was found significantly down-regulated at all three stages, and its function on cell senescence is discussed.

Conclusions
The altered metabolic pathways found in the present study may have severe consequences for heart function in rats with metabolic syndrome and T2DM and they may be linked to CVD.

P-280.00

NUCLEAR PROTEOMIC STUDY OF C2C12 MYOTUBES WITH ALTERNATED INSULIN SENSITIVITY INDUCED BY PALMITIC ACID

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Obesity has a tight association with type 2 diabetes mellitus (T2DM) and elevated plasma free fatty acid level induced insulin resistance is believed as the link between obesity and T2DM. However, the detailed mechanism of the changes in plasma free fatty acid level result in insulin resistance remains to be elucidated.

In this study, insulin desensitization was induced in C2C12 myotubes via palmitic acid treatment. To focus on the changes of nuclear proteome, nuclei of C2C12 myotubes were isolated for two-dimensional gel electrophoresis based proteomic study. Result demonstrated that four nuclear proteins showed changes in expression after palmitic acid treatment; nuclear factor NF-kappa-B (NF-?B) p65 subunit and 60S acidic ribosomal protein P0 were upregulated, while peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PPARGC-1?) and cleavage and polyadenylation specificity factor subunit 5 (CFIm25) were downregulated.

Whereas, inhibiting NF-?B p65 subunit nuclear translocation can prevent the palmitic acid induced deleterious effect on insulin sensitivity, implied that NF-?B p65 subunit play a key role in palmitic acid induced insulin desensitization.

P-281.00

IMPLICATIONS OF METHYLTHIOADENOSINE PHOSPHORYLASE DEFICIENCY IN LIVER INJURY

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Introduction and objective: Methylthioadenosine phosphorylase (MTAP), a key enzyme in the adenine and methionine salvage pathways, catalyzes the hydrolysis of methylthioadenosine (MTA), a compound suggested to affect pivotal cellular process. MTAP is expressed in a wide range of cell types and tissues, deletion of MTAP is commonly reported in cancer studies and is one of the genes that have been involved in liver injury. The aim of this study is to investigate the molecular mechanisms triggered by MTAP deficiency that participate in the progression of liver disorders, using proteomics technologies.

Methods: We used a combination of biochemical and differential proteomic techniques, including 1/2D-Western blotting, immunoprecipitation, HPLC-LC/MS/MS and iTRAQ coupled with bioinformatics, to dissect cellular processes deregulated by a loss of MTAP activity that may explain, at least partially pathogenic mechanisms of chronic liver ailments.

Results and discussion: Mice with MTAP deficiency displayed increased sensitivity upon challenging with CCl4. The differential phenotype of MTAP+/- mice is likely due to a threefold accumulation of MTA in hepatocytes. To characterize the mechanisms involved, SK-Hep1 and SK-Hep1MTAP+ cells were studied. The results were in line with our previous results, showing a twofold decrease in MTA levels upon MTAP expression. iTRAQ analysis was performed resulting in the identification of 216 differential proteins (p<0.05) that suggest deregulation of cellular pathways as those mediated by ERK, AKT or NFκB. Moreover, our results suggest that protein-methylation profile is modified by accumulation of MTA, a well-known inhibitor of PRMTs. Stimulation of AML12 cell with MTA induced a decreased in the overall protein-methylation state. Likewise, up-regulation of protein methylation in SK-Hep1MTAP+ was observed as well as a differential recovery upon AdOx exposition.

Conclusion: Our data support that MTAP deficiency leads to MTA accumulation and deregulation of central cellular pathways, in part due to differential protein methylation.

P-282.00

PROFILING OF CELL SURFACE PROTEOME AND METABOLOME FROM AN IN-VITRO MODEL SYSTEM, USING LC-MS TECHNOLOGIES

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Introduction and Objectives

Cell surface membrane proteins play a predominant role in cellular signaling processes. Membrane specific receptor proteins serve as cellular markers and prime drug targets to several pharmaceutical agents. We adopted the chemical biotinylation enrichment methodology (Bernd Wollsheid et al, 2009), coupled with LC-MS technologies to analyze spatio-temporal changes of protein expression along with endo-metabolome signatures from EGF induced epidermoid carcinoma cell lines-A431.

Methods

A431 cells were cultured in DMEM medium as described (YanMin Zhang et al, 2010) and EGF stimulated for 14 hours. Chemical biotinylation was carried out following the recommendations from Pierce chemicals. The adherent monolayer was treated with Sulfo-NHS-SS-Biotin at a conc of 0.25mg/ml for 30 min. Biotinylated proteins from total cell lysate were affinity purified using Agilent automation solutions. The eluted protein mixture was digested with trypsin/Lys-C mix, resulting peptides were analyzed using a microfluidic-based nanoflow LC coupled to Q-TOF MS. Data reprocessing was performed using software for protein database search for protein analysis.

Results and Discussion

Preliminary analysis of chemical enrichment strategy has resulted in the identification of several cell surface membrane proteins including extra cellular matrix proteins, moderately abundant proteins including pancreatic marker protein, plectin-1 along with F-Box Leucine rich repeat protein-2, beta actin, PGK-2, annexin-2, etc. Identified endo-metabolites include L-leucine, L-phenylalanine, succinic acid and adenosine. Cell surface proteome complemented with endo-metabolome would provide a deeper understanding of the spatio-temporal events of the cellular machinery and an insight into potential metabolite signatures upon drug treatment. Metabolome profiling and data anlaysis is currently underway.

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Topic 6

Phosphoproteomics

OP026 - PHOSPHOPROTEOMICS OF ADENOVIRUS TYPE 2 PROTEINS

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Phosphoproteomics of Adenovirus type 2 proteins

Introduction and objectives

Protein posttranslational modifications (PTMs) are essential steps for in regulating protein structure and function. We were the first group that investigated the presence of PTMs in an intact virus particle by mass spectrometry (MS). In the adenovirus type 2 (Ad2) particle, especially phosphorylations but also nitration and acetylations were detected [1,2]. In this study we apply different strategies to reveal phosphorylations of Ad2 non-structural proteins. These proteins have important roles for virus replication in host cells and their phosphorylations are likely to modulate their cellular localization and function.

Methods:

Human cells were infected by Ad2 resulting in a dual-specie sample of both human and Ad2 proteomes. Traditional 1-D-SDS-PAGE was complemented with strong cation exchange (SCX) and electrostatic repulsion-hydrophilic interaction chromatography (ERLIC) for sample fractionation. Further enrichment for phosphorylation was performed with TiO2. Peptides were analyzed with nano LC (reversed-phase liquid-chromatography) -high resolving MS and searched for a number of different modifications. Novel sites of phosphorylation were manually verified in MS/MS spectra.

Results and discussion:

Initial experiments showed highest phospho-enrichment efficiency for the SCX and TiO2 combination. So far, phosphorylation sites have been detected on proteins that play very important role in infection such as that promote viral DNA replication, DNA binding, DNA packing and inhibit host protein translation. Particularly adenovirus DNA-binding protein is highly phosphorylated with 15 detected phosphosites.

Conclusions:

Combining powerful tools of fractionation and detection to investigate the phosphoprofile of Ad2 proteins is proved to be a successful approach. The presence of new phosphorylation sites in Ad2 proteins demonstrates that the infection mechanism is more complex than hitherto believed.

References:

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OP027 - PHOSPHOPROTEOMIC PROFILING OF POST-NATAL HEART DEVELOPMENT UNRAVELS A NOVEL MITOCHONDRIAL PROTEIN TO BE ASSOCIATED WITH THE MINOS COMPLEX

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Introduction and Objectives
The mammalian heart appears to possess a limited capacity for regeneration shortly after birth. To date, the regulatory expression profiles of proteins and phosphorylation sites during post-natal cardiac development remain unexplored. In this study, we performed a global unbiased screening of mouse heart proteome and phosphoproteome with in vivo quantification and searched for novel regulators during post-natal development.

Methods
Here we used a shotgun proteomics approach using liquid chromatography-mass spectrometry (LC-MS/MS) to sequence the proteome and phosphoproteome of developing mouse heart. Heavy heart protein standard from SILAC mice was mixed with light heart proteins extracted at 2, 10 and 20 days post birth. A portion of each mixture was fractioned using SDS-PAGE and digested in-gel using LysC. The remaining protein mixtures were digested in-solution, fractionated using SCX chromatography and phosphopeptides were enriched using titanium dioxide beads.

Results and Discussion
We report the most comprehensive dataset of the proteome and phosphoproteome of mouse heart during post-natal development, including 8985 proteins and 22,675 phosphorylation sites, with the identification of 6922 novel sites. Cluster analysis showed reduced phosphorylation of several CDK1/2 substrates after P10. We also identified some transcription factors, kinases, phosphatases and ubiquitin ligases to be differentially regulated during development. Moreover, the molecular spring titin is dynamically phosphorylated with 247 phosphorylation sites. In addition, we identified an uncharacterized protein which shows a significant upregulation (p

Conclusion
Integrated analysis of proteins and post-translational modifications helps to better understand the physiological mechanisms of events during cardiac development. High-throughput unbiased screening allows identification of interesting novel regulators.

OP028 - QUANTITATIVE PHOSPHOPROTEOMICS ON A µG-SCALE - A STRAIGHTFORWARD AND HIGHLY SENSITIVE ERLIC-SCX/RP-LC-MS STRATEGY

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Introduction: Large-scale phosphoproteomics usually requires milligrams of protein, hampering applicability in clinical research with limited sample amounts. We developed a novel and highly sensitive 2D-bottom-up strategy combining considerably improved fractionation by ERLIC, a simple SCX/RP-SPE-step and LC-MS/MS analysis. We evaluated our ERLIC-SCX/RP-LC-MS strategy with respect to sensitivity and complementarity to other strategies. In subsequent SILAC-experiments, we demonstrated its applicability to study differential protein phosphorylation with low sample amounts.

Methods: Tryptic peptides (100µg non-stimulated Hela and 150 µg SILAC-labelled NIH-3T3 cells 5 h after infection with cytomegalovirus vs controls) were pre-fractionated by ERLIC. Depending on the fraction's buffer composition, peptides were purified either with SCX- or RP-spin tips. Subsequent LC-MS/MS analysis was done in 36-45 h and spectra were searched against the Uniprot database using Mascot. PSMs were filtered at a 1% FDR and only phosphopeptides with all phosphorylation sites >90% phosphoRS-probability were considered.

Results and Discussion: Using ERLIC-SCX/RP-LC-MS, we identified >7,500 confident, non-redundant phosphorylation sites of 3,013 phosphoproteins, using only 100 µg tryptic HeLa peptides. Using the 2-plex-SILAC sample, we quantified ~3,300 unique, phosphopeptides from 1,636 proteins with only 150 µg per condition. Calculations of phosphoproteins' copy numbers revealed unbiased coverage of the entire dynamic range from millions down to a few copies per cell. We observed large complementarity to affinity-based strategies, a result of ERLIC's ability to enrich acidic and longer phosphopeptides.

Conclusions: With 75 identified phosphorylation sites and 22 quantified phosphopeptides per µg, ERLIC-SCX/RP-LC-MS is among the most efficient strategies to date. It has a high sensitivity and shows complementarity to affinity-based strategies. ERLIC-SCX/RP-LC-MS enables efficient phosphoproteomics in µg-scale experiments and can be easily applied to all kinds of biological, biomedical and clinical research.

OP029 - THE DNA DAMAGE-INDUCED PHOSPHOPROTEOME IS MODULATED BY INHIBITION OF THE RECEPTOR TYROSINE KINASE MET

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Ionizing radiation (IR) is frequently used in the treatment of a variety of malignant tumors of different origins and stages. In recent years, numerous studies have demonstrated that the pharmacological interference with signaling via growth factor receptor tyrosine kinases (RTKs), can increase the sensitivity of certain tumors to IR.

The RTK for hepatocyte growth factor, MET, is aberrantly activated in numerous types of human malignancies. MET inhibition (METi) has been shown to synergize with DNA damaging agents in generation of DNA damage and interfere with damage repair.

In this study, we aim to explore how the cellular response to ionizing radiation is modulated by MET inhibition. We have recently conducted an immunoaffinity-based LC-MS/MS phosphoproteomics survey study to explore the cellular phosphoproteome following exposure of MET-addicted cancer cells to METi alone and in combination with IR. Analysis of the survey data has identified more than 300 phosphopeptides, which have changed by at least 4-fold in one experimental condition or more. Among these, we have identified using clustering analysis a group of phosphopeptides, in which the phosphorylation is increased by the combination of METi and IR compared to IR alone.

Several of these phosphorylation changes have been confirmed and further investigated in selected cell lines by targeted proteomics using selected reaction monitoring (SRM). We have identified a sub-network of the DNA damage response (DDR) that is activated in MET-addicted cancer cells upon DNA damage and MET inhibition, and could be responsible for synergism between these two modalities.

We expect that these results will aid in understanding how MET signaling crosstalks with the DNA damage response with subsequent translational therapeutic clinical applications.

OP030 - DYNAMIC PHOSPHOPROTEOMICS ANALYSES OF COLON CANCER CELLS REVEAL DIFFERENTIAL REGULATION OF SIGNALING PATHWAYS UPON RAF INHIBITION

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Introduction. Ras-regulated signaling affects downstream effectors that control key cellular processes such as actin cytoskeletal integrity, cell proliferation and differentiation, and apoptosis. Raf kinases are downstream from Ras and can harbour gain of function mutations that represent ~50% of human melanomas. Different RAF inhibitors are approved for clinical trials against metastatic melanomas though several inhibitors can enhance RAF dimerization and activity in the presence of active RAS, resulting in paradoxical effects on signaling pathways.

Method. Global changes in protein phosphorylation were obtained on cell lines Colo205 (RAF V600E mutation) or HCT116 (K-RAF mutation) treated with 3 uM PLX4032. Temporal profiles of protein phosphorylation (TiO2 extracts) were acquired every 5 min over a 60 min period using metabolic labeling (SILAC) and LC-MS/MS analyses on an orbitrap Q-Exactive mass spectrometer. LC-MS/MS data were analyzed using Maxquant and bioinformatics analyses were performed using DAVID and String database.

Results and discussion. Comprehensive data mining analyses and filtering techniques were applied to identify phosphosites showing differential regulation. We identified 37910 phosphosites in HCT116 and Colo205 cell lines, of which 278 sites were found to be regulated in both cell lines upon treatment with PLX4032. We confirmed the inhibition of mitogen-activated protein kinase (MAPK) pathway in the Colo205 cell line whereas a 8-fold activation of ERK was observed for HCT116. We identified unexpected targets such as Cdk7 not previously known to be modulated by changes in the MAPK activation pathway. Temporal changes in protein phosphorylation upon RAF inhibition enabled the identification of other regulated proteins involved in RNA splicing and transcriptional regulation, leading to a better understanding of the impact of drug treatment on different cellular pathways.

Conclusion. Dynamic phosphoproteomics was used for the first time to unravel paradoxical effects of RAF inhibition and provided unprecedented details on the differential regulation of the MAPK signaling pathway.

P-283.00

COMPARISON OF AN ORBITRAP FUSION SYSTEM PERFORMANCE WITH AN ORBITRAP VELOS PRO USING SOLID PHASE AMINO-LABELLING WITH TMTS ON COMPLEX DIGESTS FOR SUBSEQUENT PHOPHOPEPTIDE ENRICHMENT

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We present a novel Tandem Mass Tag Solid Phase Amino Labelling (TMT-SPAL) protocol using reversible immobilization of peptides onto C18 matrices. This method reduces the number of steps required in complex protocols saving time and potentially reducing sample losses particularly for phosphopeptide profiling. TMT-SPAL requires modified labelling conditions. Completeness and equivalence to solution phase labelling is demonstrated.

Human breast cancer cell line samples (MCF7) were labelled with 8-plex TMT. Reduction, Alkylation and Trypsin digestion of all samples was identical. Digested samples were reacted with NHS-TMTs in solution using a standard protocol or loaded onto Waters SepPak tC18 cartridges for TMT-SPAL.

We demonstrate a shortened TMT-SPAL workflow for global Phosphoproteomic analysis and compare with our solution phase protocol. We compare performance of the labelling protocols on two LC-MS systems: the EASY nLC II & LTQ Orbitrap Velos Pro versus the EASY nLC1000 & Orbitrap Fusion.

With both protocols, we see similar overall rates of protein identification but we observe differences in the actual proteins identified (Protein Identification by TMT-SPAL: 4,691 proteins on the Orbi-Velos Pro system and 19,643 proteins on the Fusion system; Protein identification by Solution Labelling: 4,364 proteins on the Orbi-Velos Pro and 19,268 proteins on the Fusion). 15,246 proteins are identified by both workflows on the Fusion. We detect more peptides from EGF Receptor, Estrogen Receptor (ER) and Receptor tyrosine-protein kinase erbB-2 by TMT-SPAL on the Fusion system than with solution phase labelling. We detect EGFR by TMT-SPAL on the Orbi-Velos Pro system, but not ER. We detect a higher number of phosphoproteins with TMT-SPAL on the Fusion (TMT-SPAL: 10,299; Solution: 9,962).

Robust TMT-SPAL labelling conditions are demonstrated, significantly reducing time requirements for our global Phosphopeptide workflow. TMT-SPAL on the EASY nLC1000 & Orbitrap Fusion provides significantly improved overall system performance.

P-284.00
**EXPANDING THE MYCOBACTERIUM TUBERCULOSIS
PHOSPHOPROTEOME CATALOGUE AND IDENTIFICATION OF
TYROSINE-PHOSPHORYLATION THROUGH LABEL-FREE
PHOSPHOPROTEOMIC ANALYSIS OF A CLINICAL ISOLATE**

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Introduction and objectives

Mycobacterium tuberculosis can show phenotypic differences due to protein function variations caused by structural alterations, regulatory changes or post-translational modifications, impacting interaction with substrate targets. Reversible protein phosphorylation mediates a switch between protein activity and cellular pathways that contribute to a large number of cellular processes and is regulated by protein kinases. The M.tb genome encodes for 11 Serine/Threonine kinases with close homology to eukaryotic kinase. The objective of this study was to use a mass spectrometry-based proteomics approach to identify novel phosphopeptides for potential drug targets. Characterisation of novel bacterial phosphorylation sites offers the potential to identify drug targets with novel mechanisms of action which will not influence or affect the host kinase activity.

Methods

To elucidate the phosphoproteomic landscape of a clinical isolate of M.tb, we performed a high throughput mass spectrometric analysis of proteins extracted from an early-log phase culture. Whole cell lysate proteins were processed using the filter-aided sample preparation method, followed by phosphopeptide enrichment of tryptic peptides by strong cation exchange and TiO2 chromatography. Bioinformatics tools, MaxQuant were used to identify proteins from the LC-MS/MS and statistical analysis was performed using Persues.

Results and discussion

Our label-free quantitative analysis identified 571 serine/threonine/tyrosine phosphorylated sites present on 454 peptides mapping to 261 M.tb proteins. Seventy-eight of the phosphoproteins identified in our study had been previously described, confirming previous reports. The remaining 189 phosphorylated proteins were newly identified in this study. We identified 8 novel tyrosine phosphorylated proteins.

Conclusion

Our results confirmed previously described as well as identified novel phosphorylated Serine/Threonine sites. For the first time we identified novel tyrosine phosphorylation events. These findings expand our current understanding of the protein phosphorylation network in clinical M.tb, and further extend and complement previous knowledge regarding phosphorylated peptides and phosphorylation sites in M.tb.

P-285.00

PHOSPHOPROTEOMIC ANALYSES REVEAL THAT GALECTIN-1 AUGMENTS THE DYNAMICS OF B-CELL RECEPTOR SIGNALING

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B-cell activation is important for mounting humoral immune responses and antibody production. Galectin-1 has multiple regulatory functions in immune cells. However, the effects of galectin-1 modulation and the mechanisms underlying the coordination of B-cell activation are unclear. To address this issue, we used label-free quantitative phosphoproteomic analysis to investigate the dynamics of galectin-1-induced signaling in comparison with that following anti-IgM treatment. Mouse splenic B cells were treated with galetin-1 or anti-IgM for 10 or 30 min and followed by protein extraction. Sample was digested and phosphopeptides were purified by IMAC purification and analyzed by LTQ-Orbitrap.

Peptide quantification was computed by our previously reported IDEAL-Q software. A total of 3247 phosphorylation sites on 1245 proteins were quantified, and 70¡V80% of the 856 responsive phosphoproteins were commonly activated during various biological functions. The similarity between galectin-1- and anti-IgM-elicited B-cell receptor signaling (BCR) signaling was also revealed. Additionally, the mapping of the 149 BCR-responsive phosphorylation sites provided complementary knowledge of BCR signaling. Compared to anti-IgM induction, the phosphoproteomic profiling of BCR signaling, along with validation by western blot analysis and pharmacological inhibitors, revealed that the activation of Syk, Btk, and PI3K may be dominant in galectin-1-mediated activation.

We further demonstrated that the proliferation of antigen-primed B cells was diminished in the absence of galectin-1 in an animal model. In conclusion, the current study revealed the first systematic phosphorylation-mediated signaling network and its dynamics in B-cell activation. These findings provided evidence for a new role and insight into the mechanism of how galectin-1 augments the strength of the immunological synapse by modulating BCR signaling.

Keywords: B-cell activation / B-cell receptor / galectin-1 / phosphoproteome

P-286.00

SIMPLIFIED AND MINIMIZED FRACTIONATION USING STAGETIPS FOR IN-DEPTH PROTEOME AND PHOSPHOPROTEOME ANALYSIS

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StageTips are widely used for desalting and purification of peptides for various proteomic analysis. However, StageTips are not commonly used for peptide fractionation especially for phosphopeptides fractionation. We developed simple, reproducible and MS-friendly fractionation method using StageTips applicable for both peptide and phosphopeptide fractionation for in-depth proteome and phospho proteome analysis.

Combination of C18-SCX disks (C18-SCX StageTip) was employed for stepwise fractionation. Although salt and pH gradient are often used for cation exchange chromatography, we used counterion gradient in addition to salt and pH gradient. 25 μg of tryptic peptides from Hela-S3 cell lysate was applied to C18-SCX StageTip for proteome analysis. Phosphopeptides enriched by Fe-IMAC method from 2 mg lysate was applied to C18-SCX StageTip for phosphoproteome analysis. Each sample was separated to seven fractions, dried up and applied to LC-MS/MS (Q-Exactive, 3h run/fraction). Elution buffer consists of volatile reagents, thus it was possible to skip desalting step after fractionation.

For proteome analysis, more than 85000 non redundant peptides, 6700 proteins were identified at FDR < 0.01. For phosphoproteome analysis, more than 25000 phosphorylation sites with a localization probability > 0.75 were identified. These results were reproducible between experimental replicates. Moreover, minimized loss and overlap across the fractions resulted in high detection efficiency over 10.0 (phosphosites ID/sample amount (ƒÊg)). Compared with previous large-scale phosphoproteome studies using extensive fractionation by SCX or HILIC or ERLIC, we minimized process steps, fraction numbers and LC-MS/MS runtime without sacrificing large-scale identification. Furthermore, it is easy to fractionate many samples (maximum 40 samples in our lab) in parallel for large-scale experiments.

In conclusion, we developed simple, reproducible, low-cost and MS-friendly fractionation method using C18-SCX StageTips for in-depth proteome and phosphoproteome analysis.

P-287.00

IRON-TRIGGERED SIGNAL TRANSDUCTION IN THE PARASITIC PROTOZOAN TRICHOMONAS VAGINALIS

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As a major risk factor in promoting transmission of human immunodeficiency virus, trichomoniasis caused by urogenital infection of Trichomonas vaginalis poses an imminent threat to public health. Iron is a key determinant in monitoring expression of multiple virulence phenotypes in the human pathogen.

Our previous studies on the mechanism underlying regulation of iron-inducible expression of an adhesion protein have revealed that iron might trigger a PKA-centered signal transduction pathway leading to rapid nuclear influx of a Myb3 transcription factor in this organism. In the present report, the effects of iron on cell signaling were studied exploring a phosphoproteomics approach, in which cells were replete with iron for 3 min and lysed in 6 M urea.

Proteins from control and treatment were each digested by trypsin, dimethyl-labeled, and mixed. The peptides were separated by SCX. Each eluted fraction was enriched by IMAC and analyzed by tandem mass spectrometry using Orbitrap Elite. Increasing phosphorylation of many proteins, including various protein kinases and other regulatory proteins involved in several cellular functions, were identified and motif analysis of these phosphopetide were performed. Our results suggest that iron probably triggers multiple signal transduction pathways to adapt to iron overloading, which is cytotoxic in most organisms.

These observations provide information to further address cellular responses elicited in the parasite to counteract deleterious effects of iron overloading.

P-288.00

A PHOSPHOPROTEOMIC VIEW OF CHICKPEA EXTRACELLULAR MATRIX ILLUSTRATE COMBINATORIAL INTERPLAY OF PREDICTED AND UNEXPECTED COMPONENTS OF CELL WALL INTEGRITY SIGNALING IN PLANT

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Introduction and Objectives

Cell wall integrity (CWI) signaling orchestrates complex regulatory mechanism that control mechano-sensing and turgor driven deformation within the dynamic milieu of extracellular matrix (ECM). However, this modulation in plant is largely unknown. Reversible protein phosphorylation, a sub-stoichiometric PTM is known to regulate signaling pathways and protein function.

Methods

The proteome and phosphoproteome was developed with ECM enriched fraction using combination of 1 DE, 2 DE and subsequent staining with Pro-Q Diamond, SCX, IMAC and MOAC. Proteins were identified using MALDI-TOF/TOF, ESI-MS/MS and Triple TOF MS. 2-DE immunoblot analysis was performed for hydroxy amino acid phosphorylation. Further, a protein-interactome model was developed.

Results and Discussion

To better understand the role of protein phosphorylation in CWI signaling, we have developed the ECM phosphoproteome and proteome of a crop legume, chickpea (Cicer arietinum). Mass spectrometric analysis led to the identification of more than 150 phosphoproteins and 400 proteins, presumably involved in a variety of biological functions viz. cell wall remodeling, mechanical signaling, innate defense, protein folding and degradation. Data analyses revealed new ECM phosphoproteins of unknown functions vis-à-vis the presence of many known cell wall proteins along-with the presence of unexpected phosphoproteins which have never been associated with ECM. In silico prediction and mass spectrometric identification of site-specific phosphorylation of amino acid residues indicated their possible effect on ECM signaling network, which include both the regulatory as well as the functional proteins. Further, we interrogated the dataset using cluster and network analyses that identified significant protein modules and small correlation groups.

Conclusion

Our study provides novel imminent in cell wall dynamics and elucidated the phosphoprotein network that branches to several hormonal and signaling pathways. To our knowledge, this is the first report on the comprehensive understanding of the complex phosphoprotein network in CWI signaling operating in plant extracellular matrix.

P-289.00

MASS-SPECTROMETRIC IDENTIFICATION OF AMPK PHOSPHOSITES IN RAT MITOCHONDRIAL FUMARATE HYDRATASE

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AMP-activated protein kinase (AMPK) is a cellular and whole body energy sensor with manifold functions in regulating energy homeostasis, cell morphology, motility and proliferation in health and disease. An in vitro screen for novel AMPK substrates in rat liver revealed tumor suppressor fumarate hydratase (FH; Klaus et al., 2012, J Proteomics, 75:3304-13) without localizing the involved phosphorylation sites.

To identify these sites, we performed nanoLC-ESI-MS/MS analysis of in vitro phosphorylated full-length FH by using an 4000 ESI-Qtrap mass spectrometer (AB Sciex) connected to a nano-chromatography system (Thermofisher).

By this approach we identified three putative phosphorylation sites, two serines and one threonine. In vitro phosphorylation assays, performed with FH proteins mutated at these sites, confirmed major phosphorylation at one serine located in the cleavable N-terminal mitochondrial targeting peptide, and lower level phosphorylation at a threonine in the FH C-terminal domain 3. Thus, the initially observed activation of FH due to phosphorylation may be an indirect effect, and secondary to effects on mitochondrial targeting.

In conclusion, combining nanoLC-ESI-MS/MS analysis and in vitro studies allowed an unambiguous identification of an AMPK phosphorylation site in the prepeptide of rat FH that targets the protein to mitochondria.

P-290.00

EFFECTS OF A TOXOPLASMA GONDII ROP KINASE ON THE HOST CELL PHOSPHOPROTEOME

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Introduction and objectives

Toxoplasma gondii infection can lead to severe and lethal encephalitis. Upon invasion of the host cell, the parasite releases kinases that modulate the host immune response and metabolism. The main host immune response involves interferon-gamma signalling. Here we used a quantitative phosphoproteomic approach to firstly characterize the effects of interferon-gamma on a mouse fibroblast cell line. We then compared this with the phosphoproteome of this cell line infected with a ROP18 knockout-strain and the ROP18 complement-strain to identify further targets of the parasite ROP18 kinase.

Methods

A SILAC approach was used for quantitation. Fractionation of peptides prior to analysis on the mass spectrometer was performed using high pH RP fractionation and SCX. Phosphopeptides from the SCX fractions were enriched using Titanium-IMAC. LC-MS/MS experiments were performed using a QExactive mass spectrometer. The data was analysed using MaxQuant and bioinformatic analysis was performed using various R packages.

Results

A protocol has been established for robust phosphopeptide enrichment. This protocol was used to analyse the effects of interferon-gamma on mouse fibroblasts, that are used here as a model for Toxoplasma gondii infection. 4400 phosphopeptides were quantified in three replicates with 80-90% enrichment specificity. Moreover, we quantified 4000 proteins in the three replicates and thus were able to distinguish changes in phosphorylation from changes in total protein abundance. The data elucidated which pathways are activated by interferon-gamma in mouse fibroblasts and the targets of phosphorylation mediated by a Toxoplasma gondii kinase.

Discussion

A phosphoproteomic approach has been established including SILAC, RP- and SCX-fractionation and Titanium-IMAC enrichment, to enrich phosphopeptides with high efficiency and specificity. This approach has been used to analyse the global changes in the proteome and phosphoproteome induced by interferon-gamma and to investigate more specifically the targets of a kinase secreted by Toxoplasma gondii.

P-291.00

DEEP PROTEOME MAPPING OF MOUSE KIDNEY BASED ON OFFGEL PREFRACTIONATION REVEALS REMARKABLE PROTEIN POST-TRANSLATIONAL MODIFICATIONS

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Introduction and objectives
Performing a comprehensive nonbiased proteome analysis is an extraordinary challenge due to sample complexity and wide dynamic range, especially in eukaryotic tissues. Thus, prefractionation steps conducted prior to mass spectrometric analysis are critically important to reduce complex biological matrices and allow in-depth analysis.

Methods
Here we demonstrated the use of OFFGel prefractionation to identify low abundant and hydrophobic proteins than in a nonfractionated sample. We examined the capability of OFFGEL prefractionation for detecting PTMs when coupled with targeted enrichment strategy such as TiO2 phospho-enrichment.

Results and Discussion
OFFGel prefractionation of a kidney protein sample was able to unveil protein functional relevance by detecting PTMs, especially when prefractionation was augmented with a targeted enrichment strategy such as TiO2 phospho-enrichment. The OFFGel- TiO2 combination used in this study was comparable to other global phosphoproteomics approaches (SCX-TiO2 , ERLIC-TiO2 , or HILIC-TiO2). In addition, OFFGel prefractionation showed improvement in detecting low abundance proteins for deep proteome analysis.

Conclusion
The detailed mouse kidney proteome with the phosphopeptide enrichment presented here serves as a useful platform for a better understanding of how the renal protein modification machinery works and, ultimately, will contribute to our understanding of pathological processes as well as normal physiological renal functions.

P-292.00

COMPLEMENTARY PROTEASES SUBSTANTIALLY EXPAND PHOSPHOPROTEOME COVERAGE

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Introduction and objective: Extensive efforts have been made to characterize the localization and frequency of phosphorylation events because of its importance in regulating biochemical systems. However, despite technical innovation in phosphoproteomics, certain phosphorylation events remain resistant to detection due to their low abundance, the complexity of biological mixtures or the physiochemical properties of the neighboring amino acids. Although tryptic digestion is capable of producing peptide pools which are analytically favorable to LC/MS, a selection bias does exists and that may preclude the discovery of biologically significant phosphorylation events. Therefore, we evaluate further the use of complementary proteases for phosphoproteomics.

Method: Prostaglandin E2 treated Jurkat T-cells are lysed and digested overnight with 5 different enzymes; trypsin, AspN, GluC, chymotrypsin and LysC, individually. We combine Ti4+-IMAC phosphopeptide enrichment with high-resolution mass spectrometry using decision tree CID-ETD. Raw data were analyzed using different database strategies for identification and site localization.

Results and Discussion: By accumulating the data from separate digestion with 5 enzymes, phosphorylation sites coverage could be dramatically increased compared to trypsin digestion alone. Excellent enrichment efficiency (95-98%) and reproducibility (average r>0.9) were achieved. Subsequently, several peptide characteristics were evaluated to understand phosphopeptide behavior such as peptide length, pI, hydrophobicity, charge, polarity, and AA composition.

Conclusions: We demonstrate that a large portion of phosphorylation sites are simply inaccessible following digestion with trypsin alone. Using multiple proteases, rather than technical replicates, provide a direct route to increase both phosphorylation site detection and phosphoproteome coverage. Taken together, our results reveal region of the phosphoproteome that are refractory to standard methods.

P-293.00

GLIOBLASTOMA PHOSPHOTYROSINE-CONTAINING PROTEINS IDENTIFIED WITH TWO-DIMENSIONAL WESTERN BLOTTING AND TANDEM MASS SPECTROMETRY

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Introduction and objectives: Protein tyrosine phosphorylation is a reversibly dynamic post-translational modification that is regulated by protein tyrosine kinases and phosphatases, that regulates multiple aspects of physiological and pathological processes, and that plays important roles in multiple signal transduction pathways. This present study aims to investigate the presence of, and the potential biological roles of, protein tyrosine phosphorylation in the glioblastoma pathogenesis.

Methods: Two-dimensional gel electrophoresis (2DGE)-based Western blotting was used to detect the phosphotyrosine immunoreaction-positive proteins in a glioblastoma tissue. Proteins from 2D gel-spots that correspond to the positive anti-phosphotyrosine Western blotting spots were subjected to in-gel trypsin-digestion and liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS) analysis to determine the amino acid sequence of a protein. MS/MS and Mascot analyses were used to determine the phosphotyrosine sites of each phosphopeptide. Protein domain and motif analysis and systems pathway analysis were used to determine the important protein domains/motifs that contained phosphotyrosine residue and signal pathway networks to in-depth clarify the potential biological functions of protein tyrosine phosphorylation.

Results and Discussion: A total of 24 phosphotyrosine-containing proteins were identified. Each phosphotyrosine-containing protein contained at least one tyrosine kinase phosphorylation motifs and a certain structural and functional domains. Those phosphotyrosine-containing proteins were involved in the multiple signal pathway systems such as oxidative stress, stress response, and cell migration.

Conclusion: 2DGE-based Western blotting, tandem mass spectrometry, and bioinformatics are a set of effective approach to detect and identify glioblastoma tyrosine-phosphorylated proteome and to effectively rationalize the biological roles of tyrosine phosphorylation in the glioblastoma biological systems. The present results provide novel insights regarding tyrosine phosphorylation and its potential role in the molecular mechanism of a glioblastoma.

P-294.00

QUALITATIVE AND QUANTITATIVE PHOSPHOPROTEOMICS ANALYSIS OF CHICK RETINA OF TIO2-ENRICHED STRATEGY

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• Introduction and objectives

To profile and quantify the phosphorylated protein changes in chick¡¯s retina in response to myopic and hyperopic eye growth using highly sensitive Triple-Tof tandem mass spectrometer.

• Methods:

White Leghorn chicks were used as animal model. Lens-induced myopia was achieved by mounting -10D lens on the right eye, while lens-induced hyperopia was achieved by fitting the left eye with +10D lens. Refractive error was measured before and after treatments for 3 days. The interocular components changes were measured using A-type ultrasonography. The chicks were sacrificed and their retina was dissected and frozen. AfterTiO2-based phosphopeptide enrichment, pooled retinal tissues were analyzed with nanoLC cHiPLC system and TripleTOF MS/MS. MS data were acquired on a TripleTOF 5600 system (AB SCIEX) fitted with a Nanospray III source. Label-free quantification was applied by performing MS/MSALL with SWATHTM acquisition at 25 amu increments. The acquired MS/MS spectra were searched using the Paragon algorithm in the ProteinPilot software (AB SCIEX). For phosphoprotein quantitation, up to four peptides (¡Ý95% confidence) with 3 transitions were selected for XICs peak area generation.

• Results and Discussion

1,631 proteins (560 phosphoproteins) were identified in myopic chick retina while 1,547 proteins (473 phosphoproteins) were identified in hyperopic chick retina samples at 1% FDR. A total of 30 phosphoproteins were found to be up-regulated and 45 were down-regulated (hyperopic/myopic eyes). Moreover, unique acetylated proteins were found in both myopic and hyperopic retina samples. Sixteen of them were screened as differentially regulated.

• Conclusions:

We attempted TiO2 phosphopeptide enrichment strategy combined with a highly sensitive mass spectrometer to characterize phosphorylated sites and performed comparative quantification of phosphoproteins between myopic and hyperopic retina. The identified phosphorylated and acetylated proteins could be involved in the pathogenesis of myopia.

P-295.00
MOTIF-BASED QUANTITATIVE PROTEOMICS FOR ABSOLUTE PHOSPHORYLATION STOICHIOMETRY MEASUREMENT

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Measurement on the phosphorylation event is often used as indicator for signaling pathway activation. Despite being very sensitive, conventional methods such as immunoassay or kinase activity assay only provides arbitrary ratio and requires control sample to compare the relative level of phosphorylation. The critical information on absolute amount of protein and extent of phosphorylation to alter signaling pathways is missing. Here, we developed a motif-based quantitative proteomic approach to calculate absolute phosphorylation stoichiometry on a large scale.

This strategy only requires the measurement of single ratio between phosphatase-treated peptides and recovered phosphopeptides from unphosphorylated peptides after kinase reaction. To our knowledge, this is the first approach to access the phosphorylation stoichiometry for large scale human proteome in single cellular status. The performance of this approach was evaluated to measure the absolute stoichiometry as well as to compare the phosphoproteomic alterations between the targeted-therapy (gefitinib) sensitive (PC9) and resistance (PC9/gef.) lung cancer cell lines.

With reproducible quantitation result (standard deviation: ¡Ó6%), more than one thousands of phosphorylation sites and their phosphorylation stoichiometry were calculated for serine/threonine phosphopeptides and especially for high percentage of low abundant tyrosine phosphopeptides. This approach not only revealed the basal level of phosphorylation stoichiometry of the two lung cancer cell lines, but also differentiated the regulation at the protein level and more dramatic phosphorylation degree associated with drug resistance in lung cancer.

The result also revealed that acidic phosphorylation motifs from CK2 had higher proportion (30%) of high phosphorylation stoichiometry (>70%) than those from MAPK and EGFR. Through network analysis, the measurement identified a substrate of CK2 as the potential drug resistant target to reverse the drug resistance in PC9/gef cells. We expect that this motif-based isotopic quantitative approach may be useful to study phosphorylation-mediated events from broad ranges of fundamental research to discovery-driven cancer research.

P-296.00

ADAPTATION OF CELLULAR SIGNALING BY INTRINSICALLY ACTIVE P38 MAP KINASES

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The p38 Mitogen Activated Protein Kinases (MAPKs) are a family of stress-activated kinases, mediating responses to osmotic shock, radiation, immune stimuli and inflammatory cues. They are induced under different conditions, such as cell cycle arrest, apoptosis, differentiation and cell proliferation.

The four known genes of the p38 MAPK family share ~60% sequence identity and share ~45% identity with the other members of the three MAPK families. Since the same MAPK-Kinases (MKKs) activate the different p38 MAPK, we aimed to define the specificities of the unique signaling cascades induced by the different p38 MAPKs. Large-scale proteomics and phospho-proteomics technologies were employed, using SILAC labeling p38β/α (-/-) mouse embryonic fibroblasts (MEFs) expressing HA-tagged: 1) wild-type p38α or p38β; 2) intrinsically active mutants of these p38β/α; 3) an empty vector.

This way, the signaling through each variant could be elucidated independently from other extracellular stimulations or upstream activations. A total of 12,234 phosphorylation sites, located in 3472 proteins were identified after TiO2 enrichment. In addition, 4617 proteins were identified in independent proteomics analyses of the same cells. Changes in the mutant/w.t. ratio of the proteins and phosphopeptides were observed, while phosphorylation sites associated with the known MAPK signaling pathway remained unchanged in the intrinsically active mutants.

We noticed an adaptation process, in which the intrinsically active mutants modulate other members of the same family mechanism. Better understanding of molecular mechanisms involved with adaptation to continuous signaling is significant for chronic inflammation and cancer.

P-297.00

PHOSPHOPEPTIDE ENRICHMENT USING SYNTHETIC BIOMIMETIC LIGANDS

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Introduction and objectives
Phosphorylated proteins and their related kinases are involved in the regulation of complex signalling networks, and have been implicated in a myriad of human diseases. The identification and quantification of these proteins has enormous social and economic impact, because of their potential as biomarkers and drug targets. However, due to their scarcity in biological samples and low phosphorylation stoichiometry, there is a need to enrich samples in phosphoproteins prior to Mass Spectrometry analysis. The vast majority of methodologies used today rely on phosphate coordination to metal ions, but lack specificity [1]. In this work, by performing structural studies of the human phosphoprotein-binding domains, we have developed three libraries of synthetic biomimetic ligands for the selective binding of phosphorylated targets.

Methods
Three combinatorial libraries of ligands have been synthesized in 96-well blocks using agarose as solid support. Amine, carboxylic acid, and aldehyde components have been selected in order to mimic key amino acids involved in phosphate recognition, such as Arg, Lys, Tyr, Ser, His, Gly, and Asn. After several steps of optimization, two lead ligands were selected. These ligands were further synthesized in magnetic nanoparticles, and screened against a semi-complex mixture of á-casein, â-casein and BSA. Samples were analysed using MALDI-TOF MS.

Results and Discussion
232 tailor-made synthetic ligands have been synthesized using three different chemistries. Lead ligands presented selective binding towards phosphorylated species at acidic pH, high binding capacities and up to 70% recovery yields upon elution. Ligands immobilized on magnetic nanoparticles present higher binding capacities (100x), but at the cost of lower selectivity.

Conclusions
Small biomimetic ligands have been developed for the specific molecular recognition of phosphorylated peptides. Lead ligands have been compared to and are competitive with a Ti4+-IMAC based approach.

[1] Batalha, I.L. et al (2012) Trends in Biotechnology, 30(2), 100-110

P-298.00

PHOSPHODISTILLER: A WORKFLOW OF THE PHOSPHORYLATION QUALITY CONTROL BASED ON THE TANDEM MASS SPECTROMETRY DATA

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Protein phosphorylations are widespread in eukaryotic cells. Tandem mass spectrometry-based proteomics technology can achieve the large-scale identification of phosphorylated proteins and peptides. Database searching is the major strategy but the quality control remains a big challenge for phosphoproteomics, especially for detecting the exact location of phosphorylation sites.

First, most algorithms were not evaluated by large-scale synthetic datasets and have no considerations for the new fragmentation modes of HCD or ETD. Second, the spectra of MS/MS may not always provide enough information to distinguish the modification sites, which resulted in some uncertain phosphorylation sites. Last, there is no integrated workflow to achieve the quality control both for phosphorylated peptides and phosphorylation sites with the protein FDR less than 1%.

Here, PhosphoDistiller, a workflow with high sensitivity and high accuracy was introduced for the quality control. For the phosphorylated peptides, by inheriting the merits of PepDistiller and considering the phosphorylation neutral loss, PhosphoDistiller could generate peptides with high sensitivity without losing the accuracy. To improve the ability to locate phosphorylation sites, a feature of kinase/substrate specificity was integrated into the A-score like algorithm.

Using a data-dependent algorithm to predict the motifs along the phosphoproteome datasets, PhosphoDistiller could determine about 30% more sites than the probability based programs. Using the synthetic datasets published by Harald et al, we demonstrate this workflow can deal with large-scale datasets and guarantee the FDR

P-299.00

SIGNALLING NETWORK PLASTICITY IN RESPONSE TO CHRONIC CLASS I PI3K OR MTORC1/2 INHIBITION

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Introduction
Small-molecule inhibitors against class I phosphoinositide 3-kinases (PI3K) and mTOR complex 1 and 2 show promise for the treatment of multiple cancer types. Here, we investigated the plasticity of a kinase signalling network in models of acquired resistance to GDC-0941 and KU-0063794 (a class I PI3K and mTORC1/2 inhibitor, respectively). To achieve this, we utilised label-free mass spectrometry-based phosphoproteomics technology to first empirically define the network and subsequently assess how it was remodelled in resistance.

Materials and Methods
By treating MCF7 cells with a panel of small-molecule inhibitors against physiologically important kinases, we inferred the topology of the kinase signalling network in these cells. Through chronically exposing MCF7 to the aforementioned inhibitors, we obtained six resistant cell-lines; three resistant to GDC-0941 and three resistant to KU-0063794. We then compared the phosphoproteomes of these resistant cells to the parental MCF7 cell-line from which they were derived.

Results
The defined network consisted of 805 phosphorylation sites having been identified as inhibitor-target activity markers (ITAMs). Quantifying and comparing the abundance of the ITAMs between the resistant and parental cell-lines revealed that mTOR and PI3K signalling remained inhibited in the resistant cells. We also observed that a number of ITAM groups representing other kinases were increased relative to the parental cells. Interestingly, the cells resistant to the PI3K inhibitor showed a distinct pattern of network activity compared to the mTORC1/2 resistant cells. Furthermore, we observed marked heterogeneity between cells resistant to the same inhibitor. This heterogeneity was reflected in the cell-lines proliferative capacities and their response to other small-molecule inhibitors.

Discussion
Chronic exposure to an mTORC1/2 inhibitor remodelled the signalling network in a distinct manner from the PI3K inhibitor. Moreover, a large amount of heterogeneity was observed in the network remodelling between cells resistant to the same inhibitor.

P-300.00

MAGNETITE-DOPED POLYDIMETHYLSILOXANE (PDMS) AS A SOLID PHASE SUPPORT FOR PHOSPHOPEPTIDE ENRICHMENT.

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Introduction: Reversible phosphorylation plays a key role in numerous biological processes. Mass spectrometry-based approaches are commonly used to analyze protein phosphorylation, but due to the low phosphorylation stoichiometry phosphopeptide enrichment is usually necessary. A range of strategies have been developed, including metal oxide affinity chromatography (MOAC). Here, we describe a new material for performing MOAC that employs a magnetite-doped polydimethylsiloxane (PDMS).

Methods: PDMS mixed at various ratios with magnetite particles was cast over a suitable mould and heat cured to generate either microfluidic channels or microwells. The surface of the PDMS was then etched back to expose the magnetite particles. The microwell format was used to enrich phosphopeptides from a casein digest, and incubation time, sample loading and elution conditions were optimized. The surface was re-etched and phospopeptides enriched with the clean surface. Finally, phosphopeptides were enriched from a HeLa cell lysate. Phosphopeptide analysis was performed by MALDI-MS and LC-MSMS.

Results and Discussion: The magnetite doped PDMS could be easily cast into a range of formats, including microfluidic channels and microwells. The surface was demonstrated to perform phosphopeptide enrichment similar to that obtained with TiO2 sorbents, including from the complex background of a HeLa cell lysate. Following re-etching the devices could be reused without observable loss of performance.

Conclusion: Magnetite doped PDMS was shown to be a suitable material for phosphopeptide enrichment. This substrate-based approach is rapid, straightforward and suitable for generation of devices for phosphopeptide enrichment in a range of formats, from microwells to microfluidics.

P-301.00

QUANTITATIVE PHOSPHOPROTEOMICS FOR BREAST CANCER VIA PHOSPHORYLATION BY AMPK

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Introduction and objectives:
The aim of the project is to understand role of global phosphorylation in attached and detached (suspension) condition for MDA MB 231 breast cancer cells using Stable Isotope Labeling by amino acid in cell culture (SILAC). Further, since the kinase AMPK gets activated immediately upon cell detachment, we would like to identify the subset of AMPK-dependent phophorylations by using pharmacological activators of AMPK in attached condition, and using pharmacological inhibitors of AMPK under detached conditions.

Methods:
We performed comparative phosphoproteomics analysis for MDA MB 231 breast cancer cells under attached and detached condition using stable Isotope Labeling by amino acid in cell culture (SILAC). Using Proteome Discover software (Thermo Scientific), we will be performed phosphopeptide quantitation and phosphoprotein identification for the statistically significantly altered phosphoproteins.

Results and Discussion:
Identity of the differentially expressed phosphoproteins will be established using nano-RPLC LTQ Orbitrap. Further, correlation between the AMPK mediated phosphorylation on global signaling network will provide mechanistic insight into the role of stress induced phosphorylation in breast cancer.

Conclusions:
AMPK, the stress kinase might be the key regulator and potential therapeutic target in breast cancer.

P-302.00
IMPROVED DETECTION OF MULTIPHOSPHORYLATED PEPTIDES USING NOVEL TIO$_2$ BASED NANOMATERIALS

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Introduction and objectives
One of the challenges in phosphoproteomics is the separation and analysis of multiply phosphorylated peptides. A widely used enrichment technique for phosphorylated peptides is technique utilizing the metal oxides, i.e. TiO$_2$. As already known TiO$_2$ is able to bind multiply phosphorylated peptides as well as monophosphorylated peptides but the efficitent elution of the multiphosphorylated peptides is difficult due to the extremely high binding affinity (1). Aim of our work is to introduce the novel nanomaterial based on TiO$_2$ which can significantly increase the number of multiphosphorylated peptides compare to today´s separation techniques.

Methods
For evaluating the enrichment performance of materials, tryptically digested phosvitin was used as a peptide mixture containing multilply phosphorylated peptides. The enrichment of phosphopeptides was performed either by TiO$_2$ microparticles (Titansphere, GL Sciences) or by novel nanomaterial. Binding of phosphopeptides was performed by 80% acetonitrile/5% trifluoracetic acid including 1M lactic acid and eluted with 1% ammonia solution. Fractions were analyzed by MALDI LTQ Orbitrap XL mass spectrometer.

Results and Discussion
Mass spectra of fractions eluted from our new nanomaterial contained significantly higher ratio of multiphosphorylated peptides compared to widely used commercial TiO$_2$ beads. Number of monophosphorylated peptides was the same for both carriers. These results indicate high selectivity and excellent elutibility not only for mono- but mainly for oligo- or multiphosphorylated peptides.

Conclusions
Novel nanomaterial based on TiO$_2$ showed us the binding selectivity not only for mono- or diphosphorylated peptides but also for multiphosphorylated peptides while maintaining the same proportion to the original ratio in the analyzed material. This phosphoprofiling can be carried out in one step using only one type of carrier and during the enrichment step the level of phosphorylation is not altered and trace the phosphoproteome in studied organism.

1.Thingholm, T. E., et al. Molecular & Cellular Proteomics 7, 661–671(2007).

P-303.00

SEA URCHIN PHOSPHOPROTOEMICS REVEAL THE NEW LIGHTS ON EGG ACTIVATION

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All activities involving the transition from a mature oocyte to a embryogenesis capable egg are named of the events of"egg activation". Very limited transcription and new protein synthesis happen at the early stage of the egg. Instead, the early embryonic development was driven by the changes of maternal mRNAs and protein degradation in mature oocytes. Thus, the post translational modification (PTM) of the proteins, especially phosphorylation, might be the key player at this stage. The dramatic phosphorylation changes during egg activation have been found in sea urchin, Xenopus and D. melanogaster. Thus, the driven pathways during egg activation could be better understood with the comprehensive and systematic evaluation of global phosphoproteomics changes, which has not been fully studied.

Here we applied a large-scale, TiO2-based chelate phosphopeptide affinity enrichment strategy with 2D LC-MS/MS screening to evaluate the network of five stages of sea urchin upon egg activation: unfertilized egg (UN), 2 minutes post fertilization (PF2), 5 minutes post fertilization (PF5), 2 cells and Blastocyst (BL). We used hydrophilic interaction chromatography (HILIC) as a first dimension separation to separate and simplify protein digest mixtures into 20 discrete fractions. Phosphopeptides in each fraction were subsequently enriched using TiO2 coated magnatic beads, followed by C18 reverse phase nano-HPLC. Phosphopeptides were detected by data-dependent MS/MS Higher-energy Collision-activated Dissociation (HCD) fragmentation on a high performance Velos-Orbitrap hybrid mass spectrometer. We identified 15354 unique phosphopeptides in 3595 putative phosphoproteins.

By systematic computational analysis, we revealed biological pathways and phosphopeptides motifs enriched in PF2 that are markedly different from those observed in a similar analysis of UN stage. Most of those substrates motifs are found to related with kinases that either are significantly up or down regulated or in the differentiated pathways in post fertilization compare to unfertilization stages.

P-304.00
QUANTITATIVE PHOSPHOPROTEOMICS OF SIGNALING PATHWAYS ACTIVATED BY HEPATITIS C VIRUS CORE PROTEIN AND TGF-Â USING A TRIPLE SILAC APPROACH

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Introduction and objectives: Infection with Hepatitis C Virus (HCV) is often persistent and can progress to liver cirrhosis and hepatocellular carcinoma. The HCV core protein activates transforming growth factor â (TGF-â), a cytokine secreted in a latent biologically inactive form. Furthermore, HCV core protein switches TGF-â signaling pathways from its anti-oncogenic activity to protumoral effects. The ebb and flow of cellular life depends largely on signaling pathways that are regulated by cascades of protein phosphorylation. In this study, a quantitative phosphoproteomic approach based on stable isotope labeling of amino acids in cell culture (SILAC) is applied to investigate HCV core and TGF-â induced phosphorylations.

Methods: A triple SILAC analysis was performed using the hepatoma cell line Huh7 control, treated with TGF-â or stably expressing the HCV core protein harvested in light, medium, and heavy media, respectively. After cell lysis and enzymatic digestion, a selective enrichment of phosphopeptides was performed on IMAC resin followed by LC-MS/MS analysis using multistage activation (MSA) and electron transfer dissociation (ETcaD).

Results and Discussion: The applied state-of-the-art quantitative proteomic design allowed the identification of a subset of proteins showing a phosphorylation deregulation upon core or TGF-â treatment involved in translation initiation machinery, cytoskeleton dynamic and lipid homeostasis. By using a rational combination of quantitative proteomic profiling and antibody-based validation techniques, an overphosphorylation of mammalian rapamycin (mTOR) targeted substrates has been shown independently of phosphoinositide 3-kinases (PI3K). The readout of mTOR activity strongly suggests an important driver of cell proliferation and survival. Furthermore as previously reported, our findings revealed an activation of the extracellular signal-regulated kinases (ERK1/2) pathway by HCV core protein, controlling the balance between cell survival and apoptosis.

Conclusions: Our data shed new light on signaling pathways activated by HCV core protein and TGF-â, emphasize the role of HCV core protein in liver cancer promotion.

P-305.00
PARTICULATE MATTER DEPHOSPHORYLATES EZRIN/RADIXIN/MOESIN (ERM) PROTEIN ON BEAS-2B CELL

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ERM (Ezrin, Radixin, Moesin) proteins play role of corss-links between the plasma membrane and the F-actin and regulate the function of cell migration, morphology and adhesion and cell signal processes. The ERM proteins are consisted of three domains ; N-terminal FERM domain; a central ¥á-helical domain; and an actin-bind C-terminal domain. The ERM proteins are phosphorylated on their C-terminal threonine or serine residue are active in their cross-linking activity. Inhalation of the airborne particulate matters (PM) like titanium dioxide and silica dioxide can induce or aggravate the acute and chronic airway diseases. But, the underlying mechanisms of this response remain poorly understood. Therefore, to explain this mechanisms, we investigated the connection between the effect of PM and the dephosphorylation of ERM using a proteomics approach.

We established the epithelial cell and the animal model that was treated with PM particle. In according to treat PM particle, the state of changed phosphoprotein was estimated by western blot, immunohistochemistry staining.

We showed that phosphorylated ERM proteins indicating dose-dependent decrease on treatment of epithelial cells with PM particles. These data were validated by western blot and the degree of ERM phosphorylation was decreased in PM treated epithelial cells compared with that of the non-treated. Dephosphorylation of ERM proteins was also observed in animal model. The expression of phosphorylated ERM proteins was definitely lower in lungs of PM treated mice than those in control mice.

We have provided evidence that dephosphorylation of the ERM proteins is concomitant with the PM treatment. This process may be associated with working of various phosphatases and kinases. Therefore, further studies should be need to research about the phosphatase and kinases inhibitor related signal pathway. In that case, inhibition sites of dephosphorylation in ERM proteins may act as diagnosis maker for the pulmonary disease patients stimulated with PM.

P-306.00

IDENTIFICATION OF NEW PHOSPHORYLATION SITES IN KEY MAIZE C4 ENZYMES BY LIT-ORBITRAP MASS SPECTROMETRY

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Introduction and Objectives- Phosphoenolpyruvate carboxylase (PEPC), NADP-malic enzyme (NADP-ME), and pyruvate ortophosphate dikinase (PPDK) are key enzymes in the C4 photosynthesis. Our goal is to understand how phosphorylation regulates the activity of these enzymes in maize.

Methods- Maize leaves (3rd leaf) from 12-day-old seedlings were collected along several time points during the 16h light/8h dark photoperiod. Distinct isoforms of PEPC, PPDK and NADP-ME were detected by 2-DE/Western-blot and confirmed by MALDI-TOF-TOF analysis. A LIT-orbitrap mass spectrometer was used to identify these isoforms.

Results and Discussion- Specific staining of phosphoproteins (Pro-Q Diamond) was performed to assess differences in the phosphorylation level of these enzymes. For PEPC, a higher level of phosphorylation was detected at 4 hours of light period, decreasing until 12 hours and increasing during the pre-dawn period (30 min before light). A positional shift towards the basic end of the gel was observed (2-DE/Western-blot) in the 4 hours light sample after alkaline phosphatase treatment, as compared with an untreated sample, suggesting intense phosphorylation at this time point. For NADP-ME, five distinct isoforms were detected, with pH ranging between 4.8 and 5.2. PPDK isoforms were also detected in a pH range from 5.0 to 5.5. In order to assess the nature of the different isoforms, a phosphoproteomics analyses of 1DE gel slices containing our target-enzymes was performed in a LIT Orbitrap mass spectrometer (Thermo Scientific Orbitrap Elite). This allowed the identification of several new putative phosphorylation sites.

Conclusions- Several new putative phosphorylation sites were successfully identified by LIT-orbitrap MS, including phosphorylations in main C4 enzymes. Further validation of these new putative sites and assessment of their functional role will contribute to better understand C4 photosynthesis.

P-307.00

MONITORING KINOME ACTIVITIES USING THE HIGHLY SPECIFIC AND SENSITIVE SUBSTRATE PEPTIDES

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Kinase-substrate networks are important for cellular signal transduction. Large-scale phosphoproteomics enables us to overview protein phosphorylation events occurred in the signaling. However, it is still difficult to clarify a role or regulation mechanism of individual kinase from the phosphoproteome data. In this study, we developed a method for direct monitoring of kinase activity at the kinomewide scale using kinase-specific substrate peptides.

To decide the kinase-specific substrate peptides for each kinase, in vitro substrates of recombinant human kinases were identified by using in vitro kinase assay combined with LC-MS/MS based quantitative phosphoproteomics. Furthermore, phosphorylation stoichiometry in each substrate peptide was investigated about some kinases. Based on the in vitro kinase-substrate relationships, the most suitable substrate peptides which show the highest specificity and sensitivity for monitoring of kinase activity were determined in combination with other information such as protein-protein interaction and cellular localization. We also applied machine learning techniques to design artificial substrate peptides. Finally linearity, sensitivity and recovery rate for each substrate peptide were evaluated by a spike and a drug-inhibition assay using a crude cell extract.

As a result, we profiled 430 kinds of recombinant human kinases by using in vitro kinase assay, and then more than 180,000 kinase-substrate relationships were obtained. Based on the kinase-substrate relationships, about 1,500 phosphorylation motifs were extracted. Most of the obtained motifs were well-known, however, novel phosphorylation motifs were extracted by focusing on highly phosphorylated substrate sequences. Using the predicted kinase-substrate relationships and phosphorylation stoichiometry, kinase-specific substrate peptides were designed. In vitro kinase assay using synthetic peptides showed these designed substrate peptides were exclusively or highly-selectively phosphorylated with a specific kinase. We successfully quantified kinase activities in the crude cell extract with high accuracy.

These results suggested the kinase-specific substrates based on the in vitro profiling are useful for high-throughput monitoring of kinome activities.

P-308.00

PLK2 SPECIFICITY AND SUBSTRATES IDENTIFICATION BY PROTEOME-DERIVED PEPTIDE LIBRARIES AND QUANTITATIVE PROTEOMICS

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Recent studies on the pathogenesis of Parkinson's disease and other synucleopathies have highlighted the key role played by the Polo-like kinase 2 (PLK2), the protein kinase responsible for the phosphorylation of α-synuclein at S129, both in vitro and in vivo. Although little is known about the function of this kinase within the cell, the role of PLK2 in different biological processes is now emerging, suggesting that this enzyme is involved in oncogenesis, synaptic regulation of the brain, and cell division.

However, the precise functions of this kinase remain elusive, as, with few exceptions, its main cellular targets are still unknown. To gain important insights into the cellular processes in which PLK2 is involved, it is therefore essential to identify the physiological substrates of this kinase. For this reason we took advantage of an approach based on an in vitro kinase assay, combined with a mass spectrometry-based quantitative phosphoproteomics strategy. We used a proteome-derived peptide library obtained from neuronal human cells. Proteins were digested with trypsin and peptides were dephosphorylated by lambda phosphatase and a phosphorylation reaction was then performed with or without the presence of recombinant PLK2. A dimethyl labeling-based quantitative phosphoproteomics strategy was then applied to identify the phosphopeptides specifically generated by PLK2. A total of 88 unique PLK2-dependent phosphosites from 79 unique proteins were identified by LC-MS/MS.

The WebLogo analysis of these phosphosites allowed us to precisely determine the consensus sequence recognized by PLK2. Additionally, a list of potential high-confident PLK2 substrates was obtained by matching the identified phosphosites with those collected in the PhosphositePlus database. Bioinformatics and pathways analysis performed on the collected data suggest novel functions for PLK2, thus paving the road towards a deeper understanding of the physiological role played by this kinase.

P-309.00

THE PHOSPHOPROTEOME OF JURKAT T-CELLS UPON COSTIMULATION WITH ANTI-CD3/ANTI-CD28

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Introduction and objectives

Phosphorylation is one of the most abundant post-translational modifications of proteins and plays a vital role in a wide range of cellular processes. In most phosphoproteomics studies, physiological cell stimulation is performed using anti-CD3 antibodies. T cell receptor (TCR) and CD28 costimulation, which better represents physiological conditions, produces an enhanced phosphorylation of the PLC-γ/Shc/Grap2/Vav-1 and downstream proteins with incubation times of 5 min [1]. CD28 is suggested to cause mostly quantitative changes on the phosphorylation profiles induced via CD3, but also specifically affects proteins involved in cytoskeletal rearrangement. In order to gain insight on the phosphoproteome evolution upon costimulation, we are studying the effect of antiCD3/CD28 activation at times ranging from 15 min to as long as 24 hours in Jurkat E6.1 (wild type) and Jurkat gamma1 (PLC-γ null) clones. Here we describe our early results on these studies.

Methods

Jurkat cells were activated by anti CD3/CD28 antibodies at different time points (15 min, 30 min, 60 min, 120 min and 24 hours). Tryptic peptides from each time point were labeled with TMT, pooled, and fractionated by SCX before phosphopeptide enrichment by IMAC and TiO2. All fractions were analyzed by LC-MSn using an LTQ-Orbitrap XL instrument. Mass spectra were searched using multiple search engines (Sequest, Omssa and Easyprot). Quantitative and statistical analyses were carried out by the DanteR software and our Lymphos workflow [2].

Results and Discussion

Until now, more than 4000 unique phosphopeptides have been identified and quantified from Jurkat E6.1 cells. Analysis of this data, together with the results being obtained from the Jurkat gamma1 clone are presented. Our studies are expected to contribute to the knowledge on the medium-term effects of TCR/CD28 costimulation on the phosphoproteome of these cells.

P-310.00

QUANTITATIVE PROTEOMICS APPLIED TO THE STUDY OF THE MAMMALIAN CIRCADIAN PROTEOME AND PHOSPHOPROTEOME

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P-311.00

EVALUATION OF PHOSPHOTYROSINE IMMUNOPRECIPITATION FOR LABEL-FREE PHOSPHOPROTEOMICS USING CANCER CELL LINE MODELS

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P-312.00

ROBUST TIO2-BASED ENRICHMENT FOR SINGLE-SHOT PHOSPHOPROTEOMICS; APPLICATION TO COLORECTAL CANCER CELL LINES REPRESENTING DIFFERENT SUBTYPES

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Introduction and Objectives

There is a need for robust phosphopeptide enrichment methods to allow signaling network analysis in cancer cell lines and tissues with minimal fractionation. Here we assess reproducibility and depth of phosphosite identification and label-free quantification at different input levels by single-shot LC-MS/MS. We have profiled the baseline global phosphorylation of 8 colorectal cancer cell lines representing different subtypes using TiO2-based phosphopeptide enrichment.

Methods

Global phosphopeptide enrichment was performed on 500 µg peptide input using TiO2 based on the method developed by Ishihama and coworkers using lactic acid to minimise binding of non-phosphorylated peptides. All steps were performed in a stage-tip format by centrifugation. Phosphopeptides were measured in a 2 hr gradient on a Q Exactive mass spectrometer using a top-10 data-dependent acquisition method and data was processed using MaxQuant 1.4.1.2.

Results and Discussion

Workflow and analytical performance of TiO2 based phosphopeptide enrichment were assessed using a desalted CRC cell line (HCT116) sample. Workflow and analytical reproducibility were >76% for all duplicate analyses with 50% reproducibility for all samples (n=8 injections). Subsequently, baseline global phosphopeptide enrichment of 8 Colorectal cancer (CRC) cell was performed on biological triplicates per cell line using the TiO2 method. Biological reproducibility of phosphopeptide identification ranged from 55%-63% for biological triplicates with 5340-5813 phosphopeptides in 3/3 bio replicates with an average CV of phosphopeptide quantitation ranging from 51%-55% with lower CVs for higher intensity phosphopeptides.

CRC subtype related differences were found in phosphokinases and associated signaling pathways. De results will be presented.

Conclusions

We have streamlined and assessed performance for global phosphopeptide enrichment coupled to single-shot LC-MS/MS and uncovered subtype-related phosphorylation-based signaling in a panel of colorectal cancer cell lines. With the current state of the art MS instrumentation single shot LC-MS/MS analysis allows for deep phosphoproteome analysis at clinically relevant protein input levels

P-313.00

IS LESS ENOUGH? SCALING DOWN PROTEIN INPUT FOR PHOSPHOPROTEOMICS BASED TREATMENT SELECTION IN PATIENTS WITH ADVANCED SOLID TUMORS

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Introduction and objectives
Mass spectrometry-based phosphoproteomics of cancer cell- and tissue lysates provides a unique approach to evaluate the cell signaling network, revealing information on aberrantly activated signaling pathways and potential drug targets. To enable phosphoproteomics-based treatment selection for improved efficacy of targeted therapies in patients with advanced solid tumors, needle biopsies should provide reproducible profiles, representative of the individual tumor phospho-proteome. We have assessed reproducibility and tumor heterogeneity using limited input material, including needle biopsies from patients.

Methods
Phosphopeptide immunoprecipitation (IP) using P-Tyr-1000 anti-phosphotyrosine-coated beads (PTMScan®, CST) was performed using 1, 5 and 10 mg protein from lysates of the colorectal cancer (CRC) cell line HCT116 and 3 patient-derived tumors (n=3 per protein input level). In addition, 14-gauge core needle biopsies from 3 additional tumorswere analyzed. After measurement by LC-MS/MS, MaxQuant database searching and analysis were applied for phosphopeptide identification (ID), ion-intensity-based quantification and phosphosite localization.

Results and discussion
Phosphoproteomics of the cell line triplicates of 1, 5 and 10 mg protein input yielded a total of 454, 559 and 664 unique phosphosites, and a median number of 345 (range:266-376), 435 (236-448) and 501 (476-618) per replicate, respectively. The ID-reproducibility was 45% for 1 mg and 58% for 10 mg protein replicates. In tissue, a median total of 622 (427-754) unique phosphosites were identified per tumor, with a median number of 190 (91-353), 404 (199-512) and 546 (252-638) per sample using 1, 5 and 10 mg protein, respectively. Analyses of needle tumor biopsies are in progress and will be presented at the meeting.

Conclusions
This scale-down study demonstrates the feasibility of phosphoproteomic analysis of patient tumor biopsies, by showing that hundreds of phosphopeptides can be profiled at low-level protein input. We are currently further developing phosphoproteomics-based targeted therapy selection using tumor needle biopsies from patients with advanced cancer.

P-314.00

PHOSPHOPROTEOMICS OF A PANEL OF AML CELL LINES

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Introduction and objectives Acute Myeloid Leukemia (AML) is a malignancy of the bone marrow and is characterized by the presence of undifferentiated myeloid cells. Initial treatment is similar for all patients, generally consisting of intensive chemotherapy, whereas subsequent consolidation therapy is based on classification into risk-groups. Though the initial response to therapy is good , long-term survival is generally low around 30%.

In recent years clinical success has been achieved in the treatment of several cancer types with kinase inhibitors (KIs) and they may prove to be a valuable addition to standard therapy in AML. However, a proper rationale to select a suitable KI is still lacking. Phosphoproteomics is a powerful tool in discovery, and could help identify aberrancies in signal transduction pathways and associated key kinases that may be suitable for targeted treatment with KIs. The aim of this project is to characterize 16 AML cell lines on the phosphoproteomic level by means of phosphotyrosine immunoprecipitation (pTyr IP) and LC-MS/MS.

Methods We enriched our AML cell line samples for pTyr peptides using the pY1000 antibody in an IP experiment. A single sample per cell line was processed, using 10 mg protein from each cell lysate. Both whole lysate and pTyr IP fractions were measured using nanoLC-MS/MS (2-hour gradient, QExactive). MaxQuant version 1.4.1.2 was used for phosphopeptide identification and label-free quantification.

Results and discussion The first six of the AML cell line panel (EOL-1, KG1, KG1a, ME-1, MM6, and NB4) have currently been measured. Identified peptides numbers are similar between cell lines, with numbers between 700 and 3500 pTyr peptides. pTyr enrichment varies between cell lines with the enrichment ranging from 5 to 21%, potentially related to differential kinase driver activity. The analyses of the data are currently in progress, and results will be presented.

The proteome quest to understand biology and disease

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New trends in biomarker discovery

OP022 - EXCLUSION OF CEREBRAL LESIONS IN MILD TRAUMATIC BRAIN INJURY USING PLASMA NUCLEOSIDE DIPHOSPHATE KINASE A (NDKA)

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Background. CT scan is used for the detection of cerebral lesions after mild traumatic brain injury (mTBI). However, CT scans are expensive and harmful for the patients. Despite several years of research on blood biomarkers, no alternative to the CT scan is yet available. The most promising biomarker remains S100b with specificity around 30% and sensitivity close to 100%. In brain injury models, the proteins GSTP1 (glutathione S-transferas pi), NDKA and H-FABP (heart-type fatty acid binding protein) have previously been discovered as potential biomarkers by a proteomics-based strategy. Here, we investigate whether these proteins, in comparison to S100b, could improve the rule-out of mTBI patients and thereby avoid unnecessary CT-scans.

Methods. The plasma level of S100b, GSTP1, NDKA and H-FABP was measured by immunoassays on more than one hundred mTBI patients recruited within the first 6h after trauma. The patients were dichotomized into CT-negative and CT-positive groups for statistical analyses. The predictive performance of these biomarkers was established using Mann-Whitney U tests and ROC curves.

Results. Out of the four proteins, only S100b and NDKA were significantly increased in CT positive patients (p

Conclusions. The present pilot study demonstrated that NDKA might be a useful plasma biomarker to reduce the number of unnecessary CT-scans in mTBI and save hospitalization costs.

OP023 - BLOOD BASED PROTEOMIC AND GENOMIC BIOMARKERS FOR CARDIAC TRANSPLANTATION: FROM DISCOVERY TO CLINICAL IMPLEMENTATION.

Robert McMaster[1], Gabriela Cohen Freue[1], Zsuzsanna Hollander[1], Janet Wilson-McManus[1], Robert Balshaw[1], Paul Keown[1], Christoph Borchers[2], Raymond Ng[1] Scott Tebutt[1]
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Solid organ transplantation is often the only treatment for end stage organ failure and has shown a dramatic increase in outcomes over the past 20 years. However, acute graft rejection remains an important clinical concern and is an adverse predictor for long-term graft survival. The current gold standard diagnostic for acute rejections remains repeated tissue biopsies – a highly invasive procedures with associated risks. Using advanced proteomics, genomics and computational biology, a series of blood based diagnostic biomarkers for acute heart allograft rejection have been developed and are currently being clinically implemented to monitor timely and effective therapeutic intervention to minimize graft damage and enable knowledgeable adjustment of immunosuppressive therapy.

Using a series of blood samples from over 400 transplant recipients with or without acute rejection, panels of biomarker correlating with acute rejection were generated using leukocyte quantitative plasma proteomics and mRNA and whole genome microarrays. Biomarker development can be divided into 3 phases: discovery, qualification, and validation.

To achieve maximum plasma proteome coverage different qualitative proteomic platforms were utilized for each development phase: non-biased isobaric labeling for discovery - identifying a number of potential biomarker panels; targeted MRM for qualification - resulting in panels of less than 10 proteins; a combination of MRM and ELISAs for validation and clinical implementation on standard clinical laboratory platforms. The biomarkers were then validated using a separate series single time point samples from a cross-Canada cohort of recipients. The resulting validated panel of 6 proteins and 10 genes exhibit excellent performance with 100% sensitivity and 91% specificity and 0.91 AUC (100% NPV and 32% PPV).

Multiplex assays for both genomic and proteomic biomarkers are currently being developed for FDA approval for implementation in clinical laboratories and routine transplant monitoring.

OP024 - GERM CELL-SPECIFIC PROTEINS MEASURED IN SEMINAL PLASMA EMERGE AS MARKERS OF SPERMATOGENESIS AND MALE INFERTILITY

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Introduction and objectives

Non-invasive methods for differential diagnosis of male infertility present urgent unmet needs in the urology clinics. Previously, we verified and validated by SRM 30 seminal plasma (SP) proteins and identified two proteins, germ cell-specific TEX101 and epididymis-specific ECM1, which differentiated between normal spermatogenesis and azoospermia forms with near absolute specificities and sensitivities. TEX101 also differentiated between histopathological subtypes of Sertoli cell-only, maturation arrest and hypospermatogenesis. As a result, we proposed a 2-biomarker algorithm for differential diagnosis of azoospermia forms and subtypes (Drabovich et al. Sci. Transl. Med., 2013, 5, 212ra160). Unlike TEX101, which was expressed at multiple stages of spermatogenesis, we hypothesized that other secreted germ cell-specific proteins measured in SP may emerge as stage-specific markers of spermatogenesis.

Methods and Results

Our work on TEX101 provided us with unique criteria to identify germ-cell specific proteins in SP. First, we analyzed raw data available in the Human Protein Atlas (HPA) and selected 85 proteins with an exclusive expression in seminiferous tubules. Second, we retained 22 secreted and membrane-bound proteins which were identified in our in-house SP proteome of 3,141 proteins. Following that, we developed SRM assays and verified 18 proteins in pre- and post-vasectomy SP samples. Fourteen proteins were not detected in the post-vasectomy SP, which experimentally confirmed their exclusive expression in the testis tissue. Upon examination of HPA immunohistochemistry staining, we selected cell type-specific proteins secreted exclusively by spermatogonia (n=1), spermatocytes (n=1), spermatids (n=5) and spermatozoa (n=2).

Conclusions

We identified and verified in SP 9 stage-specific markers of spermatogenesis and 14 novel biomarkers of male infertility. Pending further validation, these markers may pinpoint stage-specific termination of spermatogenesis leading to male infertility and facilitate development of a non-invasive test to predict the success of testicular sperm retrieval, thus increasing the reliability of assisted reproduction techniques.

OP025 - DIAGNOSIS AND PREDICTION OF PROGRESSION OF CHRONIC KIDNEY DISEASE BY ASSESSMENT OF URINARY PEPTIDES

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Introduction: Chronic kidney disease (CKD) is a major health problem affecting approximately 10% of the population. Albuminuria/proteinuria and/or eGFR (based on serum creatinine), although routinely used in the clinic, detect the disease only at a late stage. Hence, there is a need for biomarkers detecting CKD more early to allow timely interventions, and improve the risk stratification for patients.

Methods: The urinary proteome from 1990 individuals, including 522 individuals with 54±28 months follow-up data was studied with capillary electrophoresis coupled to mass spectrometry.

Results, Discussion: We first validated a previously established multi-peptide classifier (CKD273) that displayed a significantly higher correlation with baseline and follow-up eGFR (rho=-0.437 and rho=-0.395, respectively) than urinary albumin levels (rho=-0.339 and rho=-0.290, respectively). The classifier was also more sensitive for rapidly progressing CKD patients. Compared to the combination of baseline eGFR and albuminuria (AUC=0.758), the addition of the multi-peptide marker classifier significantly improved CKD risk prediction (AUC=0.831) as assessed by net reclassification index (NRI=0.303±-0.065; p Correlation of individual urinary peptides to CKD-stage (advanced-stage CKD, eGFR≤45 ml/min/1.73m2, n=321; moderate-stage CKD patients, eGFR>45 ml/min/1.73m2, n=1669) and progression showed that peptides associated with CKD, irrespective of CKD-stage or CKD-progression, were either fragments of major circulating proteins (beta-2-microglobulin, apolipoprotein A-I, alpha-1-antitrypsin, serum albumin) suggesting failure of the glomerular filtration barrier sieving properties, or different collagen fragments (collagen alpha-1(I) and (III)) suggesting accumulation of intra-renal extracellular matrix. Besides these findings, protein-fragments associated with progression of CKD originated mostly from proteins related to inflammation (CD99-antigen, complement factor B, protein S100-A9) and tissue repair (clusterin, membrane-associated progesterone receptor component-1, annexin A1).

Conclusions: This study suggests that urinary peptidome analysis can significantly improve the current state-of-the-art of CKD detection and outcome prediction and that identification of urinary peptides allows obtaining insight in various on-going patho-physiological processes in CKD.

P-315.00

DEVELOP A MALDI-IMAGING MASS SPECTROMETRY METHOD IN MOUSE SKIN TISSUE PEPTIDE DISTRIBUTION STUDY.

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MALDI-imaging mass spectrometry (IMS) can be used to find the distribution of lipid, peptide and protein in tissues. In our study, we report some peptides specific distribution in mouse skin. Methods: Frozen mouse skin tissue samples were cut as 10 mm sections using a cryostat onto a glass slide, dry in vacuum, and sprayed 40 g/l DHB matrix using a SunCollect MALDI Sprayer.

All experiments are performed on a Thermo Scientific MALDI LTQ Orbitrap XL Mass Spectrometer. Glass slides in plates with matrix-sprayed flakes are scanned with a commercial scanner and regions of interest as well as the entire flake area are selected along with laser raster step size (20um). These position files are treated similar to autosampler positions in LCMS. Experiments with regard to an FTMS full scan. Raw file acquisition for MALDI Imaging monitors the corresponding x-y position for every scan. With Thermo Scientific ImageQuest software the mass spectral raw data is displayed in a spatially resolved manner. Result: In FTMS Scans, peptide 565 especially high express in epidermis. Peptide 768, 664, 774 especially high express in dermis. Subsequently a MS/MS scan was executed on these peptides detected in the full scan to

Identify the sequnces.Signal in 765 was identified as a peptide of the protein: Vacuolar protein sorting-associated protein 11 homolog. Peak 774 was identified as a peptide of the protein Zinc finger protein 865. The MALDI-imaging mass spectrometry of mouse skin tissue found some peptides specifically distribute in different skin regions. That will help us in the study of skin disease further.

P-316.00

POTENTIAL BIOMARKERS BETWEEN BURKHOLDERIA PSEUDOMALLEI WILD TYPE AND ITS RPON2 MUTANT STRAIN USING MALDI-TOF MASS SPECTROMETRY APPLICATION

Kitima Sresanga[1], Suthamat Niyompanich, Sittiruk Roytrakul, Sumalee Tungpradabkul
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Melioidosis is a disease infected by Burkholderia pseudomallei, which is a motile, Gram-negative bacillus. It can survive under various environmental stress conditions and invade to several kinds of host cells, including phagocytic and non-phagocytic cells. Previous studies have been reported that this bacterium can switch colony morphology under starvation, which is classified into seven types. The rpoN2 mutant strain has been constructed and shown its importance to control some virulent genes.

In our experiment, B. pseudomallei wild type and rpoN2 mutant were cultured on Ashdown's agar for observing colony morphology changes and used the whole-cell matrix-assited laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) to identify biomarkers. This technique is currently used as a rapid tool for discovering the unique biomarkers in biological samples. The results showed that colony morphology of the rpoN2 mutant was similar to the parental strain as well as the protein profiles obtained from whole-cell MALDI-TOF MS. In addition, we also demonstrated the potential seven candidate biomarkers mass ions at m/z 3,519, 3,689, 4,158, 6,323, 7,435, and 7,650 for discriminating these two strains.

Therefore, our present study shows that the mutated rpoN2 gene slightly affects the changes of colony morphology compared to wild type, resulting in similar colony appearances. However, whole-cell MALDI-TOF MS still has ability to provide the efficient candidate biomarkers for differentiating these two strains.

P-317.00

RELATIONSHIP BETWEEN BIOMARKER DISCOVERY AND COLONY MORPHOLOGY OF BURKHOLDERIA PSEUDOMALLEI BY WHOLE-CELL MATRIX-ASSISTED LASER DESORPTION IONIZATION TIME-OF-FLIGHT MASS SPECTROMETRY (MALDI-TOF MS)

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Burkholderia pseudomallei is a soil-dwelling Gram-negative bacterium and the causative agent of melioidosis, which is widely dispersed among Southeast Asia and northern Australia. Variability in colony morphology of B. pseudomallei can be observed in vitro on Ashdown's selective agar and is divided into seven different morphotypes, type I-VII, leading to the hypothesis that distinct colony morphotypes possess of altered surface protein expression.

To our work, we also observed the different appearances of colony morphology between PP844 wild type and rpoS mutant strains. Due to the importance of RpoS in B. pseudomallei, it is an alternative sigma factor that plays a crucial role in gene regulation in response to various stress conditions and is proposed to be involved in pathogenicity of bacteria. Interestingly, colony morphotype obtained from rpoS mutant, differs obviously and cannot be categorized into those seven types, has never previously been reported. Therefore, this present study employed whole-cell matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) to detect the potential biomarkers to differentiate these two strains, correlated to colony morphology. Bacterial colonies were mixed with matrix solution, spotted on the target plate, and then analyzed by MALDI-TOF MS. The results showed that the potential biomarkers at m/z 4,709, 6,642, 7,561, 8,120, and 8,250 demonstrated significant differences between mass spectra of these two strains.

Hence, our findings provide the novel candidate biomarkers differentially among mass fingerprints of B. pseudomallei PP844 wild type and rpoS mutant, for future aspects, protein/peptide verification of candidate biomarkers may contribute to the knowledge of proteomic alterations with regard to RpoS-regulated gene expression involving in colony morphology changes.

P-318.00

IDENTIFICATION OF POTENTIAL BIOMARKERS OF HEPATOTOXICITY BY PROTEOMIC ANALYSIS OF BLOOD PLASMA IN A LOWER VERTEBRATE EXPOSED TO ARSENIC

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Introduction and objectives: Arsenic (As) is a toxic environmental contaminant and is a potential human carcinogen. Chronic intake of As-contaminated water leads to arsenicosis that has become a major public health problem. Early detection of toxicity provides great benefits to patients for prevention of further complications. Developing bbiomarkers could be useful in therapeutic intervention and developing mitigation measures and the omics technologies offer the ideal platforms for biomarker discovery. Blood plasma contains a plethora of proteins and as it circulates through the tissues, the plasma picks up proteins that are released from their origin. Specific disease processes are often characterized by plasma proteome signatures. The potential for proteomic technologies to identify and quantify novel proteins in the plasma that can function as biomarkers of the presence or severity of clinical disease states holds great promise for clinical application. In the present study, we investigated the plasma proteome changes in As-exposed Labeo rohita, a cyprinid like the Zebrafish (Danio rerio), with the objective of identifying biomarkers of arsenicosis.

Methods: Proteomic changes were investigated using gel-based proteomics technologies like 2-D GE, MALDI-TOF-MS and LC-MS/MS.

Results and discussion: Based on gel image analysis of plasma proteome changes following arsenic exposure, 14 protein spots were picked up and were identified by MALDI-TOF-TOF-MS and validated by LC-MS/MS. The unique proteins identified include Apolipoprotein-A1 (Apo-A1) (6 out of 14 spots identified), $f\tilde{N}$-2 macroglobulin-like protein (A2ML) (2), transferrin (3) and warm-temperature acclimation related 65kDa protein (Wap65) (1). Highly up regulated protein spots identified in plasma proteome (> 50%) were found to be liver-specific.

Conclusions: Up regulation of liver-specific proteins like Apo-A1 (> 10 fold), A2ML (7 fold) and Wap65 (> 2 fold) indicate liver damage following arsenic exposure at higher concentrations. Combination of these novel biomarkers could be utilized as biomarkers of hepatotoxicity and chronic liver disease.

P-319.00

LABEL-FREE QUANTITATIVE PROTEIN PROFILING OF VASTUS LATERALIS MUSCLE DURING HUMAN AGING

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Sarcopenia corresponds to the loss of muscle mass occurring during aging, and is associated with a loss of muscle functionality. To better understand the mechanisms involved in muscle aging, we performed a proteomic analysis of Vastus lateralis muscle in mature (53.0 ± 3.5 years (n = 6)) and old (77.6 ± 2.0 years (n = 4)) women. For this, a shotgun proteomic method was applied to identify soluble proteins in muscle, using a combination of high performance liquid chromatography and mass spectrometry Nano-LC-ESI-MS/MS analysis. A label-free protein profiling was then conducted to quantify proteins in mature and old women, using Progenesis LC-MS software (version 4.1, Nonlinear Dynamics, Newcastle, UK).

This quantitation analysis, based on 366 proteins identified, showed that 35 proteins intensities were linked to aging in muscle. Most of the proteins were over-represented in mature women compared to old women. Western-Blot analysis was used to validate some of the potential biomarkers of sarcopenia. We built the functional interactions network linking the differentially expressed proteins between mature and old women. The results revealed that the main biological functions involved in muscle aging were defined by proteins involved in energy metabolism, actin myofilament, proteins involved in detoxification and cytoprotection and protein turnover. The label-free quantitative proteomics was applied for the first time in the study of aging mechanisms. This approach highlights new elements for elucidating muscle evolution during aging and may lead to sarcopenia biomarkers.

P-320.00

A NOVEL APPROACH FOR PROCESSING LC - ION MOBILITY - MS METABONOMICS DATA SETS

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MS interfaced with LC and ion mobility (IM) is routinely used to measure the level and variation of metabolites within biofluids as data generated through metabolomics studies may yield insight into disease onset and progression. LC-IM-MS based metabolomics generates large and complex data sets with analysis and interpretation of the results being the rate determining steps. This has led to a demand for improved data analysis, including processing and advanced multivariate approaches, which are described for the large scale analysis of metabolomics datasets.

Urine from a healthy individual was centrifuged and the supernatant diluted. The urine was divided into control, low dosed (LD) and high dosed (HD) groups. To create a sample set, 11 different drugs were differentially spiked into LD and HD urine, contrasted with blank urine. A reversed phase gradient was applied and MS data acquired in positive ion data independent (LC-DIA-MS) and mobility assisted data independent (LC-IM-DIA-MS) modes.

Distinguishing biological variation and metabolic change from analytical interference is key to data processing and analysis. Samples were randomized and measured six times, including QC runs, to ensure statistically valid analysis. LC-MS data were retention time aligned and deconvoluted to produce a feature list. Identified features were compound searched and interrogated with multivariate statistics to provide marker ions of interest. Relative high abundance levels of the standards were reported for LD and HD compared to controls, confirmed by trend plots analysis showing an increase in LD and HD groups compared to control. The standards were identified with an average score of 91 and mass error of 1.2 ppm. Three sample clusters were produced with the standards being the most differentiating features (top 20 based on q value) between groups. Functionality of the software will be demonstrated using biological samples.

P-321.00

DEVELOPING A HIGH-THROUGHPUT, LABEL FREE TARGETED-MS APPROACH FOR VALIDATION OF PLASMA PROTEIN CONCENTRATIONS ASSOCIATED WITH DISEASE RISK.

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Introduction and Objectives. Clinical proteomics generates large amounts of data, usually on small sample numbers. Candidates therefore require verification in larger sample cohorts before being considered for proper clinical testing. While this step commonly uses ELISA, antibodies are not always available, or specific. Selected Reaction Monitoring (SRM) MS is a viable alternative. Here, we describe a low cost, robust and reproducible label-free SRM-based assay to measure isoforms of the pregnancy-specific glycoprotein (PSG) family, which discovery experiments suggested had power in predicting risk of pre-eclampsia (PE) in asymptomatic women undergoing their first pregnancy, but for which no isoform-specific antibodies are available.

Methods. SRM transitions were optimised in Skyline. Peptides were selected on the basis of digestion reproducibility and peptide recovery. Loading on-column was optimised and specificity determined using a range of dilution-series experiments. For the final assay, 108 samples were analysed, containing both technical and full replicates and samples from women who had a healthy or PE-affected pregnancy (n=42/group). ELISA was used to assess another of our target proteins, NAP-2, and compared to the SRM data.

Results and Discussion. Our assay detects a 10-20% change in protein level, with a C.V. of 12-25% for 19 peptides from eight full replicates analysed up to 2 weeks apart. NAP-2 ELISA and SRM values are highly correlated, validating our method. PSG-5 and -9, but not other isoforms, predict PE risk in plasma from asymptomatic women, with AUROC equal to that of the best current PE risk marker. Correlation analysis showed this effect was independent of processing variables. PSG-5 and –9 are promising candidates for further study.

Conclusions. Our protocol provides a relatively rapid, robust and inexpensive method for the targeted measurement of proteins in moderate but clinically relevant numbers of plasma samples, allowing candidate biomarkers to be quickly prioritised for properly powered clinical assessment.

P-322.00

PROTEOMICS-DRIVEN SELECTIVE MITOCHONDRIAL TARGETING FOR TREATING ANXIETY DISORDERS

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Introduction and objectives
Anxiety disorders are the most prevalent psychiatric diseases worldwide. Since a third of the patients do not respond to current anxiolytic treatment, hypothesis-free approaches to identify novel drug targets are pivotal for improved response rates.

Methods
We developed a quantitative proteomics platform based on in vivo 15N metabolic labeling and quantitative mass spectrometry to identify differences in mice selectively bred for high vs. low anxiety. We then pharmacologically manipulated the identified differences in vivo with appropriate compounds in order to assess whether selective pharmacological targeting exerts an effect on the behavioral phenotype of high anxiety mice.

Results and Discussion
Our hypothesis-free approach revealed altered brain mitochondrial pathways in high vs. low anxiety mice. These mitochondrial pathways were selectively targeted in vivo by MitoQ, a mitochondria-targeted antioxidant. We observed a decreased anxiety-related behavior in MitoQ-treated compared to untreated high anxiety mice. We then analyzed the molecular correlates of this anxiolytic effect with immunoassays, biochemical assays and targeted metabolomics.

Conclusions
This is the first time that a proteomics-driven, mechanism- rather than symptom-based approach is used to manipulate a behavioral phenotype. Our findings emphasize the potential of proteomics-driven approaches for drug target discovery and highlight the therapeutic potential of mitochondrial targeting for treating brain disorders.

P-323.00

NOVEL BIOMARKERS OF ANDROGEN DEFICIENCY FROM SEMINAL PLASMA PROTEOMIC PROFILING USING HIGH-RESOLUTION MASS SPECTROMETRY.

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Introduction and objective

Epididymis, seminal vesicles and prostate are androgen-dependent organs having important but poorly defined functions in establishing sperm function and male fertility. Aim of this study was to identify new seminal biomarkers for male hypogonadotropic hypogonadism (HH) using proteomic profiling.

Methods

Twenty male patients affected by HH were studied. Ten patients were re-evaluated after 6 months of testosterone replacement therapy (TRT). Ten normogonadic men were enrolled as control group. An aliquot of each seminal sample was subjected to in solution digestion and analyzed by an Ultimate 3000 Nano/Micro-HPLC apparatus equipped with an FLM-3000-Flow manager module, and coupled with an LTQ Orbitrap XL hybrid mass spectrometer.

In order to analyze the effect of HH in inducing proteic secretion impairment we evaluated, among the proteins identified in normogonadic fertile men, which ones were absent in the group of hypogonadic patients and subsequently the list of absent proteins in hypogonadic patients which were identified after TRT.

Proteins identified by SEQUEST were analyzed using the PANTHER classification system. The interaction network of the differentially expressed proteins was built by the STRING system including the androgen receptor (AR).

Results and discussion

A lower number of proteins was identified in hypogonadic patients compared with normogonadal men, confirming the role of HH in reducing protein synthesis overall. 33 proteins were absent in hypogonadic patients. Fourteen out 33 absent proteins were identified in samples after 6 months of TRT, representing an array of proteins related to testosterone action on accessory glands. Functional annotation analysis revealed that binding and enzymatic activities are mainly deficient in HH. 7 differentially expressed proteins can fall into one interaction network, including the AR.

Conclusion

A proteomic approach was firstly used to decribe the alterations of seminal seminal proteome in HH. These proteins represent putative physiological in vivo targets for androgen deficiency.

P-324.00
INCREASING DEPTH OF COVERAGE IN DATA INDEPENDENT ACQUISITION

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Data independent acquisition (DIA) strategies have been used to increase the comprehensiveness of data collection while maintaining high quantitative reproducibility. In DIA, larger fixed-width Q1 windows are stepped across the mass range in an LC timescale, transmitting populations of peptides for fragmentation, and high resolution MS and MS/MS spectra are acquired. Previous work has shown that using more narrow Q1 windows can improve peptide detection and increase sample coverage. Here both method and instrumentation advancements will be explored to continue to increase depth of sample coverage.

The MS analysis was performed on a modified quadrupole time of flight instrument equipped with an ADC detection system. DIA data collection was done using SWATH™ acquisition with prototype acquisition software to explore a variety of acquisition strategies. The DIA data was interrogated using a comprehensive yeast spectral library created from many data dependent experiments. Results assessment was performed using Excel tools.
Original work exploring variable window size and more narrow windows demonstrated that increasing the number of total Q1 windows from 24 to 60 windows provided an increase in confident peptide detections with good quantitative reproducibility of ~15%. To enable higher sample loads, the dynamic range of the detection system was extended by switching from a TDC based detection system to an ADC based system on a modified TripleTOF system. At the higher sample loads, we next applied increasingly narrow Q1 windows during SWATH acquisition to continue to improve the S/N in MS/MS.

The number of windows was extended from 60 to 100 windows across the 400-1250 precursor m/z range while maintaining a cycle time of 3.2 secs. This provided a 20% increase in confident peptide detections with 20% or better CVs across replicates. Further optimization of longer chromatography and investigations of impact on other proteomes will be discussed.

P-325.00

THE DISCUSSION ABOUT RESULTS FROM IMMUNOASSAYS AND TARGETED PROTEOMICS - NEVER-ENDING STORY OF THE QUANTIFICATION OF PROTEINS

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Introduction

Selected reaction monitoring (SRM) has become an important method of the targeted proteomics for protein detection and quantification in complex samples with high sensitivity, selectivity and good reproducibility. This method is often presented as a powerful toll which could advantageously replace the widely used and still more sensitive immunoassays. Often the immunoassay results are used for verification of SRM results or to their comparison.

However, first we should answer some basic questions, e.g. how to compare results from these methods when they are focused on the different targets (proteins and peptides) or how to choose and use one or several peptides to the quantification of the whole protein when you know that they give opposite results? The main aim of this poster is to use a small example to show at least some of the problems associated with targeted proteomics.

Methods

Study group included 17 patients with hypertrophic cardiomyopathy and 17 healthy control subjects with similar characteristics.
Plasma concentration of soluble fibronectin was determined by commercial ELISA kit. GraphPad Prism software was used for statistical analyses.
Trypsin digested and stable isotope spiked samples were analyzed on QTRAP mass spectrometer. Absolute quantification of fibronectin and antithrombin peptides was performed in Skyline software.

Results and Discussion

ELISA results of fibronectin and the quantification of the two choosen peptides from fibronectin by SRM are comparable in the trend but not in the absolute quantities. Absolute quantification of the antithrombin peptides showed different results. We suggest that the differences are not caused by the preanalytical part or statistical discrepancies but are based on the biological or pathological processes of the studied proteins.

Conclusion

The truth of the measurement result is not just a number on the screen.

P-326.00

SPIKEMIX PEPTIDES – A NOVEL APPROACH FOR SYNTHETIC PREPARATION OF LOW COST & SMALL SCALE PEPTIDE POOLS FOR MS-BASED PROTEOMICS

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Introduction and objectives

Targeted proteomics is dependent on the availability of peptide standards for assay development and protein quantification. Recently, SpikeTidesTM were reported as small-scale, inexpensive, and heavy labeled standards for relative and absolute protein quantification using mass spectrometry (1). While the individual synthesis of SpikeTidesTM peptides sufficiently supports discovery and targeted proteomics studies, for large scale approaches including thousands of peptides, handling efforts (pooling) and costs remain significant. Here we present an advanced procedure for the preparation of synthetic heavy labelled peptide pools with increased efficacy and throughput.

Methods

In contrast to the standard methodology, two major parameters were changed in the synthesis procedure: 1) The density of the peptides during synthesis was increased; 2) After synthesis, the peptides were simultaneously cleaved off the membrane, yielding all desired peptides in a single pool. The resulting peptide mixtures can be directly analyzed via LC-MS/MS for presence of assembled peptides.

Results and Discussion

Results from several projects are presented.

A) ProteomicsDB is a new publicly available database which contains high-quality shotgun proteomics data for the whole human proteome (2). Reference standards to support weak peptide identifications were developed and synthesized using the new SpikeMixTM technology.

B) Cytokines are important regulators of immune processes. We prepared 461 proteotypic peptides, allowing the simultaneous detection and relative quantification of more than 200 cytokines.

C) An ABRF cross species proteomics standard, derived from proteins that are conserved across the three most commonly analyzed species and consisting of 1,000 stable isotope labeled peptides was developed.

Conclusions

The new SpikeMixTM method enables the chemical synthesis of complex proteotypic peptide pools at unprecedented speed and cost efficiency.

P-327.00

TEAR PEPTIDOMICS: IDENTIFICATION OF TEAR NATURALLY OCCURRING PEPTIDES BY CID, HCD AND ETD

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Introduction and objectives:
Endogenous peptides and peptides derived from the naturally occurring cleavage of proteins are valuable targets for the analysis of biological processes. However, MS-analysis of endogenous peptides has proven difficult because of their size heterogeneity and multiple internal basic residues. Despite its physiological relevance, little is known about tear fluid endogenous peptides content. In this work we characterize the naturally occurring peptides from human basal tears using CID, HCD and ETD fragmentation methods as a first step towards in depth characterization of the tear peptidome.

Methods:
Samples from 5 age-matched individuals (3 male and 2 female, age range 35-45) were obtained from the inferior temporal tear meniscus using 10 ìl calibrated glass microcapillary tubes (Blaubrand intraMark, Wertheim, Germany). After collection, the tear samples were pooled, reduced and alkylated, acidified and subjected to stage-tip Reversed-Phase C18 (Millipore).

Peptides were separated by on-line NanoLC (nanoACQUITY UPLC, Waters), and analyzed using electrospray tandem mass spectrometry (LTQ Orbitrap XL ETD, Thermo). Sample was concurrently characterized by CID, HCD and ETD-based acquisition methods, carried out in triplicate independent runs.

Results:
Preliminary characterization of the sample led us to the identification of 140 peptides mapping to 19 different proteins. Proteins and peptides related to the defense response and protective role of tear fluid were identified, among others. CID, HCD and ETD revealed to be largely complementary, and each of them contributed with the identification of 39, 20 and 23 exclusive peptides. Further approaches are currently being carried out for a deeper characterization of the basal tear peptidome.

Conclusions:
Our results expand current knowledge on basal tear naturally occurring peptide content, and reveal that the concurrent use of CID, HCD and ETD may result in an increase in sample analysis and coverage for this kind of studies.

P-328.00

NEOPTERIN PLASMA LEVELS PREDICT INFECTION DEVELOPMENT IN ANEURYSMAL SUBARACHNOID HEAEMORRHAGE PATIENTS

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Background. Aneurysmal subarachnoid haemorrhage (aSAH) is associated with high rates of mortality and morbidity. Inflammatory events as vasospasm or infection occurring after the haemorrhagia are the main cause of outcome worsening. Until now, no biomarker is available for the early detection of these complications. The aim of this study was to use omics-based strategies to find biomarkers for infection prediction in aSAH patients.

Methods. Plasma samples from aSAH patients (N=61) were collected daily from the arrival at the hospital to ten days after. Patients' outcome was established 12 months after the haemorrhagic event using the Glasgow Outcome Scale and were classified as poor (GOS 1, 2, 3) or good (GOS 4, 5) depending on the grade of dependence. Onset of any infection was determined through a positive bacterial culture. 'Omics techniques were used to compare the samples of infected patients (n=3) from those of non-infected ones (n=3), in order to identify differentially expressed markers. Among them, neopterin was selected for further ELISA validation on a larger cohort (N=42 infected and 19 non infected patients). The predictive performances were established using Mann-Whitney U tests and ROC curves.

Results. At the day of infection (mean day after admission=5), neopterin levels were significantly increased in infected patients (14.1nM/L) than in non-infected ones (10.2nM/L) (p

Conclusion. Our data suggest that neopterin is a potential important outcome and infection predictor after aSAH. Its measurement could potentially lead to an earlier antibiotherapy to decrease the development of nosocomial infections in aSAH patients.

P-329.00

SEARCH FOR NOVEL ALLERGEN OF HEN□FS EGG ALLERGY USING WESTERN BLOT ANALYSIS

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Food allergy is a serious health issue affecting roughly 4% of children, with a substantial effect on quality of life. Chicken egg allergy is frequently observed in infants, some of who therefore have to avoid hen□fs egg from daily diet to eliminate unfavorably allergenic symptoms. Hen□fs egg is composed of two soluble parts; one is egg white which has been characterized as major in allergenic portion, while the other is egg yolk which is estimated as miner in that, regardless of less accumulation of research in allergenicity of egg yolk proteins. Only two allergen from the egg yolk, a-livetin (Gal d 5) and YGP42 protein (Gal d 6) has been described thus far.

A new egg yolk allergen was detected studying 14 egg allergic patients. The study was performed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and IgE-immunoblotting assay. The new allergen detected was characterized by LC-MS/MS. A total of 3 of the 14 patients (21.4%) detected a yolk allergen of apparent molecular weight of 39.0 kDa by SDS-PAGE.

Identification of these egg yolk allergens are currently underway to clarify the significance and mechanisms of the observation made in this study. Also, larger-scale study is planned to further validate the utility of measurement of these egg yolk allergens.

P-330.00

INTEGRATION OF SWATH AND MRM FOR BIOMARKER DISCOVERY OF ESOPHAGEAL SQUAMOUS CELL CARCINOMA

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Introduction and objectives
It is gradually recognized that biomarker discovery based on DDA may meet the technique challenges in acquiring peptide MS/MS and accurate quantification. Recently DIA has proven useful for combined qualitative/quantitative analysis to proteomes. Herein, we proposed an approach that integrates SWATH and MRM for biomarker discovery and verification.

Methods
The ESCC and the adjacent tissue lysates were tryptic digested, and all DDA and SWATH data were acquired by 5600 TripleTOFTM MS coupled with Eksigent400 NanoLC. The peptides identified from DDA were taken to build the ion library, and the analysis of SWATH data was implemented by Peakview V2.0. The differential proteins were evaluated through SRMstats and transitions extracted from SWATH were used to develop MRM assay on QTRAP5500.

Results and Discussion
Discovery of the ESCC-related proteins with SWATH: With SWATH, 2157 unique proteins with 10198 peptides and 1955 unique proteins with 9708 peptides were identified in ESCC and the adjacent tissues, respectively. According to the statistical analysis by SRMstats, total of 294 unique proteins were found with their abundance in the pooled ESCC tissue lysate significantly up-regulated from that in the pooled lysate of the adjacent tissue.

Verification of the ESCC-related candidate with MRM: With stringent criteria to select the ESCC-related proteins for MRM assay, total of 146 unique proteins with 505 peptides were selected from the 294 up-regulated ones. The MRM method was conducted to detect the targets in the individual serum collected from the 10 ESCC patients who received surgery. 28 proteins were found in lowering protein abundance after operation, indicating that these proteins in serum were tingly correlated with the tumor removal.

Conclusions
Herein, we developed a novel pipeline to integrate SWATH and MRM for biomarker discovery and verification. The results demonstrated the combination of SWATH/MRM is feasible for ESCC biomarker discovery.

P-331.00

STOP ENZYMATIC ACTIVITY AND PRESERVE PROTEOME INTEGRITY OF TISSUE SAMPLES BY HEAT STABILIZATION

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The action of proteolytic and other protein-modifying enzymes rapidly change the composition of the proteome and post translational modifications (PTM) after sampling. Subsequent analytical results reflect a mix of the in vivo molecular status and degradation products and display increased inter-sample variation. Effective enzyme inactivation and standardization of sample handling eliminate this problem.

A heat-stabilization system has been used to generate rapid, homogenous thermal denaturation of enzymes to stop degradation in tissues. Comparisons were made to snap-freezing and inhibitors, and in time study manner, compared with different post-mortem intervals. Using mass spectrometry, Western blot, RPPA and activity assays, the protein and peptide content, including PTM's were examined.

The results show rapid changes in phospho-states on a variety of different proteins detected only minutes after excision whereas after heat-stabilization, phospho-levels remain unchanged during 2 hours in room temperature. In three minutes post-mortem both proteins and endogenous peptides/neuropeptides, including PTM's, are subjected to substantial degradation. Conversely, amounts and identities of the detected proteins/peptides in heat-stabilized samples show maintained integrity. Similarily, levels of pCREB, pGSK3â and pERK1/2 were unchanged for 2 hours, whereas snap-frozen samples showed a dramatic decrease in levels after 10 min in room temperature.

Post-mortem changes may distort our view of in vivo protein expression. Adequate suppression of both phosphatases and kinases is important for phospho-state analysis. Heat-stabilization stops activity thereby enables sample analysis to reflect the in vivo status as closely as possible. This approach may be of great help in research on neurodegenerative disease such as Alzheimer and Parkinson to differentiate true biomarkers from those proteins found in any situation where cells are under stress.

P-332.00

DIFFERENTIAL MOBILITY SPECTROMETRY FOR GAS-PHASE FRACTIONATION INCREASES PROTEOME COVERAGE AND IMPROVES ION LIBRARY CREATION FOR SWATH™ ACQUISITION

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To deeply interrogate a complex proteomic sample, fractionation is often required to dig deeper, to create more simple sub-samples that can be effectively analyzed by LC/MS. Typically that is done using an offline or online strategy (SCX, OGE, high pH-LC) upfront of the analytical LC-MS analysis. In this work, a gas phase fractionation strategy was explored using a planar differential mobility separation device (DMS) installed in-front of a QqTOF instrument. DMS separates peptides based on differences in their chemical properties, prior to entering the instrument orifice, providing an orthogonal level of selectivity. The utility of this gas-phase fractionation for increasing proteome coverage was assessed.

The MS analysis was performed on a TripleTOF® 5600+ system (AB SCIEX) equipped with a proto-type DMS device. Data collection was performed using both data dependent and data independent acquisition strategies. For each acquisition, the peptides were separated into fractions ahead of the MS orifice by setting the DMS at a fixed compensation voltage (CoV). A range of CoV values and total fractions were explored. Proteins were identified using ProteinPilot™ software and results were assessed in Excel.

Using discrete CoV steps across the CoV range for peptides, yeast was fractionated in the gas-phase before data-dependent analysis. Using data from 8 DMS fractions, the number of peptides tripled and the number of proteins doubled, providing a significant increase in proteome coverage relative to a single injection. Using the lowest resolution setting, ~50% of peptides were found in 1 fraction and 20% in only 2 fractions indicating good results in the fractionation. More optimization will be done to determine the optimal balance between the resolution of the DMS separation. Finally, using the more comprehensive ion library generated with DMS fractionation, SWATH acquisition data was interrogated and a significant increase in reproducibly quantified peptides was observed.

P-333.00
CRITICAL COMPARISON OF SAMPLE PREPARATION STRATEGIES FOR SHOTGUN PROTEOMIC ANALYSIS OF FORMALIN-FIXED, PARAFFIN-EMBEDDED SAMPLES

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Introduction and objectives
The growing field of formalin-fixed paraffin-embedded (FFPE) tissue proteomics holds promise for improving translational research. Worldwide archival tissue banks hold a significant number and variety of tissue samples, as well as a wealth of retrospective information regarding diagnosis, prognosis, and response to therapy. This makes them an important resource for protein biomarker discovery and validation. Direct tissue trypsinization (DT) and protein extraction followed by in solution digestion (ISD) or filter-aided sample preparation (FASP) are the most common workflows for shotgun LC-MS/MS analysis of FFPE samples, but a critical comparison of the different methods is currently lacking.

Methods
DT was preceded by homogenization in ammonium bicarbonate, while ISD and FASP comprised protein extraction in SDS based-buffer, followed by SDS depletion with Detergent Removal Spin Columns and Microcon Ultracel YM-30 filtration devices, respectively. The three workflows were applied to consecutive tissue sections cut from an FFPE liver tissue block, and peptide mixtures were finally analyzed according to a label-free quantitative MS approach. Data were evaluated in terms of method reproducibility and protein/peptide distribution according to localization, MW, pI and hydrophobicity.

Results and Discussion
DT showed lower reproducibility, good preservation of high-MW proteins, a general bias towards hydrophilic and acidic proteins, much lower keratin contamination, as well as higher abundance of non tryptic peptides. Conversely, FASP and ISD proteomes were depleted in high-MW proteins and enriched in hydrophobic and membrane proteins; FASP provided higher identification yields, while ISD exhibited higher reproducibility.

Conclusion
These results highlight that diverse sample preparation strategies provide significantly different proteomic information, and present typical biases that should be taken into account when dealing with FFPE samples. When a sufficient amount of tissue is available, the complementary use of different methods is suggested to increase proteome coverage and depth.

P-334.00

INVESTIGATING BIOLOGICAL VARIATION IN HUMAN HEPATOCYTES OF PHASE I AND II DRUG METABOLISM ENZYMES

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Measurement of drug metabolizing enzymes responsible for phase I and II biotransformations is a fundamental aspect of assessing drug-drug interactions, and evaluating drug safety and efficacy. In this work, we used SWATH Acquisition, a data independent acquisition method, to analyze protein expression levels of many of the enzymes involved in the drug metabolism.

A spectral ion library containing more than 2000 proteins was generated from data dependent analysis of a pooled sample. In the SWATH data generated using a TripleTOF® 5600+ system, an average of 1987 proteins, including 19 CYP proteins, 12 UGT proteins, and 7 GST proteins, were quantified across the 13 samples. The quantitative interpretation of SWATH data was achieved by automatic extracting characteristic fragment ions for each identified peptide from high resolution TOF MS/MS spectra. A set of protein/peptides of interest obtained from SWATH acquisition was then further analyzed with MRM using a QTRAP® 6500 System.

Quantitative comparison of two phase II metabolism enzymes, EST1 - liver carboxylesterase 1 and EST2 - cocaine esterase, showed 40% variations across 13 samples. The relative intensities of 4 individual peptides of each protein showed very good agreement.

P-335.00

IMPROVING PREDICTION OF IVF SUCCESS - LOOKING FOR PUTATIVE BIOMARKERS IN IVF-MEDIA UPON EMBRYO CULTIVATION

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Embryo selection for transfer is the most important step of assisted reproductive technology. The determination of the viability of embryos is performed using morphological criteria like cleavage rate and blastomere symmetry. Because morphology alone cannot determine the molecular signature of an embryo, multiple implantations are necessary in order to increase the probability of successful fertilization.

The secretome of human embryos was compared between embryos that were labeled as morphologically positiv, negativ and mixed on different days of cultivation. By comparing identified proteins in the embryo secretom, the differences in the pattern of secreted proteins were observed in morphologically different embryos.

We can conclude that the proteome based analyses of embryonaly secreted proteins can provide a useful tool to understand the processes during early embryo development. It may also help to define embryo derived distinct protein profiles causative for embryo pathologies.

P-336.00

HIGH-THROUGHPUT/HIGH ACCURACY PROTEIN BIOMARKER DISCOVERY FOR DEVELOPMENTAL NEUROTOXICITY AFTER INSECTICIDE EXPOSURE BY APPLICATION OF ISOBARIC REPORTER ION QUANTIFICATION (TMT10PLEX) IN COMBINATION WITH HCD-MS3 ACQUISITION

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In biomarker discovery studies, appropriate throughput is a crucial requirement. TMT6plex reagents allow the simultaneous analysis of up to six samples at reasonable costs and time. Isobaric TMT quantitation in complex samples may be compromised by co-isolation of unrelated analytes in the MS precursor ion selection window. HCD-MS3 almost completely eliminates interference.

Various studies have indicated that exposure to low doses of insecticides and heavy metals during human development have adverse effects on cognitive development in childhood. Here, we show proof of performance data for the enhanced throughput using TMT10plex reagents studying a large sample set for developmental neurotoxicity effects of insecticide at low levels.

Cell-lines/animals were exposed to various doses of insecticide (organophosphates, carbamates, pyrethroids) and heavy metals (methylmercury). Samples were homogenized and labeled with TMT6plex or TMT10plex reagents. MS were acquired in the Orbitrap and TOP10 MS/MS were acquired for peptide identification and quantitation. For MS2 quantitation, HCD-MS2 was acquired in the Orbitrap. For MS3 quantitation, MS2 was acquired in the ion trap using CID for identification. MS2 fragments ions were selected for further HCD-MS3 quantitation in the Orbitrap. MS data was processed with Proteome Discoverer and applied to statistical analysis (PCA, PLS & ANOVA).

In an in vivo model, the total number of identified proteins was 877 for TMT6 and 891 for TMT10. Reporter ions presence in HCD-MS3 spectra was similar for TMT6 (84.4%) and TMT10 (84.2%) labeled samples. Quantitative values for MS3 are closer to theoretical values compared to MS2 in spiked-in experiments which highlight the MS3 method accuracy. Use of an online multi-notch MS3 algorithm resulted in 74% increase of quantified peptides. This TMT10plex-based workflow enables high-throughput and high accuracy at reasonable costs/time: we identified 33 to 80 differently expressed proteins as putative biomarkers for developmental neurotoxicity after insecticide exposure.

P-337.00

NOVEL BIOMARKERS FOR BLADDER CANCER: EVALUATION OF ELISA ASSAY PERFORMANCE IN URINE

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Introduction and objectives

Bladder cancer (BC) is the second in incidence and mortality among cancers of the genitourinary tract and accounts for more than 350,000 new cases worldwide per year. Non-invasive approaches for early diagnosis and detection of progression and recurrence of the disease are needed because cystoscopy (endoscopic examination of the bladder) is invasive and urine cytology has low sensitivity for low grade tumors. Several biomarker candidates were identified by analyzing the proteome of urine samples from bladder cancer patients and controls. In order to investigate the clinical application of BC biomarker candidates the performance of ELISA assays in urine was evaluated.

Methods

ELISA analytical characterization was performed for the following proteins: SURVIVIN, SLIT-2, NIF-1, H2B, PROFILIN-1. The parameters used to evaluate the performance of each ELISA assay were: standard curve validation, recovery of standards spiked in urine, reproducibility, sensitivity and linearity.

Results and Discussion

The NIF-1 assay has recovery above 200% and poor reproducibility (CV=24%). The H2B assay has low sensitivity and unacceptable reproducibility (CV=97%). The PROFILIN-1 assay did not perform well in the linearity test ($r2=0.275$). The SURVIVIN and SLIT-2 assays gave satisfactory results in all analytical performance tests.

Conclusions

The NIF-1, H2B and PROFILIN-1 ELISA kits used are not compatible with urine samples. The compatibility of other ELISA kits will be evaluated. SURVIVIN and SLIT-2 ELISA assays are compatible with urine samples and will be used for diagnosis and detection of recurrence of BC.

P-338.00

DOWNSCALING TISSUES PROTEOMICS, TOWARD PRECIOUS FFPE TISSUE SAMPLES PREPARATION. APPLICATION TO EARLY EVENTS OF CERVIX CANCER DECIPHERING.

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Introduction and objectives

Formalin Fixed and Paraffin Embedded (FFPE) tissues handling has long been a major issue in tissue proteomics. This requires dedicated procedures to access the identity of the largest panel of proteins. Some proteomics experiments need working with restricted tissues sizes, especially when proteomes of cancers of early stages are explored. We designed different procedures in the context of sample amount downscaling.

Methods

Several extraction and digestion approaches, adapted from previous tissue proteomics procedures, were tested on breast cancer FFPE tissue samples obtained by laser micro-dissection (LMD) and bearing approximately 2500 cells. The preparations were followed by nanoLC MS/MS analyses using Waters nanoACQUITY UPLC and Thermo QExactive instrumentations.

Results and discussion

First, experiments sets suggested that raw LMD sample has to be kept during sample preparation. Proteolytic digestion occurs directly on tissues and proteins are not fully extracted. The number of steps has to be limited to the minimum to retrieve an adequate number of proteins. Trypsin solution for on-tissue proteins digestion also has to be set to high concentrations to compensate the fact that digestion is rather performed on raw samples than on proteins extracts. Finally, an adapted procedure of the Citric Acid Antigen Retrieval approach, combined with adequate 2D-UPLC separation, allowed us to retrieve more than 1000 proteins on these samples of limited size. These developments were applied to the analysis of proteomes of early stages of cervix cancer, restricted to few thousand of cells. This gave us the first insights of early events occurring for the development of this affliction, supported by the identification of several involved biomarkers.

Conclusions

These procedures highlight essential issues for FFPE tissue preparation, regarding sample loss. This will open new opportunities for the study of limited amounts of tissue such as early development of cancerous afflictions.

P-339.00

DATA DEPENDENT VERSUS DATA INDEPENDENT ACQUISITION APPLIED TO SAMPLES OF DIFFERENT PROTEIN DYNAMIC RANGE

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Introduction and objectives

Label-free differential proteomics might be performed using two acquisition method types: Data Dependent Acquisition (DDA) and Data Independent Acquisition (DIA). DDA implies ions isolation prior MS/MS but has some limitations (MS speed acquisition and lack of reproducibility between runs). DIA process partially overcomes these limitations because all MS signals are further submitted to MS/MS. This study compares the efficiency (number of proteins identified and repeatability) of DDA and DIA analysis with a common 2D-UPLC system separation.

Methods

Two samples exhibiting significant differences in protein concentration dynamic range were analyzed in triplicates to challenge both acquisition methods: raw or depleted serum and total protein extract from colon biopsy. 20 µg were purified and digested using trypsin. Samples were then analyzed on a Synapt G2 HDMS (Waters) in DIA mode or on a Q Exactive (Thermo) in DDA mode. Raw data were respectively processed with PLGS 3.01 or Proteome Discoverer 1.4.

Results and discussion

Analysis of the raw serum allowed identifying 127 (191 after depletion) and 211 (249 after depletion) common proteins to triplicates using DIA and DDA, respectively. These results highlight the difficulty to analyze samples with large protein concentration dynamic range but show the clear advantage of DDA. For the sample having a lower protein concentration dynamic range, around 3200 proteins were identified using the DDA and will be compared to the analysis using DIA.

Conclusion

This comparison allowed determining that DDA using a Q Exactive leads to identify a higher number of proteins within the high protein concentration dynamic range sample.

P-340.00

PROFILING OF HUMAN PLASMA CLUSTERIN USING A GLYCOPROTEOMICS APPROACH TO DISCOVER POTENTIAL BIOMARKERS OF ALZHEIMER'S DISEASE

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Clusterin is a highly glycosylated secreted protein implicated in the pathogenesis of Alzheimer's disease (AD). Expression of the clusterin gene is significantly elevated in AD brain (May et al., 1990) and levels of plasma clusterin correlate with AD progression (Thambisetty et al., 2010). Since glycosylation plays an important role in the physiological functions of clusterin (Stuart et al., 2007), we have developed an IP-LC/MS/MS workflow and a selected reaction monitoring (SRM) assay to perform detailed profiling of plasma clusterin glycosylation, including determination of glycosylation sites, structural characterisation of glycosylated peptides and investigation of the correlation between the glycosylation pattern of clusterin and AD progression.

Plasma clusterin from 28 patients was enriched using our established workflow, and analysed via LC-MS/MS using nanoflow reverse-phase chromatography on the Orbitrap Velos, Orbitrap Fusion and TSQ Vantage (ThermoScientific, USA). Glycopeptides were manually identified by the presence of glycan-specific oxonium ion fragments, m/z 204.08 for N-acetylhexosamine (HexNAc), m/z 366.14 for hexose-N-acetylhexosamine (Hex-HexNAc), and m/z 657.24 for N-acetylneuraminic acid-hexose-N-acetylhexosamine (NeuAc-Hex-HexNAc) in the MS/MS spectra.

Using our IP-LC/MS/MS workflow, all six known N-glycosylation sites of clusterin were identified, such as α64N, α81N, α123N, β64N, β127N, and β147N. In addition, we have determined the differences in the glycoforms associated at each of the different glycosylation sites. We are currently comparing the clusterin glycoform distribution in plasma obtained from subjects of low atrophy (n=14) and high atrophy (n=14). The results of this analysis will be presented at the meeting. Furthermore, we have demonstrated our developed SRM assay could measure specific clusterin glycoforms in depleted plasma, to achieve a robust workflow suitable for rapid verification of clusterin glycoforms to aid AD biomarker discovery.

A thorough characterisation of the clusterin glycopeptide profile using LC/MS/MS and SRM will be a useful tool, allowing potential clusterin glycoform biomarkers of AD progression to be identified.

P-341.00

TIO2 ENRICHMENT REVEALS LOW-ABUNDANCE, CANCER-ASSOCIATED PROTEINS IN HUMAN SERUM

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Glycosylation is a key post-translational modification in the study of cancer progression and transformation. Enriching glycosylated peptides from biological fluids is a strategy to detect low-abundance proteins by mass spectrometry. In this study we have applied a TiO2 enrichment protocol to the isolation of sialylated glycopeptides from sera of patients diagnosed with Colorectal cancer (CRC) and Prostate cancer (PCa) against healthy controls.

Methods:
In this work we studied 3 serum pools in triplicates from CRC, PCa and control candidates. Proteins were reduced, alkylated and subjected to tryptic digestion. After overnight digestion and SPE purification, the peptide mixtures were subjected to TiO2 enrichment of sialylated glycopeptides, followed by de-glycosylation by PNGase F. The enriched samples were fractionated using SCX (6 fractions). The resulting fractions were analyzed using nanoLC-MS/MS (Q-Exactive). Targeted MS/MS mode was performed for a subset of the identified peptides in a single nanoLC-MS/MS analysis without SCX fractionation, in order to demonstrate the detectability of the peptides in a shorter analysis time.

Results and Discussion:
The discovery experiment on sample pools allowed us to identify over 700 glycosylation sites. Several of the identified proteins were reported to be present at low ng/mL concentration in serum/plasma according to the Plasma Proteome Database. Furthermore, many of those low abundance proteins have never been detected as glycoforms in human serum/plasma by mass spectrometry, such as: EGFR, MET, MMP8, MMP9, MFAP3, TGFBI. Targeted MS/MS experiments confirmed the detectability of the identified proteins in a single nanoLC-MS/MS run.

Conclusions:
Fast and easy-to-automate methods for the detection of low abundance proteins are a strong need in serum cancer biomarker discovery, especially concerning the validation phase. In this work we prove that TiO2 enrichment can be a very valuable approach to detect low abundance, cancer- associated proteins. The enrichment protocol is already suitable for future high-throughput experiments.

P-342.00

A NEXT GENERATION PROTEOMIC APPROACH FOR BIOMARKER DISCOVERY LINKING MULTIPLEXED SINGLE BINDER ASSAYS TO MASS SPECTROMETRY (MS) FOR DIRECT MOLECULAR VERIFICATION AND INVESTIGATION OF CANDIDATE BIOMARKERS.

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Affinity-based protein profiling of biological fluids allows high throughput analysis of samples to suggest panels of candidate biomarkers in a broad range of human diseases. Nevertheless, antibody selectivity needs to be confirmed in any given sample, otherwise the identity of the biomarker may be difficult to verify. Multiple antibodies with concordant performance characteristics for the same target are often not available. Thus a strategy for the direct molecular verification of candidate biomarkers, identified using Suspension Beads Array (SBA), is being developed.

In an SBA assay, the relative concentration of a protein in solution is retrieved from the number of molecules detected by every bead from a population of antibody-coupled beads. Differently, in MS, protein identifications, relies on the total number of molecules immune-captured and analyzed. Using model proteins of known concentration, we investigated how number of beads and volume of crude plasma applicable for SBA assays should be scaled up for MS identification. Then, we aimed to verify the identity of a candidate biomarker detected by a single antibody from the Human Protein Atlas. Protein captured by this antibody was analyzed and identified by LC-MS/MS and LC-MS-MRM.

In conclusion, we proposed and applied successfully a strategy based on straightforward combination of SBA and MS which represent a promising tool to support and complement affinity-based biomarker discovery.

P-343.00

DESCRIPTION OF BAL PROTEOME IN LUNG TRANSPLANT RECIPIENTS: OPTIMIZATION OF SAMPLE ANALYSIS AND COMPARISON WITH HEALTHY VOLUNTEERS

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With more than 39,000 procedures performed around the world in the last 30 years, lung transplantation (LT) has become a validated therapeutic option for terminal lung diseases. The long term survival remains limited because of Chronic Lung Allograft dysfunction (CLAD) onset, affecting 50% of LT recipients within 5 years post-LT. Bronchoalveolar lavage fluid (BAL), is a major tool for LT follow-up as it represents a unique description of lung allograft microenvironment. Modifications of BAL proteome have been studied in several lung diseases but little is known about its changes after LT and during CLAD.

This study aimed to describe BAL proteome in stable LT recipients, as a first step of SysCLAD, a FP-7 European program, which will build a systemic model for CLAD prediction. BAL samples were collected from a European prospective cohort of LT recipients and treated with Amicon 3 kDa ultrafiltration before OFFGEL fractionation and nano-HPLC. Data from quantitative iTRAQ-MALDI-TOF/TOF MS and MS/MS analysis were compared following 2 bioinformatic approaches : ProteinPilot™ software v. 4.0 with the Paragon™ Algorithm (AB Sciex) and MASCOT v. 2.4 (Matrix Science). 539 non redundant proteins were identified in BAL from 2 stable LT recipients. After merging with data from literature, 698 proteins appeared to compose BAL proteome of stable LT recipients. Comparison with previous descriptions of BAL proteome from healthy volunteers showed that, among proteins present in at least the half of the samples, 93 proteins were represented in both populations. 80 proteins were specific for LT recipients while 102 were only represented in healthy people. Ontologic analyses showed that differentially expressed proteins were mainly involved in the innate immune system.

In conclusion, this investigation provides an exhaustive description of BAL proteome in LT recipients and constitutes an indispensable step in CLAD biomarker identification, the main objective of SysCLAD project.

P-344.00

IDENTIFICATION OF HUMAN TEAR FLUID BIOMARKERS IN DIFFERENT DRY EYE DISEASE SUBGROUPS USING LABEL-FREE QUANTITATIVE PROTEOMICS

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The study of dry eye syndrome (DES) is gaining in importance owing to the high frequency of occurrence, and limitations in classifying and treating the disease. Definitive diagnostic test paradigms that can identify specific dry eye classes and monitor response to treatment are thus required. The major objective of this study was to examine the tear proteome profiles in different subgroups of dry eye patients, with the ultimate aim to discover new biomarkers for the pathology.

Twenty healthy subjects (CTRL) and 60 patients with DES were recruited. Dry eye patients were subdivided into aqueous-deficient dry eye (DRYaq: N=20), lipid-deficient dry eye (DRYlip: N=20), and a combination of the two (DRYaqlip: N=20). Tear samples were collected using Schirmer's strips. Three independent label-free quantitative experiments based on one-dimensional gel electrophoresis combined with LC-MS system was employed to identify candidate biomarkers from the tear samples. In total, 205 tear proteins were identified. Statistical and hierarchical clustering analysis identified 39 proteins which, could be employed as potential biomarkers to distinguish different DES subgroups. Generally, decrement of lacrimal gland-specific proteins and increment of inflammatory proteins were observed in DRYaq and DRYaqlip compared to the CTRL group.

Conversely, there were only slight differences in the tear proteome of DRYlip compared to the CTRL group. Among the differential proteins, proline-rich protein 4 and zymogen-granule-protein-16-homolog-B were found to be significantly down-regulated in both DRYaq and DRYaqlip groups. The major regulation levels of these proteins reflect the aqueous secretion deficiency by lacrimal gland and thus, are highly regarded as potential biomarkers for DRYaq.

This study demonstrated that specific alterations in the tear proteome are associated with the different subgroups of DES. These results, when extrapolated to clinical application, can provide invaluable hints on advanced therapies and are of great importance for the treatment of the specific DES.

P-345.00
IDENTIFICATION OF POTENTIAL ISCHEMIC STROKE BIOMARKERS THROUGH SECRETOME ANALYSIS OF A BRAIN ENDOTHELIAL CELL LINE USING SILAC

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Introduction and objectives
The integrity of the blood-brain barrier (BBB), primarily composed of endothelial cells with tight intercellular junctions, is compromised during cerebral ischemia. The immortalized cell line of human cerebral microvascular endothelial cells hCMEC/D3 is considered an in-vitro human model of the BBB. We aimed to describe changes in the secretome of hCMEC/D3 cells derived from ischemia and the BBB disruption in order to find new potential biomarkers of cerebral ischemia.

Methods
Cells were cultured (40000cells/mL) with RPMI-1640 SILAC medium. Proteome was labeled with heavy/light lysine and arginine and a cross-labelling replication step was performed. After 6h of oxygen and glucose deprivation (OGD)(0.5% O_2, 5% CO_2, 94.5% N_2, without glucose, T=37ºC) or normoxia/normoglycemia (21% O_2, 1% CO_2, 78% N_2, glucose 2g/L, T=37ºC) conditioned media were collected, concentrated, mixed 1:1 (OGD:normoxia/normoglycemia), trypsin digested and analyzed by nanoLC-MS/MS. Proteins were identified using MASCOT and quantification of heavy/light pairs was performed using ProteinScape3.1. PANTHER was used to classify proteins with Gene Ontology terms. Altered pathways due to OGD were analyzed using Ingenuity Pathway Analysis (IPA) database. A replication step was performed by western blotting in independent cell cultures.

Results
We identified 717 proteins from cell secretome analysis. A total of 19 proteins were found differentially secreted when OGD and normoxia/normoglycemia were compared (FoldChange>|1.5|, CV1). PANTHER analysis showed important catalytic and ligand roles of most of these proteins. IPA analysis revealed their involvement in processes such as cell death/survival and cell growth/proliferation after OGD; and in neurologic and metabolic disorders. Replication is ongoing by western blot.

Conclusions
We identified changes in the secretome of hCMEC/D3 cells after OGD. If validated, these results could represent a basis for the discovery of potential biomarkers of cerebral ischemia and BBB disruption, or for the identification of new therapeutic targets.

P-346.00

IMMUNO-LASER MICRODISSECTION COUPLED TO LABEL-FREE PROTEOMICS FOR THE ANALYSIS OF HUMAN BRAIN CELLS TO IDENTIFY POTENTIAL STROKE BIOMARKERS

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Introduction and objectives: The identification of changes in the human brain proteome after an ischemic stroke has allowed the discovery of new blood biomarkers to predict long-term functional disability. Further analysis of the neurovascular unit components such as neurons and vessels, could give further insights into stroke pathophysiology.

Methods: Neurons and blood vessels from infarcted and healthy contralateral areas of 7 patients who died because of stroke were isolated from brain slices by immunofluorescence staining followed by laser microdissection. LC-MS analysis was performed on an ESI-LTQ-OT, selecting 4 different m/z ranges in MS1 for gas-phase fractionation. Protein identification was done using Mascot whereas quantification and statistical comparison were performed with Progenesis® software. PANTHER was used to classify proteins according to Gene Ontology terms. Ingenuity Pathway Analysis (IPA) database was searched to find altered pathways after ischemia.

Results and Discussion: A total of 768 proteins in neurons and 1078 proteins in vessels were identified and quantified. When paired infarcted and contralateral areas were compared, 58 proteins from neurons and 24 proteins from vessels were differentially expressed ($p \leq 0.05$, fold-change ≥ 2, and peptide count ≥ 2).
PANTHER and IPA analyses revealed that differential proteins in neuron samples were predominantly involved in membrane trafficking, cell-to-cell signaling, interaction processes and neurological diseases. In contrast, proteins from brain vessels were rather associated with nucleic acid binding and mainly related to cell death and survival.

Conclusions: We were able to describe protein level changes in human neurons and vessels after stroke, with differential findings regarding the type of cell. If confirmed, these results could highlight novel candidates to be further explored as therapeutic targets or biomarkers for the diagnosis or prognosis of stroke.

P-347.00
MALDI IMAGING MASS SPECTROMETRY IN SUBTYPING BREAST CANCER: A PILOT STUDY

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Breast cancer is a complex disease: the response to the treatment, as well as disease free and overall survival, is not the same for all patients with similar tumor histological characteristics. Even genomic tests are not helpful in determining the appropriate therapy for almost 40% of patients with G2 classified breast cancer (intermediate risk).

The proteomic profile could provide a complementary tool to genomic studies in better classify subtypes of breast carcinoma. In order to determine differences in the proteomic profile of formalin fixed paraffin embedded (FFPE) breast cancer tissues, a specific subset of tumors (HER2- ER+ G2), from patients enrolled in a clinical trial performed at the IRCCS AOU San Martino-IST, was analysed using MALDI Imaging mass spectrometry (IMS). First, sample preparation protocol was developed to "unlock" the proteomic content of the FFPE tissues, since formaldehyde crosslinking complicates MALDI imaging: different HAIR (heat antigen induced retrieval) treatments were tested and the procedure optimized. After in situ trypsinization and matrix deposition, IMS data from 30 patients' tissues were collected at MUSC Proteomics Center on a Ultraflex III Smartbeam (Bruker Daltonics) mass spectrometer in positive linear mode in the 750-5000 Da mass range and 200 µm image resolution.

Preliminary statistical analysis (PCA and clustering) based on the proteomic profile was able to distinguish benign from cancer regions, confirming the reliability of the approach. However the sub-classification of the whole data set couldn't explain the biological characteristic of the tumors. The classification was improved including in the analysis G3 tissues (carcinoma with worst prognosis) from patients enrolled in the same clinical trial. Further analysis and eventually validation using 2D-HPLC-ESI/TOF MS alternative approach, are in progress for a better classification. This work could help in better determining risk categories for a subtype of breast carcinoma for which the therapy choice is still controversial.

P-348.00
DETECTION OF TEAR PROTEIN BIOMARKERS FOR DIABETIC RETINOPATHY SCREENING

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Introduction:
Diabetic retinopathy is the most common diabetic eye disease and a leading cause of blindness among patients with diabetes. As the advanced proliferative stage with high risk for vision loss can develop without any serious symptoms. A noninvasive method to detect biomarkers characteristic for diabetic retinopathy from the tear fluid would be of high importance in screening.

Methods:
Basal tears collected from patients with normal, non-proliferative and proliferative stages of diabetic retinopathy were subjected to mass spectrometry analysis. iTRAQ labeling followed by nanoHPLC coupled ESI-MS/MS mass spectrometry was used to identify proteins differentially expressed in tears of patients with proliferative stage of diabetic retinopathy. SRM methods were designed to some of the potential biomarker proteins and used to evaluate their levels in tears. The proteomics data were combined with digital microaneurysm detection on fundus images and machine learning algorithms to improve the results of already used image processing methods in retinopathy detection systems.

Results and Discussion:
Six proteins were shown to have significantly higher levels in proliferative stage of the disease compared to the normal. The lipocalin 1, lactotransferrin, lacritin, lysozyme C, lipophilin A and immunoglobulin lambda chain were identified as possible biomarker candidates with significantly higher relative levels in the tears of patients with diabetic retinopathy.

Conclusion:
A noninvasive method to detect biomarkers characteristic for diabetic retinopathy from the tear fluid was developed. This tool can be preferably used to improve the results of image processing methods as a complementary tool in automatic or semiautomatic systems.

P-349.00

HIGH THROUGHPUT MRM TARGETED PROTEIN QUANTIFICATION USING MICRO LC

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Introduction
Nano LC in combination with electrospray ionization (ESI) has been used for a long time in proteomics research due to the increase in sensitivity observed at lower flow rates. Nano LC runtimes can be long (often greater than 30 minutes). Micro LC runs between 5 - 50 µl/min and often is a lot faster due to the decreased impact of dead volume on the run time but still has a higher ESI sensitivity than analyses run at higher flow rates (> 250 µl/min). This study was therefore run to evaluate Micro LC as an alternative to Nano LC for peptide quantitation.

Methods
In this work peptides present in a commercially available kit (iRT) were spiked into human plasma to produce a set of defined test samples. These samples together with samples spiked with Cetuximab (Erbitux) were used to evaluate sensitivity and analysis time of a micro LC system (35 µl/min flow rate) and compared to that of a nano LC (300 nl/min flow rate). The Micro LC was then evaluated for linearity and robustness by repeat analysis over 2 days.

Results
It was found that loading 1 µL of matrix sample (1 µg of protein) was the maximum loading amount possible on the nano LC while 10 µL (40 µg of protein) was possible using Micro LC before chromatographic performance deteriorated. The relative responses of these samples were very similar but the Micro LC was running 4 times faster. When the Micro LC was evaluated over a 2 day period CVs of 5% or below were obtained for peptides.

Conclusion
This study has shown that Micro LC is robust and can offer similar sensitivities to nano LC (using larger loading volumes) but offers a large improvement in throughput.

P-350.00

QUANTIFYING DRUG INDUCED ORGAN INJURY BIOMARKERS ACROSS THE SPECIES BARRIER

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During the drug development process, drug induced organ injury (DIOI) is assessed in animal models to predict possible toxicity in humans. However, current approaches are limited to monitoring only one or a few toxicity marker per trial and experience ethical and financial constraints.

Our Triple-X-Proteomics (TXP) MS-based immunoassay strategy allows highly sensitive and selective mass spectrometry-based quantitation of proteotypic peptide from multiple biomarker in tissue, plasma or urine with a single immunoprecipitation step. Applying our TXP strategy across pharma industry-relevant model organisms will allow a more rapid and cost-efficient monitoring of DIOI.

Here, we developed a quantitative TXP-antibody-based immunoenrichtment MS strategy applicable for screening plasma and urine for liver (DILI) and kidney (DIKI) injury biomarkers across 5 species; human, cynomolgus, mouse, rat and dog. Plasma and urine of each species were tryptically digested. The tryptic digests were spiked with isotopically labeled peptides to allow accurate target peptide quantification. The protein-relevant signature peptides and standards were enriched using peptide group-specific TXP-antibodies. Accurate identification and quantification were done via nanoLC-Parallel Reaction Monitoring-MS.

We demonstrate i) the capability to identify multiple biomarker in plasma and urine across species using a single TXP-antibody, ii) the validation parameters (dynamic range, LOD, LOQ, reproducibility) of the quantitative analysis of Paraoxonase 1, 2 and 3 in human plasma and iii) the quantitative screening of healthy and DILI patient on Paraoxonase 1, 2 and 3 plasma levels. Our further goal is to integrate multiple TXP-antibodies for maximum multiplexing and simultaneous DIOI marker screening to increase throughout and reduce screening costs.

P-351.00

CROSS-SECTIONAL SALIVA PROTEOMICS REVEALS ASSOCIATIONS BETWEEN PROTEIN ABUNDANCE AND PERIODONTAL AND NON-PERIODONTAL PHENOTYPES

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Introduction and objective

The potential of large-scale human saliva proteomics for disease-specific biomarker screening has received considerable interest in the last decade. In this context, human saliva proteomics was applied to screen for protein signatures with specific information for the characterization of oral and non-oral (systemic) diseases.

Material and Methods

We analyzed whole human saliva of 120 subjects recruited from the population-based, cross-sectional Study of Health in Pomerania (SHIP), which in addition to standardized medical diagnostics received a dental examination. Samples were subjected to liquid chromatography-mass spectrometry based bottom-up proteomics. Individual LC-MS/MS-data were recorded on a LTQ-Orbitrap Velos and label free relative quantitation was carried out using a GeneData software package.

Results and Discussion

Characterization of the salivary proteome across all saliva samples resulted in identification of 902 human proteins from 2,297 unique peptides across all samples (mascot score > 20). For label free quantitation 403 proteins identified with at least two unique peptides were considered for calculation of associations between protein abundance and dental and non-dental phenotypes.

We discovered a set of proteins which correlated to indicators of periodontal diseases like periodontal pocket depth, clinical attachment loss and bleeding on probing. Functionally these proteins are related to immune cell trafficking, host response and inflammatory processes reflecting the characteristics of periodontitis. Furthermore, proteins were detected in whole saliva the abundance of which shows association to age and body mass index.

Conclusion

Cross-sectional saliva proteome analysis showed the capability to discover proteins the level of which associates with chronic periodontitis phenotypes. Functional annotation of this specific protein signature provided a link to periodontal disease status. Furthermore, the results obtained from association analyses to non-dental phenotypes emphasize the value of whole saliva for biomarker screening not only for oral but also systemic diseases.

P-352.00

DATA INDEPENDENT ACQUISITION - PACIFIC – FACILITATES IN-DEPTH PHENOTYPE ANALYSIS OF IRRITABLE BOWEL SYNDROME

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Precursor Acquisition Independent From Ion Count (PAcIFIC) is a method used in shotgun proteomics that is capable of detecting the largest dynamic range of all currently available data-independent acquisition (DIA) techniques as sited by Gillet et al. (Mol Cell Proteomics 2012). PAcIFIC operates by successively interrogating predefined 2.5 m/z isolation widths, or channels, by tandem-MS across a user-defined m/z range (typically 1000 m/z). The time to complete a full cycle is dependent on the number of channels chosen and the scan rate of the instrument. Here we present results showing that coupling PAcIFIC to label-free quantification facilitated in-depth interrogation of irritable bowel syndrome (IBS), a chronic functional gastrointestinal (GI) disorder.

Urine from 10 subjects representing each sub-symptom group was pooled for proteomic analysis. Only first morning void urine with no presence of blood and urinary creatinine level $\geq$ 100 mg/dL were included in the study. The urine proteome was analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) using the PAcIFIC that allowed extended detectable dynamic range. Dif-ferences in protein quantities were determined by peptide spectral counting followed by validation of select proteins with ELISA or a targeted single reaction monitoring (SRM).

IBS patients were classified into distinct subgroups based on symptom profile. Both GI (diarrhea, constipation, abdominal pain) and psychological (anxiety, depression) distress symptoms were considered to look for logical clustering of women with IBS. Four IBS sub-symptom groups were selected: 1) constipation, 2) diarrhea + low pain, 3) diarrhea + high pain, and 4) high pain + high psychological distress. A fifth group consisted of healthy control subjects. Trefoil Factor 3 (TFF3), for which quantitative assessments were possible for the whole cohort (40 IBS + 10 healthy) and 68 independent IBS cohort, analysis shows it holds promise for identifying IBS.PAcIFIC and label-free quantification facilitated identification putative biomarkers for IBS.

P-353.00

PREPARATION OF ANTIBODY COATED NANOPARTICLES FOR HIGH SPEED PROTEOMICS ANALYSIS

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Proteomic analysis of human samples and protein identification have an increasing impact on life sciences, biomedical, and diagnostic application. The human plasma contains large number of proteins with different size and functions. The peptidome is thought to be a rich source of biomarkers that can be used to examine organ functions, get information about status of diseases, or analyze clinical samples during the IVF procedures. Usually these biomarkers have low abundance or small molecular weight that makes difficult to detect especially at the presence of high abundance proteins, such as serum albumin and immunoglobulins.

Thus prior fractionations or other enrichment procedures are applied that makes the analysis to time consuming process. The main goal of our project is to produce nanoparticles with immobilized antibody that can be applied in on-line depletion/digestion of serum albumin from human plasma or cell cultures from IVF procedures. The conjugated nanoparticles - having TiO2 core - will be used to develop monolithic separation columns for diagnostic purposes. In this paper we present the synthesis and characterization of surface modified TiO2 nanoparticles in different stages of the preparation using IR spectroscopy and ICP AES measurements.

Biotinylation and the streaptavidin adsorption had been checked by CE measurements. The albumin binding capacity had been tested after each modification, and it was found that nanoparticles bind HSA only after antibody immobilization, so that can be used for albumin depletion.

P-354.00

MONOLITHIC COLUMNS WITH IMMOBILIZED TIO2 FOR TRAPPING PHOSPHOPEPTIDES FROM HUMAN-EMBRYO-SECRETED PROTEINS

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Protein phosphorylation is one of the most common posttranslational with great importance in many biological processes.

IVF (In-Vitro Fertilization) involves implantation of fertilized oocytes following long medical preparation of patients. Usually, several fertilized oocytes are cultivated and the physician must decide which oocyte will be transferred according solely to their morphology.

During the cultivation and growth, fertilized oocytes secrete proteins depending on their condition and health status into surrounding medium. Analysis of secreted proteins can help providing more accurate prediction of the implantation success. We assume that analysis of phosphorylated proteins secreted at the beginning of embryo development into cultivation medium will shed more light on the processes and help isolate a potential biomarker for prediction of implantation success.

However, the complexity of the sample (cultivation medium) must be reduced prior to analysis of phosphorylated peptides.

In order to isolate phosphopeptides we have depleted high abundant proteins from spent cultivating medium by applying MW-Cut-Off filtration, collected fractions were digested with Lys-C/Trypsin, and phosphopeptides were trapped on monolithic columns with immobilized TiO2. Upon elution, peptides were separated using nano HPLC and detected with mass spectrometry.

A number of phosphopeptides was identified in both morphologically "good" and "poor" embryos indicating differences in embryo development.

P-355.00

PATIENT-DERIVED MELANOMA CELL LINE PROFILING BY MULTIPHASE-CHIPLC COUPLED SWATH CORRELATES WITH SENSITIVITY TO MEK INHIBITION

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Introduction and Objective
Melanoma is the most common cause of skin cancer related deaths, especially in younger people.

Methods
We cultured 9 patient-derived melanoma cells with different MAPK pathway mutations (3 BRAFmut, 3 NRASmut, 3 BRAFwt/NRASwt) where 1 cell line of each group displayed insensitivity to MEK inhibition (MEKi). Trypsin digested cell lysates were submitted to information dependent acquisition (IDA) on an ABSCIEX TripleTOF 5600 MS with Eksigent nanoLC with cHiPLC utilizing a novel multiphase-chip trap column enabling online strong cation exchange fractionation followed by 60 min acetonitrile gradients. Combined IDA data were used as spectral library for RP-chip-SWATH profiling.

Results and Discussion
Multiphase-chip IDA experiments (8 hr each) revealed in total 2350 proteins with 7400 peptides (FDR¡Ý0.01) present among 9 cell lines. SWATH acquisition was performed using non-fractionated RP separation and retention time recalibration due to differences in dimension of the multiphase and the RP-chip traps. SWATH data processing revealed 1700 proteins quantifiable throughout triplicate analysis of the cell lines. Besides different protein expression between the 3 groups (ANOVA, p¡Ý0.01), principle component analysis of SWATH data explicitly separated MEKi sensitive from MEKi insensitive cells and revealed distinct marker proteins for these phenotypes which were confirmed by selected reaction monitoring.

Conclusions
Multiphase-cHiPLC separation coupled with SWATH enabled sensitivity to MEK inhibition prediction in patient-derived melanoma cells and is thus an approach for rapid cell phenotyping.

P-356.00

DEVELOPMENT OF DIAGNOSTIC BIOMARKERS FOR DIABETIC RETINOPATHY AT EARLY STAGES USING ABSOLUTE QUANTITATIVE PROTEOMIC METHOD

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Diabetic retinopathy (DR) is a common microvascular complication caused by diabetes mellitus (DM) and is a leading cause of vision loss and visual disability among adults. Although the regulation of systemic risk factors such as hyperglycemia, hypertension, and dyslipidemia have been shown to improve clinical therapies for diabetes-induced vision loss, more effective clinical therapies for DR patients are needed. In this study, we have performed a comprehensive proteome analysis for the discovery of a biomarker for DR diseases.

First, to identify possible biomarker candidates that are specifically expressed in human vitreous (MH, NPDR, and PDR), we performed data mining on the previously published DR-related studies and our experimental data; 125 proteins were then selected. For verification and validation of the selected biomarker candidates in plasma, candidates were selected, confirmed, and validated in the plasma of patients in the No DR, Mi, and Mo NPDR groups using preliminary multiple reation monitoring (Pre-MRM) and stable-isotope dilution multiple reation monitoring (SID-MRM) analysis.

Further, we performed a multiplex assay using 15 biomarker candidates measured from the SID-MRM analysis, which resulted in a merged AUC value of 0.99 (No DR versus Mo NPDR) and 0.93 (No DR versus Mi+Mo NPDR), respectively. Although we acknowledge that the model requires further validation in a large sample size, the 4-protein marker panel (A001, COO1, COO2 and IOO1) can be used as baseline data for the discovery of novel biomarkers of the early stage of DR.

P-357.00

AUTOANTIBODY PROFILING OF MENINGIOMAS TO IDENTIFY BIOMARKERS USING HUMAN PROTEOME ARRAYS

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Introduction and objectives

Neoplasms evoke the immune response for the production of autoantibodies against autoantigens or tumour associated antigens (TAAs) and these autoantibodies can be used for the early detection of cancers. Meningiomas are the intracranial tumours, which constitute upto 20% of all intracranial neoplasms and 35.5% of all central nervous system (CNS) tumours. In this study, we performed screening of sera from healthy controls, different grades of meningioma patients using human proteome arrays for the identification of potential biomarkers.

Methods

The screening of sera from 7 healthy control and 8 meningioma patients was performed using ProtoArray V5.0 (Invitrogen). These protein microarrays harbour more than 9000 unique GST-tagged human proteins. The microarray slides were blocked and then incubated with serum samples. The identification of sero-reactive proteins was performed using anti-human IgG conjugated with Cy5. The signal intensities were captured using GenePix 4000B microarray scanner. The data obtained was subjected to background correction and normalization and obtained signal intensities were subjected to further statistical analysis.

Results and discussion

Our analysis suggested modulation of several autoantibodies, including interesting proteins like complexin 1 (CPLX1), and regulator of calcineurin 1 (RCAN1) transcript variant-1 to be up-regulated by 6.6 folds and 2.5 folds, respectively. Both of these proteins are associated with nervous system. CPLX1 is known to be regulator for a late step in synaptic vesicle exocytosis and RCAN1 plays a role in stress-induced apoptosis.

Conclusion

This is first comprehensive analysis of human meningiomas to identify the autoantibody markers using human proteome arrays. Levels of significantly modulated proteins need to be further validated to establish its potential as candidate biomarkers.

P-358.00

CHARACTERIZATION OF PROTEIN ISOFORMS PREDICTIVE OF CANCER USING IMMUNO-AFFINITY CAPTURE AND FAST LC-MS.

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Biomarker evaluation studies rely on true quantification measurements performed on large sets of clinical samples. There is thus an urgent need for the development of high-throughput and reproducible analytical platforms. The development of an integrated analytical platform based on immuno affinity enrichment, fast LC and high resolution mass spectrometry was applied to the high throughput quantification of the isoforms of predictive lung cancer markers (EGF receptor and RAS family) in plasma samples.

Lysates from lung cancer cells lines were purified by immuno affinity enrichment using disposable micro columns packed with protein A/G beads and loaded with antibodies selective for RAS proteins or EGF receptor. After elution and proteolysis, the peptides mixtures were separated by a 5 min length gradient on a prototype nano-HPLC system using nanobore reverse phase column packed with a sub-2µm particles. Mass spectrometry analysis was performed on a quadrupole-orbitrap instrument operated in either single MS or parallel reaction monitoring (PRM) modes.

The performance of the LC-MS system was tested, including the linear response of the signal, the retention time reproducibility and the sample carry-over. A lowest limit of quantification of 80 attomoles/µl was demonstrated for EGFR peptides in low complexity samples. The MS analysis performed by high resolution PRM combined high selectivity with accurate sequence characterization, which is critical for protein isoforms discrimination. The analytical workflow applied on cell lines extract enabled the detection of EGFR and RAS mutations from as little as 80'000 cells.

A new integrated platform based on short nano-LC gradient was combined with a fast scanning quadrupole-orbitrap in order to accelerate the throughput of proteomics analyses. The use of high throughput and robust LC-MS platforms is promising approach for providing access to mass spectrometry in clinical environment.

P-359.00

CHALLENGES OF BIOMARKER DISCOVERY IN HUMAN DISEASES IN DEVELOPING WORLD

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Introduction:
One of the most promising applications of proteomics in clinics is identification of next generation diagnostic, prognostic or disease biomarkers, which can effectively improve diagnostics and therapeutics. The need for next-generation biomarkers is particularly emphasized in case of low and middle income nations, which bears around 90% of the global burden of the infectious diseases. Challenges like economic crunches, lack of education and awareness, lack of biobanking practises, enormous diversities in socio-epidemiological backgrounds, environmental risk factors, lifestyle and social and ethical glitches cripple healthcare research in these nations. In this study we intended to provide a comprehensive comparative analysis of differentially expressed serum/plasma proteins in various infectious diseases and human cancers and categorize the protein markers associated with generic or specific responses.

Methods:
We have performed comparative proteomic analysis of different tropical infectious diseases like malaria, dengue and leptospirosis, and different types of brain tumours including different grades of glioma and meningioma to investigate the proteome patterns under various diseased conditions and identify specific or non-specific signatures.

Results and Discussion:
Serum/plasma proteins such as α-1-antichymotrypsin, α-1-antitrypsin, serotransferrin, serum albumin, α-2-HS-glycoprotein, which exhibit similar trends of differential expression in multiple infections, are basically non-specific indicators of inflammation or stress response and are not promising from a diagnostic/prognostic point of view. Serum/plasma proteins, like haptoglobin and serum amyloid A, which show opposite trends of differential expression in some diseases compared to other types of disorders, are promising candidates for diagnosis and discrimination analysis.

Conclusion:
Biomarker candidates which are commonly altered in multiple diseases cannot effectively discriminate between different clinical manifestations. Therefore, analysis of suitable disease control is essential for establishment of any reliable biomarker for a specific diseased state. We feel that the combination of clinicopathological parameters with serum/plasma markers can provide improved prediction accuracy for most of the diseases.

P-360.00

DEVELOPMENT OF NOVEL BIOMARKER COMBINATIONS FOR THE DIFFERENTIAL DIAGNOSIS OF LUNG CANCER AND LUNG DISEASES USING MULTIPLE REACTION MONITORING

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Introduction and objectives

Lung cancer still shows highest mortality in caner related death. This is due to the low symptomatic lung cancer specific characteristic and lack of diagnosis tools in early stage with high sensitivity. In addition, to overcome the current misdiagnosis problem of lung cancers from other respiratory lung diseases, development of serum-based differential diagnostic biomarkers is on high demand. In this study, clinical serum samples of 99 non-cancer lung diseases and 99 lung cancer patients were subjected for LC-MRM-MS analysis on multi-lung cancer biomarker candidates.

Methods

Fifteen biomarker targets were selected from our previous lung cancer studies and references of other lung cancer cell line secretome data. Out of fifteen potential targets, MS detectable 7 targets were verified on the clinical samples using LC-MRM-MS. The data was analyzed to select best combination of biomarkers using logistic model, a type of probabilistic statistical classification model.

Results and Discussion

MRM analysis showed that the serum levels of two target proteins showed statistically significant changes in between lung cancer and other lung disease patients. C4BPA and SERPINA4 were significantly low, respectively, in the sera of lung cancer patients, compared to lung disease patients. However, other target protein did not show any statistical difference independently between two groups. Therefore, we made multiple combinations using the results of seven candidates via logistic model – and found a combination of four target proteins showing best differential diagnostic capability. The combination of four biomarkers, C4BPA, SERPINA4, CLUS and PON1, showed enhanced sensitivity and specificity between two groups.

Conclusions

Our results suggested that the combination of potential several biomarkers via statistical model provide better diagnostic specificity and sensitivity than a single biomarker for the differential diagnosis between lung cancer and lung disease patients.

P-361.00

PROTEOME-WIDE STUDY OF NEW POTENTIAL CEREBROSPINAL FLUID BIOMARKERS FOR BIPOLAR DISORDER WITH MULTIPLEXED SEMI-QUANTIFICATION BY TANDEM MASS TAG (TMT-6) LABELING IN COMBINATION WITH HIGH-RESOLUTION LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY

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Aims and purpose

In this project we aim to explore the underlying molecular mechanisms for common neuropsychiatric disorders as Bipolar disorder (BD) and to find new biological markers for disease diagnosis and for prediction and evaluation of treatment responses. A valuable source for biomarkers for brain disorders is cerebrospinal fluid (CSF), as it is in close contact with the central nervous system (CNS) and contains large numbers of endogenous proteins with great potential value as biomarkers.

Methodology

Labeling with isobaric Tandem Mass Tag (TMT) reagents enables multiplexed semi-quantification from small quantities of CSF (50 ul). Six different TMT reagents (TMT 6-plex), each with a unique reporter mass, were used in this study. High-resolution liquid chromatography-mass spectrometry (LC-MS) analysis of the trypsin digested peptides was performed on a Q-Exactive instrument. This methodology was employed on a CSF patient material from 15 bipolar disease (BD) patients and 15 controls (Ctrls).

Results

In this exploratory proteome-wide study, approximately 600 endogenous proteins were identified and semi-quantified. 37 proteins showed significantly (Mann-Whitney, p

Conclusions

This study clearly demonstrates a promising approach for finding relevant biomarkers for BD and other neuropsychiatric disorders. A future panel of biological markers would be very helpful in the diagnostic assessment of patients and to explore treatment responses.

P-362.00

INTEGRATED GENE AND PROTEIN TISSUE EXPRESSION ANALYSIS FOR ADJUVANT TREATMENT DECISIONS IN EARLY STAGE LUNG CANCER

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Introduction and objectives

Outcome of early stage lung cancer (stage I/II) may be improved by adjuvant chemotherapy treatment after complete surgical resection. However, some patients are cured without adjuvant therapy and some relapse and die in spite of it. A biomarker signature predictive for likelihood of relapse without therapy and likelihood of benefit from it would be a valuable support for clinical decisions.

Methods

To identify such a signature, we have performed whole transcriptome sequencing (RNAseq) and parallel shotgun proteomics profiling of resected lung cancers. The clinical discovery cohort consisted of forty-four early stage lung cancers with equal numbers of chemotherapy treated and chemotherapy naïve patients presenting disease free survival and recurrence after treatment.

Results and Discussion

Transcript mutation analysis revealed almost three million sequence variants, among them more than ninety-five thousand in coding regions. Multidimensional separation and LC-MS/MS analysis of formalin fixed paraffin embedded samples revealed more than one thousand six hundred protein groups with a minimum of two unique peptides (0.05 FDR threshold). Molecular phenotypic expression of gene variants potentially associated with treatment sensitivity or disease outcome were investigated at the gene and protein level in the clinical discovery cohort.

Conclusions

Parallel investigations of the genotype and the molecular phenotype of early stage lung cancer are expected to describe individualized mechanisms of benefit from adjuvant chemotherapy. Integration of these complementary levels of information represents a potential strategy for personalized treatment decisions in early stage lung cancer.

P-363.00

PROTEOMICS APPLICATION OF CLINICALLY ARCHIVED FORMALIN-FIXED PARAFFIN EMBEDDED TISSUES: QUALITY EVALUATION BY 2-D PAGE

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Introduction and objectives

The vast number of disease samples stored in hospitals worldwide preserved by formalin fixation and paraffin embedding (FFPE) hold valuable information about disease progression, patient's response to therapies, disease outcome and survival. Detection and characterization of disease biomarkers in FFPE tissues will greatly aid the understanding of the diseases mechanisms and help in the development of diagnostic and prognostic markers. The aim of this study was to access the level of comparability of total proteomes isolated from clinically archived FFPE tissues with the matching fresh tissue proteomes.

Methods

We compared the total protein profiles of fresh/frozen and matching FFPE tissues by conventional 2-D PAGE analysis. The evaluation was done based on two types of tumor tissues (breast and prostate) using two extraction protocols with highest reported yield and quality.

Results and Discussion

The mean number of the detected spots in FFPE maps compared to the matched fresh/frozen maps was three fold lower. The comparison of the 2-D patterns showed that only 7-10% of proteins from frozen tissues can be matched to proteins from FFPE tissues. Most of the spots in the 2-D FFPE's maps had pl 4-6, while the percentages of proteins with pl above 6 were 3-5 times lower in comparison to the fresh/frozen tissue. The majority of protein spots in FFPE (67-78%) could not be matched to the corresponding spots in the fresh/frozen tissue maps indicating irreversible protein modifications.

Conclusions

Using the presented extraction methods the obtained proteomes differ significantly from the matching fresh tissue proteomes, both quantitatively and qualitatively. The inability to completely reverse the cross-linked complexes and overcome protein fragmentation with the present day FFPE extraction methods stands in a way of effective use of these samples in 2-D gel based proteomics studies.

P-364.00

IDENTIFICATION OF CANDIDATE BIOMARKERS FOR CNS RELAPSE OF DIFFUSE LARGE B CELL LYMPHOMAS USING LABEL-FREE QUANTITATIVE PROFILING

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Dissemination inside the central nervous system (CNS) occurs with a variable frequency in adult patients affected by non-Hodgkin lymphoma and represents a devastating and usually fatal complication, with a median survival of 4-5 months. Aggressive lymphomas like diffuse large B cell lymphomas (DLBCL) are more likely to involve the CNS, and various risk factors for CNS recurrence have been described such as an involvement of certain extranodal organs, localisation in the anatomical regions near to the base of the skull, or an elevated serum lactate dehydrogenase (LDH).

Prevention of this complication requires a strong prophylaxis in the selected high-risk patients, involving for example intrathecal administration of methotrexate, which impair the quality of life of patients during treatment and may be associated with rare but major side effects. It is thus important to identify patients who present the highest risk of CNS relapse and will really have benefit of CNS prophylaxis. However, to date, no suitable biomarker is available to stratify patients and predict, at the time of diagnosis, the CNS relapse of the DLBCL.

Here we present a discovery clinical study to identify such biomarkers in the cerebrospinal fluid (CSF) of patients with DLBCL. CSF samples from patients presenting high-risk criteria were collected in different clinical centers and submitted to proteomic analysis. Each sample was treated to immunodeplete albumin and IgG, tryspin-digested, and analyzed on a Q-exactive mass spectrometer. In average, about 500 proteins were identified in each sample, and more than 1300 proteins were identified from the entire study involving 109 patients. Protein relative levels were profiled across the cohort by label-free quantification using the MaxQuant software, and statistical analysis was used to identify candidate markers associated with increased probability of CNS relapse over a 24 month-period.

P-365.00

LOW ABUNDANT PROTEINS ENRICHMENT OF URINARY SAMPLES FROM NEFROLYTHIASIS PATIENTS UNDERGOING LITHOTRIPSY THERAPY: PROTEOMINER TECHNIQUE VS ACETONE PRECIPITATION FOR URINE PROTEOME ANALYSIS.

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Introduction and objectives: Urinary proteomics represents an important clinical research field able to identify novel biomarkers for early and accurate diagnosis of human kidney diseases (1). Urine an ideal biological source for non-invasive sample collection, presents experimental problems mainly due to the inter- and intra-individual variabilities (urine volume, total protein concentration) (1). A novel methodology applied to urine proteome study to overcome sample preparation difficulties is Proteominer (trade name) (2). The aim of our research is to define proteome urinary changes for the low abundant protein expression in response to the renal effects of Extracorporeal Shock Wave Lithotripsy (ESWL) on nephrolythiasis patients.

Methods: The first step of our research was focused on choosing the best analytical proteomics procedure comparing Proteominer sample preparations with the protein extraction via acetone precipitation on urinary samples from untreated patients. Then we applied Proteominer to urinary samples from patients following ESWL and carried out a comparative proteomic 2DE analysis before and post ESWL at 24 h and 28 days.

Results and Discussion: Proteominer treatment of the urinary samples has shown its efficiency in low abundant protein enrichment due to an increase of reproducible and detectable protein spots (1313 ±27) when compared to those obtained from precipitation exctraction. Interestingly in urine samples post ESWL we found a down regulated isoform 2 of dimethyl arginine dimethyl aminohydrolase (DDAH2) involved in the degradation pathway of dimethyl arginine, known as a risk factor of cardiovascular death and renal chronic disesase.

Conclusion: We conclude that the Proteominer approach allowing us to discover molecular markers of renal dysfunctions related to short and long time effects of ESWL represents an invaluable tool enabling extensive recovery of the urinary proteome under pathological conditions.

P-366.00

MONITORING THE STATUS OF INFANTS BEING ON NURSING IN INCUBATORS
BY URINE PROTEOME ANALYSIS.

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Introduction

The most complicated medical problems associated with premature neonates nursing arise due to insufficient data about the dynamics of change. This is largely due to the invasiveness of the applied methods of research. Thus, there is a need to develop new approaches to monitoring such patients using new multifactorial approaches based on non-invasive methods. One of the promising methods for noninvasive monitoring is the monitoring of the urine proteome composition.

Methods

Random urine samples (1-2 ml) were collected from 30 newborns (2-3 days life) nursing in incubators at the Department of neonatal intensive care at the Research Center for Obstetrics, Gynecology and Perinatology, Moscow, Russia. Total protein concentration was monitored at all preparation steps using Bradford assay, Bio-Rad. Proteins were precipitated (ice-cold acetone) and digested (trypsin) and analyzed in triplicate by nanoflow LC-MS/MS. Proteins were identified against human IPI human database (version 3.82) using Mascot Server 2.2 software (Matrix Science, London, UK). Bioinformatics analysis including GO annotation, proteins quantification was carried out using Scaffold 4.0 software (version-01_07_00, Proteome Software Inc., Portland, OR).

Results

Protocol for urine preparation and protein extraction were optimized with the aim to minimize loss. A series of comparative studies to determine differences in the urine proteome of full-term and premature infants, changes in the composition of urine in the process of child development and the formation of pathologies, as well as study the variability determined in the urine as for the individual patient (daily, weekly) and groups (evidence)were performed. Totaly 456 proteins were identified in the data set (0.1% FDR). Comparative analysis revealed 87 proteins (>95%) consistently present in urine. On the basis of the received data significant indicative parameters will be identified, which can be used for continuous monitoring of the condition of patients.

P-367.00

NEW PARKINSON'S DISEASE EARLY BIOMARKERS? A DYNAMIC INTERACTOME APPROACH TO BETTER ELUCIDATE DJ-1 ROLE IN OXIDATIVE STRESS

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Parkinson´s disease (PD), the second most common progressive neurodegenerative disorder, is a multifactorial disease caused by both genetic and environmental factors. Among the genes associated with PD, DJ-1 is a multifunctional protein involved in oxidative stress response and neuroprotection. The dynamic interactome of DJ-1 under oxidative stress conditions and its comparison with specific mutations was never addressed and should increase our knowledge on the early mechanisms of PD.

Using affinity purification combined with SWATH (AP-SWATH), a dynamic interactomic screening of endogenous DJ-1 was performed to identify and quantify DJ-1 interactions under resting and oxidative stress conditions.
To study the role of extracellular DJ-1 and to evaluate the role of some specific DJ-1 residues, recombinant DJ-1 proteins (WT and mutants) were used. Pull-down assays of these proteins allow us to establish the importance of DJ-1 in stress regulation and PD mutations impact on the newly interactions/mechanisms identified. Data was used in gene ontology tools as well as hierarchical clustering and PCA analysis based on quantification profile under oxidative stress.
The results increase our knowledge on the main mechanisms of DJ-1 action, monitoring the interactions changes and highlight groups of proteins with similar behavior, and identify DJ-1 interactors that are responsible for the discrimination between the different stress conditions.

A wide number of binding partners were identified and quantified by AP-SWATH, and these interactors have a broad range of functions, including cellular response to oxidative stress. This characterization of DJ-1 dynamic interactome, both intracellular and extracellular, comprises the most comprehensive elucidation of DJ-1 function and reveals the broad range of DJ-1 interactors under oxidative stress conditions. Some of these interactors were validated and can be targeted as potential predictive biomarkers.

P-368.00

MEASUREMENT OF GLYCOSYLATED ALPHA-FETOPROTEIN IMPROVES DIAGNOSTIC POWER OVER NATIVE FORM ON HEPATOCELLULAR CARCINOMA

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Serum alpha-fetoprotein (AFP) has long been used as a diagnostic marker for HCC, but with controversies. Although the protein is still widely and currently used in clinics, the use of AFP in diagnosis of HCC has recently been challenged due to the associated false positive and false negative findings.

In this study, we have tried to develop the effective analyzing method for measuring of total AFP and glycosylated AFP using a multiple reaction monitoring (MRM)-MS method and verified total amount of AFP (nonglycopeptide level) and the degree of glycosylation level of AFP (unglycosylated glycopeptide level) in patients with normal group (n = 60) and HCC group (n = 60). In our MRM-MS analysis, nonglycopeptide showed 56.7% sensitivity, 68.3% specificity and AUC of 0.692 when the normal group (n = 60) was compared to HCC group (n = 60), while unglycosylated glycopeptide showed 93.3% sensitivity, 68.3% specificity and AUC of 0.859. These data demonstrate that the discrimination power of the unglycosylated glycopeptide showed better performance than nonglycopeptide.

Consequently, we finally suggest two types of peptide marker (nonglycopeptide and unglycosylated glycopeptide) which are able to distinguish cancer status between normal group and HCC group. However, we think that verification in a larger sample size might contribute to more reasonable understanding of statistical analysis and correlated variables and would be aided for better development of biomarkers for HCC.

P-369.00
MULTIPLEXED QUANTIFICATION OF PRETERM INFANT PLASMA PROTEINS USING HEAVY PROTEIN STANDARDS AND MS-BASED IMMUNOASSAYS

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Conventional biochemical methods for monitoring multiple plasma biomarkers in preterm infants are not applicable due to the low amount of blood (ca. 125 mL) of preterm infants. However, routine analysis of plasma proteins would allow monitoring e.g. organ development or presence of inflammation and could prevent severe damage, consequently improve preterm infants' overall health.

Advanced MS instrumentation allows targeted detection and quantification of analytes in low sample volumes. However, the complexity of plasma as sample matrix requires targeted analyte enrichment to enable the analysis of hundreds of clinical samples per day. Our Triple-X-Proteomics (TXP) MS-based immunoenassay strategy allows the selective enrichment of tryptic peptide groups containing the same C-terminal sequence and identification of multiple analytes using one antibody enrichment step. To date, stable isotope labeled peptides are the primary choice as quantification standards in targeted quantitative ms-based approaches. However, this may lead to skewed results if the proteolytic sample digestion is incomplete. Using Stable Isotope Labeled recombinant Protein standards (SILP), adding them to the sample prior to processing, would compensate for sample processing errors.

Here we developed a TXP-MS based immunoassay for the identification and accurate quantification of plasma proteins in 5 µL plasma. This low volume meets the sample requirements to monitor plasma proteins in preterm infants. We combined an optimized tryptic digestion protocol with a single step immunoaffinity enrichment followed by high resolution Parallel Reaction Monitoring (PRM) MS analysis We present i) the quantitative assay validation parameters (dynamic range, LOD/LOQ, reproducibility) of plasma proteins, ii) the first quantification of these markers in neonatal and preterm infants plasma samples and iii) the feasibility of using SILP for accurate quantification of preterm infant plasma proteins. Our workflow and the generated quantitative data are to our knowledge the first and largest screening strategy of preterm infant plasma proteins.

P-370.00

CHANGE IN BLOOD CAN BE MORE SENSITIVELY DETECTED IN URINE

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The most fundamental property of biomarker is change. Biomarker studies commonly monitor the composition of plasma, which is under strict homeostatic control. Urine has no homeostatic mechanism and accumulates lots of changes.

We hypothesize that change in blood can be more sensitively detected in urine. Here we introduce changes by two anticoagulants respectively which all resulted in coagulation status changes.
Plasma and urine protein composition were analysed by LC-MS/MS and some proteins were validated by Western blot in six SD female rats before and after treatment with heparin or argatroban. With same LC-MS/MS strategy and same thresholds, in argatroban treated group, 62 proteins changed in urine, only one of which changed in plasma. In heparin treated group, 27 proteins changed in urine but only three other proteins changed in plasma. LC-MS/MS and Western blot analyses demonstrated drug-induced increases in transferrin and hemopexin levels in urine but not in plasma.

Our data indicates that urine may serve as a source for more sensitive detection of protein biomarkers than plasma. The age of urinary biomarkers is coming.

P-371.00
NOVEL BIOMARKER DISCOVERY FOR ACUTE MYOCARDIAL INFARCTION: PROTEOMIC ANALYSIS OF FORMALIN-FIXED PARAFFIN-EMBEDDED TISSUE FROM AUTOPSY

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Introduction
Few proteomic studies have examined human cardiac tissue following acute lethal infarction. Here, we applied a novel proteomic approach to formalin-fixed paraffin-embedded (FFPE) human tissue and aimed to reveal the molecular changes in very early phase of acute myocardial infarction (AMI).

Methods
Heart tissue samples were collected from forensic autopsies of 5 AMI patients who died within 7 hours of symptoms and from 5 age- and sex-matched control cases. Infarcted and control myocardia were histopathologically diagnosed, and sampling areas were captured using laser microdissection. Proteins were extracted from the tissue using an originally established method and digested with trypsin. Obtained peptides were analyzed using liquid chromatography–tandem mass spectrometry (LC–MS). The abundance of each cardiac protein was calculated as the total sum of peptides in label-free quantitation.

Results
10,396 peptides and 734 proteins were identified in FFPE cardiac tissues. The signal intensity levels of 21 proteins differed significantly between AMI and control tissues. A sarcoplasmic protein, sorbin and SH3 domain–containing protein 2 (SORBS2), which is known to be abundant in cardiac muscle and scarcely exists in skeletal muscle, was also significantly reduced in infarcted myocardia. Immunohistochemical analysis of cardiac tissues confirmed that this cytoskeletal protein localized at Z-lines and intercalated disks in normal hearts, and remarkably reduced in AMI myocytes. Moreover, in Western blotting of the serum, SORBS2 was significantly increased in AMI patients (n = 10) compared with control cases (n = 11).

Conclusions
Our advanced comprehensive analysis using patient tissues and serums indicated that sarcoplasmic SORBS2 is released from damaged cardiac tissue into bloodstream upon lethal AMI. The proteomic strategy presented here is based on precise microscopic findings and quite useful for candidate biomarker discovery using human tissue samples stored in depositories.

P-372.00

PROTEOME ANALYSIS AND COMPARATIVE STUDY OF NORMAL ENDOMETRIAL STEM CELLS, ENDOMETRIOSIS STEM CELLS AND BONE MARROW MESENCHYMAL STEM CELLS

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Introduction and objectives

The related diseases of endometrial proliferation are common, which leads to endometrial hyperplasia, endometriosis, and endometrial cancer. Our hypothesis suggests that endometrial progenitor and stem cells play key roles in the beginning of these endometrial proliferative disorders. Moreover, in endometriosis, endometrial stem /progenitor cells may be migrate into the pelvic cavity by retrograde menstruation to establish endometriotic lesions.

Methods

Flowcytometry and quantitative real-time PCR were used to detect the expression of Stemness-related target genes and stem cell surface marker in Endometriosis endometrial stem cells (EESCs), normal endometrial stem cell (ESCs), endometrial lesions stem cell (ELSCs) and bone marrow Mesenchymal stem cell (MSCs). Proliferation of all stem cells was observed by MTT assay. The differentiation potential was evaluated by alizarin red, oil red O and RT-PCR method. The karyotyping was performed on EESCs and ELSCs at passage 20. In this regard, all of groups were analysis for proteome patterns by 2-DE and MALDI-TOF/MS.

Results and Discussion

Although these cells expression of stemness-related genes are similar to each other, they also have unique patterns. Spindle-like morphology, normal karyotype, Adipogenic and osteogenic potential, expression of Oct4, Nanog, Klf4 and ERAS genes and CD44, CD105, CD90, CD73 and CD146 specific surface markers, in EESCs and ELSCs was observed. Furthermore, rate of proliferation and expression of these genes like SALL4, DPPA2, TCL1 and Sox2 were significantly higher than ESCs and MSCs. Proteomics results show that GAPDH, Glutathione-s transferase, Albumin, KRT 1, Heat shock protein beta-1 significantly different expression pattern with together.

Conclusions

According to our data, EESCs and ELSCs may be belonging to the stem cell categories. Moreover, endometrial stem/ progenitors may be valuable targets for early genetic or epigenetic alterations, leading to the appearance of endometrial disorders.

P-373.00

QUANTITATIVE PROTEOMIC ANALYSIS OF VITREOUS IN DIABETIC RETINOPATHY USING SWATH

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Introduction and objectives:
Diabetic retinopathy (DR) is an ocular pathology that is caused by both Type 1 and Type 2 diabetes. This study aims to compare the proteomics profile of the vitreous in patients with diabetic retinopathy against patients without diabetic retinopathy.

Methods:
Vitreous from eight patients with proliferative diabetic retinopathy (DR group) and eight patients with retinal detachment (non-DR group) were collected during surgery. Vitreous proteins were tryptically digested and analyzed using a 2-hour nanoLC-MS/MS and MS data was acquired using MS/MSALL with SWATH acquisition on a TripleTOF 5600 system. Data from three information dependent acquisition (IDA) experiments were combined and used as ion library for subsequent SWATH data processing. Several protein candidates were verified using high-resolution MRM (HR-MRM).

Results and Discussion:
In total, 343 vitreous proteins were identified and quantified using SWATH. Of these 343 vitreous proteins, 51 proteins were up-regulated and 75 proteins were down-regulated (ratio of DR vs non-DR > 1.5 or < 0.67). Altered expression of some proteins are noticeable including: FN1 (ratio of DR vs non-DR: 2.38), LOXL4 (2.94), C6 (2.70), AFM (0.26), FRZB (0.48), N4BP2 (0.48) and TIMP2 (0.66). FN1 is considered angiogenic and LOXL4 can be induced by hypoxia. Complement activation inhibits angiogenesis. Both FRZB and TIMP2 suppress angiogenesis. FN1 and C6 were also confirmed by HR-MRM.

Conclusions:
The differentially expression of several vitreous proteins between DR and non-DR may provide us with valuable information regarding the pathogenesis and possible new treatment of DR.

P-374.00

PROTEOSEPSIS: PROTEOMIC ANALYSIS OF PLASMA PROTEINS DURING THE COURSE OF SEPSIS.

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Sepsis is the most frequent complications in the surgical patient and a leading cause of death in intensive care units with an overall mortality around 50%. Sepsis is estimated to affect approximately 18 million people worldwide annually. Despite the recent efforts to standardize diagnosis and therapy of sepsis, no biomarker-based test is available for screening, diagnosing, monitoring septic patients or for guiding molecularly targeted therapy and assessing therapeutic response. There is therefore a great need to identify biomarkers for diagnosis and prognosis in sepsis.

In the present study, we aimed to identify markers that reflect sepsis progression and response to extracorporeal hemoperfusion cartridge therapy for endotoxin removal. To this end, we collected blood from patients at 3 time points – following patient ICU admission, after hemoperfusion therapy and upon patient ICU discharge. In addition, we collected plasma samples from healthy donors.

Using a strategy that combines immunoaffinity depletion of 14 high-abundance plasma proteins and 2- and 1-DRPLC-MS/MS, we found a complex protein expression pattern in sepsis progression. In addition to the known proteins C-reactive and Neutrophil gelatinase-associated lipocalin, which are used as biomarkers for sepsis, more than 100 proteins were found having a deregulated level of expression.

P-375.00

SUBREGIONAL PROTEOMIC ANALYSIS OF HIPPOCAMPI FROM PATIENTS WITH ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is the most common form of dementia and affects people around the world. Studies of AD are currently ongoing in numerous laboratories but the disease mechanism and valid biomarkers remain unclear. In particular, biological functions underlying the wide-proteome changes that have been associated with AD pathology in the CA4 have not been reported.

In the current study, we have concentrated on the CA4 and DG subregions of the hippocampus in order to elucidate mechanisms that are involved in the onset and development of AD pathology when accumulation of neurofibrillary tangles is first detected. To identify proteins differentially expressed in AD, we performed mass spectrometry-based proteomic analysis combined with label-free quantification for identification and estimation of proteins from AD and control samples.

Using these combined approaches, we identified 4,328 proteins. Among them, 113 proteins were expressed more than twofold higher or lower in AD hippocampi than in control tissues. Notably, 75% of the 113 proteins were identified for the first time as being AD-related, and only 60% of the 113 proteins were reported as being brain-related. Among these proteins, five proteins were chosen as putative biomarkers for AD (MDH2, PCLO, TRRAP, YWHAZ, and MUC19). The five proteins were cross-validated using western blot, multiple reaction monitoring, and MALDI mass spectrometry imaging. Thus, using this approach, we have been able to demonstrate a new platform that may aid in the diagnosis of AD. Moreover, by studying functionally relevant areas from human brain tissue, we have expanded on our understanding of the molecular pathology underlying this disease.

P-376.00

PROTEOME ALTERATION INDUCED BY DIFFERENT-SIZED SILVER NANOPARTICLES (AG NPS) IN AN INTESTINAL CO-CULTURE MODEL

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Introductions and objectives: This study aims at establishing a more physiological intestinal co-culture model with the use of Caco-2 and HT29 cells in order to evaluate the effects of Ag particles (20nm and 200-nm) on the intestinal compartment in vitro.

Methods: Metabolic activity was evaluated by alamar blue assay, intracellular oxidative stress was evaluated by DCFH-DA assay and pro-inflammatory cytokine release (IL-8) was measured by ELISA. 2D-DiGE/MALDI-Tof/Tof approach was used in order to identify possible mechanisms of toxicity. The uptake and localisation of the particles and ions was assessed by Secondary Ion Mass Spectrometry (NanoSIMS50). Contribution of Ag ions to toxicity was also evaluated (ultrafiltration and ICP-MS).

Results and discussion: AgNO3 induced a reduction in metabolic activity in a dose dependent manner whereas no reduction was observed in the case of Ag 20nm and 200nm. The presence of mucus showed a protective effect against oxidative stress upon exposure to H2O2. Ag 20 nm led to an increase in IL-8 release (5-fold). Ag was found to be distributed homogenously in the cell with aggregates observed in specific locations in the case of Ag 20nm.The proteomic data revealed that AgNO3 and Ag particles induced an up-regulation of oxidative stress pathways (PRDX-6, PDI) and a modulation of cytoskeleton machinery (ACTB, VIL-1 or Gelsolin) and apoptosis-related proteins (ANXA4, PDCD5). Ag 200nm and 20nm were found to behave in different manner compared to in solution ions. Interestingly, a size dependent effect was observed: the 20nm particles seemed to be more effective than Ag 200nm, that were found to be close to the negative control.

Conclusions: We described a co-culture model for intestine that is more physiological and relevant for toxicological studies compared to Caco-2 cells alone. Observed differences in effects cannot be attributed solely to ions while the effects were also particle size dependent.

P-377.00

IDENTIFICATION OF NEW POTENTIAL MARKERS OF PRETERM BIRTH IN AMNIOTIC FLUID

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Introduction and objectives

Around 10% of neonates worldwide are born preterm, that is before the 37th week of gestation. Preterm birth accounts for 70 – 80 % of perinatal mortality and more than half of the long-term morbidity. It increases risk of neurodevelopmental and respiratory complications. The precise mechanism of this multifactorial syndrome is still unknown despite advancing knowledge of risk factors and mechanisms related to preterm birth. A reliable screening tool that could reduce the incidence by early diagnosis was still not found.

The aim of this project is to use the shotgun proteomics to identify putative predictive markers of preterm birth in amniotic fluid.

Methods

Sixty amniotic fluid samples from patients undergoing transabdominal amniocentesis in 16th week of gestation were retrospectively classified based on the outcome of the pregnancy into two groups, 23 patients delivered preterm, and the other 37 delivered at term. Using immunoaffinity chromatography, high abundance proteins were removed and the depleted samples were digested with LysC and trypsin, all samples were prepared for iTRAQ quantitative analysis. Twenty multiplexes were prepared, where each contained three patient samples and one global internal standard. All multiplexes were analyzed using nanoLC-MS.

Results and Discussion

On average, 847 proteins and 5815 peptides respectively, were identified per multiplex. Statistical analysis of results showed 11 significantly ($p < 0.05$) altered protein levels between both groups. Our workgroup focused on two proteins; glycodelin and neutrophil defensin 1 due of their possible relationship to preterm birth pathophysiology.

Conclusions

Proteomic analysis of 60 samples of amniotic fluid revealed 11 proteins significantly altered due the pregnancy outcomes. The verification of these results is currently under way.

P-378

IDENTIFICATION OF HUMAN HEMOGLOBIN-ACETAMINOPHEN ADDUCT

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Metabolism can detoxify or bioactivate xenobiotics to more toxic. For many drugs, pathways of metabolism can be divided into two groups: Phase I and phase II. Phase I reactions involve formation of a new or modified functional groups (oxidation, reduction, hydrolysis). Phase II reactions involve conjugation with an endogenous substance (eg, glucuronic acid, sulfate, glycine). The main blood proteins, serum albumin and hemoglobin, which easily interact with electrophilic compounds through their amino acid residues, are the most available for research. Analysis and identification of blood proteins adducts (non-standard PTM) is an important aim of pharmacokinetics and pharmacodynamics.

Our goal was to develop a method for identification of human hemoglobin adducts with metabolites of drugs, for example acetaminophen (APAP, paracetamol). After the first phase acetaminophen formed N-acetyl-p-benzoquinone imine (NAPQI). Oxidized form of paracetamol was obtained using electrochemical cell (ROXY, Antec). Paracetamol with its oxidized metabolite NAPQI were added to human hemoglobin solution and this mixture was incubated, treated by trypsin and analyzed by MALDI-MS and HPLC-MSMS.

In mass spectra of tryptic digest of hemoglobin were observed signals, which corresponded to peptides no modified MH+ 2529.2 Da (fragment 84–96 of â-subunit) and modified by NAPIQ MH+ 2678.3 Da. Modified peptide was identified using ETD and CID fragmentation. NAPIQ binds to Cys-94 of beta subunit hemoglobin as result gives mass shift 149.07 Da.

Method to identify human hemoglobin adducts with metabolites of drugs for example acetaminophen was developed. The measurement of NAPQI – protein adducts in the blood of patients with significant paracetamol exposures holds promise as a confirmative biomarker in the study of poisoning and toxicity.

P-379.00

ISOLATION AND IDENTIFICATION OF SERUM PEPTIDES - A USEFUL TOOL FOR IDENTIFICATION OF NOVEL DISEASE BIOMARKERS FOR ALZHEIMER'S DISEASE

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Introduction
Low-molecular weight (LMW) proteolytically-generated peptides are an important source for identification of novel disease biomarkers. Separation and enrichment of LMW proteins and peptides are hindered by their low abundance and as they account for 1% of the serum protein content. In the present study a fractionation and enrichment method for identification of serum peptides is presented to identify novel biomarkers for Alzheimer's disease.

Methods
Sera from fifteen 15 Alzheimer's patients and fifteen healthy subjects were included. Serum samples were separated by ultrafiltration (MW < 20 kDa) and low molecular weight fraction was further fractionated by reversed phase liquid chromatography (LC) coupled with step-wise elution. Samples without any prior enzymatic digestion were subjected to LC-MS/MS.

Results
The complexity of the serum samples was significantly reduced and the most abundant proteins were depleted after the ultrafiltration. It was possible to identify several peptides including peptides from proteins not reported in the Human Proteome Organization (HUPO) Plasma Proteome Project datasets.
A number of peptides belonging to neuronal proteins were also identified and the relation of these to Alzheimer's disease will be further studied.

Conclusions
The ultrafiltration and fractionation method was effective and has a low cost when compared to other serum separation and enrichment techniques. Furthermore, the LC-MS/MS analysis was conducted without enzymatic digestion of the samples, which allows identification of disease-specific peptides derived from proteolysis during the disease pathology.

P-380.00

3D MALDI IMAGING OF MOUSE HEART AFTER MYOCARDIAL INFARCTION

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Introduction and objectives

MALDI Imaging (MSI) is now maturing into a tool for three-dimensional analyses. We integrate magnetic resonance imaging (MRI) and 3D MSI and apply it to a mouse model of myocardial infarction. The entire MS data set was co-registered with MRI data and histological images to construct a 3D representation of the multi-modal data.

Methods

Myocardial infarction was induced in a C57BL/6J mouse. After sacrifice, MRI data was acquired on the heart which was then embedded in paraffin, sectioned and mounted onto conductive glass slides. After de-paraffinization, low-res optical images were acquired and MSI analysis was performed on a MALDI-TOF mass spectrometer operating in linear mode. After MS acquisition, all sections were stained with H&E for detailed histological examination

Results and discussions

Combining classical histology, MRI data and MSI, we elucidated proteomic signatures specific to the different areas of the infarcted myocardium.

In practice, we encountered several challenges to this approach Standard deposition of sinapinic acid matrix proved to be insufficient for small structures such as mouse heart. We show how á-CHCA matrix, applied with a modified protocol, allowed us to analyze the sample in sufficient detail.

Our previously established registration method to reconstruct a 3D object proved insufficient for handling the fine detail of the mouse heart. We refined the procedure and show a process of initial stacking, rigid and elastic registration of the images. Due to the large amount of data generated by even a single 3D analysis, we have developed new approaches for data. In particular, we show the application of a novel, spatially-aware peak picking algorithm to 3D MSI data

Conclusions

We could detect proteomic signatures of a myocardial infarct in 3D MALDI Imaging data.

P-381.00

IN-DEPTH IDENTIFICATION OF PROTEIN IMAGES BY COMBINING HIGH MASS RESOLUTION MALDI FTICR IMAGING AND HIGH PERFORMANCE QTOF NLC-MS/MS

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Introduction and objectives

In a MALDI imaging study assigning protein identity to peaks can be challenging. We have previously described a workflow where serial sections are digested under identical conditions; one section is imaged while the other is extracted and analyzed by LC-MALDI TOF/TOF. A script matches identified proteins with corresponding peptide images. This approach is limited by the Tof's relatively low MS resolution and precursor isolation window. In this study we examined if the number of proteins imaged and identified could be increased by combining high resolution MALDI-FTICR imaging with high-performance qTOF LC-MS/MS.

Methods

Serial sections of fresh-frozen rat brain and FFPE human kidney tumor were cut to 10-12 micron and mounted as paired sections on ITO slides. The FFPE sections were. All samples were then digested with trypsin and HCCA matrix was applied on one section for MALDI image analysis by either 7T FTICR (solarixXR, Buker)or a MALDI TOF (Autoflex speed, Bruker). The companion section was extracted and the collected tryptic peptides were analyzed by MALDI TOF/TOF (ultraflextreme, Bruker) or UHRQ-TOF MS (impact HD, Bruker.

Results and discussions

FFPE human kidney tumor section was imaged using both TOF and FTICR. The TOF data set contains approximately 80 discrete images in the range of 700-1400 m/z after deisotoping. In contrast, the FTICR image dataset exhibits mass resolution in excess of 200k which allows for more than 3290 unique monoisotopic images, in the same m/z range, 700-2500.

The nano-LC UHRQ-TOf analysis of the extracted peptides, lead to the identification of ? peptides corresponding to ? proteins. ? of theaccurate mass peptides could be correlated to the accurate mass peptides detected in FT-ICR.

Conclusions

The combined use of high-resolution FTICR imaging and of nano-LC UHRQ-Tof analysis enable to dramatically boost the number if identified proteins on a tissue section

P-382.00

PROTEOMIC PATTERNS IN THE NCI60 CANCER CELL COMPENDIUM COMPLEMENT GENOMIC FEATURES IN PREDICTIVE MODELING OF ANTICANCER DRUG SENSITIVITY

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Introduction and objectives

Cancer cell's response to drugs can be predicted using molecular features from DNA, RNA and proteins. Recently, there has been a surge of activities to build predictive models for anticancer drug sensitivity based on genomic data. To date, few models have been built on proteomic data, mainly because of difficulties in high-throughput and accurate quantification of cancer proteomes.

In this study, we aimed at performing absolute quantification of NCI60 proteomes to support predictive modeling of anticancer drug sensitivity based on proteomic patterns. We hypothesize that the integration of genomic and proteomic patterns results in superior modeling of drug sensitivity compared to genomic classification alone.

Methods

Proteomes of 60 human cancer cell lines (the NCI60 panel) were analyzed using pressure cycling technology SWATH-MS (PCT-SWATH). Synthetic heavy-labeled AQUA peptides of known concentrations were spiked-in to calculate the absolute amount of peptides and proteins. Data were acquired with technical replicates. Genomic data of the NCI60 cells were obtained from CellMiner (http://discover.nci.nih.gov/cellminer).

Results and Discussion

We measured the absolute quantity of over 5000 Swiss-Prot proteins from more than 44,000 peptides in the NCI60 cells. The characterized proteomes included > 400 cell surface proteins, > 120 kinases, and > 500 drug bank protein targets. Protein quantities from technical replicates were highly reproducible ($R2>0.93$). Leukemia cells displayed distinct proteomic profiles compared to solid tumors.

We built predictive models of the sensitivity of the NCI60 cells to a number of anticancer drugs using both proteomic and genomic features. Our models showed how different compounds can be better predicted from distinct types of molecular features, and allowed us to identify best biomarkers.

Conclusions

We presented high quality absolute quantitative proteomic data set of NCI60 cells. The proteomic features complement genomic features in predictive modeling of anticancer drug sensitivity.

P-383.00
PROTEOMIC LANDSCAPE OF THE HUMAN CHOROID-RPE

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Introduction and Objectives: Geographical protein expression differences in the human choroid-retinal pigmented epithelium (RPE) complex may explain molecular predisposition of regions to ophthalmic diseases such as age-related macular degeneration.

Methods: Experimental study of choroid-RPE tissue from three non-diseased eyes. The choroid-RPE was biopsied from beneath the foveal, macular, and peripheral retina. Protein fractions were isolated and subjected to multidimensional liquid chromatography and tandem mass spectrometry. A bioinformatic pipeline matched peptide spectra to the human proteome, assigned gene ontology, and identified protein signaling pathways unique to each of the choroid-RPE regions.

Results and Discussion: We identified an average of 4,402 unique proteins in each of the foveal, macular, and peripheral choroid-RPE tissues. There were 671 differentially expressed proteins that included previously known risk factors for retinal diseases related to oxidative stress, inflammation, and the complement cascade. Gene ontology analysis showed the foveal and macular regions had more immune process proteins as well as catalytic proteins and membrane bound proteins. The peripheral region contained more antioxidant activity and ribonucleoprotein complexes. Many proteins had the highest expression in the foveal or macular regions, including inflammation related proteins HLA-A, HLA-B, HLA-C, ICAM-1, S100, transcription factor ERG, antioxidant SOD1, ion channel CLIC6, activators of the complement cascade C1q, C6, C8, and complement inhibitor CFH. Proteins with higher expression in the periphery were BEST1, transcription factor RBM39, inflammatory mediator MIF, antioxidant SOD3, ion-channel VDAC3, and complement inhibitor CD55.

Conclusions: This proteomic analysis identifies novel molecular signatures in anatomically sensitive regions of the choroid-RPE. The findings give mechanistic insight into choroid-RPE function, reveal important choroid-RPE processes, and prioritize new pathways for therapeutic targeting. This study provides functional explanation of genetic risk factors.

P-384.00
SCREENING FOR NEW FIBROSIS BIOMARKERS IN RAT BRONCHOALVEOLAR LAVAGE FLUID

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Introduction

Mass spectrometry (MS) has become a common tool in the identification of new biomarkers for various tissues disorders. Tissues disorders includes pulmonary fibrosis with mortality rates similar to most cancer types. Vast efforts have been made to quantify the protein levels of lung proteins and the trend is now directed towards the smaller protein fragments that may be differently derived during lung fibrosis. The objective of this study was to induce pulmonary fibrosis to rats with bleomycin and to identify the peptides in the collected bronchoalveolar lavage fluid (BALF) and compare to the peptides identified in BALF from saline induced rats.

Methods

BALF samples were collected from rats induced with bleomycin (n=10) and saline (n=10). Regenerated cellulose (MW ≤ 10 kDa) was used to separate fragments from proteins by ultrafiltration (18,000 G). Further separation was performed by reverse phase chromatography with stepwise elution. Samples were subjected to liquid chromatography tandem mass spectrometry (LC-MS/MS) without any in vitro cleavage.

Results

The sensitivity was significantly increased after ultrafiltration and chromatography with stepwise elution. Several peptides derived from extracellular matrix proteins were identified. Peptide belonging to different proteins was identified when comparing the BALF of bleomycin treated rats with saline treated. Peptides not belonging to the proteome of Rattus norvegicus were also identified.

Conclusion

Ultrafiltration was an efficient method to removed larger proteins and has a low cost compared to other known enrichment methods. As no proteases was applied prior to purification and mass analysis a more disease specific identification of the BALF peptides was conducted. Although different peptides were observed from bleomycin treated rats compared to saline treated larger studies are needed for a more statistical separation of peptides in healthy and disease BALF samples.

P-385.00
CHARACTERIZATION OF AMNIOTIC FLUID PEPTIDOME IN PPROM PREGNANCIES WITH INFECTIOUS AND INFLAMMATORY COMPLICATIONS

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Introduction
Preterm prelabour rupture of membranes (PPROM) complicated by microbial invasion of the amniotic cavity followed by histological chorioamnionitis (MIAC/HCA) is associated with a high risk of neonatal morbidity and mortality. Consequently, reliable markers are required for improving clinical management of such pregnancies. Our recent proteomic study revealed dysregulation in a large number of amniotic fluid proteins potentially associated with MIAC/HCA, including several distinct proteases (Tambor et al., Plos ONE 2012). Altered levels of proteases suggest that characterization of amniotic fluid peptidome may reveal molecules with diagnostic potential in MIAC/HCA too. Hence, the aim of this work was to develop a protocol for the enrichment of native peptides from amniotic fluid and to highlight their dysregulation due to MIAC/HCA.

Methods
The native peptides were enriched using protocol based on triple solid phase extraction (C18, SCX, C18) followed by LC-MS/MS identification. Initial experiments were focused on the optimization of the procedure. Subsequently, this protocol was applied to detect dysregulation of amniotic fluid peptidome associated with MIAC/HCA.

Results
The viability of the protocol and efficient identification of amniotic fluid native peptides were proven through the initial LC-MS/MS analyses. In the pilot application of the protocol, we pointed out on several native peptides quantitatively altered in the presence of MIAC/HCA. Besides, to extend and refine the list of potential peptide markers associated with MIAC/HCA, analysis of multiple individual samples is currently in progress.

Conclusion
A viable protocol for characterization of amniotic fluid peptidome was developed, using which few hundreds of native peptides were identified. Furthermore, the application of the protocol brought new information about dysregulation of native peptides due to MIAC/HCA in PPROM.

P-386.00

IN-DEPTH ANALYSIS OF THE HUMAN VITREOUS PROTEOME USING A RESIN DEPLETION-BASED METHOD: APPLICATION TO THE DISCOVERY OF NEW BIOMARKERS FOR PROLIFERATIVE DIABETIC RETINOPATHY.

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Introduction and objectives:

Vitreous body is a promising source of biomarkers for vitroretinal pathologies. Nevertheless, as for blood, the dynamic range compromises the identification of less abundant proteins. We propose here to evaluate the gain brought by a resin depletion-based method and to assess the improvement in term of global identification and biomarkers discovery for proliferative diabetic retinopathy (PDR).

Methods:

Vitreous samples were collected from retinal detachment (RD) (n=16), PDR (n=8) and control patients (n=4). Resin-depletion of the 12 most abundant proteins (R&D system, Proteom PurifyTM12, ref 893677) was performed on 450μg of samples for identification (exp.1-2) and 300μg for quantification (exp.3-4). Different proteomics experiments were then applied on depleted or non-depleted vitreous samples (Offgel electrophoresis (exp. 1-4) - Gas Phase Fractionation (exp.1-4) and Tandem Mass Tag (exp.3-4)). Identification and isobaric quantification were assessed using Easyprot platform (IsoQuant, 2 peptides, 1% FDR). Gene ontology was obtained using DAVID software (6.7 version, p-value<0.05).

Results and discussion:

Extensive identification of vitreous proteome leads to a total of 991 proteins. Considering only identification experiments (exp.1-2), 40% proteins were gained with the depletion method, including 209 proteins not reported in the litterature*. Interestingly, in quantitative experiments (exp.3-4), 23 proteins were found differentially expressed between RDP and control patients only in the depleted experiment. 12 proteins including 3 lysosomal acid hydrolases were down-regulated (ratio1.5).

Conclusions: Our results show that depletion method associated to quantitative proteomics could open potentially new avenues for the diagnosis and a better understanding of the pathophysiological process taking place in diabetic retinopathy.

P-387.00

NEW APPROACH TO THYROID-ASSOCIATED ORBITOPATHY DISEASE IN TEARS BY QUANTITATIVE PROTEOMICS

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Background: Thyroid-Associated Orbitopathy (TAO) is part of an autoimmune process that can affect the extraocular muscles and periorbital tissue. The diagnosis is only based on clinical evaluation of signs, brain imaging and blood analysis (thyroid hormones). Therefore, we proposed a proteomics analysis of TAO tears in order to identify biomarkers that could help the diagnosis, prediction of the development of TAO and finally allow a better knowledge of the pathological events.

Methods: The tear fluid from TAO patients (N =3, 2 females, mean age ($\pm$SD): 52 years ($\pm$6) and controls (N=2, 1 female, mean age ($\pm$SD): 67.5 ($\pm$6)) was collected with Schirmer test. Then, two isobaric (Tandem Mass Tag, TMT)) proteomics experiments performed. The samples were fractionated by Off Gel Electrophoresis (12 fractions, pH3-10) and analyzed by LC-ESI MS/MS and gas phase fractionation (GPF) on a linear trap quadrupole (LTQ) Orbitrap Velos Pro. Proteins identification and quantification were done with Easyprot platform software. The biological process was obtained using DAVID software (6.7 version and p-value <0.05).

Results: On the overall tear proteome identified with both experiments (539 proteins with 2 unique peptides, 1% FDR), a total of 184 tears proteins were differentially expressed. Among them, 40 common proteins appeared particularly interesting with 2 down-regulated (Phospholipase A2 and β-2-microglobulin) (ratio< 0.66, p-value 1.5). Interestingly, into the up-regulated proteins, we found 13 proteins directly or indirectly related to glycolysis pathway (gluconeogenesis, pentose metabolism and tyrosine metabolism).

Conclusions: Our results confirm that tears could be a promising source of biomarkers for TAO. In addition, the number of differential proteins strongly connected with the glycolysis, open a new strange borderline that could be interesting to analyze, for a better knowledge of the disease, in future experiments.

P-388.00

REDOX-STATE OF PENTRAXIN 3 AS A NOVEL BIOMARKER FOR RESOLUTION OF INFLAMMATION AND SURVIVAL IN SEPSIS

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Severe sepsis is a common acute illness in intensive care units (ICU) with high mortality rates and chronic morbidity. A key unmet clinical need is the availability of a biomarker that predicts myocardial dysfunction early, monitors response to treatment and thus identifies a cohort of patients at higher risk of septic shock to aid in targeted interventions and improve outcome.

In an endotoxaemic model of sepsis, mice were injected with lipopolysaccharide (LPS) or saline and sacrificed 6-8 hours later. The Triton-insoluble fractions of mouse heart homogenates from control and septic group were analysed by gel-LC-MS/MS. The quantitative difference was validated by immunoblotting. Validation of our proteomics findings in the preclinical model was then performed using samples from sepsis patients admitted to ICUs.

Proteomic analysis showed the long pentraxin 3 (PTX3) was the most pronounced change and accumulated in LPS-treated mice as an octamer in heart, kidney and lung – common organ dysfunctions seen in patients with sepsis. Oligomeric moieties of PTX3 were also detectable in the circulation. The redox-state of PTX3 was quantified over the first 11 days in critically ill adult patients with sepsis. On admission day, there was no difference in the redox-state of PTX3 between survivors and non-survivors. From day 2 onwards, the conversion of octameric to monomeric PTX3 was consistently associated with a greater survival after 28 days of follow-up. In comparison to the conventional measurements of total PTX3 or NT-proBNP, the redox-sensitive oligomerization of PTX3 was more dynamic and a superior predictor of disease outcome.

Our findings suggest that the transformation of octameric to monomeric PTX3 in survivors may be a surrogate end point for the inflection point where oxidative stress subsides, and inflammation turns into resolution / repair.

P-389.00

PROTEOMICS OF RED BLOOD CELLS FROM PATIENTS WITH OBSTRUCTIVE SLEEP APNEA

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Introduction and Objectives: Obstructive sleep apnea (OSA) is a common public health concern in many countries, including Portugal, causing deleterious effects on metabolic and cardiovascular health. The molecular mechanisms and specific genes/proteins associated with such processes remain poorly defined. Using 2-DIGE -based proteomics evaluation of red blood cells, we plan to identify dysregulated proteins that could be useful as candidate biomarkers of diagnosis/prognosis of OSA.

Methods: Red blood cells were collected from peripheral blood of patients with severe OSA (n=12) or simple snore (n=12) at pre-(evening) and post-night (morning) lab-sleep polysonography study, so that proteome variations between these time points could be also assessed. RBC samples were randomized pooled (n=4/pool) to constitute three biological replicates per group of patients/conditions. The RBC-soluble fractions were depleted of hemoglobin using HemovoidTM system, protein quantified and analysed by 2DIGE, using 24cm IPG strips (3-10NL) and 12.5% SDS-PAGE. Images were analysed by Progenesis SameSpots software (NonLinear version 4.5).

Results and Discussion: Fifty seven protein spots differentially expressed (p

Conclusion: We anticipate that the complete validation and interpretation of those proteins/pathways will provide better understanding of OSA pathology that ultimately can be translate into newly effective diagnosis tools.

P-390.00
SECOND-HAND TOBACCO SMOKE EFFECTS EVALUATED BY PROTEOMICS

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Introduction and Objectives: Second-hand smoke (SHS) is responsible for more then 600000 premature year deaths. In the European Union, 14% of Non-Smokers (NS) are exposed to other individuals' tobacco smoke at home and 30% are exposed at the workplace. Towards their protection several European countries, except Portugal, move to a total tobacco ban in indoor public places. In 2008, a project aiming to analyze the impact of partial smoking ban in Portuguese public venues started. After our previously evidence of both SHS air contamination in non-smoking areas and inhalation of tobacco smoke by the venues workers, we aimed to study resultant biochemical and molecular changes at both systemic and respiratory level.

Methods: Ninety six workers participated in the study. A detailed lifestyle survey and clinical evaluation, which included spirometry were performed. 81 and 62 consented to provide blood and nasal epithelia (NE) cells, respectively. The obtained plasma samples were immunodepleted (14-MARS-affinity column, Agilent Technologies). Plasma and NE were analysed by 1D-SDSPAGE followed by in-gel digestion and LC-MS/MS.

Results and Discussion: At a clinical level all NS workers were considered healthy and recorded normal lung function as well as biochemical oxidative biomarker parameters. However at a molecular level a number of identified proteins were differentially expressed in those that are SHS exposed. Pathway Analysis is still in process.

Conclusions: Altogether these findings might contribute for disclosing early SHS induced pathogenic mechanisms and constitute a useful tool for monitoring the early effects of SHS on occupational exposed individuals, preventing the onset of related diseases.

P-391.00

DEEP PROTEOME PROFILING USING HR/AM SELECTED ION MONITORING AND DATA INDEPENDENT ACQUISITION ON THE ORBITRAP FUSION TRIBRID MS

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Quantification at proteome level is crucial for biomarker discovery but lacks selectivity when performed at full scan MS level. Although the concept was introduced several years ago, Data Independent Acquisition (DIA) strategies for proteome profiling and relative quantification have gained new attention with mass spectrometers capable of scanning at higher frequency.

The introduction of a quadrupole ion trap Orbitrap instrument scanning above 15 Hz at high resolution opens new perspectives in this application. Here we benchmarked several deep proteome profiling workflows where both precursors and fragments information can be monitored with the simplicity of a full scan MS analysis and the selectivity and precision of SRM analysis. Sequential SIM scans with different width and resolution were used to cover all precursor ions from 400 to 1000 m/z. In parallel, sequential ion trap or FT detected HCD MS/MS scans with 2 to 16 amu isolation windows were acquired to cover the same mass range.

For analytical performance comparison, FT detected HCD MS/MS scans with 2 to 6 amu windows were acquired with a targeted mass list. For evaluating the analytical precision and the sensitivity of the different workflows, 15 isotopic labeled peptides were spiked into 500 ng of human HeLa cell digests at levels from 10 amol to 10 fmol. The quantitative data for targeted peptides was extracted from the SIM and FT MS/MS spectra using ± 5 ppm mass window, and from ion trap MS/MS spectra using ± 0.5 Da mass windows. The ultra high resolution SIM data provided sensitivity over classical full scan MS data while MS/MS from fixed, small m/z isolation windows provided additional selectivity when required to confidently confirm and quantify the targeted peptides.

In conclusion we showed that these strategies offer improved quantitative performances with peptides detected at attomol level while maintaining simplicity in method set-up.

P-392.00

BRONCHOALVEOLAR LAVAGE FLUID PROTEIN PROFILE IN HEMATOPOIETIC STEM CELL TRANSPLANT RECIPIENTS

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Introduction: Idiopathic Pneumonia Syndrome (IPS), a form of non-infectious lung injury, is a devastating complication in Hematopoietic Stem Cell Transplant (HSCT) recipients. Our objective is to characterize the global Bronchoalveolar Lavage Fluid (BALF) protein expression of IPS to identify proteins and pathways that differentiate IPS from infectious lung injury.

Methods: Patients with lung injury within 180 days of HSCT were classified as IPS by current diagnostic criterion. BALF was depleted of high abundance proteins, treated with trypsin and labeled with iTRAQ® 8-plex reagent for 2D capillary LC and data dependent peptide tandem MS on an Orbitrap Velos system in HCD. Protein identification was performed using target decoy strategy. Relative abundance of the proteins was determined with reference to pooled BALF from patients with respiratory failure and no history of HSCT.

Results: BALF is available on 21 patients with infectious lung injury after HSCT and 11 patients with IPS. No statistically significant difference was seen in mean duration of transplant to BALF collection, age, serum creatinine, BALF leukocyte, neutrophil and lymphocyte count. BALF protein profile is available from 4 patients with infectious lung injury and 2 patients with IPS. We identified 542 proteins at a global FDR of 1%. These proteins represent diverse biological process such as programmed cell death, proteasome-ubiquitin dependent protein catabolism, cellular ion homeostasis, response to oxygen radicals, carbohydrate catabolism, actin filament polymerization, plasma protein involved acute inflammation and protein remodeling. Of these, 16 proteins demonstrated differential expression in subjects with IPS when compared to infectious lung injury. These proteins will be explored as potential diagnostic biomarkers for IPS.

Conclusion: High-resolution MS platforms provide extended coverage in BALF. BALF Protein expression profile represents the pathophysiologic mechanisms involved in development of IPS. Early findings suggest presence of candidate biomarkers in the BALF for rapid diagnosis of IPS.

P-393.00

SMFAP4 – A NOVEL BIOMARKER FOR GRADING AND MANAGEMENT OF HEPATITIS C INFECTION

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Introduction

The human microfibril-associated glycoprotein 4 (MFAP4), which in the liver is mainly produced by activated myofibroblasts, is a ubiquitous protein playing a potential role in extracellular matrix (ECM) turnover. MFAP4 was recently identified by a proteomics approach as a serum biomarker (sMFAP4) for hepatic fibrosis in hepatitis C patients.

Methods

The aim of the present study was to elucidate the potential of sMFAP4 as biomarker for hepatic fibrosis discriminating the stages F0-4 of hepatic fibrosis and the activity grades A0-4 (Desmet-Scheuer-Score) in a large cohort of hepatitis C patients (n=552) who underwent liver biopsy. sMFAP4 levels were measured using AlphaLISA immunoassay.

Results

Regarding the different stages of hepatic fibrosis sMFAP4 serum concentrations were 9.3 ± 6.9 U/ml in F0; 10.5 ± 8.2 U/ml in F1; 12.3 ± 9.9 U/ml in F2; 21.1 ± 15.9 U/ml in F3 and 24.6 ± 15.8 U/ml in F4. sMFAP4 reached a sensitivity of 80%, a specificity of 82% for discriminating fibrotic stages F0 and F4 and had a sensitivity of 67% and a specificity of 80% in the differentiation of the activity grades A0 and A4 in hepatitis C infected patients.

Conclusions

sMFAP4 was confirmed as novel, reliable biomarker with high diagnostic accuracy in separating stages F1-4 [except of F0 vs F1 ($p = 0.12$, n.s.)] and reflecting inflammatory activity in hepatitis C. It reaches remarkable levels of statistical significance, which so far were not demonstrated for any other single biomarker for hepatic fibrosis. As a robust and valid biomarker for grading and monitoring, it could be a valuable tool for the responsible, economic clinical management of hepatitis C infection in the era of exploding costs for the new direct acting antivirals (DAAs).

P-394.00
AFFIMER ARRAYS FOR BIOLOGICAL AND BIOMARKER DISCOVERY

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Introduction and objectives
Proteome wide discovery is dominated by mass spectrometry, with 2D-DIGE and protein microarrays playing only minor roles to date. All three technologies suffer from significant limitations- high capital cost and specialised skills for mass spec; loss of protein complexes in 2D-DIGE; and uncertainties around the folding, orientation and specificity of capture reagents in protein arrays.
To overcome these limitations, we have developed a new protein microarray platform, comprising chemically-active microscope slides on which we immobilise large and diverse libraries of non-antibody binding proteins called Affimers. Each Affimer is immobilised in a distinct feature on the array and is in theory capable of recognising a specific partner protein, protein isoform or protein complex.

Methods
We have established a high throughput protein expression system capable of performing more than 4000 automated His-Nickel purifications a week. We print 25,000 Affimers at a time on batches of slides. Proteins in test versus control samples are differentially fluorescently labelled. Samples are incubated together on each slide, allowing the identification of differential protein behaviours as molecules or complexes in each sample compete for binding to cognate Affimers. Affimers that underpin the signature are then used to affinity-purify target proteins or complexes from the sample for unequivocal identification by mass spec.

Results and Discussion
We have used this platform to discover binders to individual purified peptides and proteins, to proteins that are differentially expressed in HeLa cell lysates following drug treatment, to candidate biomarkers (including the clinical gold standard biomarker, CRP) in serum from patients with inflammatory disease and to multiple proteins in tissue (placenta) homogenates.

Conclusions
Affimer Discovery Arrays represent an unbiased, inexpensive yet powerful new tool for the identification of protein-protein interaction networks and individual biomarkers in cells, tissues and biological fluids.

P-395.00

HIGH THROUGHPUT PROTEIN PURIFICATION: 235,000 PROTEINS A YEAR, AND COUNTING

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Protein microarrays, defined as large numbers of proteins immobilized on a two-dimensional surface, represent a major opportunity for the interrogation of biological samples and systems. Unfortunately, they also represent a number of challenges, including complexity and cost.

Affimers are combinatorial engineered proteins, alternatives to antibodies, that are uniquely stable when immobilized on a range of inorganic materials. Here, we describe the establishment of a new, robust, high throughput protein expression, purification and quality control pipeline that is currently producing 4,608 Affimer proteins a week.

The process includes automated colony picking into 96 well plates, overnight growth with built-in temperature ramping and auto-induction, a manual freeze/thaw step and automated, magnetic-bead-based Affimer purification. Greater than 85% of the purifications have yields that meet or exceed the levels and purity required for microarray printing.

P-396.00
ROLE OF COMPLEMENT COMPONENTS IN RETINOPATHY OF PREMATURITY?

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Purpose: Retinopathy of Prematurity (ROP) is a proliferative retinal vascular disorder affecting the eyes of premature babies with low gestational age (=32 weeks) and birth weight (=1,700g). An earlier study on a mouse model of ischemia induced retinopathy suggested a protective role for complement C3a and C5a in neovascularisation, whereas complements areknown to mediate angiogenesis in age- related macular degeneration (AMD). Thus, the exactinvolvement of complements in ROP pathogenesis is still unclear. The present study was aimed to understand the role of complement components in the pathogenesis of ROP.

Methods: Vitreous humor (50-100µl) was collected with prior informed consent from patients (n=40) with stage IV and V of ROP (classified as per ICROP guidelines) along with infants with congenital cataract as control subjects (n=40) undergoing vitrectomy. Prefractionated proteins were subjected to trypsin digestion either in-gel or in solution and the resulting peptides were analysed on a FT LTQ Orbitrap Velos mass spectrophotometer. The obtained mass spectra were searched against the SwissProt database using the Peak studio search engine. Following this strategy, we examined the complement components in ROP and control vitreous. Additionally, the levels of complement component in the vitreous were assessed by multiplex ELISA and further validated by western blotting.

Results: Proteomic analysis of in-gel trypsin digested proteins of selected gel pieces (160kDa, 63 kDa and 45 kDa) could detect 371 spectra in ROP and 95 spectra in controls for the C3 fragments, and 323 spectra in ROP and 102 spectra in controls for the C4 fragments.These results indicated elevated levels of complement component C3 and C4 in ROP patients which was further confirmed by a parallel multiplex ELISA and in-solution digested total proteome analysis. Analysis of in gel pieces of 63 kDa and 45 kDa for the activated C3 fragments, indicated 182 spectra in ROP and 24 spectra in the controls. Increased number of spectra for activated C3 and the cleavage of C5 into C5b in ROP vitreous were confirmed by western blotting.

Conclusions: The elevated levels and activation of complement factors indicated their abnormal immune activity in ROP. Hence, activation of complement pathway might be playing an important role in angiogenesis in ROP patients.

P-398.00

STROMAL MARKERS OF BREAST CARCINOMA IDENTIFIED BY PROTEOMICS OF LASER MICRODISSECTED TISSUE

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Introduction and Objective: Mammary stroma plays a significant role in the development, growth and metastasis of breast carcinoma. Our goal was to characterize the stroma in proximity to breast cancer (DCIS).

Method: Using both a tissue culture approach and laser microdissection of archival breast cancer tissue we have determined the proteomes of stromal extracellular matrix produced in vitro from fibroblasts stimulated by different breast cancer cell conditioned media as well as the proteomes of normal breast stroma and stroma adjacent and distal to breast carcinoma.

Results and Discussion: The matrix proteome from stimulated fibroblasts is similar but not identical to that from breast carcinoma stroma and is related to the invasiveness of the cell line. Several proteins in the stroma adjacent to breast carcinoma tissue appear be markers for reactive breast stroma. Furthermore these proteins suggest an altered functional stroma adjacent to tumors that may support tumor growth and metastasis.

Conclusions: Host response to tumors may in fact play a supportive role for tumor growth and metastasis and suggests that methods to "normalize" the tumor adjacent matrix could provide an adjuvant cancer therapy.

P-397.00
CHARACTERIZATION OF UROKINASE VIA ELECTROPHORESIS AND MASS SPECTROMETRY ANALYSES

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Urokinase (uPA) is a serine protease belonging to the peptidase S1 family and specifically cleaving the zymogen plasminogen into the active form, plasmin, which in turn degrades the fibrin clots. For this property uPA is linked to cancer and vascular diseases [1-2], as fibrinolytic agent in thrombolytic therapy. The binding of uPA to its specific surface receptor (uPAR) amplifies the cell-surface plasminogen activation, thus enhancing pericellular proteolysis.

uPA is a glycoprotein [3], present in human urines in two active forms: high molecular-mass urokinase (HMr uPA) and low-molecular-mass urokinase (LMr uPA). Urokinase is used clinically as a thrombolytic agent in the treatment of severe or massive deep venous thrombosis, pulmonary embolism, myocardial infarction, and occluded intravenous or dialysis cannulas. It is also administered intrapleurally to improve the drainage of complicated pleural effusions and empyemas. It is fundamental for pharmaceutical industries to have reliable analytical technologies able to demonstrate the conformity of their products with codes for medicinal products for human use.

The aim of this study was to certify the identity of urokinase produced by a Swiss company with commonly commercialized products by using analytical techniques, such as mono- and bi-dimensional (2D) electrophoresis, and mass spectrometry analysis performed via a LTQ-XL mass spectrometer. Via 2D mapping, uPA was resolved into 42 spots, which were all identified, via MS, as isoforms and/or cleavage products of intact uPA. These conventional technologies have been highly effective in the quality control of this pharmaceutical product.

P-399.00

POTENTIAL BIOMARKER DISCOVER OF THE WILSON'S DISEASE BY TOP-DOWN PROTEOMIC INVESTIGATION OF WHOLE SALIVA

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Introduction and objectives. Wilson disease (WD) is a rare inherited disorder of hepatic copper metabolism characterized by hepatic, neurological and psychiatric manifestations. The cause is a deficiency of the P-type copper-transporting ATPase (ATP7B) resulting in an overload of free copper in liver, subsequent hepatic injuries and progressive copper accumulation in extra-hepatic organs. Early diagnosis is complicated by symptoms often non-specific and confusing and, currently, a unique diagnostic test is not applicable. We investigated the salivary proteome/peptidome of WD patients with the aim to characterize new biomarkers.

Methods. A Top-down approach, complemented by bottom-up strategies, based on RP-HPLC-ESI-MS and MS/MS analysis, was applied for the structural characterization and label-free-quantification of proteins/peptides. Clinical parameters (AST, ALT, ceruloplasmin, cupruria, albumin) were correlated with protein/peptide levels.

Results and Discussion. WD patients exhibited significant higher levels of S100A9 and S100A8 proteins and their differently oxidized derivatives with respect to healthy controls. Oxidation occurred on methionine residues, on the unique cysteine residue (glutathionylated, cysteinylated, sulfinic and sulfonic acid derivatives, disulfide dimers), and in S100A8 also on tryptophan-57. Oxidative state of S100A8 and S100A9 is an alert of the oxidative stress typical of the disease and in the same time confirms their redox scavenger role. Moreover, WD saliva showed high levels of two new fragments of polymeric immunoglobulin receptor (pIgR), and of ?-defensins 2 and 4. Transaminases correlated with pIgR fragments, probably associated to hepatic damage, while ceruloplasmin and albumin correlated with several oxidized forms of S100A8 and S100A9. ?-defensins 2, 4 correlated with cupruria and seem to be involved in the disease exacerbation.

Conclusions. Oxidative stress and inflammatory conditions typical of WD reflect on the salivary proteome of these patients. S100A8, S100A9 and their oxidized derivatives, as well as pIgR fragments and ?-defensins 2 and 4 could be sensitive potential biomarkers of the pathology.

Topic 8

Non-human and food proteomics

OP035 - PLANT PROTEOMICS: ITS APPLICATION TO CLARIFICATION OF FLOODING TOLERANCE MECHANISM IN SOYBEAN

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Climate change poses tremendous global challenges for agriculture and environmental stresses severely diminish crop productivity. One of them, flood causes a serious problem for crop cultivation because it reduces grain yields. Soybean is sensitive to flooding, which markedly reduces growth.

To clarify the flooding tolerance mechanism of soybean, the flooding-tolerant mutants were screened and analyzed using proteomic techniques. Based on proteomics results, abundance of proteins related to glycolysis, development, protein synthesis/degradation, secondary metabolism, and cell wall changed in wild-type under flooding stress; however, these proteins did not differ in the mutant under flooding stress. The root tips of mutant were not affected by flooding stress, even though the wild-type had damaged root. Physiological and proteomic results indicated that ubiquitin/proteasome-mediated proteolysis did not occur in the root tip in the mutant. Furthermore, involvement of abscisic acid (ABA) in soybean response to flooding stress was analyzed by proteomic and biochemical techniques.

Growth was resumed after water removal when ABA was added during flooding treatment though it was not possible to grow after flooding without ABA. The abundance of proteins related to cell organization, vesicle transport and glycolysis decreased with ABA treatment compared with those in root of soybeans flooded without ABA. Additionally, nuclear proteomics revealed that cell division cycle 5 protein, C2H2 zinc finger protein, CCCH zinc finger protein, and transducin were significantly decreased under ABA treatment compared with those under flooding without ABA treatment. ABA mediated regulation of these proteins might be involved in the enhancement of flooding tolerance of soybean. These results suggest that ABA might be involved in the enhancement of flooding tolerance of soybean through the control of energy conservation via glycolytic system and the regulation on transcription factors.

References: (1) Komatsu et al., J Proteome Res, 2013, 12:4769-4784. (2) Komatsu et al., J Proteomics, 2013, 79:231-50.

OP036 - MARINE PROTEOMICS: FINDING MECHANISMS OF ADAPTATION IN LARVAE OF RED SEA BARNACLE BALANUS AMPHITRITE

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Introduction and objectives: The potential of barnacle larvae adapt to extreme conditions of red sea often depends on their ability to change gene or protein expression patterns. We focused on a newly hatched barnacle larvae, neuples II, to investigate the molecular mechanisms underlying adaptive regulation and examine whether these mechanisms differ between genetically similar larval populations of Hong Kong coast.

Methods: RNA sequencing was performed on an Illumina HiSeq2000 genome analyzer and sequences were used as a reference genome. Isobaric tags (iTRAQ)-based quantitative proteomics coupled to LTQ-Orbitrap mass spectrometer was used to identify differentially expressed proteins (DEPs).

Results and Discussion: A neuples larval EST database, containing 92117 sequences, was constructed and annotated. Genes responsive to salinity and temperature were cataloged. A total of 2000 proteins were identified, of which 1380 were quantified. The two populations exhibited drastically different protein expression patterns. The red sea larvae showed up-regulation of proteins involved in stress response, osmoregulation, and cytoskeletal development, while the proteins related to energy homeostasis and transport were down-regulated when compared to larvae of Hong Kong coast. Hyper salinity tolerance is apparently achieved through regulation of Na+/K+-ATPase or V-type proton ATPase. The synergic overexpression of heat shock proteins and chaperones may facilitate larvae to counter balance thermal stress. The results illustrate the mechanisms of larval tolerance to osmotic and thermal stress to enhance survival in the extreme red sea marine environment.

Conclusions: We demostraste that quantitative proteomic tools can be employed for better understanding of regulatory mechanisms behind non-model marine species.

OP037 - SHOTGUN PROTEOMICS OF DOG CEREBROSPINAL FLUID

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With an increasing number of fully sequenced animal genomes and advancements in mass spectrometry, there is now a great opportunity to increase the knowledge in animal proteomics. This research area is further stimulated by a growing interest from veterinary medicine and from the pharmaceutical industry in animal healthcare research. In this study, mass spectrometry was used to explore the dog cerebrospinal fluid (CSF). In diseases related to the central nervous system (CNS), CSF changes in composition and is therefore a very good source for better understanding of such diseases both in animals and humans. Four different high abundant protein depletion kits, developed for human plasma/serum depletion, were evaluated. A shotgun proteomics approach, based on nanoLC-Orbitrap MS/MS, was applied to analyze the different fractions. Thus, it was investigated if those methods could be successfully applied on and optimized for dog CSF. A volume of 400 μl dog CSF sample was processed with each depletion kit. Depleted and non-depleted samples were run on a SDS PAGE gel and each lane was cut into 3 pieces and a test with slicing the lanes in 10 pieces was also evaluated.

In total, using all approaches, over 950 proteins of the dog CSF proteome were detected. All four evaluated methods were able to deplete dog CSF samples, resulting in an improved coverage of the dog CSF proteome. Even if all methods could deplete sample, there were large differences between the different methods. To the best of our knowledge, this is the first shotgun proteomics study of the dog CSF proteome. It was shown that it is possible to use commercially available depletion kits, primarily developed for human plasma samples, to prepare dog CSF samples with an improved coverage of the dog CSF proteome.

OP038 - DEVELOPMENT OF AN ABSOLUTE AND MULTIPLEX MS-BASED QUANTIFICATION METHOD FOR E. COLI CARBON CENTRAL METABOLISM PROTEINS: A TOOL TO FEED DYNAMIC PREDICTIVE MODELS.

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Introduction and objectives

Green chemistry aims at designing and inventing the next generation of daily products by reducing or eliminating the use of fossil resources. The strategy consists in developing high-performance production strains using biomolecular engineering and metabolic network models. Metabolic network models allow absolute fluxes through larger networks of central carbon metabolism to be determined. However, these stoichiometric based-models only set aside static prediction. To obtain dynamic prediction models, quantitative "omics" data must be integrated into a systems-oriented framework together with enzymology data. The challenge of the present study is to obtain highly accurate absolute quantification of dozen of proteins in a single SRM analysis without any decomplexification for modelling puposes.

Methods

To accurately quantify protein, we used the PSAQ strategy (Brun et al., 2007; Protein standard Absolute Quantification), based on full length isotope labelled protein, which was described as being the most accurate. In order to quantify E. coli carbon central metabolism proteins, full length 15N isotopically labelled protein were produced. In addition, scheduled SRM (scSRM) analysis was chosen to accurately and specifically quantify proteins in E.coli lysates.

Results and Discussion

The assay was applied to obtain accurate quantification of 22 key enzymes of central metabolism, resulting to more than 720 transitions monitored during a single LC-SRM run. Using this workflow, we investigated two E.coli strains genetically modified to induce an increased NADPH/NADP+ ratio (Auriol et al, 2011). Thus accurate enzyme concentrations were determined and used in fluxes models particularly to assess the levels of regulation of some metabolic reactions.

Conclusions

With such an accurate quantification method, it becomes possible to obtain enough quantitative proteomics data of different E. coli strains for providing dynamic prediction models with accurate measurements.

OP039 - ONE HEALTH PROTEOMICS: MECHANISM OF BACTERIAL COMPETITION IN FOOD SAFETY OF DAIRY PRODUCTS

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Introduction

One of the key pillar of one health initiative organization that promotes the collaboration between veterinary, medical and environmental disciplines, is the counteraction of emerging infectious diseases that include food-borne pathogens. Lactic acid bacteria (LAB) are part of the typical microbiome of dairy products, including cheese. They are some well-known activities of inhibition of growth of foodborne pathogens as Listeria and E. Coli, but especially for what it concern Listeria monocytogenes often they are not always able to block the growth at refrigerator temperature.

Aim

Study the secreted proteomes in in-vitro experiments of Listeria monocytogenes and two different strains of Lactic Acid Bacteria to evaluate proteins and metabolites that are important to understand the mechanism of bacterial competition to improve food safety

Methods

Two stains of Lactococcus lactis were used in co-colture experiments with Listeria monocytogenes. Filtered culture and spent medium were lyophilized and proteins separated by 1D – Tricine gel and 2D electrophoresis coupled to a LC-MS/MS shotgun approach. Metabolites from the same media were analysed by GC-MS .

Results and discussion

Enolase of Listeria is one of the major Moonlighting proteins revealed in secretome of cocolture, together with the metabolite gamma-aminobutyric acid (GABA) that correlate with the major metabolic activity of Listeria in respect to Lactococcus.

Conclusions

Our results are in accord with experimental observation where Lactococcus in complex mixtures is able to control but not to completely reduce Listeria. Starting from those interesting results, we explain some key points for the modulation of Listeria metabolism.

OP040 - PROTEOMICS WORKFLOWS AND PROTOCOLS FOR THE STUDY OF ORPHAN SPECIES

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Proteomics research on biomedical and model systems has benefited greatly from the availability of high coverage, high quality gene and protein sequence databases. Basic tasks like protein identification and quantitation have become routine due to high matching rates between experimental data and database entries. The same is not the case though for many species of high biomedical or economical significance, e.g. microorganisms or crop plants. The genomes and proteomes of these "orphan species" are only partially covered in existing databases, and there is no clear assessment of the quality of the existing references or the biological homogeneity. Consequently even proteomics tasks that are considered routine in model systems require a high degree of customization for orphan species.

Potential remedies for this challenge are discussed using Holm oak (Quercus ilex) as an example. It is the predominant Mediterranean forest tree species and has received interest both due to its environmental (conservation and reforestation programs) and economic importance (as part of agroforestry efforts, Spanish "dehesa"). We have been using proteomics approaches to characterize its natural variability, seed germination, seedling growth and responses to environmental stresses e.g. drought and fungal diseases.

We present recent technological advances in the proteome analysis of Q. ilex as an orphan species, and discuss their impact on the analysis of other orphan species especially from the Viridiplantae clade:

- the extension of a previously published substitution-tolerant database searching approach from 2-DE samples to GeLC/MS/MS and shotgun LC/MS/MS analyses;

- the use of customized, species-specific protein sequence databases derived from publicly available RNA sequences, mostly ESTs and NGS reads.

We demonstrate that both approaches have the potential to improve depth of protein identification and quantitation by mass spectrometry.

P-400.00

RAPID DIRECT DETECTION OF THE MAJOR FISH ALLERGEN BY HIFU-SMIM

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Fish is one of the most frequent causes of IgE-mediated food allergy. The symptoms appear within 60 min of exposure (urticaria, nauseas). Besides, anaphylaxis shocks can potentially life threatening. Parvalbumins beta (β-PRVBs), are considered as the major fish allergens. The allergenic properties of these proteins are related with their heat and gastrointestinal enzymes resistance. To guarantee the security to the consumers, a number of regulations have been implemented (D2007/68/EC). As consequence, accurate, sensitive and fast detection methods that permit the direct recognition of allergens in food samples are highly recommendable.

Here, we present a new strategy for the fast and direct detection of the major fish allergen by selected MS/MS ion monitoring mass spectrometry (SMIM).

This strategy integrates three main steps:

(a) the purification of β-PRVBs by a short heat treatment followed by centrifugation of the sarcoplasmic fraction (Time: 45 min)

(b) their accelerated in-solution tryptic digestion using high intensity-focused ultrasound (HIFU) (Time: 2 min)

(c) the monitoring of 19 common β-PRVBs peptide biomarkers by selected MS/MS ion monitoring (SMIM) in a linear ion trap (LIT) mass spectrometer (Time: 60 min).

A total of 16 different raw fish species, 6 commercial sea-foodstuffs and 6 non-fish food species were used to validate this strategy. These were purchased from local markets and were selected in order to include the most commonly consumed fish species in Europe.

Therefore, with this strategy the direct detection of fish β-PRVBs in any sea-foodstruff, including processed and precooked products, can be achieved in less than 2 h.

P-401.00

QUANTITATIVE PROTEOME ANALYSIS OF THE ENDOSPERM OF DEVELOPING SEEDS OF JATROPHA CURCAS L.

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Physic nut (Jatropha curcas L.) is a potential source of raw material for the production of biodiesel. Exploitation of this potential is hampered by the limited knowledge on the biosynthesis pathways of fatty acids (FA) and other important seed constituents, such as the phorbol esters (PE). With this as motivation, we are driving efforts towards establishing the proteome of the endosperm of developing seeds in order to identity proteins involved in the synthesis of FA and PE and in determining the deposition patterns of these proteins and of other important classes such as that of the storage proteins.

For such, we used a label-free quantitative shotgun proteomics approach to study the proteomes of endosperm isolated from seeds at five developmental stages, ranging from seeds in early stages of endosperm development (Stage 6) to mature seeds (Stage 10). This resulted in the identification of the 1517, 1256, 1033, 752 and 307 proteins, from Stage 6 to 1010, respectively, summing up 1760 different proteins. Proteins with similar expression pattern were grouped into five clusters and protein quantification based on spectral counting was determined.

We identified several proteins involved in the biosynthesis of FA and proteins involved in the biosynthesis of carbohydrates, amino acids and a whole range of seed storage proteins. Although several enzymes belonging to the biosynthetic pathway of diterpenoid precursors were identified, we were unable to find any terpene synthase/cyclase, indicating that the synthesis of PE does not occur in seeds. In conclusion, we disclose the first in depth proteome analysis of the developing endosperm, providing an important step toward understanding the enzymatic machinery devoted to the production of C and N sources to sustain seed development.

P-402.00
METABOLOMIC PROFILE IN XYLITOL INTOXICATION OF DOGS

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Introduction
Xylitol is a naturally occurring 5-carbon sugar alcohol that is used as a substitutive sweetener in chewing gums, candies, and other foods. The use of xylitol in humans is increasing due to its positive effect on dental caries prevention and diabetic diet. Dogs are sensitive to xylitol, and it causes 2.5-7-fold increase in insulin secretion compared to glucose. Ingestion of xylitol can lead to intoxication in dogs characterized by depression, vomiting, ataxia, and seizures.

Material and methods
Blood samples were collected from 12 dogs with xylitol intoxication and treated in the intensive care unit of the Veterinary Teaching Hospital at the University of Helsinki in years 2010-2011. More than 1 year after the intoxication, control blood samples were collected. The aim of the study was to identify plasma metabolites associated with xylitol intoxication in dogs using non-targeted metabolite profiling. Ultra-performance liquid chromatography quadruple time-of-flight mass spectrometry (UPLC-QTOF/MS) metabolite profiling was applied to plasma samples.

Results and Discussion
More than 30 metabolites were found to be uP- or downregulated after acute xylitol intoxication compared to the control plasma samples in the same dogs.

Conclusions
The non-targeted metabolite profiling proved to be useful approach to identify plasma metabolites after xylitol intoxication in dogs.

P-403.00

PROTEOMIC ANALYSIS OF ESCAMOLES (LIOMETOPUM APICULATUM MAYR, LARVAE), THE "MEXICAN CAVIAR": A NEW MODEL FOR DISEASE RESEARCH?

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Entomophagy or insect consumption is a habit that has significantly increased worldwide, either for pleasure or to satisfy the food needs in developing countries. Worldwide, there are approximately 2,000 species of edible insects distributed in 120 countries. From these, approximately 540 are located in Mexico; being the most consumed maguey worms (Comadia redtembacheri), grasshoppers (Sphenarium histrio mexicanum), and escamoles (Liometopum apiculatum) ant larvae. Escamoles are considered as a sought-after delicacy and have been called the "Mexican caviar".

The most important recollection zones are located in the States of Mexico, Hidalgo, Tlaxcala, Zacatecas, and San Luis Potosi, during short periods from february to april. In this study, the proteome profile of escamoles was analyzed using 2DE and LC-MS/MS. Out of 380 spots analyzed by LC-MS/MS and using the MASCOT search engine against the Hymenoptera subset of the NCBI protein database, 174 protein spots corresponding to 118 different proteins were identified.

The identified protein spots were classified into 11 groups with regard to their main known function involving: cell structure (7%), transcription and translation (13%), metabolism and energy production (18%), protein folding and degradation (19%), cell signaling (2%), antioxidant activity (5%), transport (3%), oxidation-reduction processes (8%), other processes (14%), unclassified function (3%) and unknown function (8%). In addition, de novo sequencing and homology-based protein identification was carried out allowing the identification of 36 more protein.

The results of this study provide a valuable resource for molecular analysis of the "Mexican caviar" and moreover the functions assigned to the identified protiens suggest that L. apiculatum larvae could be a novel model of study several human diseases.

P-404.00

PROTEIN PROFILING ACROSS THE DEVELOPMENT OF THE ORIENTAL FRUIT FLY

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Introduction and Objectives: The Oriental fruit fly, Bactrocera dorsalis (Hendel), has been a very important pests worldwide. Changes that reduce the fitness of an organism are called stress such as climate changes, control strategies. How insects respond to stress at a molecular level is very critical while it is not well known. There could be gene amplification; gene product alteration; or genetic codes rewritten. In order to identify all these changes, we first need to understand how this insect developed molecularly. We previously identified molecular markers of nutrient deficiencies in larvae, pupae, F1 eggs and adults of larvae reared on media lacking wheat germ oil (WGO) and the WGO effect was peak at the age when females begin reproductive maturation (day 8). Subsequently, we identified differentially expressed proteins between chemosterilant or radiation treatment compared to controls. We addressed the idea that the whole animal proteome changes dynamically with different developmental stages and ages. It would be beneficial if we have a better understanding of their protein profiling.

Methods: We reared fruit fly larvae on media with WGO and analyzed protein expression from larval age 0-10-d-old, pupal age 0-11-d-old, females and males age 0-10-d-old, and F1 eggs age 0.5, 2, 6, 12, 24, 36, and 48-h-old using standard protein extraction protocols and 2D gel electrophoresis. Database searches were performed with Mascot search engine v. 2.4 on an in-house server against the updated Bactrocera dorsalis (2013) custom protein database, the NCBInr Metazoa protein database.

Results and Discussion: Proteins from different developmental stages and ages are differentially expressed dynamically, especially between stages. They were demonstrated in gels. A more complete profiling of their functionality and identification are ongoing.

Conclusions: The results confirmed our hypotheis. With these piece of information on hands, any changes due to the stress elements will be easily identified.

P-405.00

PROTEOMICS OF RICE AND COCHLIOBOLUS MIYABEANUS FUNGAL INTERACTION: INSIGHT INTO PROTEINS AT INTRACELLULAR AND EXTRACELLULAR SPACES

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Necrotrophic fungal pathogen Cochliobolus miyabeanus causes brown–spot disease in rice leaves upon infection, resulting in critical rice yield loss.

To better understand the rice-C. miyabeanus interaction, we employed proteomics approaches to establish differential proteomes of total and secreted proteins from the inoculated leaves. 2-DE approach after PEG-fractionation of total proteins and coupled with MS (MALDI-TOF/TOF and nESI-LC-MS/MS) analyses led to identification of 49 unique proteins out of 63 differential spots. SDS-PAGE in combination with nESI-LC-MS/MS shotgun approach was applied to identify secreted proteins in the leaf apoplast upon infection and resulted in cataloging of 501 unique proteins, of which 470 and 31 proteins were secreted from rice and C. miyabeanus, respectively. Proteins mapped onto metabolic pathways implied their reprogramming upon infection.

The enzymes involved in Calvin cycle and glycolysis decreased in their protein abundance, whereas the enzymes in the TCA cycle, amino acids, and ethylene biosynthesis increased. The differential proteomes also generated distribution of identified proteins in the intracellular and extracellular spaces, providing a better insight into defense responses of proteins in rice against C. miyabeanus. Hence, the establish proteomes of the rice-C. miyabeanus interaction serve not only a good resource for the scientific community but also highlight its significance from biological aspects

P-406.00

COMPARATIVE METABLOMICS AND PROTEOMICS ANALYSIS REVEAL CHANGES OF ISOFLAVONE PATHWAY FLOW IN SEED COAT OF GLYCINE MAX CV. MALLIKONG AND MALLIKONG MUTANT

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The present study was carried out to investigate comparative proteomics analysis of soybean seed coat from Mallikong (M) and Mallikong mutant (MM), which is naturally mutated and exhibited interesting phenotype with brown colored seed coat, during different development stages.

To investigate comparative protein changes between M and MM soybean seed coats, we analyzed 2-DE. Total 231 protein spots were identified by MALDI-TOFTOF mass spectrometry, followed by gene ontology analysis (ufo.gobics.de, bioinfo.cau.edu.cn/ agriGO/).

Out of successfully identified 186 proteins, 59 and 127 proteins were uP- and down-regulated in MM soybean seed coat, respectively. Among them, expression patterns of dehydrins and typsin inhibitors were uP- and down-regulated, respectively, and they were verified by Western blot analysis showing consistent with that of 2-DE. Also, isoflavone reductase proteins were down-regulated in MM compared to the M. Furthermore, metabolomics analysis revealed that total isoflavone contents and individual their components by using reverse-phase HPLC were reduced in MM soybean seed compared to the M seed coat. Thus, these results suggest that changes in metabolome and proteome in two soybeans (M and MM) should be altered by natural mutation, even though it still remains further study.

P-407.00

INVESTIGATION OF NUTRIENT DEFICIENCY RESPONSES OF POTATO ROOTS USING PROTEOMICS AND METABOLOMICS APPROACHES

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Introduction
Nitrogen is the major macronutrient limiting crop plant yield worldwide. The resulting usage of nitrogen as a fertilizer can result in nitrate leaching and strong environmental pollution. Therefore, breeding of crop plants with higher nitrogen use efficiency is of importance for future agronomical performance.
Knowledge about responses of potato to N-limitation is only elementary. We present a combined 'omics' approach allowing analysis of changes in the proteome, phosphorylation pattern, and of primary and secondary metabolites in potato roots tissue of genotypes with contrasting nitrogen use efficiency.

Methods
Plants are cultivated in an in vitro system for 10d in full medium and are then subjected to low nitrogen medium. Roots are collected after 10, 30, 60min, and after 3, 24, 48, 72h. For preliminary experiments proteins were separated by 2D SDS-PAGE, and stained for phosphoproteins. Main studies apply LC separation coupled to UHR-Q-TOF MS to analyze changes in phosphoproteome and secondary metabolite profiles. Primary metabolites are monitored using GC-MS.

Results and Discussion
We established an in vitro culturing system to modify growth conditions of potato plants for investigation of early and long term nitrogen deficiency stress responses. We observed macroscopic symptoms of stress after 24h of treatment, including reduced growth, chlorosis and synthesis of anthocyanins.
Already after stress treatment for 10min we could observe changes in the phosphorylation pattern, most likely corresponding to signaling events. Comprehensive studies using LC-based methods were initiated to investigate the impact of nitrogen limitation on the proteome and metabolome level. In addition data is analyzed by a metabolic pathway driven targeted approach enabling a rapid evaluation of changes in the flavonoid biosynthetic pathway.

Conclusion
Combination of the different 'omics' techniques, applied to investigate different time-points of nitrogen deficiency stress responses, is be the basis for an in-depth understanding of stress signaling in potato roots.

P-408.00

QUANTITATIVE LABEL-FREE SHOTGUN PROTEOMIC ANALYSIS OF RED AND WHITE GRAPEVINE EXPOSED TO HIGH AND LOW TEMPERATURE STRESSES.

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Drought and extreme temperatures are abiotic stresses that can trigger significant complex responses in grapevines. Global warming and expanded cultivation range expose grapes to temperature stresses. This project investigates proteomic changes induced by the impact of high and low temperature stress on cultured Cabernet Sauvignon and Chardonnay cells. The aim of this quantitative label-free shotgun proteomic experiment is to provide insights into the targeted proteins and metabolic pathways that are related to temperature stress in grapevine.

Grape cells grown at 26°C were exposed at 34°C and 42°C for high, and 18°C and 10°C for low temperature stresses, for 14 hours. Cells from five temperatures were harvested in biological triplicates, proteins extracted using guanidine hydrochloric acid, digested by filter aided sample preparation and trypsin digested. Proteins were identified using LC-MS/MS spectra acquired by gas phase fractionation (GPF) in four mass ranges with m/z values of 400 – 506, 501 – 658, 653 – 913 and 908 – 1600 from a Velos-Pro linear ion-trap mass spectrometer. Peptides were searched against the UniProtKB Vitis vinifera genome and quantified by spectral counting.

The number of reproducible proteins identified in the three biological replicates of each temperature point ranged from 1056 to 1167 with a protein false discovery rate of < 1%. High reproducibility of peptide counts was observed across all replicates and the five temperature points, and ranged from 23815 to 35125. Peptide FDR was < 0.2%. Gene Ontology annotations of the uP- and down-regulated proteins compared to control revealed the crucial role of proteins involved in biosynthetic processes, amine metabolic processes, cellular amino acid processes, secondary metabolic processes, oxoacid metabolism and carbohydrate metabolism. This is the first label-free shotgun proteomic study on grape cells exposed to high and low temperatures.

P-409.00
UNDERSTANDING NUTRIENT-RESPONSIVE PROTEIN AND METABOLIC NETWORKS IN FOOD CROPS

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Introduction and Objectives
Nutritional quality and agricultural productivity are the two key issues to the sustainable food production worldwide. Composition of nutrients in the storage organs greatly influence the organ development and determine the nutritive quality. Seed storage proteins in plants, rich in essential amino acids are thought to play key role in growth and meet the major dietary protein requirement, while the plant metabolite oxalic acid is increasingly recognized as food toxin with negative effect on human nutrition. Nutrient response in plant is a complex phenomenon and the exact physiological relevance and functional modification caused as a result of nutrient and/or anti-nutrient accumulation is poorly understood. Thus, identification of nutrient-responsive proteins is a crucial step towards understanding of their cellular function/s.

Methods
The proteins were resolved by 2-DE and differentially expressed protein spots were identified using MALDI-TOF/TOF and ESI-MS/MS, while metabolite analysis was performed using GC-MS. Further, a protein-interactome model was developed.

Results and Discussion
To better understand the regulatory networks and metabolic pathways involved in increased protein synthesis and reserve accumulation and decrease in anti-nutrient in plants, we have developed comparative tuber and fruit proteomes of wild-type and genetically modified potato and tomato, respectively. The nutrient-responsive comparative proteomics revealed a coordinated response, which involves both the functional as well as signaling proteins. Further, metabolome study indicated uP-regulation of metabolites paralleling the proteomics analysis. Network modeling based on our datasets illustrates nutrient-responsive gene regulatory functional modules, novel pathways, and metabolic consequences upon sensing a storage protein and reduction in oxalate level and aim to show how target proteins might work in coordinated fashion and attribute to enhanced nutritional quality.

Conclusion
These findings will not only impact plant biology, but in near future would be useful for identifying biomarkers, prioritize molecular targets, and pathway bioengineering for food crop improvement.

P-410.00

PROTEOME ANALYSIS OF PROGRAMMED CELL DEATH FOLLOWING SPINAL CORD INJURY

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Introduction and objectives:

Spinal cord injury (SCI) can lead to paraplegia or quadriplegia and there remain no effective treatments to reverse paralysis following SCI. Traumatic injury to the spinal cord can kill cells and disrupt axons immediately, but also is followed by a spreading of secondary tissue damage that expands from the injury epicentre that involvesboth necrotic and programmed cell death. Several programmed cell death forms have been shown to participate in the secondary damage such as apoptosis or autophagy. Unlike those cells directly involved in the primary injury (necrosis), it is currently possible to develop strategies for promoting the survival of cells undergoing cell death programs. In order to better understand how these forms of cell death evolve after injury, we aim to characterize key proteins involved in apoptosis and autophagy as well as to determine the expression levels following neurotrauma.

Methods: MRM pilot software (ABSciex) was used to calculate theoretical transitions of 17 target proteins and they were tested and confirmed in a mouse spinal cord sample using a Triple quadrupole-Linear ion trap mass spectrometer (4000QTrap).

Results and Discussion:

99 optimized transitions from the proteins involved in authofagic and apoptotic cell death were validated in mouse samples of spinal cord. Relative quantitative analyses of these transitions were then employed to detect concentration changes in spinal cord protein samples 1, 3 and 7 days post-injury. These results might clarify the evolution of these forms of programmed cell death over time after injury and provide a better understanding how the spinal cord responds to trauma.

Conclusions: MRM analyses of protein involved in cell death are a useful tool for mapping changes in proteins after spinal cord trauma in order to identify new therapeutic targets.

P-411.00

PEPTIDOMICS OF YOUR SARDINIAN SNACK: ENRICHMENT AND CHARACTERIZATION OF PEPTIDES IN ARTISANAL CHEESE AND BEER

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Introduction and objectives
The food peptidome describes the complete set of peptides generated in food from raw components during processing, ripening, and storage. Adding to its interest for product characterization, food peptidomics can have reflections on human health, since many peptides possess bioactive properties impacting body functions or conditions, including antihypertensive, immunostimulating, antimicrobial, and opioid activities. This study investigated the peptide profile of two typical products of Sardinia, Fiore Sardo cheese and Zàgara beer. Fiore Sardo is a hard cheese produced from raw sheep milk, whereas Zàgara is an artisanal amber ale enriched with organic orange flower honey.

Methods
Peptides were extracted from different cheese and beer samples, then purified and enriched using an FPLC system connected to a Superdex Peptide 10/300 GL column. Fractions were desalted using solid-phase extraction (SPE) cartridges and then analyzed by mass spectrometry. Abundance of the purified peptides from cheese and beer was assessed by normalized spectral index (SIN) for each peptide, while the biological activity was determined using bioactive peptide prediction software and customized databases.

Results and Discussion
In cheese, LC-MS/MS analysis identified 1554 peptides, many of which were predicted to possess different biological activities, such as antibacterial, immunomodulating, ACE-inhibitor, and antioxidant. In beer, we identified a total of 510 peptides generated during fermentation from raw ingredients. These were mainly derived from the degradation of 175 proteins distributed in three taxonomy classes (Hordeum vulgare, Saccharomyces cerevisiae and Apis mellifera).

Conclusions
The peptide enrichment method applied to artisanal cheese and beer enabled to reach a profound peptidomic characterization of these two foods. Useful data were obtained concerning the production variables impacting on the final peptide profile, on the protein regions deriving from raw materials that can still be found in the final, ripened product, and on the potential bioactivity of these peptides for the consumer.

P-412.00

GEL-BASED PROTEOMIC ANALYSIS OF RNA-DEPENDENT PROTEIN STABILITY IN *ESCHERICHIA COLI*

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Since 1980s, the mechanism of folding and quality control of proteins in vivo has been studied based on the view of molecular chaperones. Accumulating evidence, however, suggests that molecular chaperones play only limited role in protein folding.

There are new suggestions that nucleic acid; especially RNAs have a lot of possibilities to influence on the destiny of protein. With the same opinions, discovery of intrinsically disordered proteins (IDPs) has brought skepticism that protein¡¯s folded structure is not necessary for to act its own function for the cellular processes. And many patients who suffered from neurodegenerative diseases have dysfunctional proteins in their neuronal cells with aggregated forms. In this study, we performed a proteome-wide analysis of potential role of RNAs in folding and stability of proteins. Soluble extracts of E. coli were depleted of RNAs by RNase A treatment and fractionated into soluble and insoluble forms.

After performing 2D gel-based proteomic analysis, the identities of the proteins enriched in insoluble fraction were determined by MALDI-TOF-MS.

The results show that RNAs are involved in maintaining the stability of a repertoire of proteins, including ribosomal proteins. Subsequent analysis on the degree of structural disorder is expected to enhance our understanding of the role of cellular RNAs on the stability and the quality control of IDPs.

P-413.00

IDENTIFICATION OF CHITINOLYTIC PROTEINS IN THE SECRETOME OF CELLVIBRIO JAPONICUS

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Introduction and objectives

Cellvibrio japonicus is a gram-negative bacterium long known for its capability to degrade cellulose. The organism contains a diverse collection of enzymes able to degrade the different polysaccharides in plant cell walls. Genomic analysis has confirmed the presence of 123 glycoside hydrolases as well as 14 predicted polysaccharide lyases, 18 carbohydrate esterases and 2 auxiliary activities. Closer inspection of these enzymes shows that the bacterium also harbours what appears to be a complete chitinolytic machinery. Building on this observation, we sought to evaluate the ability of C.japonicus to utilize chitin as sole carbon source and identify secreted proteins involved in this process.

Methods

C.japonicus Ueda107 was grown in chitin containing M9 media and the sectretome was collected by culture filtration at different time points. Proteins were identified by bottom-up mass spectrometry using a QExactive (Thermo) coupled to a nanoHPLC (Dionex), and relative protein abundances were calculated using label-free quantification with the MaxQuant software.

Results and Discussion

Several carbohydrate-active enzymes (CAZymes) were identified in the secretome of C.japonicus, including chitin specific proteins such as chitinases from glycoside hydrolase (GH) family 18 and 19, proteins from auxiliary activity family 10 (lytic polysaccharide monooxygenases) and a chitosanase (GH46). In addition, we identified two polysaccharide deacetylases (carbohydrate esterase family 4), potentially able to deacetylate chitin to yield chitosan.

Conclusion

C.japonicus is able to utilize chitin as sole source of carbon and energy by efficient substrate degradation using a complex chitinolytic machinery.

P-414.00

POTEOMIC MILK AND BLOOD ANALYSIS OF GREEK DOMESTIC SHEEP BREEDS

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Background/Aim: Goat farming constitutes an important agricultural activity in Greece whilst the milk of these animals is the main content in numerous dairy products. The present study aimed at investigating the proteome of both milk and blood of three indigenous Greek breeds of sheep, targeting at elucidating factors directly related to aminal productivity.

Materials and Methods: Blood plasma from healthy sheep of Karagkouniko, Boutsiko and Chios breed (n=15 from each breed) were investigated by 2-DE and MALDI-TOF-MS. While several (n=20) milk samples were analyzed via LC-MS/MS using an Orbitrap Elite instrument.

Results: One hundred and sixty different proteins were identified in animal plasma, with 23 being common to all three breeds (e.g. A1AT, APOA1, APOA4, b -2 glycoprotein, Gelsolin) and 62 common to two breeds only. More specificaly, 40 proteins were found in Karagouniko breed (e.g. FABPH, FGG, proenkephalin A) 13 proteins were found in Boutsiko breed (e.g. clusterin, SUMO3, actinin A heart muscle) and 22 in sheep of Chios breed (e.g. phakinin, b1 subunit of proteasome). Regarding milk samples, caseins and lactoglobulins were identified as major components of sheep milk, while other proteins such as beta-2-macroglobulin, lactoperoxidase, BRE1A, POP5, annexins, tafazzin, SPG21 and PALD were also identified.

Conclusion: The above findings represent reference data for Greek domestic sheep breeds. Detailed description of proteomic characteristics of breeds will provide a baseline dataset for development of knowledge concerning their genetic characterization, furthermore elucidate on how rural products production will be enhanced   and   ameliorated

P-415.00

MULTIPLE REACTION MONITORING ANALYSIS REVEALS CHANGES IN S1P-S1PRECEPTOR AXIS FOLLOWING SPINAL CORD INJURY

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Introduction and objectives: Trauma to the spinal cord causes permanent disabilities in approximately 160,000 people every year worldwide. The pathophysiology of the spinal cord injury (SCI) is a complex set of events, responses, and processes that affect the nervous, vascular and immune systems during the months following the damage. The sphingolipid metabolite sphingosine-1-phosphate (S1P) regulates different processes of the SCIthrough binding to G protein-coupled receptors (S1PRs).Characterization of the changes in the S1P-S1PRs axis after SCI is basic to determineits role and therapeutic potential for SCI. To contribute to the understanding of the S1P-S1PR axis we aim to characterize the expression of the S1P receptors and the enzymes involved in forming and degrading S1P following SCI.

Methods: MRM transitions of 10 target proteins were predicted in silico using MRMPilot software (ABSciex) and validated in mouse spinal cord samples using a 4000QTrap instrument. Optimized transitions were then used to determinechanges of protein concentration in spinal cord samples obtained 1, 3 and 7 days after an experimental model of moderate contusive SCI.

Results and Discussion: 57 transitions from the 10 proteins involved in the S1P-S1PR axis were validated. Relative quantifications of all transitions showed minor expression changes except for the S1P degrading enzyme S1P lyase (SPL) and the S1P receptor 4. Additional absolute quantification of SPL using an isotopically-labelled peptide confirmed an early expression increase followed by a later reduction back to control values which negatively correlates with the S1P profile. This observation suggests that SPL is modulating S1P concentration after injury and thus constitute a potential target to modulate the processes regulated by S1P.

Conclusions: MRM analyses of spinal cord samples identify changes in the S1P-S1PR axis after SCI that may constitute important therapeutic targetsfor this pathology.

P-416.00

DE NOVO SEQUENCING AND QUANTITATIVE PROTEOMICS IN A NON-SEQUENCED SPECIES AS A WAY TO DISCOVER NEW ANTI-OBESITY MECHANISMS

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Brown adipose tissue (BAT) plays a key role in the transformation of food into heat. A central issue, still debated, is that BAT could protect the whole organism against obesity. Here we report BAT proteome changes in wild-derived common voles (Microtus arvalis) artificially selected for low and high BAT activity levels and subjected to high-fat diet-induced obesity.

After tissue grinding, proteins were extracted and liquid digested (trypsin). Tryptic peptides were analysed on a BRUKER Impact HD QToF equipped with a captive spray source and nanoBooster. Mascot was used as the main search algorithm to identify proteins and de novo sequencing was used to increase proteome coverage. The benefit of using a large (i.e. mammals) vs. restricted (i.e. mouse) database was examined in terms of the number of identifications. The skyline software allowed protein quantification using MS1 XICs. Finally, the stability of the nanoLC-MS system and reproducibility of XICs were controlled by using iRT peptides (Biognosys) spiked in all samples and by injecting repeatedly a same pool of samples throughout the whole duration of the experiment.
About 1000 BAT proteins were identified (FDR

In conclusion, we show that robust de novo sequencing and label-free quantitative proteomics data of samples collected in a non-sequenced species were obtained using the BRUKER Impact HD QToF. The biological meaning of the differences between voles according to their BAT activity level is discussed with regard to possible anti-obesity effects.

P-417.00

APPLICATION OF CESI-MS ON SILAC-BASED QUANTITATIVE PROTEOMICS

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Introduction:
Stable isotope labeling with amino acids in cell culture (SILAC) relies on metabolic incorporation of amino acids with nonradioactive isotopes like 2H, 13C, and 15N. The SILAC approach needs no multi-step labeling protocols or purification steps to remove excess of labeling reagent as required for other strategies in quantitative proteomics. It is the preferred method to quantitate the abundance of proteins in comparative proteomics due to the highly uniform and efficient labeling.

Method:
The suitability of a sheathless capillary electrophoresis-electrospray ionization-mass spectrometry (CESI-MS) interface with a porous tip as nanospray emitter for the use in peptide quantification was evaluatd. Protein extracts of two yeast strains, one heavy-lysine labeled and one normal strain, were mixed 1:1 and enzymatically digested using Lys-C. Peptides were separated by RP-HPLC and the fractions collected were further analysed by CESI-MS. To evaluate the accuracy of our data, a comprehensive comparison experiment was performed using one dimensional SDS-PAGE followed by in-gel digestion using Lys-C and LC-ESI-MS analysis of the resulting peptides.

Results:
182 HPLC fractions were analyzed by CESI-MS, yielding 33,656 identified peptides. A total of 28,536 peptides were quantified, corresponding to 3,272 quantified proteins with at least 2 unique peptides and 2 peptide H/L ratios. In comparison, 42 gel fractions were analyzed in duplicate by LC-ESI-MS yielding 2,455 quantified yeast proteins, which is roughly 25% less compared to the CESI-MS approach. Merging both results, a total number of 3,517 proteins could be quantified. 133 proteins were found to be differently regulated of at least +/- 50%, 41 of these proteins could only be quantified by CESI-MS and 16 by LC-ESI-MS. A further valuation of the data concerning statistical analysis of the protein ratios, variability of the peptide ratios will be discussed.

Conclusion:
Sheathless CE-MS can be considered as an alternative approach in quantitative proteomics.

P-418.00

IDENTIFICATION OF WELFARE BIOMARKERS IN PIGS USING PERIPHERAL MONONUCLEAR CELLS (PBMCS) AND DIFFERENTIAL GEL ELECTROPHORESIS (DIGE)

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Introduction: Animal welfare is a topic of high interest due to its implications for animal health and because of economic consequences. Peripheral mononuclear cells (PBMCs) are adequate for proteomic studies since they can be easily obtained and do not contain highly abundant proteins as plasma. The objective of this work was to identify protein markers for welfare assessment.

Materials and Methods: Two groups of pigs (3-month old, n=15) were subjected to different management conditions. One group was kept under commercial farm conditions ("non-treated", NT) and the other one was subjected to close human-animal relationship ("treated", T). Serum and PBMCs were collected at 0, 1 and 2 month. Biochemical nutritional parameters, acute phase proteins, antioxidant enzymes and cortisol were determined in serum to control the health status of the animals. PBMCs were purified using ficoll gradient tubes and differences in protein expression between groups were analyzed by DIGE using 4 animals from each group, at time 0 and 2 months. Proteins were extracted by sonication, precipitated with Clean Up (GE Healthcare™) and resuspended in urea-thiourea buffer.

Results: When comparing differences in protein expression between t=0 and t=2, 305 differential spots were identified in non-treated animals and 153 in the treated group (P< 0,05, 1,5 fold-change). Eighty-five spots overlapped between NT and T groups, whereas 220 spots were differentially expressed only in the NT group and 68 spots in the T group. The identification of these proteins is under way.

Conclusions: Differences in protein expression between t=0 and t=2 are probably due to age since the animals were still young and growing. A larger number of changes were found in non-treated animals than in animals subjected to a close relationship to humans, suggesting that some of the changes were due to management conditions and not only to age.

P-419.00

MINING OF PROTEINS INVOLVED IN NUTRITION AND TRANSDIFFERENTIATION IN BOVINE SKELETAL MUSCLE

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The purposes of this study are to identify the difference in the protein expression from the blood and longissimus dorsi muscle (BLDM) using the proteomics and metabolomics method in order to discover specific causable biomarker proteins and metabolites involved in nutrition and transdifferentiation.

In the 2-DE and LC-Mass analysis of the blood, the 16 protein spots and 10 lipoidal hormones were found to be differentially expressed with changes in the density of twP-fold or more, including down or uP-regulation. Also in the 2-DE maps of the BLDM, the 13 protein spots were found to be differentially expressed with changes in the density of twP-fold or more, including down or uP-regulation.

To confirm the tendency of these proteins and lipoidal hormones, we used two bovine cell lines, BEFS-PPAR¥ã2 and BEFS-MyoD, which are derived from spontaneously immortalized bovine embryonic fibroblasts (BEFS). In a real-time PCR cell culture study of BEFS-PPAR ¥ã2, we found an increase or decrease in 2 proteins upon adipogenic differentiation into adipocyte.

Also in the cell culture study of BEFS-MyoD, we found an increase or decrease in 3 proteins upon myogenic differentiation into myoblast. In the conclusion, this finding suggests that the identified proteins may help to understand the induction of transdifferentiation and adipogenesis in MLMD.

P-420.00

PROTEOMIC ANALYSIS OF ARTHROBACTER SP. MWB-30, OIL-BIODEGRADING MICROBE ISOLATED FROM CRUDE-OIL CONTAMINATED SEASHORE

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Arthrobacter sp. MWB-30 was isolated from crude oil contaminated seashore caused by oil spill incident in Taean, Korea. Arthrobacter sp. MWB-30 exhibited by 99.99% of complete 16s rRNA sequence similarity with Arthrobacter nicotinovorans DSM 420 (x80743).

The strain was able to degrade and utilize diesel and kerosene, as well as crude oil as a sole carbon source for growth. Genome sequencing analysis shows approximately 4.6 Mbp of genome size, 4,466 of predicted ORF, and 63.0% of GC contents, respectively. Especially, this strain encodes single copy of alkane monooxygenase (MWB30_00280), which in turn alkane components were totally decreased in the oil containing media via Arthrobacter sp. MWB-30 compared to controls. Additionally, genes for degradation of polyhydrocarbon components (naphthalene, xylene, toluene, etc.) presented in then genome.

We conducted global proteomics on respective oil containing media with either Arthrobacter sp. MWB-30 or not, using LTQ- and TOF-MS. Based on the global proteomics, we addressed the enzymes that are comprehensively or substrate specifically responded to the crude oil, diesel and kerosene. Therefore, we evaluated the catabolic enzymes of MWB-30 composing of global and specific correlation for biodegradation of respective oil and their metabolism.

P-421.00

THE NUTRACEUTICAL POTENTIAL OF QUERCUS ILEX L. REVEALED BY PROTEOMIC ANALYSIS OF ACORNS AND ROOTS. IMPACT OF THE DROUGHT STRESS.

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Introduction and objectives: Quercus ilex Holm oak is dominant tree in the Mediterranean forest with key importance for the Spanish "dehesa". Its acorns are main dietary constituent of free-ranged Iberian pigs and are a rich source of phenolic compounds and fatty acids. Besides, acorns have been part of the local diet of the poorer classes of Italy and Spain, consumed in the form of bread cake and as a coffee substitute. Holm oak tissues have the potential be used for functional foods preparation. However, this value added application requires systematic study and basic knowledge of the active substances - producing potential and how it is affected by adverse environmental conditions. Proteomic study was undertaken to characterize the protein complement of holm oak acorns and the impact of drought stress at earliest developmental stage on the protein pattern, focusing mainly on the enzymes involved in the primary and secondary metabolism.

Methods: Gel based and gel free label free proteomic approaches were combined. As Quercus ilex is an orphan species with non-sequenced genome, protein identification was based on homology and on home-made EST quercus database. Acorns and germinated plants were used for the study. Drought stress was induced by water limitation. Proteomic data were supported by analysis of some metabolites of interest. Results and Discussion: The use of home-made EST quercus database significantly improved protein identification. Identified proteins mainly belonged to primary and secondary metabolism, protein biosynthesis and proteolysis, stress defence, and intracellular transport. In roots secondary metabolism was stimulated while in cotyledons carbohydrate and aminoacid metabolism were changed under drought. Total phenolic and sugar content were highly responsive to stress.

Conclusion: The obtained results provide an insight on the enzymes building the rich composition in bioactive substances of holm oak acorns, and on the metabolic adjustment as a response to drought.

P-422.00

PROTEOMICS APPLIED TO OENOLOGY, CHARACTERIZATION OF AUTOCHTHONOUS WINE YEAST STRAINS

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Typically, Saccharomyces cerevisiae is the typical wine fermentations yeast. However, after several years of yeast population studies has been well settle the crucial role that "others yeast" has during wine making process, being crucial to acquire wines specificity and distinction. Our group found different population of wine yeast during different vintages of red grapes, showing that, the wine yeast Saccharomyces bayanus var uvarum produce a specific profile of organoleptic properties of special relevance. Moreover, this strain has the ability to work nicely at low temperature, increasing the formation of volatile compounds responsible for the different wine organoleptic characteristics.

We had developed a differential proteomics analysis to elucidate the proteins involved in the winemaking process at low temperature by comparing the profiles obtained at 13ºC (low temperature) and 25ºC (high temperature). We studied the proteome by twP-dimensional electrophoresis (2DE) and MALDI TOF/TOF, the secretome by LC-MS/MS analysis and the membrane fraction by isobaric tagging to obtain a wide point of view of the different proteins involved in the whole process.

Through a detailed study of detected differences in the expression profiles, we will be able to elucidate the role of each of them during fermentation, which could be the basis for new strains selection criteria and/or the quest of biomarkers of wine quality.

P-423.00

PROTEOMICS ANALYSIS OF BOTRYTIS CINEREA SIGNALING CASCADES: THE MEMBRANOME

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Botrytis cinerea is a model phytopathogenic fungus that affect to more than 200 different relevant crops, being classified in the "top ten" fungal plant diseases. B. cinerea exhibits a wide arsenal of tools to infect plant tissues. Most of these factors, are related to signal transduction cascades, where membrane proteins, must play key role as a bridge between environmental conditions and intracellular molecular processes.

This work describes the first proteomic approach to study the membrane proteins expressed by B. cinerea under different pathogenicity conditions. Membrane protein extraction was optimized under two different pathogenicity states by using different plant-like elicitors; a) Glucose as a constitutive response and b) desproteinized Tomato Cell Wall as a virulence state inductor. 2-DE protein profiles were obtained, showing, in both stages, a subproteome located between 4 and 8 of pI with a molecular weight from 116 to 14 kDa.

Analysis of these profiles showed a differential expression of membrane proteins. We detect common and specific spots in each assayed carbon source, which has been analyzed by MALDI TOF/TOF. Due to the high hydrophobicity showed by this proteins, a wide "shotgun" analysis of the obtained proteins extracts by LC-MS/MS was used, resulting in a large number of detected peptides (7507) and identified proteins (2203).

Due to most of the identify B. cinerea virulence/pathogenicity factors seems to be related with signaling cascades mechanisms, we will analyze the role of this identifications in the infection cycle to unravel its potential role in the signaling network, and its use as new therapeutics targets for fungicide desing.

P-424.00

PROTEOMIC ANALYSIS OF CARDIAC TISSUE FROM RESISTANCE TRAINED RATS REVEALS CHANGES COMPATIBLE WITH HEART FAILURE

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Regular exercise is highly effective for prevention and treatment of many common chronic diseases and has already been shown to improve cardiovascular health. However, high intensity resistance training (RT) may induce pathologic structural remodeling of the heart, leading to acute injury and even cardiac sudden death. To evaluate the modulation of a RT protocol on the expression of proteins of the cardiac tissue, male Wistar rats were divided into 2 groups: Trained (T) and Sedentary (S).

Animals from T group underwent a RT protocol jumping into the water 5x/week, for 6 weeks with progressive overload attached to the chest (50-70% body weight). After this period, heart was excised for proteomic analysis by UPLC-MSE. The analysis detected 187 proteins, and of these, 132 were identified. Furthermore, 52 proteins were expressed only in the T group and 8 in S group. Quantitative analysis of proteins on T and S groups identified that 58 proteins were up regulated and 14 down regulated on T group. Based on Gene Ontology we identified the biological processes in which these proteins were involved: 1) There was a significant modulation of RT on proteins related to cardiac metabolism, shifting heart fatty acid metabolism to glycolysis, 2) Increased expression of proteins involved in the degradation of reactive oxygen species, 3) Expression of proteins related to apoptosis processes and 4) Expression of some heat shocks proteins, as HSP20, related to protective mechanisms against cardiac tissue injury.

The data presented here show, for the first time, that this RT protocol impairs heart metabolism and induces the expression of several antioxidant proteins. Furthermore, results from this study corroborate with previous data from our research group, revealing structural heart remodeling, with pathological hypertrophy, a profile compatible with heart failure.

P-425.00

SEMINAL PLASMA PROTEOMES OF RABBITS, BOARS AND BULLS AND THEIR ASSOCIATIONS WITH SPERM PARAMETERS.

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Seminal plasma is mainly formed by secretions from the epididymis and accessory sex glands. Presently, we describe the seminal plasma proteome of domestic species and their associations with semen parameters. Based on a study with 18 rabbits, zeta-globin protein and annexin 5 comprise more that 50 % of all seminal proteins, as evaluated by 2-D SDS-PAGE and mass spectrometry.

Other components, including the nerve growth factor were also detected in the rabbit seminal fluid. In a second study, we determined that spermadhesins PSP-I, PSP-II, AQN1, AQN3 and AWN1 represent the major proteins of the boar seminal plasma. Based on evaluations of semen samples from 12 boars, the percentage of sperm with midpiece defects was related to the amount of CH4 and secreted domains of swine IgM and fibronection 1, IgG-binding protein, complement factor H and lactadherin ($R^2 = 0.45 – 0.61$; $p < 0.05$). The percentage of sperm with tail defects and sperm motility had negative and positive associations with seminal plasma lactadherin ($R^2 = 0.74$ and 0.48, respectively; $p < 0.05$). In a third experiment, we collected and froze semen samples from 13 Bos indicus bulls. After semen thawing, bulls with average sperm motility of 52.4 ± 20.5 % were defined as the high freezability (HF) ones.

Bulls with sperm motility of 13.7 ± 3.9 % represented the low freezability group (LF; $p < 0.05$). Seminal plasma osteopontin, deoxyribonuclease gamma precursor and DNASE1L3 had greater expression in animals with HF semen. Conversely, acrosin inhibitor 1, glutathione peroxidase 3, metalloproteinase inhibitor 2, ephirin-A1, annexin A1 and platelet-activating factor acetylhidrolase had greater expression in LF bulls.

In conclusion, seminal plasma proteomes show unique content for each species. Nevertheless, their components function for sperm protection, maturation, capacitation, acrosome reaction and fertilization. Seminal proteins are potential markers of the sperm fertilizing capacity.

P-426.00

LACTOBACILLUS REUTERI SUB-PROTEOME STUDY TO ELUCIDATE UPTAKE, METABOLISM AND RELEASE OF SELENIUM FOR NUTRACEUTICAL APPLICATION

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Introduction and objectives: Selenium (Se) is an essential dietary trace element for humans and microorganisms. It is called "essential toxin" since it is fundamental at low concentration but toxic at higher levels. Se assumption depends on diet supply and its deficiency is associated with various chronic diseases such as oxidative stress, cardiovascular diseases and some kinds of cancer. It generally may be considered an anticarcinogenic and antioxidant agent. The use of Se-enriched lactobacilli is an innovative approach to counteract selenium-deficiency: microorganisms, grown in presence of inorganic (toxic) forms of selenium, are able to convert it into more biP-available organic forms, introducing it into proteins, called selenoproteins.

Methods: 2DE gels coupled with MS on different cellular districts (in toto proteome, envelope enriched fraction, extracellular proteome) and pI ranges (4-7 and 6-11) and LA ICP-MSI analyses were used on the probiotic Lactobacillus reuteri Lb2 BM DSM 16143 in order to understand the effects of Se on the strain and to determine in which proteins and specific amino acids it is inserted.

Results and Discussion: From these analyses emerged the ability of the strain to metabolize Se and to fix it into selenocysteine in 7 cytosolic proteins. Se also induces the uP-regulation of the sugar metabolism and a certain degree of stress. Two of these SeCys-containing proteins were also detected in the extracellular environment being useful for nutraceutical applications, since they allow to release Se at human gut level also before cell lysis.

Conclusions: This L. reuteri strain is a good candidate to be used as nutracelutical supplement since it combines its probiotic features with its ability to generate organic biP-available Se forms.

P-427.00

TYPE I COLLAGEN COMPOSITION IN THE BONY FISH DANIO RERIO

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Introduction and objectives: Classical osteogenesis imperfecta (OI) is caused by a dominant mutation in COL1A1 or COL1A2, encoding α1 and α2 chains of type I collagen, respectively. The bony fish Danio rerio (zebrafish) recently emerged as a good animal model for bone disorders and some mutants have been generated, e.g. the Chihuahua (Chi+/-) fish which resembles a classical OI form. Zebrafish skeletal system shows membranous and endochondral bone formation and the key regulators of bone development are highly conserved between teleosts and mammals. Moreover, zebrafish is an ideal drug screening tool owing to its high fecundity, rapid ex-utero development and transparency. In higher vertebrates collagen I, the main bone structural protein, is a heterotrimer composed of two α1 and one α2 chains.

The existence of three genes encoding three different α(I) chains, α1, α2 and α3, was described in zebrafish, but the exact collagen I composition is still unclear. To use zebrafish as OI model in drug screening, it is absolutely necessary to elucidate collagen I composition in this teleost.

Methods: Acid and pepsin soluble collagen I was purified from bone, skin, and scales of adult fishes and from whole embryos at 48hpf, 5dpf and 10dpf. Purified collagen was then electrophoretically resolved and identified by MS.

Results and discussion: In adult fish α1 and α3 chains comigrated, while in embryo some α1 isoforms singularly resolved and some other comigrated with α3 chain. These data suggest the existence of $[\alpha1(I)]2\alpha2(I)$ and $\alpha1(I)\alpha2(I)\alpha3(I)$ heterotrimers in embryos and of $\alpha1(I)\alpha2(I)\alpha3(I)$ in adults, although the presence of the $[\alpha3(I)]2\alpha2(I)$ form cannot be excluded in both developmental stages.

Conclusions: We proved, for the first time, the existence at the protein level of α3 chain in zebrafish and we contributed to collagen I characterization in Danio rerio at different development   stages.

P-428.00

DYNAMIC CHANGES OF CONA-ENRICHED URINARY PROTEINS IN A FOCAL SEGMENTAL GLOMERULOSCLEROSIS RAT MODEL

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Introduction and objectives: Compare to blood, which has mechanisms to maintain homeostasis, urine is more likely to reflect changes in the body. As urine accumulates all types of changes, identifying the precise cause of changes in the urine proteome is challenging and crucial in biomarker discovery. To reduce the effects of both genetic and environmental factors on the urinary proteome, this study used a rat model of adriamycin-induced nephropathy resembling human focal segmental glomerulosclerosis (FSGS) development. FSGS is a common cause of nephrotic syndrome and end-stage renal disease, and there is an urgent need for diagnostic and prognostic urinary biomarkers.

Methods: Urine samples were collected at before adriamycin administration and day 3, 7, 11, 15 and 23 after. Urinary proteins were profiled by LC-MS/MS. To reduce albumin abundance suppression and enhance sequence coverage of low abundance proteins, concanavalin A-enrichment strategies were applied.

Results and Discussion: Of 25 changed proteins with disease development, 20 have human orthologs, and 13 proteins were identified as stable in normal human urine, meaning that changes in these proteins are more likely to reflect disease. Sixteen of the identified proteins have not been established to function in FSGS development. Seven proteins were selected for verification in ten more rats as markers closely associated with disease severity by western blot.

Conclusions: We identified proteins changed in different stages of FSGS, which may aid in biomarker development and the understanding of FSGS pathogenesis.

P-429.00

PROTEOMIC IDENTIFICATION OF POTENTIAL LIVER BIOMARKERS IN AYU, PLECOGLOSSUS ALTIVELIS AS AN AGING ANIMAL MODEL

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Aging refers to a set of processes that lead over time to a gradual increase in vulnerability to be damaged and the probability of death. Fish is a promising model animal for aging research. It is well known that ayu (Plecoglossus altivelis), a common freshwater fish of East Asia, die after spawning and have a life span of only 1 year. The determinants for such a short life span are still ambiguous, but are probably connected with spawning. In the present study, we attempt to identify potential biomarkers in ayu as an aging animal model using proteomics approaches. Healthy ayu was collected from a fish farm and was grouped by gender and three growth stages: before, during, and after spawning.

After measuring the biometric parameters, liver protein samples were extracted and cleaned-up, then subjected to twP-dimensional electrophoresis (2-DE) analysis in triplicate experiments. Gel images were analyzed by Prodigy SameSpots software, and protein spots significantly altered by at least twP-fold among three spawning stages were selected for LC-ESI-Q-TOF MS/MS analysis.

Among nine proteins identified, betaine-homocysteine S-methyltransferase 1, triosephosphateisomerase, and glyceraldehyde-3-phosphate dehydrogenase were uP-regulated, while apolipoprotein AI, 94 kDa glucose-regulated protein, 78 kDa glucose-regulated protein, tumor necrosis factor receptor, aldehyde dehydrogenase, and phosphoethanolaminemethyltransferase were down-regulated.

We found that these proteins were associated with lipid transport and metabolism, carbohydrate metabolism, and protein folding-related diseases. Relationships of these liver proteins with the rapid death of ayu after spawning are now under investigation, and they may serve as potential markers for aging research.

P-430.00

UNDERSTANDING THE INTERACTION OF ORGANISMS WITH THEIR BIOLOGICAL ENVIRONMENT – INITIATIVE ON MULTI ORGANISM PROTEOMES (IMOP)

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Proteome studies in organisms like bacteria, invertebrates, or plants hold great potential to deliver essential knowledge transferable to human biology/biological processes. Moreover, due to the lower biological complexity of these organisms compared to mammals, and the possibility to grow and study them under defined conditions, such organisms are important tools for proteomics method development and validation.

Many species studied as model organisms are directly involved in homoeostatic and pathogenic processes in humans or serve as source of nutrition for humans. In this respect, the need for understanding the complex molecular interactions between microbiomes and its human host becomes increasingly important in biomedical research. Numerous model organisms are investigated extensively at the level of classical biology but also at the molecular level, such as the genome, transcriptome, metabolome and the proteome level. Despite the extensive knowledge collected within the different model organism communities, the transfer and cross-leverage of data between these communities and the translation from model organisms to humans remains challenging.

Several research groups working on different non human organisms and focusing on different aspects of interactive and comparative proteomics have established the HUPO initiative on multi organism proteomes (iMOP) (www.imop.uzh.ch) to facilitate knowledge and data exchange between the communities.

P-431.00

A VALIDATED METHOD FOR COW MILK DETECTION AND QUANTIFICATION IN BAKERY PRODUCTS BY LC-MS/MS.

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Introduction and objectives

Adverse reaction to cow milk affects many children and adults. The term "adverse reaction" include both cow milk allergy (CMA) and milk intolerance (Crittenden RG, 2005). Actually, for CMA sufferers avoidance of dietary milk proteins remains the only effective management strategy (Fiocchi A, 2010).

Currently milk allergen detection is based on immune assay method such as ELISA. However, positive ELISA results preferably require confirmatory analysis by non-immunological techniques, such as polymerase chain reaction (PCR) or mass spectrometry (MS) (Heick J et al. 2011).

In this work, a simple LC-MS/MS method was set up and validated for the detection of milk contamination in bakery products.

Methods

Incurred materials were prepared at eight levels of milk contamination. All LC-MS/MS analyses were performed on an HP 1100 HPLC (Agilent) coupled on line with a XCT-Plus Ion trap mass spectrometer (Agilent). Transitions related to two peptides from bovine α-s1 casein were monitored and used for limit of detection (LOD) and limit of quantification (LOQ) calculation. Validation of the whole analytical method was performed, following both Eurachem guidelines and EMEA guidelines in terms of linear dynamic range, LOD, LOQ, accuracy, precision and recovery. After method validation, the procedure was applied to some commercial bakery products in order to evaluate the presence of milk traces.

Results and discussion

Remarkable results were obtained for LOD and LOQ: 1.3 and 4.0 mg milk/Kg cookies, respectively. Sensitivity was around 1.0 mg milk/Kg cookies, while precision was calculated in term of intra-day (RSD in the 5-20% range) and inter-day repeatability (RSD never exceeding 12%).

Conclusion

The method validation, performed by a LC-ESI-MS/MS system, equipped with a widely diffuse ion trap analyser, allowed us to obtain an easy and reliable tool for identification and quantification of milk contamination in cookies.

P-432.00

PHENOTYPING CHICKEN MALE FERTILITY BY INTACT CELL MALDI-TOF MASS SPECTROMETRY

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Introduction and objectives: The chicken is a valuable animal model for reproductive biology, since ovulation/fertilization prediction is very precise, and the measurement of egg fertilization rate and embryonic development is highly standardized. Measuring reproductive ability trough the latter is more accurate than in vitro sperm quality tests, although its routine use is cumbersome and expensive. Here, we used a new tool based on intact cell MALDI-TOF mass spectrometry (ICMS-MS; recently proven useful for fast, high-throughput chicken fertility evaluation) to evaluate the proteomic differences between fertile and sub-fertile spermatozoa from 2 common chicken breeds for a better characterization of this species as a reproduction model.

Methods: Roosters of 2 genetically divergent breeds were classified depending on their reproductive quality based on in vitro and in vivo fertility tests. Fresh cells (three ejaculates per male) were mixed with sinapinic acid. Spectra were acquired (20 replicates) using a Bruker UltrafleXtreme MALDI-TOF-TOF instrument and analyzed with Progenesis MALDI v1.2. Targeted proteomics using toP-down MS approach was performed to identify significantly different m/z peaks (p

Results and Discussion: Fertile and sub-fertile males were successfully discriminated for each genetic line, thus confirming the suitability of ICM-MS approach to evaluate chicken fertility. ICM-MS analysis revealed characteristic profiles for each genetic line with specific molecular signatures, but also common markers for sub-fertiles animals. Thus, targeted proteomic allowed us to identify peptido/proteoforms (endogenous species) corresponding to protein degradation products which may contribute to impaired fertility.

Conclusions: Intact Cell MALDI-TOF Mass Spectrometry is an innovative "phenomic molecular tool" that may help discriminating avian males on their reproductive capacity. Furthermore, ICM-MS combined to a top down proteomic approach allowed characterization of several molecular species related to fertility.

P-433.00

CHANGES IN THE PROTEIN PROFILES OF PLASMA MEMBRANE PREPARATIONS FROM BETA VULGARIS ROOTS AS AFFECTED BY FE DEFICIENCY

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Iron deficiency is a worldwide problem that often results in decreases in crop yield and quality. Although Fe is ubiquitously present in soils, its bioavailability in high pH, calcareous sols is often compromised. To take up Fe from the soil, plants utilize a number of components, many of them localized in the root plasma membrane (PM).

When Fe in the medium is low, the plant root PM undergoes changes that facilitate Fe uptake. The aim of this work was to characterize changes induced by Fe deficiency in the protein profile of highly pure root PM preparations of Beta vulgaris using shotgun proteomics. PM preparations were obtained using aqueous two phase partitioning (polyethylene glycol and dextran). Six independent biological replicates per treatment (PM from roots of control and Fe deficient plants) were analyzed using nanoLC-MS/MS. Spectra were processed with Progenesis software and protein identification was carried out using MASCOT and a custom made Beta vulgaris database containing UniProtKB entries and the recently published sugar beet genome (http://bvseq.molgen.mpg.de/index.shtml).

A total of 278 proteins were identified in root PM samples, with 66 of them showing changes in abundance as a result of Fe deficiency (7 increasing and 59 decreasing when compared to the Fe-sufficient controls) when using ANOVA test (p1.5 as selection criteria. A preliminary analysis of these protein species reveals that significant changes in the root PM proteome occurs when plants are grown in Fe deficiency conditions. Decreases in abundance were found for proteins involved in transport of ions (ammonium, nitrate and K), aquaporins and signal transduction proteins.

Increases in abundance were found for proteins related with C metabolism, cell division and secondary metabolism. Changes in abundance were also found for proteins with unknown function.

P-434.00

PROTEOMIC STUDY OF BOVINE ENDOMETRIAL CELL STIMULATED WITH LPS

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INTRODUCTION One of the major cause of cattle infertility is represented by uterine infections. Uterine epithelial cells play e a key role in protection against bacterial pathogens infection forming a mucosal barrier that avoids their infiltration and promotes the movement of IgA and IgG from the submucosa to the lumen. The different response of uterine epithelial cells to a microbial challenge could be the cause of the differences in cattle fertility and/or susceptibility to uterine infections. Lipopolisaccharide (LPS) represents a choice methods to reproduce in vitro the infection with gram-bacteria and can be easily used to have reproducible experiments.

AIM Aim of this work was to identify differentially expressed proteins after challenge of endometrial cell with different concentration of LPS in order to evaluate the epigenetic effects of infection on this cellular type.

METHODS Uterine bovine epithelial cells have been challenged with different concentrations of LPS. Proteomic analysis (2D electrophoresis/Maldi TOF MS and shotshug MS analysis) was used in order to highlight differential protein expression putatively linked to epigenetic modulation.

RESULTS AND DISCUSSION Stimulation of cell growth following exposure to 8 µg/ml LPS was associated to significant differential protein expression of Interferon-induced dynamin-like GTPase, Protein disulfide-isomerase A3, and of transketolase.

CONCLUSION These preliminary results indicates that LPS-induced proliferation is associated with changes in protein profiles linked to resistance to pathogens, general metabolism, membrane activity and regulation of DNA methylation Work supported by 7FW KBBEPluridisciplinary study for a RObust and sustainabLe Improvement of Fertility In Cows-Prolific

P-435.00

META-OMICS ANALYSIS TO INVESTIGATE MICROBIAL CONSORTIUM IN CHEESE PRODUCTION

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Introduction- The application of integrated meta-omics in expecially in food, is essential to investigate specific microbial functions in complex microbial communities, to assess their effects in term of safety, in product transformation and to improve also the quality and the taste.Infact, bacteria and moulds are vital to the ripening and aroma of many cheeses.

Aim-To apply both metagenomic and metaproteomic techniques to a typical, Italian Cheese, Grana Padano DOP,s a regional speciality exported worlwide to investigate the characteristic microflora correlated to the geographic area and the ripening conditions.

Methods- Investigations were made on 12 months Grana Padano PDO cheese from different geographic area (TN/MN). Bacterial DNA and proteins were extracted and subjected respectively to NGS using illumina HISEQ 2000 and metaproteomics by 2D-nLC MS/MS. Metagenomic data were analyzed using MetaPhlAn and the relative abundances of the reads at various taxonomic levels, were graphically plotted using two different visualization tool (GraPhlAn and Krona). Metaproteomic analysis on high quality MS/MS data (FDR

Results and Discussion: A deep taxonomic view associated to normal and blowed cheese was obtained using a comprensive meta-omic approach

Conclusions The resulting meta–omics catalogue is useful to better undestand genomic and proteomic features that are able to identify the production zone of and to give lights in this important functional community within the cheese microbiota.

P-436.00

PROTEOMIC APPROACH TO INVESTIGATE THE ESCHERICHIA COLI MEMBRANE
PROTEOME IN SEVERE ENVELOPE STRESS CONDITIONS.

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Introduction and Objectives

Lipopolysaccharide (LPS) is an essential constituent of the outer membrane (OM) of Gram-negative bacteria. The mechanism to export LPS to the cell surface has been well characterized in Escherichia coli and it involves seven essential proteins LptABCDEFG [1]. Depletion of any Lpt component causes a severe OM biogenesis defect, such as the accumulation of de novo synthesized LPS in a novel membrane fraction (hIM) [2]. Here, we aimed to understand, at proteomic level, pathways and strategies adopted by E.coli for responding to OM stress caused by the block of LPS transport upon LptC depletion

Methods

Inner (IM), Outer (OM) and novel (hIM) membrane were separated and their protein profiles were characterized by means of MudPIT (Multidimensional Protein Identification Technology) approach [3].
The evaluation of differential expressed proteins among the analyzed conditions was obtained through a label-free quantitative approach [4].

Results and Discussion

By MudPIT, 1300 non-redundant proteins were identified. For OM and hIM samples about 5000 redundant peptides were identified, while 3000 resulted for IM. Above 71% of the proteins with known subcellular localization were membrane proteins, underling the aptitude of MudPIT to identify proteins without restrictions of pI, MW and hydrophobicity. Of note, the identified proteins belonged to pathways controlled by signalling systems whose activation is related to an alteration at envelope level.

Conclusions

In addition, the label-free quantitative approach, applied to characterize highly hydrophobic proteins (like
membrane ones), led to the identification of proteins that contribute to repair the OM and restore its properties; in particular, these proteins are involved in maintaining OM asymmetry and in the synthesis of phospholipids and exopolysaccharides. Finally, this work represents the first proteomic evaluation of
hIM fraction, that appears to be enriched in proteins normally localized in IM and related to transport systems. The main results will be presented.

P-437.00

PROTEOMIC INSIGHTS INTO SULFUR METABOLISM IN THE HYDROGEN-PRODUCING HYPERTHERMOPHILIC ARCHAEON THERMOCOCCUS ONNURINEUS NA1

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Thermococcus onnurineus NA1, a hydrogen-producing hyperthermophilic archaeon, is capable of utilizing elemental sulfur (S0) as a terminal electron acceptor for heterotrophic growth and reduce it to H2S. To gain insight into the sulfur metabolism, the proteome of T. onnurineus NA1 cells sampled under sulfur culture condition has been quantified and compared with those under formate, CO and starch culture conditions. Using a label-free nanP-UPLC-MSE-based comparative proteomic analysis, we found that approximately 38.4 % of the total identified proteome (589 proteins) were significantly uP-regulated (¡Ã1.5 fold) under sulfur culture condition.

Many of these proteins are functionally associated with sulfur reduction, Fe-S cluster biogenesis, ATP synthesis, protein glycosylation, CO2 fixation, and amino acid metabolism. In contrast, SurR, Mbh and other key enzymes for H2-production and-recycling were generally down-regulated in sulfur-grown cells, consistent with a role of SurR as a redox switch in response to S0. In addition, enzymes involved in oxygen detoxification such as SOR, FdpA, NROR and rubrerythrin were also strongly down-regulated in sulfur-grown cells.

Our data suggest that oxygen resistance-linked H2 production and sulfur metabolism are oppositely controlled through regulatory actions by oxidized SurR in the presence sulfur. Furthermore, enzymes of the sulfur assimilation and cysteine biosynthetic pathway were also differentially down-regulated in response to sulfur. Our results revealed that many previously uncharacterized proteins play a key role in sulfur metabolism and in other metabolic pathways, and provide further insights into the metabolic strategies adapted by hyperthermophilic archaea under sulfur-rich environment.

This study will contribute to a better understanding of the mechanisms and physiology of T. onnurineus NA1 cells in response to sulfur, in addition to opening novel avenues to identify suitable targets for genetic manipulation that would lead to development of more effective and sustainable H2 production.

P-438.00

A PROTEOMIC APPROACH UNRAVELS GENOTYPE DIFFERENCES IN GRAPEVINE RESISTANCE TO DOWNY MILDEW

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Introduction and objectives

Grapevine downy mildew is an important disease affecting crop production and causing severe losses. To identify genotype-dependent responses towards this pathogen and to explore the molecular mechanisms involved in grapevine-P. viticola resistance, we conducted proteomic analysis of leaf samples directly comparing resistant (Regent) and susceptible (Trincadeira) grapevine genotypes prior (0h) and post-inoculation (6, 12 and 24h) with the pathogen. Both grapevine genotypes were in the same developmental stage and suffered the same environmental changes, so by directly comparing one genotype against the other the obtained protein modulation illustrates the genotype-specific differences in protein modulation.

Methods

Total protein extracts were separated and differentially expressed protein spots were detected by 2D-DIGE. Protein identification has been performed by MALDI-TOF/TOF MS.

Results and Discussion

Ninety-five grapevine proteins differentially expressed were successfully identified across all comparisons. As expected, at 0h, grapevine genotypes were constitutively differentiated by 24 proteins spots, being the majority related to photosynthesis and carbohydrate metabolism. Upon inoculation with the pathogen, the resistant genotype presented upregulated proteins mainly included in the functional categories of photosynthesis, carbohydrate metabolism and defence, indicating that the resistant variety is more efficient in transforming light into chemical energy, in CO_2 assimilation and obtaining intermediate metabolites from photoassimilates needed for biosynthetic pathways and subsequent defence responses. Surprisingly, most down regulated proteins (ie more expressed in the susceptible genotype) were related to redox homeostasis and defense responses suggesting that susceptible plants appear to mount an attempted resistance reaction, which is neither fast nor robust enough to prevent the pathogen from spreading.

Conclusions

Our data reveal the genotype-specific proteins that may account for the successful resistance of Regent plants and provide insights into the underlying molecular processes, which may eventually yield novel strategies for pathogen control.

P-439.00

PROTEOMICS TO UNRAVEL MECHANISMS OF NANOPARTICLE TOXICITY

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Manufactured nanoparticles
 Here we explore potential phytotoxicity of functional silica nanoparticles and its mechanisms using Arabidopsis cells. In order to characterize the growth inhibition capacity of CMB clusters and/or SiO_2 nanoparticles compared to $CMB@SiO_2$ nanoparticles, concentration–response curves have been constructed at different exposure times. We finally used proteomics (2-DE and MS/MS analysis) as an approach to study changes occurring in plant cell metabolism after exposure to different nanoparticles.

Cell growth, viability, pigment content and photosynthetic yield was affected after exposure to CMB clusters, but so far SiO_2 or $CMB@SiO_2$ nanoparticles did not show any toxicological potential on Arabidopsis cells. After these preliminary tests, we deeply analyzed molecular mechanisms of plant cell response to clusters and nanoparticles using proteomics. In the light of these results, we will propose and discuss different hypothesis for explaining cluster and nanoparticle impacts.

As a matter of fact, the silica encapsulation of the clusters protects the plant cells by avoiding direct contact of harmful clusters with cellular structures.

P-440.00
PROTEOMICS OF THE AGING LIVER MITOCHONDRIA

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Mitochondria play a dominant role in energy metabolism. Mitochondrial dysfunction has been linked to chronic metabolic disorders such as type 2 diabetes, neurodegenerative diseases. Accumulation of mitochondrial damage has been associated with the aging process and is therefore of significant interest to clinical researchers.

To extensively mine the liver mitochondrial proteome, mitochondria were isolated; proteins were extracted and digested with proteolytic enzymes; peptides were labeled with Tandem Mass Tags (TMTs); and - prior to nanoLC-MS/MS - phosphP- and acetyl-modified peptides were enriched. The goal was to study changes to the mitochondrial proteome, acetylome and phosphoproteome in relation to aging using a rodent animal model.

Liver mitochondria were isolated. Proteins were extracted, reduced, alkylated and digested with trypsin in combination with Lys-C. Samples were labeled with TMT 10-plex and analyzed on a Orbitrap Fusion¢â Tribrid¢â mass spectrometer using DDA synchronous precursor selection (SPS) MS3. In addition, Electron Transfer Dissociation (ETD) was used to localize phosphorylated amino acids. Data were processed with Proteome Discoverer for identification, quantification and verification of modified sites. We have already demonstrate that the TMT-MS3 approach (a) greatly reduced interferences resulting from cP-isolation of precursor-ions, thus improving quantitative accuracy; and (b) showed excellent correlation with label-free quantitation, with the advantage of higher throughput via multiplexing. In the present study, the analysis of the liver mitochondrial proteome led to the identification of > 2600 protein groups, of which > 2200 could be quantified with TMT. Of those, 817 mitochondrial protein groups were annotated and 631 were quantified using ¡Ã 2 unique peptides/protein. Significant protein changes were evidenced between rats of 3 age groups. The PTM-ome analysis is expected to deliver further insights into aging mechanisms.

Application of TMT MS3 enables comprehensive and accurate quantitative analysis of the mitochondrial proteome, acetylome and phosphoproteome, expected to reveal adaptations to biological aging.

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CHARACTERIZATION OF PROTEINS AND PUTATIVE FUNCTIONAL PATHWAYS IN ROTIFER ENCYSTED DORMANT EMBRYOS (RESTING EGGS)

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Introduction and objectives

Several organisms display dormancy and developmental arrest at embryonic stages and long-term survival in dormant forms is usually associated with desiccation. However, numerous aquatic invertebrates displaying dormancy during embryonic stages, survive for tens or hundreds of years in a hydrated form, raising the question whether survival of non-desiccated forms is associated with proteins or pathways similar to those of desiccated tolerant forms. Rotifers are minute invertebrate metazoans that enter long-term dormancy in a hydrated or desiccated state. In search for functional pathways associated with dormancy, we have compared the resting eggs and the amictic eggs of the rotifer (Brachionus plicatilis) as a model.

Methods

Protein extract of resting eggs (encysted dormant embryos; RE), amictic eggs (with non-dormant embryos; AM) and resting eggs ready for hatching (E0 eggs), were trypsinized. The resulting tryptic peptides were analyzed by LC-MS/MS and quantified by label free analysis. The mass spectrometry data from three biological repeats was analyzed and quantified using the MaxQuant software.

Results and Discussion

We identified 2,928 protein groups and 2,482 were annotated to known protein sequences. Comparison between proteins in AM vs. RE revealed 1,263 differentially expressed proteins), including 556 proteins specific to egg-type or with higher than ten-fold change. Some proteins were unannotated, suggesting their specificity to rotifers. Surprisingly, similar putative functional pathways were identified in all egg types, but RE or E0 eggs displayed a significantly lower number of proteins than AM eggs or displayed different proteins within these pathways. Among the most abundant proteins in RE or E0 were proteins (e.g. LEA family proteins; ferritin) with high abundant mRNAs. The putative role of these mRNAs during dormancy is intriguing, as the mRNAs and the corresponding proteins degrade upon hatching. A comparison with proteomes of other organisms displaying dormancy will be presented and discussed.

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CHARACTERIZATION OF PLANT GLYCATED PROTEOME AND ITS CHANGES DURING AGEING AND UNDER ENVIRONMENTAL STRESS CONDITIONS

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Protein glycation is formed by reaction of reducing sugars with amino groups and commonly accompanies thermal food processing. Further oxidation of resulting Amadori compounds (glycoxidation) yield advanced glycation end-products (AGEs) known for their prP-inflammatory effects in humans. However, glycation may occur also before thermal treatment, i.e. during the life time of crop plants. In this context, it is important to know, if glycoxidation is increased in crop plants affected by ageing and environmental stresses.

The models of high light and metal stress were established with Arabidopsis thaliana and Brassica napus, respectively. The leaves and roots were harvested before stress application and in multiple points throughout the stress period. Soluble proteins were isolated, digested with trypsin and the digests were analyzed by LC x LC-LIT-OrbitraP-MS/MS, using boronic acid affinity chromatography or HILIC as the first dimension and RP-nanoUPLC as the second one. Modified peptides identified by database search peptides were quantified by label-free nanoUPLC-ESI-OrbitraP-MS approach.

Both control and stressed A. thaliana and B. napus plants displayed rich patterns of glycated and glycoxidated proteins, representing mostly regulatory pathways, protein and nucleic acid metabolism. For plants, this information, as well as exact modification sites, is reported here, to the best of our knowledge, for the first time. In B. napus the product pattern dominated with triose- and tetrose-derived early glycation products and Nε-carboxymethyllysine (CML), methylglyoxal-derived hydroimidazolone (MGH), argpyrimidine. However, glyoxal-derived hydroimidazolone (Glarg)-modified peptides were more abundant in A. thaliana plants. Glycated products related to multiple intracellular proteins clearly accumulated during the plant ontogenesis. However, though levels of glycation were increased under stress conditions in comparison to controls, only certain AGE types showed higher abundance in those plants.

Plant protein glycation and glycoxidation patterns are characterized for the first time. These patterns undergo qualitative and quantitative changes during plant ontogenesis and under stress conditions.

P-443.00

LEVELS OF CHEMOSENSORY AND ODORANT-BINDING PROTEINS FROM CATERPILLAR SALIVA ARE AFFECTED BY HOST PLANT AND IMMUNE CHALLENGE

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Objectives and method
Molecules in the insect saliva have been found to be either inhibitors or activators of plant defense mechanisms against herbivory. Recently, we have found that more than half of the soluble protein fraction in mandibular glands of caterpillars was composed of a single putative chemosensory protein. We conducted feeding treatments to assess the effect on the levels of chemosensory proteins in the mandibular and labial glands in Vanessa cardui caterpillars induced by the host plant, a diet-swtch and bacteria-containing diet. After two days of feeding, the labial and mandibular glands of the larvae were dissected and extracted proteins analyzed by label free quantitative proteomics.

Results and conclusion
More than a thousand predicted proteins in hemolymph and salivary glands were identified across feeding treatments. The detection of an inducible odorant-binding protein suggests its role in transporting compounds from the diet. Host and pathogen perception may be mediated by chemosensory and odorant-binding proteins produced in the mandibular glands.

Highest levels of chemosensory protein were found in the mandibular glands and the abundance of a particular isoform was affected by host plant and immune challenge through bacteria containing food. Other proteins including immune-related, odorant-binding and glucan-recognition proteins were also found to be most abundant in mandibular glands. Since salivary factors in the mandibular glands appeared to be affected by bacterial cell walls and/or live bacteria in the diet, we consider this strong evidence suggesting that the mandibular glands have an important role in insect immunity. The study of chemosensory proteins in the mandibular glands should be directed to investigate its role in pathogen recognition.

We speculate that these findings will also contribute to the human saliva research not only on a biochemical but also biological level as similarities in mammalian and insect host defense have been reported earlier

P-444.00

FRACTIONATION OF TOTAL, SOLUBLE AND INSOLUBLE PROTEINS IN GLOEOBACTER VIOLACEUS PCC7421

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Unicellular Gloeobacter violaceus PCC7421 is a Gram-negative photoautotrophic cyanobacterium. From the phylogenetic study Gloeobacter violaceus PCC7421 was devided at very early point of the phylogenetic tree and contained very primitive and unusual features like lack of thylakoidal membrane and missing of some photosystem components. Genome sequence was completed in 2003 (Cyanobase by Kazusa Institute).

But research area was very limited due to the difficulties in cell culture and some of unique characteristics. In this study for the proteomic analysis we prepared the total, soluble and insoluble fractions and performed twP-dimensional electrophoresis (2-DE), FT-LTQ and LTQ analysis. From the 2-DE analysis 105, 71 and 20 proteins were identified from the each fraction. In detail insoluble fraction shows 3 of typical outer membrane protein, porin proteins, Gll0415, Glr0638 and Glr3954. In addition to 2-DE analysis insoluble fractions were digested using chemical and in-solution tryptic method to obtain the more peptides with trans-membrane region. From the FT-LTQ and LTQ analysis 785 proteins were identified. Among them 139 proteins with 1 or more (~14) transmembrane domains were analyzed using TMHMM program.

Considering the number of theoretical proteins (965) with 1~ TM about 14.4% of that proteins were found. Through these proteomic approaches we could determine the protein preparation methods and provide the 2-DE map of Gloeobacter violaceus PCC 7421.These result will be helpful to obtain the metabolic information of Gloeobacter violaceus PCC 7421.

P-445.00
UNDERSTANDING PHYTOCHELATIN PROFILE IN NICOTIANA TABACUM UNDER CD, CU AND AS STRESS: A STEP TOWARDS PHYTOREMEDIATION-APPLICATIONS

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Introduction and objectives

Plants obtain micronutrients, such as Cu and Zn, from aquatic and terrestrial environments that may also provide toxic metals, like Cd. These metals can be of both natural and anthropogenic origin. Plants have mechanisms that maintain physiological concentrations of essential metals, but developed defense strategies against the toxicity of other metals, keeping them below their toxicity thresholds. The objective of this work was to identify and quantify phytochelatins (PCs) involved in Nicotiana tabacum defense mechanisms against toxic levels of trace elements. Phytochelatins can sequester free metal ions from the cytoplasm into the central vacuole, where these PC–metal complexes are safely stored.

Methods

A wild type and two genetically engineered (B1F and B5H) lines were subjected to 50 mM of Cd, Cu and As. Lines B1F and B5H express higher levels of trehalose-6-P synthase than wild type plants. The plant extracts enriched in PCs were analyzed by LC-MS/MS. Levels of several PCs were compared between controls and plants subjected to metal stress for each tobacco line.

Results and Discussion

PC2 was detected in most plant lines subjected and not subjected to metal stress. PC3 was detected in all plants subjected to cadmium, all conditions for B1F line and in B5H subjected to As. Finally, PC4 was only detected in B1F subjected to Cd/As stress and B5H subjected to Cd stress. An increment of PCs levels was observed when plant lines were subjected to metal stress, especially for Cd and As. Moreover, transgenic lines seem to be more resistant to toxic levels of trace elements in comparison with Wt.

Conclusions

These results are important to understand heavy metal detoxification mechanisms in higher plants, a step towards phytoremediation-applications.

P-446.00

THE PROTEOME OF OLIVE AND THE INVISIBLE PROTEOME OF OLIVE OILS

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Olive oil consumption has been associated with the decrease of the incidence of important illnesses such as cardiovascular diseases and cancer. Despite their highly informative value and role in food stability and allergenicity, olive oil proteins have been scarcely investigated. This fact is probably due to the difficulty of working with a lipid matrix and the dramatically low abundance of the proteins potentially present. Since olive oil is manufactured by using the whole fruit, and taking into account the fact that the protein content of the seed is about 20%, the study of the proteome of the seed can be the first step in the search of olive oil proteins.

Conventional extraction methodologies have been applied in the extraction of both olive oil and olive seed proteins. Also the capture with CPLLs has been employed. Olive oil and olive seed and pulp proteins were separated by SDS-PAGE, digested, and analyzed by nanoLC-MS/MS.

This is the first time a deep study of the proteome is performed in the olive seed, as a first step in the study of the olive oil proteome [1]. A tentative identification of olive oil proteins has been possible. It is clear, though, that proteins in olive oil are only present in ultratraces and that finding additional species not yet identified in oils might be an impossible task [2]

P-447.00
PROTEOMIC FINGERPRINTING OF ITALIAN APERITIFS

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To protect consumers from any food fraud, it's important to use an efficient assay able to certify the presence of proteins and peptides belonging to natural ingredients declared by producer. Conversely their absence would suggest their preparation via addition of synthetic chemicals and flavours, thus not from natural materials.

To asses the genuiness of some Italian aperitifs [1-2], a "proteomic fingerprinting" was performed via combinatorial peptide ligand libraries (CPLLs) both in commercial drinks and in home-made alcoholic infusion. The captured proteomes were separated by SDS-PAGE, digested and identified by nanoLC-MS/MS analysis, using a LTQ-XL mass spectrometer. The genuineness was tested by comparison of identified proteins both in home made pr and in commercial ones.

The natural origin of commercial aperitifs was sperimentaly tested by a technology useful to protect consumers from adulterated products.

P-448.00

PROTEOMIC CHANGES OF CERVICAL ADENOCARCINOMA CELLS (HELA) INDUCED BY THE TREATMENT WITH A SNAKE VENOM DERIVED PHOSPHOLIPASE A2 (BOTHROPS MARMORATUS)

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Introduction and objectives: The emergence of resistance by cancer cells to chemotherapeutic drugs available makes the development of new agents of great importance and urgency. Toxins isolated from animal venoms, including snake venoms has been the subject of numerous studies due to their potential biotechnological and medical applications. We have demonstrated with a phospholipase A2 recently isolated from Bothrops marmoratus venom (BmPLA2) that it is capable to induce the apoptotic death of diverse cell lines such as cervical cancer (HeLa), skin cancer (B16F10). Furthermore, its activity was enhance in tumor cell lines compared with normal cell lines. In this study we classify the type of apoptosis BmPLA2 causes in the tumor lines and further explore the proteomic changes associated with apoptotic cell death caused by BmPLA2.

Methods: To assess the type of apoptosis BmPLA2 causes on tumor cell lines we use the following methods: To determine the intrinsic (mitochondrial) and extrinsic (death receptor) cell death pathways we performed western blot and flow cytometry analysis, using markers to mitochondrial outer membrane permeabilization and initiator caspase-9 and caspase- 8 activation. For proteomic analysis of cells undergoing apoptosis by BmPLA2 cell lysis, protein extraction and digestion were carried using standard methods in our laboratory followed by MUDPIT and LC/MS/MS.

Results and Discussion: From these studies, it appears BmPLA1 causes cell death via the extrinsic apoptotic pathway. Proteomic analyses of cells undergoing apoptosis supported the results indicating the extrinsic apoptotic pathway as well as suggested additional interesting markers for apoptosis.

Conclusions: The snake venom phospholipase BmPLA2 causes tumor cell death via the extrinsic apoptotic pathway. The proteomic analysis of tumor and normal cells treated with BmPLA2 suggests potential mechanisms for enhanced tumor cell sensitivity to the phospholipase.

P-449.00

EXTENDED-SPECTRUM ??-LACTAMASE (ESBL) PRODUCING GRAM (-) ENTEROBACTERIACEAE STRAINS IN FOODS FROM TURKEY

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Extended-spectrum ??-lactamase (ESBL) producing Enterobacteriaceae bacteria cause serious infections in urinary tract and soft tissues. The roles of foods on the human exposure to these pathogen strains have still remained unclear in Turkey. The objective of this study was to determine the occurence of ESBL-producing Gram (-) Enterobacteriaceae strains in the foods from Turkey.

In this study, 30 specimens of each of raw milk, cheese, chicken, and vegetable, a total of 120 samples, were collected from Istanbul. The samples were homogenized in LABM Enterobacteriaceae Enrichment Broth for 2 min, and incubated at 35-37 0C for 18-24 hr. For selective enrichment, Chromoagar ESBL media was used to inoculate the suspensions for performing incubation at 35-37 0C for 18-24 hr. The confirmation of Gram (-) colonies was done by using Merck Microbiology Bactident Oxidase Strips. The Gram (-) confirmed colonies were inoculated on LABM Tryptic Soy Agar, followed by an incubation at 35-37 ℃ for 18-48 hr again. After incubation, the strains of ESBL producers were identified by bioMérieux Vitek Maldi tof MS. The identified strains were subjected to agar disk diffusion test as recommended by CLSI (2009).

In this test, a combination of the discs of ceftazidime, cefotaxime, and cefpodoxime with/without clavulanic acid of MAST D67C was used. The disc inserted plates were incubated at 35-37 0C for 18-24 hr, and the zone diameters were measured. Finally, antimicrobial susceptibility testing was performed by using Merlin Micronaut-S beta-lactamase VII kit and Sifin Software integrated with a Termoscientific Multiskan FC Spectrometer.

The results showed that ESBL producing Gram (-) Enterobacteriaceae strains were found as 20 % in raw milks, 20 % in cheeses, 33.3 % in chickens, and 56.6 % in vegetables, respectively. In conclusion, ESBL producing Gram negative Enterobacteriaceae bacteria are present in foods from Istanbul, Turkey as a serious public health concern in Turkey.

Topic **9**

Immunobiology and cell signaling

OP054 - QUANTITATIVE PROTEOMICS TO STUDY PROTEIN SECRETION FROM IMMUNE CELLS

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Introduction and objectives
Secreted proteins function as key intercellular messengers in multicellular organisms. Such proteins include cytokines, interleukins, growth factors, hormones, and others that propagate biological information in the body to orchestrate immune responses and tissue homeostasis. The investigations of protein secretion have primarily relied on antibody-based methods, whose large-scale use is limited by availability, specificity, and affordability. Strategies for a comprehensive, and quantitative analysis would therefore be highly desirable, especially for a discovery-driven characterization of secreted proteins.

Methods
We developed a sensitive, mass spectrometry (MS)–based method to detect secreted proteins in complex protein mixtures with sufficient speed to investigate a large number of conditions. By combining ultra-high pressure liquid chromatography, high-resolution MS in single shot runs, computational proteomics and statistical methods, our workflow enables the discovery and quantification of secreted proteins in diverse biological settings.

Results and Discussion
We applied this proteomics workflow to identify proteins released in the context of immune responses. Achieving low picogram sensitivity, we detect secreted protein differences of more than 10,000 fold. Among the significantly released proteins, we quantified more than 50 annotated cytokines as well as proteins lacking annotated extracellular functions. Our method describes an unbiased and hypothesis-free proteomics approach that is optimally suited for a systems-wide characterization of regulated secretory programs and to complement targeted antibody-based technologies.

OP055 - A SYSTEMATIC PROTEOMICS-BASED APPROACH TO GENERATING IMMUNOTHERAPEUTICS BASED ON HIGH AFFINITY SOLUBLE T CELL RECEPTORS

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Peptides derived from cancer-associated proteins and presented to the immune system on HLA complexes represent promising targets for cancer immunotherapy. We have developed a systematic workflow to identify HLA-presented peptides from cancer-associated proteins, generate T-cell clones with reactivity toward these peptide targets and engineer T-cell receptors (TCRs) from these clones into potent soluble immunotherapeutics.

Targets are selected on the basis of differential expression in cancerous tissues by combining data from multiple sources, including;
• proteomic identification of HLA peptides presented by cancer cell lines and tissues
• proteomic evaluation of relative expression levels in cancerous and normal tissues
• expression frequency in cancer indications and normal tissues evaluated by qRT-PCR of cancer tissues and in-situ RNA hybridisation using high-density cancer arrays

Fully validated peptide targets are fed into our T-cell cloning facility to generate peptide-HLA specific T-cells. TCRs isolated from these cells are then engineered into soluble molecules (mTCRs) through the incorporation of a stabilising disulphide bond. As TCRs isolated from clones typically have a low affinity for target peptide:HLA, phage display technology is used to generate mTCRs with high affinity toward their specific peptide:HLA complex, whilst retaining specificity.

Our immune-activating therapeutics (ImmTACs) are created by coupling high-affinity mTCRs with an anti-CD3 scFv domain. Anti-CD3 mediated T cell redirection is a clinically-validated, highly potent therapeutic mechanism of action. ImmTACs are able to activate polyclonal T cell responses targeted toward cells presenting the appropriate cancer-associated antigen. Our current lead candidate, IMCgp100, is an ImmTAC targeted toward gp100280-288. IMCgp100 is currently undergoing clinical testing in a Phase IIa dose-expansion trial.

OP056 - IN-DEPTH ANALYSIS OF HLA-I PEPTIDOMES REVEALS THAT PROTEIN ABUNDANCE AND TURNOVER STRONGLY AFFECTS PRESENTATION – IMPLICATIONS FOR IMMUNOTHERAPY OF MELANOMA

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Introduction and objectives: T-cell responses against infected and cancer cells are initiated by recognition of HLA-I peptides (the peptidome) presented on the surface of nucleated cells. The repertoire of HLA-I peptides originates primarily from sampling the cytosolic degradation products of intracellular proteins. HLA-I peptides have been extensively studied in the last years because they can potentially be used directly as immunotherapy based cancer vaccines. Even more advanced cell based therapeutic applications are being developed based on cancer specific HLA-I peptides.

Methods: In this study, we used high resolution mass spectrometry and the MaxQuant bioinformatics environment to obtain a high accuracy and in-depth coverage of HLA peptidomes. We immuno-affinity purified HLA-I complexes and extracted the peptidome from cancer and primary cell lines, and from melanoma tissues.

Results and discussion: The identification of an unprecedented number of peptides enabled us to shed new light on the mechanisms governing peptidome presentation and to determine which part of the proteome is sampled for presentation. We find that globally, protein abundance correlates strongly with the degree of HLA-I sampling. When computing HLA sampling density for proteins according to their expression levels, we discovered that proteins with a sampling density five-fold or higher had high turnover-rates pointing towards efficient degradation that enable the subsequent production of multiple epitopes. We applied our approach on melanoma tumors, and identified known cancer epitopes, and most importantly many novel cancer epitopes from melanoma associated antigens.

Conclusions: Better immunotherapeutic modules could possibly be developed based on wider and more accurate repertoires of HLA-I peptides, which in turn should increase the accessibility of these therapies for a larger cohort of patients.

OP057 - OMICS ANALYSES PROVIDE EVIDENCE FOR PERITONEAL IMMUNE-MODULATION BY AN ALANYL-GLUTAMINE CONTAINING PERITONEAL DIALYSIS SOLUTION

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Glucose based peritoneal dialysis fluids (PDF) contribute to sterile inflammation, oxidative stress, and impair host defence, resulting in clinical complications such as deterioration of PD-membrane and peritoneal infections. During PD, low peritoneal glutamine levels may contribute to these pathomechanisms.

Combined proteomics, transcriptomics and metabolomics analysis was carried out, using clinical material (blood and peritoneal effluent) from an open-label, randomized, two-period, cross-over I/II trial (Eudract-2010-022804-29) was conducted at the Medical University of Vienna. 20 stable patients on PD underwent a single 4h PET either with standard PDF (Dianeal PD4, Baxter) or standard PDF with added 8mM alanyl-glutamine (AlaGln, PD-protec™). Collected sample material was tested for protein, metabolite and transcript markers of sterile inflammation, oxidative stress and reduced immunocompetence using 2D difference gel electrophoresis, a high resolution/accurate mass metabolomics approach and whole genome expression microarrays.

Transcriptomics and proteomics analysis indicated effects of glutamine supplementation related to stress response and identified a sub-group of patients, who have previously suffered from peritonitis. PD-protec™ demonstrated efficacy in this sub-group, with attenuated peritoneal levels of advanced oxidized protein products and significantly reduced interleukin 8 (IL-8 (modification of AlaGln treatment effect by peritonitis (p=0.012) with a significant decrease of 2.2 pg/ml (CI 0.1-4.3)). Untargeted metabolomics analysis identified players of the amino acids metabolism as significantly enriched in the PD effluent. Ex-vivo exposure of PBMC to PD effluent from the PD-protec™ group significantly improved LPS-induced cytokine release, associated with significantly increased intracellular glutamine levels. Safety of PD-protec™ was demonstrated by stable clinical data and absence of any drug-related adverse event.

Combined omics analysis demonstrated the safety of PD-protec™ as well as enrichment of molecular players related to cytoprotective and immunomodulatory processes and pathways countering the PD related and uremic pathomechanism of sterile inflammation, at least in the highly relevant risk population post-peritonitis.

P-450.00

MASS SPECTROMETRY ANALYSIS OF RAT ALVEOLAR MACROPHAGES NR8383 SECRETOMES: EFFECT OF PROPROTEIN CONVERTASE 1/3 (PC1/3) DOWN-REGULATION.

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The proprotein convertase 1/3 (PC1/3) is known for its role in the activation of precursor proteins within the regulated secretory pathway in the nervous system and recently for its possible implication in innate immunity 1, 2. PC1/3 knock-out mice express a dysfunctional phenotype characterized by uncontrolled cytokine secretion without stimulation 3. In a model macrophage cell line, NR8383 cells expressing PC1/3, similar results were observed 4. Secretome analyses were for a long time based on techniques employing antibodies necessitating to know what to track. In contrast, proteomic analyses offer to get a complete pattern of proteins implicated in a biological process. However, mass spectrometry based methods to detect low abundance proteins in complex secreted mixtures still remain a challenge.

Our study aims to analyse the secretomes of NR8383 knock-out for PC1/3 and NR8383 wild-type by a proteomic study in order to obtain a global vision of secreted proteins overtime with a particular attention to chemokines and cytokines considered as members of the deep proteome. A shotgun approach has been undertaken and proteins in supernatants of macrophages were digested with lysC-trypsin and mixtures were directly analyzed by nano LC-MS/MS. Results have been validated with ELISA technique.

Chemokines and some cytokines have been selectively identified in course of analyses reflecting secretory pathways dependent or independent of PC1/3. An uncontrolled cytokines secretion was highlighting in NR8383 down-regulated for PC1/3 compared with NR8383 wild-type. A peculiar attention of the function of these secreted molecules has been reached through chemotaxis assays on Th1 cells and NK cells.

P-451.00

COMPARISON OF IMMEDIATE RENAL DYSFUNCTION IN SPLIT AND PARTIAL LIVER TRANSPLANTATION VERSUS FULL SIZE LIVER TRANSPLANTATION IN SHIRAZ TRANSPLANT CENTRE

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Introduction: Renal dysfunction (RD) is a common complication following liver transplantation; it predisposes to further complications and high mortality rates. So far, postoperative renal function after split liver transplant (SLT) and (partial living related liver transplant) (LRLT) has not been well studied yet.

Patients and methods: Renal function immediately after surgery was analyzed retrospectively in 32 patients that received SLT and LRLT. Serum creatinine (SCr) was measured before surgery, and, after transplantation daily during the first week and at 14, 21, and 28 days after transplantation. Patient's medical records were reviewed to find clinical data such as Model for end-stage liver disease (MELD) score, Child-Turcotte-Pugh score (CTP) class, the length of surgery, length of anhepatic phase, hospital and ICU admission, incidence of acute rejection, renal dysfunction, and sepsis. These data compared between groups.

Results: Length of surgery and anhepatic phase was longer in SLT and LRLT group (P
Discussion: Although the number of patients studied was small, our data suggests a higher incidence of RD in patients receiving SLT and LRLT.

P-452.00

INSIGHTS INTO SIGNALLING PATHWAYS INVOLVED IN ALLERGIC IMMUNE RESPONSES THROUGH DIFFERENTIAL PROTEOME AND SECRETOME ANALYSIS OF DENDRITIC CELLS.

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Introduction and objectives

Dendritic cells (DCs) are key players of the adaptive immune response and play a fundamental role in allergen-presentation and subsequent T cell polarization. Shotgun proteomics of DCs using high-performance liquid chromatography (HPLC) and mass spectrometry (MS) enables the identification of important differentially expressed proteins, which are involved in the attraction and stimulation of other immune cells and thus give us insight into the signalling pathways of immune regulation.

Methods

For all experiments monocyte-derived DCs (moDCs) were prepared from human blood, which were seeded in serum-free DC medium and stimulated afterwards by birch pollen extract in order to cause an immune response. The collected cell lysates and supernatants were labelled using 6-plex tandem mass tag reagent (TMT), not only to identify but also to differentially quantify unique peptides. To get insight into the protein content of intracellular and secreted proteins, the cell lysates and the supernatants were analysed separately. Tryptic peptide separation was performed by micro ion-pair reversed-phase HPLC hyphenated to quadrupole-Orbitrap mass spectrometry.

Results and Discussion

Our results reveal significant differences in the proteomes and secretomes of uninduced versus stimulated moDCs. Approximately 2330 protein groups were identified in the cell lysates and about 100 protein groups in the supernatants (Protein Discoverer), wherein a multitude of identified proteins show significant differential regulation. Biochemical pathway analysis revealed that they are involved in immunologically relevant signalling pathways.

Conclusions

Our investigation shows that we are able to identify differentially expressed proteins, which are responsible for triggering an immune response. In order to get better insights into immune-relevant signalling pathways involved in T cell polarization and thus differences in triggering non-allergic and allergic immune responses, the treatment of DCs with different immune-stimulating agents is still crucial.

P-453.00

A LABEL FREE APPROACH TO CHARACTERIZE THE INTERACTION OF HUMAN NATURAL KILLER CELLS WITH POLARIZED MACROPHAGES.

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Introduction and objectives

Macrophages are the most represented leukocytes in cancer tissues and in established progressing tumors generally have M2-like phenotype. Although NK cells and macrophages are pillars of innate immunity, little information is available on their interplay. The present study was designed to systematically investigate the interaction between M2 and NK cells and the molecular pathways involved.

Methods

Our quantification strategy is based on a label free approach (LFQ), that can provide robust and precise relative protein expression. The proteomics data are interpreted by a statistical data analysis, applying tools like PCA, T-Test, ANOVA, Clustering and Gene Ontology Enrichment. Lastly, from the significative protein expression profile we build a network, based on biological processes, clinical data and functional analysis in order to discover markers and specific disease mechanism.

Results and Discussion

Analysis of the proteins rather than the mRNA levels may reflect the functional phenotype of the cells more directly. For these reasons, proteomic analysis was chosen for the evaluation of changes in protein expression in M2 cells upon exposure to NK stimuli. On the basis of this approach, we have identified a large number of proteins associated to the polarization from M2 to M1 and therefore that have acquired a antitumoral activity. Furthermore we quantified a number of proteins involved in cell-mediated pathway activation and therefore linked to immune synapses NK-M2.

Conclusions

The classification of polarization states on the basis of few cell surface markers will remain a substantial challenge. Here, we addressed how on high-resolution based proteomics data can be utilized to better understand the biology of macrophage polarization.

These experiments will provide quantitative information on molecules or ligands released by cells (e.g. consequent to functional modulation) and may allow the identification of new immune-modulatory mechanisms.

P-454.00

IMMUNOMODULATORY MECHANISM OF A NOVEL POLYSACCHARIDE FROM LENTINULA EDODES

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Introduction and objectives

The ¦Â-glucan from the fruit body of Lentinula edodes have been intensively investigated. However, the heteropolysaccharides in the mushroom Lentinula edodes were not explored well.

Methods

In this study, a new heteropolysaccharide named L2 was separated from the fruit body of Lentinula edodes, and its immunP-stimulating activities were investigated in mice, and proteomics analysis was performed for mechanism of action.

Results

L2 can enhance NK cell activity and stimulate secretions of cytokines TNF-a¡¢ IL-2¡¢IFN-r and IL-12. Proteomic analysis of colon tissue identified some differentially expressed proteins, 13 upregulations and 10 downregulations.

Conclusions

Different from traditional lentinan, L2 does not possess triple-helix structure, but it still displays potent immunomodulatory activities in vivo.

P-455.00

THE ANALYSIS OF HUMAN SPERM PROTEOME BY MEANS OF POLYCLONAL ANTISPERM ANTIBODIES.

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Introduction and objectives: One of the reasons underlying human infertility is immunological factor. Nowadays it is suggested that immunological reactions can underlie 2-30% of all reproduction failures. Antibodies directed towards sperm can be present in body fluids of both males and females. The aim of our study was to identify human sperm antigens reacting with antisperm antibodies present in blood sera of infertile patients.

Methods: Protein sperm extracts were subjected to twP-dimensional electrophoresis (2-DE) and next immune reactions (immunoblotting) were carried out with positive for antisperm antibodies and control sera samples. Immunoreactive spots were subjected to LC-MS analysis.

Results and discussion: Proteomic analysis of human sperm proteins resulted in identification of 80 sperm moieties that could be divided into three groups: antigens specific for infertile patients with antisperm antibodies (32), antigens recognized by both patients and control sera (35) and antigens detected by control sera only. Among antigens specific for infertile patients there were 12 sperm entities known to be involved in fertilization process. We have also selected three proteins identified only by sera of infertile women. Altogether, the proteomic analysis resulted in identification of 33 sperm entities not previously reported in human sperm proteome. The three subgroups of sperm antigens can suggest that some of these entities are genuine sperm immunogens while some can initiate immunological reaction by other structures and then become re-directed to sperm. These antigens may also become pathognomic for immunological type of infertility with different background.

Conclusions: Identified proteins are sperm antigens potentially responsible for immunological infertility. The study sheds a new light on the sperm antigens classification in terms of gender specificity and their origin. The investigation of human sperm proteome by the use of antisperm antibodies-containing sera of infertile individuals not only may indicate new proteins but also can draft their immunological nature.

P-456.00
IL-2 AND IL-15 SIGNALING NETWORKS: SIMILAR BUT NOT IDENTICAL

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Introduction & Objectives

IL-2 and IL-15 are critical cytokines displaying pleiotropic functions in the immune system. They share several roles that may be partly explained by the use of common signaling receptors. Both share two (IL-2Râ and IL-2RƔ) out of the three subunits that comprise the high affinity receptor required to activate signaling pathways that result in the convenient immune response. Nonetheless, in many immune responses each cytokine evokes distinct and even sometimes opposing actions.

The complete understanding of the action of both cytokines is crucial considering their implication in the development of several diseases such as diabetes and leukemia. However, attempts to unveil the paradox of how IL-2 and IL-15 induce signaling through the same receptors producing divergent outcomes still remains controversial. In order to shed light on this paradigm we aimed to dissect and compare IL-2 and IL-15-induced signaling networks.

Methods

We combined triple SILAC labeling of T-cells with immunoprecipitation of tyrosine phosphorylated proteins and TiO2-based enrichment of phosphopeptides followed by mass spectrometry and bioinformatics analysis.

Results & discussion

In the two replicas performed we quantified over 1200 proteins of which nearly 10% were regulated in response to IL-2 and/or IL-15, including numerous components of the main signaling pathways known to be initiated upon interleukin stimulation. In agreement with previously published data, we observe that signaling properties of IL-2 and IL-15 are highly similar in T-cells. In addition, we detected that several proteins involved in endocytosis were more enriched in IL-2 treated cells. Further analysis confirmed that receptor internalization was accelerated in cells stimulated with IL-2.

Conclusions

Our quantitative phosphoproteomic studies demonstrate that signaling networks initiated by IL-2 and IL-15 in T-cells are highly similar but not identical. Combination of faint differences in their transduction cascades may explain the functional dichotomy existing between both cytokines.

P-457.00

STAIN FREE TOTAL PROTEIN QUANTITATION – A METHOD FOR GREATER RELIABILITY IN WESTERN BLOT LOADING CONTROLS

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Western blotting is often used to validate protein abundance changes discovered from 2DE or mass spectrometry based investigations. Reliable assessment of changes to identified proteins of interest/POI (eg: putative biomarkers of health/disease, indicators of biological state/function, etc.) requires measurement of both the identified POI and loading control proteins in the linear dynamic range. Stain-Free technology is a novel method introduced by BiP-Rad to visualize and quantify total proteins in gels and blots.

To determine the value of this technology for quantitative western blotting, we compared the linearity of a series of dilutions measured by Stain-Free total protein measurement as well as immunodetection of three housekeeping proteins—β-actin, β-tubulin, and GAPDH—commonly used for loading controls in western blotting. We found that immunodetection-based measurements of β-actin, β-tubulin and GAPDH protein levels neither showed good linearity nor accurately indicate 10-50 ug of HeLa cell lysate loading levels. In contrast, Stain-Free total protein measurements exhibited great linearity in the same loading range and its linearity correlated very well with the immunodetection of a selection of low abundant protein targets: MEK, Akt and Erk.

Our study demonstrated that Stain-Free total protein measurement serves as a more reliable loading control than housekeeping proteins, particularly in the range of loading most commonly used for cell lysates, 10-50 µg. Thus, Stain-Free total protein normalization offers a valuable tool for researchers interested in quantitative analyses of proteins by western blotting.

P-458.00
EARLIER DIAGNOSIS AND PREDICTION OF ALLERGY TO PEANUT ALLERGENS USING ADVANCED TECHNIQUE IMMUNOCAP 250

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Introduction and objectives

Peanut allergy is the most common food related allergy. Peanuts (Arachis hypogaea) contain proteins, which encompass allergens responsible for causing most symptoms in allergic individuals. To date, 13 peanut allergens have been identified including the prognostically more significant Ara h 1 & 2. The study objectives were to evaluate novel allergen components recently produced by Phadia, to optimise specific IgE detection and to select the most reactive and prognostic allergen component sets for clinical diagnosis of peanut allergy.

Methods

Patient records were examined to recover positive patient sera samples for whole peanut allergy. The sera for these patients (n=24) was then tested with allergenic peanut proteins and peanut allergen peptide components to detect levels of sIgE, using ImmunoCap 250.

Results and Discussion

From 24 patient samples, 10 were tested against whole peanut proteins and novel Phadia allergen components Ara h 1 & 2 to identify the range of positive reactions. Results showed that sera of patients allergic to whole peanut gave high positive values to at least one of the allergen components tested.

The remaining 14 patient samples were tested additionally with Silver birch pollen extract, to identify any cross-reactivity. Results indicated the following pattern: 43% of sera had a positive reaction with Silver birch but a weaker reaction with Ara h 1 & 2; 36% samples, that had a weak or negative reaction with Silver birch, had a strong positive reaction with Ara h 1, 2 or both.

Conclusions

Measuring IgE sensitisation to peanut allergen components is a more prognostic diagnostic tool. Our results indicate that patients with severe peanut allergy show weak cross-reactivity with Silver birch proteins; this could be used for peanut allergen prognosis, i. e. strong positive reaction to Silver birch proteins would indicate better prognosis - mild symptoms of peanut allergy.

P-459.00

OPTIMIZED ANTIBODY PANEL FOR MULTIPLEXED DETECTION OF AKT/MAPK PHOSPHORYLATION EVENTS BY WESTERN BLOTTING

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The ability to multiplex monitoring of proteins and their post-translational modifications (PTM) would enhance monitoring of biological functions and signaling mechanisms in health/disease.

However, current approaches for multiplexed protein/PTM detection - especially by western blotting- suffer from poor accuracy of results and being labor/time-intensive. We describe here the development of a sensitive, fluorescence-based western blotting system to simultaneously detect and quantify multiple proteins/PTMs. As proof of principle, phosphorylation targets of the AKT- and MAPK- regulated pathways were monitored upon growth factor treatment of mammalian cells. Antibodies to target proteins were pre-screened for no cross reactivity to other proteins/PTMs and then labeled with non-overlapping fluorescent dyes for simultaneous detection of multiple targets on same blot. For loading control we fluorescently labeled bivalent human recombinant Fabs selected against housekeeping proteins from the HuCAL® phage display library expressed in E. coli.

We present here results from our optimized antibody panel - for sensitive multiplexed monitoring of quantitative changes to AKT/MAPK mediated phosphorylation event.

P-460.00

COOPERATION OF LIVER CELLS REVEALED BY CELL-SPECIFIC GENES

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Liver is the metabolic factory and the immunological organ of human body. With the increasing development of mass spectrometry and deep research of cell function in the liver, it becomes more and more important to isolate single-type cells for proteomic analysis as an essential component of systems biology. Our study aims to simultaneously isolate and purify the four types of primary cells within liver to construct protein expression profiles at cellular level. What¡¯s more, it is mainly necessary for the functional and mechanical elucidation for liver physiology and Pathology.

Here, with a systematic and detailed isolation and purification procedure, we have simultaneously isolated hepatocytes (HCs), hepatic stellate cells (HSCs), Kupffer cells (KCs) and liver sinusoidal endothelial cells (LSECs) by a combination of collagenase-based density gradient centrifugation and fluorescence activated cell sorting with high purity, viability and yield. Up to now, we have constructed the transcriptome and proteome of 4 types of liver primary cells. With high-resolution deep RNA sequencing, we detected more than 10,000 genes per cell type. With a label-free quantitative proteome method, we can reach more than 8,000 proteins from one cell type. The cellular fractionation improved liver proteome coverage and many novel proteins are identified, some of which lack functional information. With the combination of transcriptome and proteome data, we analyzed identified a set of cell-specific gene products related to cell-specific function. First, we screened cell-specific CD molecules looking for cell-specific surface markers. And we analyzed cell-specific transcriptional factor to interpret cell-specific gene regulation.

Further, we also compared KEGG pathways coverage between these cells and found some signaling and metabolic pathways that may be complementary among the parenchymal and other 3 non-parenchymal cells. This would help elucidate the cooperation of cells in liver signal transduction and metabolism.

P-461.00

LARGE-SCALE, LABEL-FREE QUANTITATIVE PROTEOMIC CHARACTERIZATION OF REGULATORY VERSUS CONVENTIONAL T LYMPHOCYTES ISOLATED FROM MOUSE

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Regulatory T cells (Treg) represent a minor sub-population of T lymphocytes of paramount importance for establishing self-tolerance, controlling inflammatory responses and maintaining immune homeostasis (1). In human, quantitative or functional defects in Treg have been associated with susceptibility to several immune diseases. These cells are characterized by the expression of the Foxp3 transcription factor, which acts as a Treg cell lineage specification factor, programming their development and suppressive activity. Although substantial progress in understanding Treg biology has been achieved in recent years, the signaling pathways and molecular mechanisms specifically involved in Treg commitment and functions remain poorly understood, and represent a critical step to manipulate those cells in a rational manner for novel therapeutic approaches.

In an initial attempt to better characterize Treg at the molecular level, we present here an extensive quantitative proteomic study of these cells. Highly enriched populations of Treg and conventional T lymphocytes (Tconv) were sorted by flow cytometry, and global proteomic analysis of these sub-populations was then performed by single-run nanoLC-MS/MS on a fast-sequencing Orbitrap mass spectrometer. The chromatographic setup was optimized to improve peptide fractionation on 50cm columns, and increase the depth of proteome analysis to more than 3000 proteins. Using label-free quantitative methods, we could achieve a detailed and large-scale comparison of protein expression profiles in Treg versus Tconv, both on freshly isolated cells or after activation.

Besides "historical" proteins that characterize Treg, our study identified numerous new proteins that are significantly up- or down-regulated in Treg versus Tconv. Functional studies on some candidate proteins suggest that they may play a role in the suppressive functions of Treg.

P-462.00

PHOSPHOPROTEOMIC CHARACTERIZATION OF INFLUENZA A VIRUS INFECTED HUMAN PRIMARY MACROPHAGES

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Influenza A viruses (IAVs) cause annual epidemics and, more rarely, worldwide pandemics of respiratory disease. The IAVs are fast mutating viruses and new strains, against which the human population lacks immunity, develop fast. Today, the viral proteins are the targets of antiviral drugs, for which the IAVs quickly develop resistance. Therefore, it is critical to identify host factors utilized by the influenza virus. The IAVs infect innate immune cells such as epithelial cells, macrophages and dendritic cells of the respiratory tract, inducing the production of antiviral and prP-inflammatory cytokines as well as apoptosis. However, the intracellular signalling pathways activated after the recognition of IAVs are not fully understood.

Here, we have characterized the phosphoproteome of IAV infected human primary macrophages combined with transcriptomics, bioinformatics and functional studies. The phosphoproteome data was processed with an in-house developed software PhosFox (Söderholm et al. submitted). We identified in total 1675 phosphoproteins with 4004 phosphopeptides and 4146 non-redundant phosphorylation sites. Of these phosphorylation sites, 587 are not included the PhosphoSitePlus, Phosida or UniProt databases. 342 proteins were phosphorylated only in the IAV infected set and 459 proteins in the unstimulated set. Additionally, the phosphorylation of 312 proteins changed after IAV infection compared to unstimulated cells. Transciptome analysis showed 657 significantly upregulated and 935 downregulated genes after IAV infection. Bioinformatic analyses revealed that signalling pathways associated with inflammatory response and apoptosis were strongly upregulated, and pathways associated with actin cytoskeleton regulation, MAPK signalling and endocytosis were downregulated.

Based on these findings, pharmacological and RNAi studies were carried out for selected host factors with potential roles in the regulation of inflammatory responses and the progression of IAV infection. In conclusion, our study provides novel information about the role and regulation of antiviral defense at early stages of IAV infection in human primary macrophages.

P-463.00

INFLAMMATORY BOWEL DISEASES - A CLINICAL PROTEOMICS STUDY SEEKING TO IDENTIFY THE UNDERLYING BIOLOGICAL TRIGGERS.

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Introduction:
Unambiguous diagnosis of inflammatory bowel diseases (IBD) represents a challenge in the early stages of the diseases. The prevalence of the diseases is increasing while the causes and etiologies remain poorly understood. We here present three key objectives: The establishment of a biobank with colonic biopsies from patients suffering from ulcerative colitis (UC), rheumatoid arthritis (RA) and healthy controls(C); The omics investigation determining proteome reflecting the diseases; Significant improvements in detection and characterizing the posttranslational modification citrullination.

Methods:
Colonic biopsies were extracted by colonscopy from patients suffering from UC, RA and healthy controls. Biopsies used for proteomics were homogenized, digested with trypsin and analyzed in triplicates using UPLC-MS on a Q Exactive MS using a 50 cm C18 column and a 240 min gradient. The raw files were analyzed with MaxQuant and the results was processed in multiple bioinformatics packages, including Progenesis LC-MS, Perseus, and SPSS statistics.

Results and Discussion
The MS driven protein identification and quantitation was optimized, and methods for identifying citrullinated peptides was developed [2]. The biopsies were analyzed accumulating more than 500 hrs LC-MS data and transcriptome profiling was determined by RNA Seq. A total of 6,302 proteins were identified (1% FDR), of which 2,868 were quantifiable across all UPLC-MS runs. Unsupervised clustering of all proteins fulfilling twP-sample t-test p

Conclusion:
We have optimized an approach for unambiguously identifying citrullinated peptides in human samples. Furthermore, we have identified a wide panel of proteins changing during the disease course of ulcerative colitis. The proteins can likely function as targets for diagnostic and prognostic markers, and help identify disease associated pathways usable in therapy.

P-464.00

IMMUNE DYSREGULATION LEADS TO PATHOLOGICAL NEOVASCULARISATION IN SUBJECTS WITH RETINOPATHY OF PREMATURITY

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Intorduction and objectives: Retinopathy of Prematurity (ROP) is a proliferative retinal vascular disorder affecting the eyes of premature babies with low gestational age (?32 weeks) and birth weight (?1,700g). Our genetic association study on ROP indicated the involvement of complement components in disease pathogenesis. However, their exact role in ROP pathogenesis is still unclear. The present study was aimed to understand the role of complement components in the pathogenesis of ROP.

Methods: Vitreous humor (50-100?l) was collected with prior informed consent from patients (n=40) with stage IV and V of ROP along with infants with congenital cataract as control subjects (n=40) undergoing vitrectomy. Prefractionated proteins were subjected to trypsin digestion and the resulting peptides were analysed on a FT LTQ Orbitrap Velos mass spectrophotometer. The obtained mass spectra were searched against the SwissProt database using the Peak studio search engine. Additionally, the levels of complement component in the vitreous were confirmed by multiplex ELISA and further validated by western blotting.

Results and Discussions: Proteomic analysis of in-gel trypsin digested proteins could detect 371 spectra in ROP and 95 spectra in controls for the C3 fragments, and 323 spectra in ROP and 102 spectra in controls for the C4 fragments. These results indicated elevated levels of complement component C3 and C4 in ROP patients which was further confirmed by a parallel multiplex ELISA and in-solution digested total proteome analysis. 182 spectra in ROP and 24 spectra in the controls were observed for the activated C3 fragments. Increased numbers of spectra in ROP were also observed for complement C5 and its activated fragments that were further confirmed vitreous by western blotting.

Conclusions: The elevated levels and activation of complement factors suggest for an important role of abnormal immune activity leading to pathological neovascularization in ROP patients

P-465.00

2-DE-BASED PROTEOMIC ANALYSIS OF THE PLATELET CLEC-2 SIGNALLING CASCADE FOCUSING ON THE PI 6-11 RANGE

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Introduction and objectives: CLEC-2 (C-type lectin-like receptor 2) is a platelet membrane receptor that plays a crucial role in thrombosis and is being studied as a potential pharmacological target. CLEC-2 is activated by the snake venom protein rhodocytin and by the endogenous ligand podoplanin. We recently analysed theplatelet CLEC-2 signalling pathway by 2D-DIGE and mass spectrometry,focusing on the pI 4-7 range[Parguiña et al. Blood 2012;120:e117-e126]. We now completed this study by analysing the pI 6-11 range of the proteome. The objective was to integrate the pI 4-7 and 6-11 data to have a complete picture of variations in thesignalling cascade upon activation.

Methods: The proteome of unstimulated platelets and platelets stimulated with rhodocytin (300 nM, 5 min)was compared. Proteins were separated by 2-DE (18 cm, pI 6-11 strips for the first dimension; 11% SDS-PAGE for the second). Gels were stained with Sypro Ruby.Image analysis was done with SameSpots software. Identifications were by LC-MS/MS and validations by western blotting.

Results and discussion: Twenty-seven differences were detected (fold change≥1.5; p Conclusions: Our study contributes to a deeper knowledge of the CLEC-2 signalling pathway and its activation mechanisms.

P-466.00

LIPOPOLYSACCHARIDE-INDUCED CYTOKINES/CHEMOKINES SECRETOME IN HUMAN NEUROBLASTOMA SH-SY5Y CELLS: A MODEL FOR NEUROINFLAMMATION

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The human neuroblastoma SH-SY5Y cell line has been widely used in experimental neurobiological studies, including neuronal differentiation, metabolism, and function related to neurodegenerative processes, neurotoxicity and neuroprotection. Sepsis ensues due to hyperactivation of the innate immune system that causes a massive production of proinflammatory cytokines and chemokines that cause septic shock. Encephalopathy is a common feature in sepsis. We investigated cytokines network in the neuroblastoma culture conditioning media after LPS treatment. The cells system can be used as in vitro model for study human brain neuroinflammation.

The undifferentiated SH-SY5Y 1*106 cells were seeded on 6-well plates, washed next day, and incubate for 4 days with sampled daily a small aliquots of culture media (DMEM-F12/10% FBS). The experimental cells were treated by LPS (10, 100 ng/ml, 1ug/ml) and small aliquots of culture media were sampled during 4 days. Forty eight cytokines, chemokines and growth factors were analyzed using the BiP-Plex Pro Human Cytokine 27-plex and 21-plex assays (BiP-Rad) and BiP-Plex 200 Luminex xMAP multiplex system.

The inflammatory response after LPS treatment was provided by up-regulated cytokines in dose-dependent manner: IL6, IL8, IL15, IP10, MCP1, RANTES, VEGF. Other group of cytokines was down-regulated: IL1a, IL2, IL2Ra, IL3, IL4, IL9, IL10, IL12, IL16, IL17, IL18, IFNa2, IFNg, LIF, MCP3, TRAIL, TNFa, TNFb, HGF, GROa, MIG, SCF, CTACK, NGF-b, PDGF-bb, SCGF-b, SDF1a, MIF. Changes in concentration of these proteins may contribute to the regulation of inflammation, and information about cytokine network profiling may be useful for development cell models in neuroimmunology, neurotoxicity and neurodegenerative diseases.

P-467.00

THE SURFACEOME OF NAÏVE T CELLS – A PROTEOMIC SYSTEMS APPROACH TO IDENTIFY NEW CELL SURFACE MARKERS IN EARLY T CELL ACTIVATION

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Introduction and objectives

Allergies are an emerging problem all over the world, e.g. at least 20% of the populations of most western countries are affected by this burden. This disease is the result of a dysregulated differentiation process of naïve CD4+ T-cells, the common precursor of all T helper cell subsets. Surface proteins play an important role in differentiation processes since the cell surface is responsible for the recognition and the response to changes in the environment by initiating further signal transduction. Therefore we created a comprehensive surface protein library for human naïve CD4+T-cells and aimed for the identification of new surface markers involved in the early time window of T-cell differentiation.

Methods

To generate the surface atlas, naïve T-cells of 3-4 blood donors were activated with aCD3/aCD28 in a time course experiment to mimic T-cell receptor engagement and the LC-MS/MS based cell surface capturing (CSC) technology, which depends on specific biotinylation of cell surface proteins, was applied. To strengthen the analysis a flow cytometry based cell surface screening investigating 332 surface antigens was performed.

Results and Discussion

The combination of both approaches led to the identification of 198 cell surface proteins. More than 20% of these proteins were not known to be present on naïve T-cells or even described in an immunological context before. The evaluation of the identified proteins also revealed that 46 proteins are already approved as drug targets.

Conclusion

The generation of this overall naïve T-cell surface proteomic resource will allow to gain a deeper understanding into mechanisms of T-cell differentiation and may led to the identification of new potential immune targets usable for the development of novel therapeutic strategies dealing with different T-cell driven diseases such as allergies.

P-468.00

PROTEOMIC PROFILE OF ISLETS OF LANGERHANS IN PRE-DIABETIC NOD MICE.

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Introduction and objectives: Type 1 diabetes (T1D) is an endocrine disease characterized by autP-immune-mediated destruction of insulin-producing beta-cells. The aim of this study was to identify early alterations in molecular pathways in the islets of Langerhans which contribute to T1D predisposition.

Methods: Proteomic profiles of islets of 3-week old female pre-diabetic non-obese diabetic (NOD), non-obese resistant (NOR) and C57Bl/6 mice were analyzed by twP-dimensional differential gel electrophoresis (2D-DIGE) (n=4). Proteins were identified by MALDI-TOF/TOF and grouped according to their biological function. Analysis of the identified proteins was performed by phenome-interactome analysis where proteins were clustered in complexes based on protein-protein interactions and ranked to their potential relevance in T1D.

Results and discussion: Proteomic analysis revealed differential expression of 162 protein-spots (p

Conclusions: Significant alterations in the islet-proteome of NOD compared with NOR and C57Bl/6 mice are present at early age before immune-cell infiltration. Differences in beta-cell pathways like metabolism and protein production may be involved in the higher susceptibility of NOD beta-cells to attack by the immune system, resulting in T1D.

P-469.00

CHEMICAL PROTEOMICS HIGHLIGHTS PROTEIN KINASES INVOLVED IN MACROPHAGE DIFFERENTIATION AND FUNCTION

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Introduction and objectives:
Protein kinase alterations during macrophage differentiation have not been interrogated comprehensively, despite fundamental links between kinase activity and cell development and specialization. We hypothesize that this approach may identify key protein kinases involved in the regulation of differentiation and macrophage-specific functions including cytokine/chemokine production and defense against pathogens.

Methods:
We applied stable isotope labeling with amino acids in cell culture (SILAC) combined with small molecule based kinase purification, phosphopeptide enrichment and high resolution mass spectrometry to study the kinome of phorbol myristate acetate (PMA)-induced differentiation of the human monocytic cell line THP-1. Macrophage-specific kinases were further characterized by ELISA, fluorescence microscopy and bacterial phagocytosis assays.

Results and Discussion:
We quantified 168 protein kinases encompassing all families of the human kinome. Interestingly, whereas more kinases were decreased (63) than increased (27) in their amount following differentiation, a general increase in overall phosphorylation was observed. Based on our expression and phosphorylation data, a number of protein kinases including MAPK13, MerTK, CaMK2b and MAP3K7 (TAK1) showed dramatic regulation suggesting vital roles for macrophage function or even differentiation. Functional analysis using the TAK1 specific inhibitor 5Z-7-oxozeaenol uncovered TAK1 as key regulator of PMA-induced differentiation in the THP-1 background. In addition, we also got indications that TAK1 is involved in efficient phagocytosis and killing of the human bacterial pathogen Staphylococcus aureus.

Conclusions
With help of chemical proteomics we could describe the reorganization of the kinome during monocyte-tP-macrophage transition and highlight protein kinases potentially essential for differentiation and specialization of macrophages. In this regard, TAK1 activity could already been identified as key regulator.

P-470.00

A SILAC-PHOS STRATEGY TO DETERMINE THE PMA-PKCA DEPENDENT SIGNALING MAP

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Introduction and objectives: Post-translational modifications such as phosphorylation are important to regulate the dynamic conformation of proteins, modulating its subcellular localization and/or regulating protein-protein interactions. Phosphorylations can be inhibitory or activating, and thus their monitorization provides a snapshot of protein activation state. We have previously reported that T cell receptor activation leads to transient translocation of the protein kinase C α (PKCα) to the immune synapse, a process regulated by phosphorylation events. To better characterize the mechanism of regulation of PKCα at this site and to determine its direct protein substrates, we designed a quantitative phosphoproteomic strategy. Our aim was to decipher key post-translational events directly regulated by PKCα. We used Phorbol 12-myristate 13-acetate (PMA) treatment, as broad PKC activator. To isolate bona-fide PKC substrates we used specific kinase inhibitors (cPKC/Go6976 and MAPK/PD98059).

Methods: T lymphocytes were SILAC-labelled and treated with PMA combined or not with inhibitors. Samples were pooled prior to phosphopeptide enrichment, which was carried out by 2D-LC-MS/MS, combining sequential basic pH-RP-HPLC peptide separation, modified IMAC purification strategy, and phosphopeptides were analysed by LC-MS/MS using a 5600 AB Sciex TTof instrument.

Results and Discussion: Our work showed great consistency with current literature and many PMA-induced phosphorylations were already reported elsewhere, such as NFκB, PKD, MAPK. However, we also identified novel sites that will be important to further characterise. Interestingly, we found a clear PKCα phosphorylation site that was regulated by PMA exposure and blocked upon cPKC inhibition. We are now carrying out site mutagenesis to better understand the impact of this phosphorylation on PKCα subcellular dynamics and function.

Conclusions: Overall, we provide a read-out of signalling pathways controlled by DAG/PKC signal axis. Better understanding of direct PKCα targets regulated during the antigenic response will be of high interest to unveil new markers of the DAG/PKC dependent response.

P-471.00
ANALYSIS OF THE SURFACEOME OF CYTOTOXIC T LYMPHOCYTES IN DIACYLGLYCEROL KINASE ZETA KO MICE

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Introduction and objectives
T cells are key players in the implementation of the adaptive immune response. After antigen presentation, T cells undergo changes in the expression of surface receptors and adhesion molecules to receive signals to migrate, activate other cells or kill. During these events, signaling dependent on diacylglycerol (DAG) plays a critical role. To get a global view on this cellular makeover and plasticity, we setup a shotgun proteomic study of membrane-protein enriched fractions from mouse T cells.
We report the surfaceome of primary mouse T-cells differentiated to cytotoxic lymphocytes (CTLs). Our aim is to complement our current knowledge of markers of T cell differentiation, critical to better track T cell sub-populations. In addition, we are using this strategy to study the direct input of DGKz activity on T cell differentiation, an enzyme that metabolizes DAG and we analyze the surfaceome of CTL derived from DGKz KO mice.

Methods
CTLs from OT-I-mice were differentiated in vitro following peptide OVA antigen stimulation and IL-2 amplification. Our experimental strategy combines biotinylation of cell surface proteins and subcellular fractionation to enrich in plasma membrane proteins.

Results and Discussion
Both the biotinylation and the subcellular fractionation methods enabled the identification of close to 2000 proteins, 20% of which were annotated as plasma membrane proteins, similar to the total putative plasma membrane proteins from the mouse (Uniprot), although subcellular fractionation enabled greater protein recovery. The combination of our experimental workflows permitted to draw a cell surface expression map of CTLs.

Conclusions
We applied a proteomic approach to perform an unbiased analysis of cell surface marker proteins in T cells, in differentiation context. We will now complement and validate our study by quantitative measurements by FACS analysis and targeted mass spectrometry to check for expression of these markers during specific models of T cell differentiation.

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Topic **10**

REDOX proteomics and mitochondrial biology

OP058 - GLOBAL PROTEIN OXIDATION PROFILING OF PODOSPORA ANSERINA MITOCHONDRIA SUGGESTS EFFICIENT REMOVAL OF DAMAGED PROTEINS EVEN AT HIGH AGE

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Introduction and objectives

The free radical biology of aging is based on the idea that reactive oxygen species (ROS) damage cellular components and lead to the accumulation of age-related protein oxidation. This study with the aging model Podospora anserina focuses on the mitochondrial proteome as main target for ROS-induced damage. The objective of this study was the development of a new proteomics workflow for global, quantitative and un-targeted analyses of oxidative protein modifications to scrutinize the ROS theory.

Methods

To account for the low amount of modified proteins and the danger of artificial protein oxidation, a workflow consisting of gel-free sample preparation and iTRAQ quantification was established. Mitochondrial proteins from four age stages were digested with trypsin using the FASP protocol and iTRAQ-labeled peptides were separated on a 25 cm column with a NanoAcquity/Orbitrap Velos system. A statistical framework was developed that allowed to quantify modified and unmodified protein species, accounting for identification rate, variance, regulation trend, and correlation in regulation profiles between modified and un-modified protein species.

Results and Discussion

This first large scale, unbiased oxidative modification-centric study for mitochondrial aging allowed the comprehensive quantification of 2352 protein species and 23 different oxidative amino acid modifications. For 746 proteins unmodified and oxidatively modified species were detected. For the majority of proteins a positive correlation of changes in protein amount and oxidative damage, and rarely age-related increases in protein oxidation were noticed. Our data suggest that P. anserina is efficiently capable to compensate ROS-induced protein damage during aging as long as protein de-novo synthesis is functioning, ultimately leading to an overall constant relationship between damaged und undamaged protein species.

Conclusion

In contradiction to the ROS theory, our results do not confirm massive increase in protein oxidation during aging and rather suggest a functional protein homeostasis mechanism even at high age.

OP059 - A DECADE OF MITOCHONDRIAL PROTEOMICS

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Introduction

Since the first discovery that mitochondria are the power plants of the cell, our knowledge about function, structure and regulation of mitochondria has been substantially extended, also with the help of proteomics. More than 99% of mitochondrial proteins are nuclear-encoded, synthesized in the cytosol and subsequently imported via membrane-bound import machineries into one of the four mitochondrial subcompartments. Owing to the central role of mitochondria for maintaining cellular homeostasis, the regulation of mitochondrial protein import and processing has direct impact on human health and can be connected to neurodegenerative and myopathic diseases, as well as cancer.

Methods

To elucidate the composition of the mitochondrial proteome, its subcompartments and the regulation of mitochondrial protein import, we utilized a variety of quantitative and PTM-based technologies, including phosphoproteomics, N-terminal COFRADIC and our recently developed ChaFRADIC strategy. We tailored specific SILAC-based approaches that allow assigning mitochondrial proteins to their subcompartiments. We validated all our MS-based findings using dedicated biochemical assays.

Results

We could not only identify "the" mitochondrial proteome with ~1000 proteins, but also for the first time revealed the proteomes of the outer membrane, the intermembrane space and very recently the matrix, including a complete mitochondrial topology. Furthermore, we could for the first time demonstrate that essential steps of the mitochondrial protein import are regulated by protein phosphorylation. We also for the first time conducted a systematic study to identify mitochondrial signaling sequences, revealing the previously unknown peptidase Icp55 that stabilizes the mitochondrial proteome in accordance with the N-end rule.

Conclusion

In one decade of mitochondrial proteomics we could demonstrate the unique power of proteomics to reveal unknown mechanisms, functions as well as temporal and spatial insights into eukaryotic organelles. The design of novel dedicated strategies holds a strong potential to further elucidate essential mechanisms that govern mitochondrial homeostasis in the future.

OP060 - PROTEOMIC CHARACTERIZATION OF CYSTEINE OXIDATION IN INS-1E CELLS IN RESPONSE TO CYTOKINES STIMULATION

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Background and aims

Oxidative stress is an essential mechanism involved in the development of insulin resistance. Reactive oxidative/nitrogen species (ROS/RNS) triggered by pro-inflammatory cytokines in diabetes can cause protein translational modifications (PTMs) and impair the insulin signaling. Among the ROS/RNS mediated PTMs, reversible cysteine oxidation attracts great attention in diabetes as it affects both the structure and function of proteins.

Materials and methods

In this work, in order to systematically characterize cysteine oxidation involved in diabetes, the iTRAQ-based quantitative proteomic strategy was employed to identify and quantify changes in cysteine oxidation in INS-1E β-cells subject to stimulations by pro-inflammatory cytokines (IFNγ and TNFα) for 12h and 24h. The free cysteine on proteins was blocked with NEM, then the reversibly modified cysteine peptides were reduced, labelled with the newly developed phospho-tag specific to cysteine and enriched by TiO2. The peptides from enrichment and flowthrough were identified and quantified using Q-Exactive mass spectrometry.

Results and conclusion

The phospho-tag based cysteine proteomic strategy was successfully applied to analyze the reversibly modified cysteine peptides from INS-1E cells with high specificity. From the enriched fractions, in total, 5050 unique peptides were identified, including 4657 cysteine peptides (92.2%) distributed in 1948 proteins. Among them, 592 cysteine peptides in 455 proteins showed significant change after cytokines stimulation, and 24h stimulation affected much more peptides and proteins than 12h stimulation, the affected proteins involved in different processes, such as metabolism, immunology, inflammatory, and redox. The IFNγ and TNFα stimulation leads to increased oxidation of peptides from STAT1 and IFN Regulatory Factor-1, which were confirmed targets of IFNγ and TNFα involved in pancreatic beta cell death.

Acknowledgements

OP061 - CONCURRENT ANALYSIS OF REVERSIBLE CYSTEINE OXIDATIONS: S-NITROSYLATION AND S-SULFONYLATION

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Introduction and objectives

Cellular redox homeostasis is essential for the normal function and survival of cells. Cysteine thiols mediate multiple signalling, transcriptional and metabolic processes in cells and redox imbalance has been recognised as pathogenic factor of numerous human diseases. We present a new strategy which provides quantitative analysis of cysteine S-nitrosylation (SNO) and S-sulfonylation (SOH) simultaneously including precise resolution of modification site.

Methods

We make use of iodoTMTTM tag.The method relies on differential reduction of "total" cysteines, SNO cysteines and SOH cysteines with TCEP, sodium ascorbate and sodium arsenite respectively followed by iodoTMTTM alkylation. Enrichment of iodoTMTTM-containing peptides is performed using anti-TMT antibody. Peptides are analysed by nLC-MSMS. In vivo model of mild oxidative stress in Escherichia coli is used. To induce endogenous SNO bacteria were grown anaerobically in minimal media supplemented with fumarate or nitrate. Short-term treatment with submilimolar levels of hydrogen peroxide was used to induce SOH.

Results and Discussion

The SNO/SOH TMT strategy was able to produce quantitative, biologically relevant data relating to SNO and SOH in E. coli under mild oxidative stress. From 3 independent biological replicates we have quantified 114 SNO/SOH modified peptides corresponding to 90 proteins. Only 6 modified peptides change significantly under mild oxidative stress. Quantitative information obtained by SNO/SOH TMT allowed us to determine heavily modified cysteine residues. We observe that high site occupancy does not necessarily associate to a modification site which responds to oxidative stress.

Conclusions

SNO/SOH TMT outperforms available strategies for cysteine oxidation analysis. It provides quantitative profiling of SNO and SOH changes simultaneously in two experimental conditions. It allows correcting modification levels by protein abundance changes and determining modification site occupancy – all in a single nLC-MSMS experiment. The method is precise and sensitive enough to detect and quantify endogenous levels of oxidative stress on proteome-wide scale.

P-472.00

GLOBAL-SCALE NITRATION IDENTIFICATION AND RELATIVE QUANTITATION USING STABLE ISOTOPE-CODED NITROTYROSINE INTERNAL STANDARDS

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Increase in protein tyrosine nitration (PTN), a general marker of oxidative stress, has been detected in numerous diseases yet our understanding of this modification as related to disease causation or progression is limited. Given the low abundance of PTN, the characterization of this modification in biological systems of nitrative stress has been a major challenge. We have developed an approach that utilizes stable isotope-coded nitrotyrosine internal standards that are generated in vitro using 15N-labeled peroxynitrite.

The resulting "heavy" nitroprotein-containing mixture serves as an internal standard that is mixed with the sample in which experimentally induced nitration will be identified and quantified. Nitropeptide pairs are distinguished by a 1 Da mass difference, where a standard isolation window allows for co-isolation and MS/MS fragmentation of both light and heavy-labeled nitropeptides. The method relies on high mass resolution and mass accuracy detection of the diagnostic nitrotyrosine immonium (or related) ion pairs. The unique 15N-labeled reporter ion facilitates detection of nitropeptides in complex cell lystate digests and also serves as an internal standard for relative quantitation.

Evaluation of the method in terms of nitropeptide detection and relative quantitation using peptide and protein standards has been accomplished. Further characterization was performed to demonstrate the utility of the method to improve the overall signal of low-abundance nitrated peptides through signal increase of the second isotope (13C) peak by the stable isotope-coded nitrotyrosine standard. We envision this method can be used to complement current approaches for PTN characterization, with the goal of identification and quantitation of nitrotyrosine formation in various cell or animal model systems of nitrative stress.

P-473.00

REDOX PROTEOMICS AND METABOLOMICS TO UNDERSTAND THE HEALTHY EFFECT OF OMEGA-3 FISH LIPIDS

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Excessive reactive oxygen species production has been described as an initial key event in the development of metabolic disorders. EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) consumption has been implicated in the prevention of these alterations, but some aspects of their action mechanisms remain unclear. This study has been focused in the determination of the optimal EPA:DHA ratio in diet to reduce oxidative stress and inflammation processes. Novel proteomic and metabolomic approaches have been developed to evaluate liver protein carbonylation and to determine lipid biomarkers of oxidative stress and inflammation in plasma.

Wistar female rats were supplemented with fish oils having different EPA:DHA ratios during 13 weeks. Soybean and linseed oils were used as controls. Liver protein oxidation was quantified by labeling carbonyl residues in vivo-generated with FTSC-tag and measuring fluorescence signal on gel-electrophoresis. Carbonylated proteins were identified by tandem mass spectrometry (LC-ESI-IT-MS/MS) after prefractionation by 2DE-gels. In addition, for the simultaneous quantification in plasma of a large number of lipid mediators, a robust and sensitive targeted analysis platform based on SPE-LC-ESI-IT-MS/MS was developed.

Carbonylation protein level was decreased in rats supplemented with fish oils ratios, above all under EPA:DHA 1:1 ratio. Specifically, several proteins were identified as main targets of EPA and DHA these healthy effects (albumin, argininosuccinate synthetase, 3-α-hydroxysteroid dehydrogenase, aspartate aminotransferase, actin). Moreover, the production of lipid mediators was highly dependent of the amount of DHA and EPA in the diet, and these results were correlated with the parameters of oxidative stress and inflammation.

The optimized methodology lends itself to proteomic and metabolomic applications targeted for biomarker discovery, and results showed the capacity of the EPA and DHA supplementation to mitigate cumulative oxidative protein damage and decrease the risk of developing metabolic disorders.

P-474.00

A NEW METHOD FOR DETECTING AND IDENTIFYING CARBONYLATED BIOMOLECULES: APPLICATION TO IN VITRO OXIDIZED TAU PROTEIN BY MALDI-MASS SPECTROMETRY.

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Introduction and objectives

Biomolecule oxidation is caused by reactive oxygen species (ROS). There is a well-managed balance between ROS formation and elimination by the antioxidant system. However, ROS excess can cause significant biomolecule damage. In this work, we focused on protein carbonylation, an irreversible oxidative modification. Several studies showed increases in carbonylated proteins during aging and related neurodegenerative processes, cancers and inflammatory diseases. Carbonyl derivatives are formed by ROS-attack on specific amino acid side chains. Carbonyls are low abundant species, therefore enrichment methods are required. We developed a new carbonyl detection method by synthesizing a compound: 4-sulfanylbenzohydrazide to detect and enrich samples with carbonylated species. We applied it on in vitro oxidized Tau, a protein implicated in neurodegenerative processes especially Alzheimer disease.

Methods

Model peptides and recombinant Tau protein were oxidized with 1 mM NaOCl solution. Samples were derivatized with 4-Sulfanyl-benzohydrazide. Carbonyls Sample enrichment was performed with thiopropyl sepharose or gold nanoparticules and eluted with a reductive agent (DTT). The method was tested on more complex samples. Eluted samples were analyzed by a high vacuum MALDI-TOFTOF instrument.

Results and Discussion

First, model peptides and proteins were derivatised with the 4-Sulfanyl-benzohydrazide leading to hydrazone formation. This led to Specific mass shifts and diagnostic ions that were monitored by MALDI mass spectrometry. The next steps consisted on using our novel enrichment method with the Tau digest which led to the detection of carbonylations at Porline 200 and 203.

Conclusions

This work shows a new method for enriching and identifying carbonylated species by MALDI mass spectrometry. We characterized for the first time carbonylation sites on oxidized Tau. The idea behind is to apply the same method on more complex samples such as cell extracts on tissues.

P-475.00

NOVEL INSIGHTS INTO THE DYNAMIC DEEP REDOXOME

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Introduction and objectives

Cardiovascular events bring about oxidative damage to proteins containing thiol-groups. However, the number of reported Cys-related modifications is scarce. We present FASILOX-B (Filter-Aided Stable Isotope Labeling of OXidized cysteines with Biotin enrichment), an improvement of our GELSILOX technique, for the simultaneous quantification of the proteome and deep redoxome to study the ischemia/reperfusion (IR) on a porcine infarction model.

Methods

Free-cysteines were alkylated during protein extraction from cardiac tissue samples. Proteins underwent reduction, second alkylation with a biotin-based Cys-reagent and tryptic digestion in filters. Peptides from infarcted and remote areas were tagged using SIL, combined, and Cys-containing peptides purified using avidin-beads prior to LC-MS/MS analysis. The non-Cys-containing peptides from the non-retained fraction provided the statistical parameters to detect significant abundance changes of reduced or oxidized peptides, and a comprehensive analysis of the whole proteome.

Results and Discussion

Initially, we applied the method for the analysis of a cell extract that was not subjected to the first alkylation step. LC-MS/MS analysis of the eluate yielded 8788 unique peptides at 1% FDR, out of which 98% were Cys-containing peptides.

We also analyzed the digestion reproducibility of the filter-aided method and its ability to discriminate between reduced and oxidized Cys-sites using protein extracts from infarcted and remote heart areas. Within identified Cys-containing peptides, 12% and 28% were found in oxidized-state in the remote and infarcted area, respectively, which confirmed method's ability to detect increases in oxidation. Statistical analysis demonstrated that the distribution of quantifications at both the peptide and protein levels followed the null hypothesis.

To characterize the dynamic behavior of the deep redoxome in response to IR, an experiment based on the porcine heart-infarction model is under way.

Conclusions

The FASILOX-B technique is a very promising approach for the simultaneous, high-throughput quantification of both the whole proteome and deep redoxome.

P-476.00

PROTEOMIC ANALYSES REVEALED RESTORING OF THE VALOSIN CONTAINING PROTEIN IN THE ISCHEMIA-REPERFUSION INJURED RAT HEPATIC CELLS BY KAEMPFEROL ENRICHED ETHANOLIC EXTRACT FROM DRY STIGMATA OF CROCUS SATIVUS L. (SAFFRON)

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Introduction: Free kaempferol and variety of its glycosides are preferentially bioaccumulated in saffron and this evidence could be stimulative for extended search of innovative application of this spice. Thus, functional proteomic tools were used to evaluate protective effect of the carefully defined kaempferol enriched ethanolic extract (KAREX), as obtained specifically from saffron, in rat animal model upon hepatic ischemia-reperfusion (IR) injury.

Methods: Histologic analyses were used for evaluation of apoptotic damage to hepatocytes in three groups of male rats: 1) sham, 2) IR, and 3) IR pretreated with KAREX. Redox proteomics assay, comprising the 2-D gel electrophoresis oxyblot followed by MALDI-MS analyses, were applied to clarify the protein carbonylation caused by oxidative stress. Mechanism associated with preventive effect of KAREX on the hepatic IR injury in rats were revealed with flow cytometry and immunohistochemistry experiments.

Results/Disscussion: Significantly increased susceptibility to liver cell damage was identified in the IR group of rats. But highly decreased level of hepatic cells apoptosis was detected in the KAREX pretreated group of rats. Carbonylation of specific chaperone proteins, functionally involved in the pro-apoptotic processes, were significantly abolished by KAREX. Under KAREX pretreatment the localization and expression levels of valosin containing protein (VCP) were restored in rat hepatic cells. In addition, the targeted depletion of VCP induced arrest of S-phase cell cycle in the HepG2 cells were also observed as the effect of KAREX. Conclusions: These results indicated that KAREX effectively prevents formation of oxidative stress in hepatic IR of rats. The potential role of VCP in modulating cell death process was also observed which could lead to develop a novel therapeutic approaches against hepatic IR injury.

P-477.00

TARGETTED MASS SPECTROMETRY METHODS FOR DETECTING OXIDATIVE POST-TRANSLATIONAL MODIFICATIONS.

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Aston University

Introduction: Oxidative post-translational modifications (oxPTMs) can alter the function of proteins, and are important in the redox regulation of cell behaviour. The most informative technique to detect and locate oxPTMs within proteins is mass spectrometry (MS). However, proteomic MS data are usually searched against theoretical databases using statistical search engines, and the occurrence of unspecified or multiple modifications, or other unexpected features, can lead to failure to detect the modifications and erroneous identifications of oxPTMs. We have developed a new approach for mining data from accurate mass instruments that allows multiple modifications to be examined.

Methods: Accurate mass extracted ion chromatograms (XIC) for specific reporter ions from peptides containing oxPTMs were generated from standard LC-MSMS data acquired on a rapid-scanning high-resolution mass spectrometer (ABSciex 5600 Triple TOF). The method was tested using proteins from human plasma or isolated LDL.

Results and Discussion: A variety of modifications including chlorotyrosine, nitrotyrosine, kynurenine, oxidation of lysine, and oxidized phospholipid adducts were detected. For example, the use of a reporter ion at 184.074 Da/e, corresponding to phosphocholine, was used to identify for the fist time intact oxidized phosphatidylcholine adducts on LDL. In all cases the modifications were confirmed by manual sequencing. ApoB-100 containing oxidized lipid adducts was detected even in healthy human samples, as well as LDL from patients with chronic kidney disease.

Conclusion: The accurate mass XIC method gave a lower false positive rate than normal database searching using statistical search engines, and identified more oxidatively modified peptides. A major advantage was that additional modifications could be searched after data collection, and multiple modifications on a single peptide identified. The oxPTMs present on albumin and ApoB-100 have potential as indicators of oxidative damage in ageing or inflammatory diseases.

P-478.00

THE REDOX INTERACTOME OF PTEN: CELLULAR AND PROTEIN SPECIFIC CHANGES

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Introduction: Phosphatase and tensin homolog (PTEN) is a redox-sensitive, dual-specificity protein phosphatase involved in regulating a number of cellular processes including metabolism, apoptosis, cell proliferation and survival, and acts as a tumor suppressor by negatively regulating the PI3K/Akt pathway. While direct evidence of a redox regulation of PTEN downstream signaling has been reported, the effect of cellular oxidative stress or direct PTEN oxidation on the PTEN interactome is still poorly defined. Here we present a correlation between cellular and PTEN redox status and PTEN protein-protein interactions.

Methods: PTEN-GST fusion was prepared in its reduced and H2O2-oxidized form and immobilized on a glutathione-sepharose-based support. The immobilized protein was incubated with a HCT116 cell lysate to capture interacting proteins. In parallel experiments, HCT116 cells transfected with a GFP-tagged PTEN were treated with H2O2 and the PTEN and interacting proteins immunoprecipated using standard methods. Bead, GST and GFP controls were also generated. Captured proteins were eluted from the beads, analyzed by LC-MSMS and comparatively quantified using label-free methods.

Results and Discussion: We were able to characterize a PTEN interactome through both immunoprecipitation from cells and resin immobilization of PTEN followed by capture from a cell lysate. These interactomes were largely similar, indicating that the immobilization affinity approach can be used successfully to identify interactomes. Some new interactors of PTEN were identified, and we were also able to show that the interactome of PTEN varies with redox status of the protein and the cell.

Conclusion: Our results suggest that the redox status of the cell or PTEN causes a functional variation in the PTEN interactome which is important for the cellular function of PTEN. The resin capture method developed had distinct advantages in that the redox status of the PTEN could be directly controlled and measured.

P-479.00

UNCOVERING THE TRACES OF OXIDATIVE DAMAGE IN OBESE PATIENTS

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Introduction

It is well known that reactive nitrogen and oxygen species (RNOS) can behave as effective second messengers under physiological conditions, and that their accumulation plays an important role in several pathologies. In obese individuals extensive accumulation of fat correlates with oxidative stress in adipose tissue due to the accumulation of RNOS, and mitochondria are thought to play a relevant role in the generation of these species. Hence, identification of redox targets and quantification of redox damage in mitochondria can help to develop therapeutic tools in the treatment of obesity.

Objectives

To investigate the role of oxidative stress in healthy obesity and in obesity linked to severe metabolic and cardiovascular disease.

Methods

We will assess the oxidation level of mitochondrial proteins from human adipocytes isolated from whole adipose tissue biopsies that are collected from morbid obese patients (BMI > 35 kg/m2) submitted for sleeve or gastric bypass surgical procedures. We will use GELSILOX (GEL-based Stable Isotope Labeling of OXidized Cys), a method that combines a robust proteomics protocol with a new computational approach that analyzes variance at the peptide level, allowing the simultaneous analysis of dynamic alterations.

Results and Discussion

We have optimized the isolation of adipocytes from the stromal-vascular fraction of human adipose tissue as well as the isolation of mitochondria from adipocytes. In a preliminary study we have identified mitochondrial proteins from adipocytes isolated from both omental and subcutaneous fat by LC/MS. An excellent enrichment for mitochondrial proteins as compared with adipocyte total protein content was found.

Conclusions

As soon as the necessary number of fat samples has been collected, we will carry out the differential protein oxidation study comparing healthy obese and obese individuals with severe comorbidity.

P-480.00

FUNCTIONAL PROTEOMICS ANALYSIS OF THE MAIN GATEWAY TO MITOCHONDRIA: DECODING THE MITOCHONDRIAL IMPORTOME IN TRYPANOSOMA BRUCEI

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Trypanosoma brucei, a unicellular parasite, is known as agent causing sleeping sickness in humans. It diverged from other eukaryotes early in evolution, which is manifested in an unusual mitochondrial biology. T. brucei features a single mitochondrion that undergoes extensive changes in the proteome during the parasite's life cycle. Analogous to other eukaryotes, ~99% of all mitochondrial proteins are posttranslationally imported. However, the mode of entry into the T. brucei mitochondrion is still poorly understood. We identified ATOM as main gateway in the outer mitochondrial membrane and exploited its import function to decode the mitochondrial importome.

Procyclic cells transfected with a plasmid for tetracycline-inducible knock-down of the target gene and untreated control cells were SILAC-labeled and mitochondria-enriched fractions were prepared. Triplicate experiments of six newly identified components of the ATOM complex including a label-switch were performed. Samples were subjected to gel-enhanced high-resolution UPLC/Orbitrap-MS followed by statistical evaluation and network analysis.

We quantified >4,000 proteins including ~1,000 proteins with known or predicted mitochondrial localization. Remarkably, knock-down of individual ATOM components led to different effects on the composition of the complex itself as well as on the mitochondrial proteome. Ablation of ATOM and POMP14 resulted in drastic changes, while protein abundances remained largely unaltered following knock-down of POMP16, a protein exhibiting functional homology to the yeast Tom20. Advanced data analysis enabled us to decipher the significance of the target proteins for the composition of a functional ATOM complex and, thus, mitochondrial protein import. Moreover, we exploited the gatekeeper ATOM to decode the mitochondrial importome and identify numerous novel mitochondrial proteins.

To conclude, we thoroughly characterized mitochondrial protein import via ATOM at a proteome-wide level, a strategy that further allowed us to decode the complete mitochondrial importome of T. brucei. Our study thus greatly contributes to a better understanding of mitochondria biology in T.brucei.

P-481.00

A NOVEL METHOD FOR ENRICHMENT OF CYSTEINE-CONTAINING PEPTIDES STARTING FROM LOW AMOUNT OF BIOLOGICAL MATERIALS

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Introduction and objectives:
The central hypothesis within this research area is that ROS/RNS mediated PTMs affect protein stability, activity, degradation and influences the physiopathological state of an organism. Cysteine residues are the primary targets of ROS/RNS species and the site-directed oxidation state of specific residues within a protein can affect its biological function. In addition, ROS/RNS mediated PTMs have a large influence on other PTMs such as phosphorylation thereby affecting signalling pathways inside these cells. To this end we wanted to develop a novel enrichment strategy based upon phosphate-like tags targeting cysteins thereby enabling easy and comprehensive characterization of cysteine containing peptides.

Methods:
Site-directed phosphate addition to cysteine residues was performed using a novel chemical tag and further enriched using TiO_2 and analysed by nLC-MS/MS. The method was initially developed for cysteine-containing synthetic peptide, in order to optimize the reaction conditions for the tagging and enrichment step. Furthermore we have extended the strategy to a single protein (bovine serum albumin) containing 35 cysteines and to a simple 12 protein mixture. Moreover, the method was extended to complex protein mixtures such as Hela cells and biofluids and shown to enrich cysteine-oxidized peptides.

Results and Discussion:
Using our strategy, we identified 9000 cysteine-containing peptides from 300ug of protein sample in a Hela cells extract. The method showed a high enrichment efficiency ranging from 87%-95%. Moreover, we were able to selectively enrich around 2000 and 1000 cysteine-containing peptides in serum and saliva, respectively.

Conclusions:
Taken together these results clearly show the development of a versatile enrichment strategy for cysteine-containing peptides by the selective chemical addition of a phosphate-like group followed by TiO_2 enrichment. The potential of this methodology is demonstrated by the facile enrichment of peptides bearing particular side-chain functionalities or PTMs from single protein to whole cell lysates as well as biofluids.

P-482.00

ANALYSIS OF CHMEICAL OXIDATION OF A DUAL SPECIFICITY PHOSPHATASE BY MASS SPECTROMETRY.

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Introduction: Protein oxidation plays an important role in both health and disease. In order to study protein oxidation, proteins are often chemically oxidised in vitro. However, many of the oxidants may not be residue specific, and may cause oxidation of a range of residues. Vaccinia H1-related protein (VHR; DUSP3) is a dual-specificity phosphatase important in controlling MAP kinase activity during cell cycle. It has an active site cysteine that acts as a nucleophile during catalysis. We have used this as a model to study the range and specificity of a number of commonly used oxidants and to correlate modification with protein activity.

Methods: VHR protein was subjected to oxidation with various concentrations of SIN-1, tetranitromethane, or HOCl. Phosphatase activity was determined by fluorimetric assay. The sites of oxidation were mapped using HPLC coupled to tandem mass spectrometry following in-gel digestion, and the percentage of oxidized peptide was determined by generating extracted ion chromatograms for the parent peptides.

Results and Discussion: More than 25 different oxidative modifications were detected, including oxidations of methionine, cysteine, histidine, lysine, proline and tyrosine. There was great variability in the susceptibility of particular types of residues, depending on their location in VHR and the oxidant used. The extent of oxidation was correlated with activity using statistical models.

Conclusion: Chemical oxidation of proteins results in very heterogenous oxidation and shows differential oxidant activity and residue susceptibility in a model signalling phosphatase.

P-483.00

PROFILING THE CYSTEINE REDOX PROTEOME: CHEMICAL VS. METABOLIC LABELING

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Reactive oxygen species (ROS) play a crucial role in the regulation of a large array of biological processes ranging from signal transduction and response to stimuli on a cellular level to the process of tissue repair. On the other hand, the accumulation of ROS induces oxidative stress, a phenomenon tied to a number of pathological conditions such as cancer and neurodegenerative diseases. At the proteome level, the presence of an increased amount of ROS leads to a number of posttranslational modifications (PTMs); notably the oxidation of cysteine residues leads to the formation of sulfenic acid (-S-OH), S-nitro groups (S-NO) and disulfides bridges, the latter known as oxidative folding.

The need of a better understanding of all these processes has led to the development of specific analytical strategies aiming to characterize and quantify PTMs on cysteine residues. However, redox proteomics remain a technical challenge due to the labile nature of thiol-redox reactions. Furthermore, compared to other PTMs, the number of modified residues per protein can be high. The low abundance of oxidized proteins, combined to the intrinsic heterogeneity of the oxidized forms is another source of complexity.

A robust shotgun proteomics strategy would take into account the change in protein expression when quantifying the change in cysteine oxidation. We already reported a proteomic strategy, called OcSILAC, allowing the profiling of protein expression and cysteine oxidation in cells. Here we present a protocol based on the use of mass spectrometry and cysteine chemical isotopic labeling. Our study evaluates to what extent our workflow could be applied to fields that are unsuitable to SILAC protocols (tissues, sera, etc.).

P-484.00

TETRAHYDROBIOPTRIN PROTECTED CARDIAC FUNCTION AND STRUCTURE IN TYPE 2 DIABETIC RAT HEART VIA MODULATION OF MITOCHODRIAL PROTEOME

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Diabetic cardiomyopathy is the major cause of mortality and morbidity in diabetes mellitus patients. Mitochondrial dysfunction has a significant role in the development and complications of diabetic cardiomyopathy. The aim of this study is to test the mitochondria mediated therapeutic potential of BH4 in the treatment of diabetic cardiomyopathy.

Fifty weeks aged LETO and OLETF rats were used as control and type 2 diabetes animal models respectively. Onset of diabetes was confirmed by intravenous glucose tolerance test (IGTT). Randomly selected OLETFs were administrated BH4 20mg/kg/day bolus i.p. during 2 weeks (OLETF/BH4). The mitochondrial BH4/ total biopterin ratio was significantly decreased in OLETF while, BH4 administration restored it. Non labeled LC-MS/MS quantitative proteomic analysis revealed differentially expressed proteins related with mitochondrial oxidative phosphorylation and proteasome activity. Functional enrichment analysis suggested that modulation of ROS generation and ATP metabolic process were significantly decreased in OLETF and restored by BH4 treatment.

As a phenotype of proteomic alteration, echocardiography revealed dilated dysfunction in OLETF and OLETF/BH4 model compared to LETO. BH4 treatment significantly increased left ventricular contractility in OLETF resulting in enhanced ejection fraction and fractional shortening. BH4 treatment also attenuated the left ventricular hypertrophy and fibrosis in OLETF. Proteomic analysis revealed intensive modulation of mitochondria respiratory chain complex and proteasome activity related proteins in OLETF and OLETF/BH4. Mitochondrial membrane potential, electron transport chain complex activity and ATP concentration were decreased in OLETF model and BH4 treatment successfully restored those. Interestingly, increased oxidative stress in OLETF heart tissues were significantly attenuated by BH4 treatment.

Proteasome activity was significantly increased in OLETF while, BH4 treatment significantly attenuated it. These results suggest that BH4 has therapeutic potential which corrected mitochondrial dysfunction resulting enhancement of LV contractility in diabetic cardiomyopathy

P-485.00

REDOX PROTEOMICS FOR EVALUATION OF PERITONEAL DIALYSIS INDUCED OXIDATIVE STRESS

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Characteristics of conventional peritoneal dialysis fluids (PDF) together with clinical factors such as time on PD, episodes of peritonitis or presence of the catheter are drivers of oxidative stress in the peritoneal cavity inducing severe alterations to proteins, thus impairing cell viability. Redox proteomics based on high-sensitive fluorescent cyanine dyes (CyDyes) and two-dimensional difference gel electrophoresis (2D-DIGE) offer a promising new tool to gain insight into the spectrum of oxidative protein modifications induced by PD fluids (PDF).

We compared protein profiles of mesothelial cells (MeT-5A), exposed for 16h to either commercial PDF, or to hydrogen peroxide (50mM H2O2) or normal growth medium as controls. Depending on the labeling protocol, the used CyDyes label reduced thiol groups of cysteines, major targets of oxidation processes (direct labeling) or previously oxidized cysteines after reversal of their oxidation status by a reductant (indirect labeling). Cell injury was monitored using lactate dehydrogenase (LDH) release. Measuring intracellular reactive oxygen species (ROS) and oxidized DNA (8-OHdG) was performed as control method.

As expected, a oxidative cell injury by PDF or H2O2 compared to control was reflected by increased intracellular ROS, 8-OHdG and LDH release,, indicating that the used assay conditions titrate the levels of oxidative stress and toxicity to a comparable level. Using direct and indirect labeling, we observed decreased abundance of reduced cysteines and increased abundance of oxidized cysteines in H2O2 and PDF treated samples. The spot pattern showed a differential oxidation status of numerous oxidation-sensitive protein spots.

Our technique successfully applies the gold standard in oxidative stress research H2O2 to establish a redox proteomics workflow in experimental PD. The ongoing identification of differentially oxidized protein spots by mass spectrometry will form the basis for bioinformatic interpretation. Application of this technique to clinical PD effluents will allow evaluation of novel interventions and biomarkers of oxidative stress.

P-486.00

LARGE SCALE PROTEOMICS REVEALS REGULATION OF MULTIPLE SIGNALLING PATHWAYS IN FIBROBLASTS FROM MAPLE SYRUP URINE DISEASE CLASSIC PATIENTS

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Introduction and objectives:

Deficiency in branched chain α-ketoacid dehydrogenase (BCKDH) causes Maple Syrup Urine Disease (MSUD), an inherited metabolic disorder with heterogeneous presentation and no tight genotype-phenotype correlation in most cases. The classical form accounts for 70% of MSUD patients and is associated with profound neurological impact and high mortality if not treated early. BCKDH is mitochondrial multienzyme complex involved in the catabolism of branched-chain amino acids (BCAA) and is encoded by four genes: E1α (BCKDHA), E1β (BCKDHB), E2 (DBT), and E3 (DLD).

Methods:

To study the molecular effects of BCKDH deficiency, we performed large scale proteomics on fibroblasts from two unrelated MSUD classic patients and two healthy individuals. The patients have null mutations in BCDKHA and BCKDHB genes, respectively, leading to instability of E1α and E1β monomers. Since BCKDH is a mitochondrial enzyme, we investigated mitochondrial-enriched samples in addition to total cell samples by an iTRAQ-based mass spectrometry strategy.

Results and discussion:

The iTRAQ studies confidently quantified more than 1800 proteins in the cell sample and 262 in the mitochondria sample, with 95 and 8 proteins differentially expressed in MSUD patients, respectively. Pathway analysis of this regulated proteins revealed a protein profile associated with different biological processes: intracellular signalling, oxidative stress, structural remodelling, and neuronal related pathways. In general metabolic pathways and mitochondrial proteins were largely unaffected. Several intracellular signalling pathways connected by MAPK were up-regulated: chemokine, neurotrophin, long-term potentiation, tight junction, and regulation of actin cytoskeleton. Up-regulation of Thioredoxin-dependent peroxide reductase (PRDX3), ferritin heavy chain (FTH1) and Cytochrome c1, heme protein (CYC1) indicates an iron-mediated oxidative imbalance. Moreover, patients´ fibroblasts showed increased mitochondrial superoxide levels (2-fold) and carbonylated protein levels (1.5-fold).

Conclusions:

These results shed light on the aetiology and symptoms associated with MSUD, as well as highlight the potency of BCAAS as regulators of different signalling pathways.

P-487.00

MITOCHONDRIAL PROTEOME CHANGES IN ETHE1-DEFICIENT MICE:INDICATION OF METABOLIC IMBALANCE

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Introduction and objectives:
Deficiency of mitochondrial sulfur dioxygenase (ETHE1) causes the severe metabolic disorder ethylmalonic encephalopathy, which is characterized by early-onset encephalopathy, chronic diarrhea, and defective cytochrome C oxidase (COX) because of hydrogen sulfide accumulation. A Biochemical hallmark is high concentration of ethylmalonic acid in urine which also is characteristic of the fatty acid beta-oxidation defect short chain acyl CoA dehydrogenase deficiency (SCADD). Although the severe systemic consequences of this sulfide detoxification dysfunction are becoming clear, the molecular effects are not well known and we therefore performed a large scale quantitative proteomics study for further elucidating the effects of ETHE1 deficiency.

Methods:
Peptide labeling with iTRAQ was performed on six wild type and KO mice, after liver tissue homogenization and two phase separation of proteins. Labeled peptides were identified and quantified by nLC-MS/MS (LTQ Orbitrap, Thermo). DAVID and STRING were used to identify enriched functional annotation terms and clustering within the data.

Results and Discussion:
Down-regulation of several proteins related to oxidation-reduction such as different dehydrogenases, cytochrome P450 members and those active in oxidative stress response like catalase and glutathione s transferase, indicate clear links between ETHE1 deficiency and redox active proteins. The ETHE1 deficiency was also shown to alter the cellular energy metabolism, in the form of up-regulation of enzymes active in glycolysis such as aldolase and pyruvate kinase together with the gluconeogenetic pyruvate carboxylase, which all in all confirmed cellular regulation to compensate the lack of energy due to electron transport chain corruption.

Conclusion:
Our data suggest that ETHE1 deficiency is clearly linked with alteration in levels of several redox active proteins and also reprogramming in metabolic pathways, and thus shed light on important functions influenced by ETHE1 deficiency and by the concomitant increase in the gaseous mediator hydrogen sulfide.

P-488.00

THIOL REDOX PROTEOMICS IN NITRIC OXIDE-INDUCED NEURAL STEM CELLS PROLIFERATION AND NEUROGENESIS

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Introduction And Objectives:
The discovery of neural stem cells (NSC) in the adult brain opened the doors for a whole new field of research dedicated to harness the power of these stem cells to replenish the lesioned brain with new neurons, either by transplantation or by stimulation of endogenous neurogenesis. However, the identification of the factors that trigger proliferation of stem cells in the brain after a lesion is still ongoing. It has been recently shown that nitric oxide (NO) is key player during brain injury and is essential for neurogenesis following excitotoxic brain lesions. One of the non-classical NO signalling modes of action proceeds through the formation of reversible oxidative modifications of protein cysteine thiols, including S-nitrosylation.
In order to understand how proliferation and neurogenesis are triggered following brain injury, our aim is to identify the S-nitrosylation and other oxidative modification protein targets of NO.

Methods:
We have applied complementary thiol redox proteomics techniques to identify targets of cysteine reversible oxidation in NSC treated with low dose of NO donors and S-nitrosocysteine.
"Redox Fluorescence Switch" (RFS) replaces reversible cysteine oxidation by a fluorophore, and differentially oxidised proteins are detected by fluorescent 2DE. With FaSILOx (filter-based adaptation of GelSILOx), reduced and oxidised cysteines are differentially labelled, and massive identification and quantification allows the estimation of the relative variation in the oxidative status of Cys-containing peptides. Protein-directed methods allow to evaluate p21Ras modifications on Cys118.

Results And Discussion:
We have identified a number of targets of S-nitrosylation and other NO-induced post-translational modifications in NSC. Among them, p21Ras S-nitrosylation can be an initial event leading to NSC proliferation and neurogenesis.

Conclusions:
The techniques we have developed can be useful for studying NO-induced reversible protein oxidation in conditions close to the physiological environment, where these modifications can be involved in cell signalling.

P-489.00

COUPLING OCSILAC WITH SUBCELLULAR PROTEIN FRACTIONATION FOR CYSTEINE-REDOXOMICS

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Introduction

Reversible cysteine oxidation is a post-translational modification serving in many cellular functions. A systematic characterization of the cysteine redox state at the proteomic scale is a key step to understand the thiol-redox based molecular mechanisms. The major issue of this kind of analysis is the high percentage of reduced cysteines in the cytoplasmic and nuclear fractions that could constitute 64% of the proteome. Our group has already developed a protocol, called OcSILAC, aimed to improve the recovery of oxidized cysteines and quantify the reversible thiol oxidation considering also protein expression profiles. However, we still observed limited recovery of proteins belonging to more oxidative cell compartments as Endoplasmic Reticulum, the secretory pathway and membranes. The aim of this study is to integrate subcellular protein fractionation into the OcSILAC workflow.

Methods

The protocol has been adapted from the commercial Thermo-Pierce "Subcellular Protein Fractionation Kit for Cultured Cells ". Reactions were optimized with 2.5×10^6 wild-type Hela cells. Reduced thiols saturation is achieved with iodoacetamide and monitored using the Dylight 550 (Thermo-Pierce Cy3-like maleimide) by SDS-PAGE.

Result and Discussion

In order to be compatible with cysteine-redoxomics, the protocol should allow the saturation of reduced thiols after cell lysis to circumvent air mediated oxidation, disulfide bonds shuffling and false positive assignations. We noticed that subcellular protein fractionation according to the commercial protocol does not yield to the complete alkylation of free thiols. Different reaction conditions (temperature, reaction time and reaction stoichiometry) were tested for each subcellular fraction to establish a final protocol.

Conclusion

The reduced thiol saturation of subcellular fractionated protein could be achieved inducing minimal impact to the quality of fractionation. Moreover, the degree of exogenous thiol oxidation induced by fractionation in comparison with direct TCA precipitation will be evaluated by OcSILAC analysis.

P-490.00

NEW FRONTIERS IN BACTERIAL INFECTION: THE 'TARGET' MITOCHONDRIA

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Introduction The central hubs of energy production is represented by mitochondria that are key cellular organelles. They dynamically change and they have different function, as apoptosis regulation, the production of biosynthetic intermediates and energy to innate immune signalling and cellular calcium buffering or the storage of pro-apoptotic components; moreover they are emerging as targets of pathogens to control the cell fate Aim The aim of this work is to investigate on the interaction of Staphylococcus aureus with the cellular and mitochondrial system of macrophages in a robust in vitro model, using a combined proteomic approach.

Methods Monocytes/macrophages coming from cattle were purified, coltured and then the purified monocytes mitochondrial fraction were analysed by nLC-MS/MS. Experimental data were elaborated using multivariate statistics and protein functional classification

Results and Discussion Several experimental workflows were optimized to obtain a detailed proteomic portrait from SA and immune cells during the host-pathogen interaction. In particular mitochondrial processes impaired by the experimental infection.

Conclusion Data obtained from this investigation address to a better comprehension of the interaction between common pathogens and host immune cells, a topic involving human healthcare as well as animal health. This knowledge will permit the development of new diagnostic biomarkers and new therapeutic resources.

P-491.00

MITOCHONDRIA PROTEIN REPERTOIRE ADAPTATION IN ANIMAL MODELS OF AMIOTROPHIC LATERAL SCLEROSIS

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Introduction and objectives : Amyotrophic lateral sclerosis (ALS) is a lethal disease characterized by motor neuron degeneration. Many etiologic factors are implicated, however it is accepted that a severe mitochondrial dysfunction leads to an unavoidable neuronal death. Until recently hydrogen sulfide (H2S) was recognized as an endogenous neuromodulator. In the brain H2S is mainly produced by astrocytes and microglia through the cystathionine-β-synthase (CBS), a cytoplasmatic enzyme that accumulates in mitochondria under oxygen sensitive conditions. H2S inhibits complex IV of the mitochondrial respiratory chain; impairments in the complex IV-drive respiration have been described in SOD1G93A mice. Therefore, the aim of this study is to investigate the hypothesis that H2S, closely related to mitochondrial disfunction, could be involved in ALS onset and/or progression.

Methods: we developed specific HPLC test to measure H2S levels in tissues and in spinal cord cultures of SOD1G93A mice. To investigate the interaction H2S-mitochondria, these results were extended to a deeper proteomics analysis by a shotgun nLC-MS/MS profiling.

Results and Discussion: We found increased H2S levels in tissues and in spinal cord cultures of SOD1G93A mice. We showed that endogenous H2S is released by astrocytes and microglia and that added H2S is toxic for motor neurons. An accumulation of CBS has been shown significant in the mitochondrial-enriched fraction, probably linked to hypoxic stress and associated to the increased production of H2S. We also found that H2S causes an increase of [Ca2+]i in single motor neurons, possibly due to inhibition of mitochondrial metabolism.

Conclusions: Based on these data, the H2S toxic effects seem to associate with phenotype development in ALS. We may infer that H2S over-production, due to the reactive gliosys, contributes to the motor neuron degeneration. We hypothesize that the increased H2S amount reaching toxic levels could further distress an already compromised mitochondrial function.

P-492.00

QUANTITATION OF OXIDATIVE STRESS RESPONSE PROTEINS IN PRIMARY CARDIOMYOCYTES USING NEUCODE SILAC

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Introduction

Primary cardiomyocytes are widely used to study the mechanisms of cardiovascular function and disease. Since primary cardiomyocytes cells do not replicate and begin to lose function after a week in culture, they are difficult to completely label using Stable Isotope Labeling with Amino Acids in Cell Culture (SILAC). To compare differences in protein expression after oxidative stress, we used a neutron-encoded (NeuCode) SILAC method to incorporate two heavy isotopologues of L-lysine into proteins for relative quantitation using mass spectrometry (MS).

Methods

The Thermo Scientific™ Pierce™ Primary Cardiomyocyte Isolation Kit was used to isolate and culture cardiomyoctyes from hearts of 1-day-old CD1 neonatal mice. Cardiomyocytes were cultured in SILAC DMEM media supplemented with dialyzed FBS and 13C615N2 L-lysine or D8 L-lysine for 3-7 days. After stable isotope incorporation, cells were treated with 10μM or 1 mM hydrogen peroxide for 2-8 hrs to induce oxidative stress response pathways or apoptosis. Cell lysate proteins were equally mixed, reduced, alkylated and digested with LysC for 16 hours at 37oC. Peptides were analyzed using a Thermo Scientific™ Orbitrap Fusion™ instrument.

Results and Discussion

Unlike traditional SILAC which uses light and heavy amino acids, NeuCode uses multiple heavy amino acids to compare protein levels. This enables relative quantitation of partially labeled heavy proteins making it an ideal method for labeling primary cardiomyoctes. LC-MS analysis of cardiomyocytes treated with and without hydrogen peroxide identified over 4000 proteins including cardiomyocyte-specific marker proteins such as cardiac troponin I, caveolin-3 and GATA-4.Stable isotope incorporation for heavy proteins ranged from 30-50% after 3 days of culture and increased to 50-70% after 7 days. Using high resolution MS, changes in protein abundance were observed for numerous proteins involved in oxidative stress and apoptosis signaling.

Conclusions

NeuCode SILAC enabled relative quantitation of protein abundance in primary cardiomyocytes following oxidative stress treatment.

P-493.00

ANALYSIS OF THE THIOL REDOX PROTEOME OF HYPOXIC TREATED HUH7 CELLS AND POST-ISCHEMIC REPERFUSION MOUSE LIVER

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Background: Oxidative stress has been increasingly recognized as a driver mechanism of acute and chronic liver disease progression. The identification of protein targets of reactive oxygen species (ROS) appears then crucial to fully understand the mechanisms orchestrating the cellular response to a redox imbalance. To Explain pathogenic mechanisms in the liver through the identification of protein targets of ROS that undergo reversible oxidation.

Methods: HuH7 cells were exposed to diamide or cultured under a hypoxic environment using a mixture of $CO_2/N_2/O_2$ at 6, 93 and 1% respectively for 2 hours. Mitosox and JC-1 reagents were used to measure mitochondrial O_2- and membrane potential in vitro. Partial ischemia was induced to mouse liver during 75 minutes followed by a 3 hours reperfusion. Protein samples were analysed using dye BODIPY Fl C1-IA and proteomic approaches.

Results: In vitro analysis showed an increase in superoxide production, carbonylation and reversible thiol oxidation. Proteins undergoing reversible cysteine oxidation were analysed by 2D electrophoresis based differential proteomics. 109 and 62 differential spots were detected upon diamide or hypoxia treatments leading to the identification so far of 24 and 15 proteins respectively. These proteins revealed impairment cellular functions including metabolism, stress response, energy production and protein degradation and provide novel insights on the mechanisms mediating the liver cell redox imbalance.

Conclusion: This study allow the identification of an important number of protein targets of ROS that undergo reversible cysteine thiol oxidation and might mediate pathogenic processes in the liver associated to oxidative stress.

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Topic 11

Top-down proteomics and macromolecular complexes

OP092 - PROTANNOTATOR: A SEMIAUTOMATED PIPELINE FOR CHROMOSOME-WISE FUNCTIONAL ANNOTATION OF THE "MISSING" HUMAN PROTEOME

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The chromosome-centric human proteome project (CHPP) aims to define the complete set of proteins encoded in each human chromosome. The neXtProt database (September 2013) lists 20 128 proteins for the human proteome, of which 3831 human proteins (~19%) are considered "missing" according to the standard metrics table (released September 27, 2013).

In support of the C-HPP initiative, we have extended the annotation strategy developed for human chromosome 7 "missing" proteins into a semiautomated pipeline to functionally annotate the "missing" human proteome. This pipeline integrates a suite of bioinformatics analysis and annotation software tools to identify homologues and map putative functional signatures, gene ontology, and biochemical pathways. From sequential BLAST searches, we have primarily identified homologues from reviewed nonhuman mammalian proteins with protein evidence for 1271 (33.2%) "missing" proteins, followed by 703 (18.4%) homologues from reviewed nonhuman mammalian proteins and subsequently 564 (14.7%) homologues from reviewed human proteins. Functional annotations for 1945 (50.8%) "missing" proteins were also determined. To accelerate the identification of "missing" proteins from proteomics studies, we generated proteotypic peptides in silico.

Matching these proteotypic peptides to ENCODE proteogenomic data resulted in proteomic evidence for 107 (2.8%) of the 3831 "missing proteins, while evidence from a recent membrane proteomic study supported the existence for another 15 "missing" proteins. The chromosome-wise functional annotation of all "missing" proteins is freely available to the scientific community through our web server (http://biolinfo.org/protannotator).

OP093 - PROTEOMICS STUDIES SUGGEST THAT THERE ARE JUST 19,000 PROTEIN CODING GENES IN THE HUMAN GENOME

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Introduction and objectives
Determining the protein coding potential of genes is a key goal of human genome annotation projects. As the most powerful tool for detecting cellular expression of proteins, peptide mass spectrometry is an attractive approach for verifying the protein coding ability of individual genes.

Methods
Here we analyse the peptides detected in 7 large-scale proteomics studies and databases. We identify protein expression for almost 60% of the protein coding genes in the GENCODE v12 annotation of the human genome.

Results and Discussion
We find that the age of the gene and its conservation across vertebrate species are key indicators of whether protein expression will be detected in proteomics experiments. The correlation between protein expression and gene age and cross-species conservation is surprisingly strong. We detect peptides that map to practically all genes that evolved before bilateria and for most highly conserved genes. Combining gene family age with conservation gives even more striking results. We detect at least two peptides for 96.5% of highly conserved Fungi-Metazoa family age genes.

By way of contrast we find little or no evidence of protein expression for recently evolved genes, those that have appeared since primates, and we find very few peptides for genes that have no protein-like features or conservation.

Conclusions
We describe a set of 2,001 genes with poor conservation, that have no protein features or that have ambiguous functional annotations. We show that the vast majority of these genes are not detected in proteomics experiments, and are do not conserve the frame in related species. Many of these genes will not code for proteins under normal circumstances and should not be included in the human protein coding gene catalogue. We estimate that the real numbers of protein coding genes in the human genome is close to 19,000.

OP094 - BIOINFORMATICS PIPELINE AND MASS SPECTROMETRY-BASED METHODOLOGIES FOR THE DISCOVERY OF MISSING PROTEINS: THE CASE OF HUMAN CHROMOSOME 2 AND 14

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The Chromosome-Centric Human Proteome Project (C-HPP) is an international collaborative effort which aims at mapping and characterizing all proteins encoded by the genes on each human chromosome. To reach this goal, one of the priorities is to identify all the proteins that currently lack evidence at protein level, called "missing proteins", by mass spectrometry (MS).

In this framework, the French and Swiss proteomics groups have adopted human chromosome 2 and 14 for which 210 and 123 proteins are still considered as missing, respectively. To tackle this challenge, the Proteomics French Infrastructure has been collecting high quality datasets encompassing 27 human samples analyzed during the last 4 years, including a series of rarely studied cell/tissue types. 36134 .dat files were reprocessed through a computational analysis including filtering criteria, leading to a list of 59 "missing proteins" with at least one associated MS/MS spectrum.

Manual verification of each spectrum allowed us to confidently map at least one unique peptide on 28 "missing proteins". These 28 proteins were considered for further MS validation. First, MS/MS spectra of the native endogenous peptides were compared to their corresponding synthetic peptides for assessing whether the fragmentation patterns were similar. In a second step, 5 proteins identified with a single unique peptide were selected on the basis of our ability to reproduce the original biological sample preparation. LC-SRM assays were developed to target proteotypic peptides from these 5 "missing proteins" using heavy labeled synthetic peptides. LC-SRM analysis following light and heavy transitions allowed unambiguously detecting the endogenous unique peptide for 3 proteins (confirmed by additional proteotypic peptides for 2 proteins).

We conclude that a rigorous step-wise approach combining bioinformatics analysis and MS-based assays is particularly suitable for "missing proteins" confirmation.

P-494.00

MONOCLONAL ANTIBODY AND RELATED PRODUCT CHARACTERIZATION UNDER NATIVE CONDITIONS USING A BENCHTOP EXACTIVE PLUS EMR MASS SPECTROMETER

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Native MS approach is now ready for the routine characterization of heterogeneous therapeutic monoclonal antibodies. Native MS has gained interest not only for intact mAb analysis but also for antibody-drug conjugates (ADCs), antibody-antigen complexes mAb/Ag, and antibody mixture characterization, mostly because of simplified data interpretation due to the presence of fewer charge states compared to classical denaturing MS. Here, a new Orbitrap based mass spectrometer- Exactive Plus Extended Mass Range (EMR), with an extended mass range of up to m/z 20,000, is used for characterizing mAbs, ADCs, mAb/Ag, and mAb mixtures under native conditions.

One single antibody standard, an ADC, one mAb/Ag sample, as well as a mixture of eleven distinct deglycosylated humanized IgG antibodies, was individually introduced using an Advion TriVersa NanoMate with chip based nanospray ionization in the positive mode to the Exactive Plus EMR MS system. Orbitrap detection parameters were set according to the type of analyte measured. Deconvolution of signals measured for determination of molecular masses and relative abundances of the analytes were carried out using Protein Deconvolution software.

The Orbitrap high resolving power can baseline resolve the native mAb's glycan peaks with an excellent mass accuracy in low ppm range. A sensitively characterize ADC complexes with mass differences between peaks corresponding to different additional number of payloads/drugs are achieved. For each set of peaks, the drug-to-antibody ratio (DAR) can be determined as well as the relative ratio of each detected compound. The number of antigens bound to mAbs and the relative abundances of mAb/Ag complexes at different stoichiometries can be achieved from MS peak intesities. The mAb mixtures are resolved and detected with an excellent mass accuracy for each individual mAb by using the high resolving power of the Orbitrap technology.

P-495.00

SIMPLIFIED MONOCLONAL ANTIBODY CHARACTERIZATION BY MIDDLE-DOWN/UP STRATEGY

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Introduction and objectives

Development of protein biotherapeutics remains a strong area of growth in the pharmaceutical industry. This is essentially accounted for by research into monoclonal antibodies (mAb) or derivatives thereof. As such, mAb characterization and analysis is a topic of intense interest and importance, not only on the R&D front, but also for QC. One facet of this characterization relates directly to protein structure, apart from any functional assessment. Analytical characterization of primary protein structure can benefit from simplification of the sample; that is, instead of focusing analytical operations on the intact mAb, fragmenting the mAb into a few component domains can simplify data analysis and interpretation. One such recent strategy employs the immunoglobulin degrading enzyme from Streptococcus (IdeS). Proteolysis of IgG by IdeS, followed by disulfide reduction, yields three similarly sized fragments of ~25 kDa (LC, Fd', Fc), which are now more amenable to top-down or bottom-up strategies for characterization.

Methods

A mAb standard is proteolyzed IdeS. Proteolysis can be monitored by size-exclusion chromatography or other chromatographic modes. Upon completion of proteolysis, protein disulfides are reduced by a small thiol. The sample is then chromatographed on a BIOshell A400 Protein C4 column, to resolve the three fragments.

Results and Discussion

In applying a conventional reversed-phase water-acetonitrile gradient mobile phase system with an acidic ion-pair reagent, high performance separation of the three mAb fragments is achieved, but only at high temperature (90° C). Any lower temperature, and peak shape suffers considerably. Temperatures >90° C are not recommended in the interest of long-term column stability.

Conclusions

High temperature reversed-phase chromatography of the reduced IdeS fragments of IgG can be efficiently achieved on a wide-pore Fused-Core column, providing a protocol for simplified subsequent characterization of mAb biotherapeutics.

P-496.00

MIGRATION ASSOCIATED PROTEINS IN HUMAN CORNEAL EPITHELIAL CELLS (HCEC) AND ITS ROLE IN WOUND REPAIR

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Introduction and Objectives:
Corneal related complications are major health concerns worldwide because its progression is associated with significant impaired vision. Therefore, there is an urgent need to develop reliable understanding of the underlying mechanism of corneal epithelial wound healing to apply therapeutic options.
• We aimed to investigate the alterations in protein expression during corneal epithelial migration.
• To demonstrate the networks of the total identified proteins with potential dual functions.

Methods
In this study, human corneal epithelial cells lines (HCEC) have been used for wound healing model. Mechanical wound was made in HCEC lines and healing was monitored at 24, 48 and 72 hours of post wounding. Epithelium was scrapped at 24, 48 and 72 hours, followed by protein quantification using BCA kit. The wounded and unwounded cells were subjected to SDS-PAGE and two dimensional electrophoresis (2DE). Mass Spectrometry (MALDI TOF) was done to identify the proteins through protein database searches. The identified protein were further analyzed and validated by western blot analysis. A further insight into the links among the identified proteins and their functional roles were analysed by STRING 8.3, KEGG and REACTOME pathway databases.

Results
A significant finding of the present study is the identification of Cdk10, EFNB3, RAB 34, RRAS, HSP22 and HSP90 in healing corneal epithelium at active phase of migration. The results were further validated using Cdk10 antibody by western blot. Interaction association network analysis further confirms the close interacting relationship among identified proteins.

Conclusion
The present communication initially provides new evidence for the potential role of identified proteins in migrating epithelial cells. We assume that these findings are one step forward in identifying the mechanism of wound repair or re-epithelialization. This study may also increase the understanding of normal and abnormal corneal function with likely relevance to corneal disease and transplants.

P-497.00

HIGH-MOLECULAR-MASS PROTEOMICS BY TWO-DIMENSIONAL GEL ELECTROPHORESIS WITH AGAROSE AND POLYACRYLAMIDE HYBRID GELS IN THE SECOND DIMENSION

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Many high-molecular-mass (HMM) proteins (MW>100kDa) are known to be involved in important biological functions, such as signal transduction, transcription and translation. Though a variety of proteomic techniques have been described, purification of a HMM protein as an intact molecule remains a difficult task.

A major limitation of MS-based proteomics is that the connection between digested peptides and their assembly into protein variants is difficult to achieve if some alternatively spliced variants encoding a protein are present. A two-dimensional gel electrophoresis method employing agarose gels in the first dimension (Agarose 2-DE) is sufficiently good at separating HMM proteins as large as 600 kDa.

However, some of the HMM proteins could not enter the polyacrylamide gels in the second dimension. When we applied agarose and polyacrylamide hybrid gels for the Agarose 2-DE in the second dimension, most of the HMM proteins were successfully analyzed. Our method would provide a means toward clarifying a comprehensive view of alternative splicing variants of HMM proteins.

P-498.00

UNDERSTANDING THE STRUCTURAL DETERMINANTS FOR THE SELECTIVE INHIBITION OF SNAKE VENOM METALLOPEPTIDASES

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Natural inhibitors of snake venom metallopeptidases (SVMP) have been isolated from the plasma of venom-resistant mammals (DM43) and reptiles (BJ46a). Due to the lack of three-dimensional information, the structural features that govern the interaction are largely ignored.

This study aims to investigate which molecular regions are important for their biological activity. Complex-type N-glycans were removed from the inhibitors under native conditions following digestions with neuraminidase, beta1-4 galactosidase, beta-N-acetylglucosaminidase and PNGase F. Deglycosylation efficiency and complex formation between the inhibitors and the SVMP were monitored by gel electrophoresis and mass spectrometry. Attempts to crystallize DM43 and BJ46a for X-ray studies have been unsuccessful. The present results show that the glycan part of the inhibitors significantly contributes to their mass heterogeneity and may hinder the formation of good-quality crystals. Glycan removal was more easily achieved with the inhibitor from reptile. Extensively deglycosylated BJ46a was able to form complex with jararhagin, effectively inhibiting its proteolytic activity. Most inhibitor studies have used the more hemorrhagic P-III class of SVMP as target toxin.

To simplify the interpretation of ongoing complementary studies on protein-protein interactions, the study has shifted focus to P-I SVMP, smaller toxins bearing only the catalytic domain of metallopeptidases. The inhibitors were tested against the following panel of P-I SVMP: leucurolysin-a, BaP1, atroxlysin-I and atrolysin c. Except for atrolysin c, native DM43 formed complex with all tested SVMP. Native BJ46a interacted with all SVMP, with the exception of leucurolysin-a. Cross-linking and hydrogen/deuterium exchange analyses combined with MS are being used to deepen our knowledge on the interaction between P-I SVMP and natural antitoxins. Glycan-free inhibitors and native complexes, made of selected P-I and DM43 or BJ46a, are now being submitted to new crystallization trials. Endogenous protein inhibitors can be used as structural templates, providing insights into the molecular determinants of selective metallopeptidase inhibition.

P-499.00

NEW WEAPONS AGAINST PERVASIVE FISH FRAUD: TOP DOWN PROTEOMICS AND A NEW HIGH THROUGHPUT LC-MS PLATFORM

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The largest seafood fraud investigation in the world to date found 33% of seafood samples were mislabeled in the U.S. Seafood fraud hurts consumers and also raises serious preservation concerns. DNA-based analytical techniques are commonly used to determine the specie that belongs to a given sample. However, the long analysis time limits its applicability.

Here, we present a protein based method that identifies most common commercially available salmon species in minutes using a simple protein extraction protocol coupled to a novel high throughput LC-system and top-down proteomics. Reference samples from species of the salmon family were compared to commercially available fish foodstuffs. Protein extraction was carried out by homogenizing 5 g of muscle. Water soluble proteins were centrifuged, the supernatant heated at 70 ºC for 5 min and centrifuged again. Soluble proteins then were loaded onto a stage tip and placed in a stage-tip based sample delivery system coupled to a high-pressure microflow LC. This system cleans-up the samples before being introduced to the LC-MS system without generating any losses. An 8 min LC-gradient was performed and the proteins were then analyzed intact or by top-down HCD using a benchtop quadrupole Orbitrap mass spectrometer. Spectra were analyzed using Thermo Scientific Protein Deconvolution 2.0 and ProSight PC 3.0 software.

For all the samples, an 11 kDa protein was the most abundant protein and identified as a parvalbumin (PRVB). This protein is highly conserved among different species. Nevertheless, slight mass differences of the PRVBs corresponding to amino acid substitutions allow for unambiguous systematic discrimination of the different species. We believe that this workflow coupled to this technology platform could represent the ultimate payoff to reliable and widely used top down proteomics to trace and authenticate food.

P-500.00
EVALUATION OF PROTEOLYTIC EXCISION ONLINE SAW-BIOSENSOR-MASS SPECTROMETRY TO MAP CONTACT SURFACES: APPLICATION TO VEGF-AVASTIN SYSTEM

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Introduction and objectives
Recently, Przybylski et al. developed an online bioaffinity-mass spectrometry system using the surface-acoustic wave biosensor (SAW) and an MS interface (MS-I). The combination SAW-MS provides detection, quantification and mass spectrometric structural characterization of affinity bound biopolymers. We describe here a targeted MS analysis of eluted VEGF (Vascular Endothelial Growth Factor protein) after interacting with Avastin antibody. In addition, we explore different strategies based on proteolytic digestion and MS-peptide mapping (epitope excision) in combination with the SAW-MS system to determine the epitope of VEGF against Avastin and evaluate these strategies as a direct methodology for epitope determination.

Methods
Online bioaffinity-MS analyses were performed with a Sam-5 biosensor (SAW-Instruments). The developed MS-I was used as the interface coupled to a LTQ-FT Ultra and a Synapt-HDMS mass spectrometer. Firstly, Avastin was covalently bound on a gold chip surface. Then, VEGF was affinity bound to the immobilized antibody. Elution of the protein was carried out with HCl and the eluate was online desalted and concentrated in the MS-I and directed to the mass spectrometer. In independent experiments, peptide digests of VEGF were bound to the Avastin immobilized chip. After washing and removing unbound digested peptides, elution was performed with HCl to determine the interacting VEGF peptides. Online proteolysis on the chip surface was also evaluated by using different proteases and conditions.

Results and discussion
VEGF was MS characterized after its interaction with Avastin by online bioaffinity MS. Different strategies based on proteolytic digestion and MS-peptide mapping (epitope excision) of VEGF were studied and the results contrasted with previously described epitope.

Conclusions
Our results suggest that the direct combination of SAW to MS, can be used as a straightforward method for the identification of epitopes and highlight the VEGF-Avastin system as an example.

P-501.00

CLINICAL SCREENING OF HEMOGLOBINOPATHIES USING TOP DOWN MASS SPECTROMETRY

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Introduction

Hemoglobinopathies include genetic defects that result in an abnormal structure of one of the globin chains of the hemoglobin molecule. Common hemoglobinopathies include sickle-cell disease and it is estimated that 7% of world's population are carriers. Thalassemias, often diagnosed using the same test, usually result in underproduction of normal globin proteins. They may overlap since some conditions, which cause abnormalities in globin proteins can also affect their production. In order to meet the requirements for a rapid, reliable screen with a low cost per test a method has been developed that does not require enzymatic digestion or chromatographic separation.

Methods. The approach selected is based on top down protein sequencing utilising ETD ion activation. After deconvolution, the multiply charged ESI derived intact globin chains from a denatured blood sample are precursor ion selected and, subjected to ETD activation. The resultant product ion spectra are analysed using in-house software routines and clinically significant variants identified. The experiments were carried out using an ion trap equipped with ETD fragmentation capability (Bruker Daltonics).
Preliminary   Data

A three-month mirrored clinical trial on 2017 antenatal patient blood samples has been carried out in which the established clinical approach based on cation exchange liquid chromatography was used in conjunction with the proposed mass spectrometry based method.. . The existing clinical method was found to give a false positive result on two samples, which were identified as HbD but were in fact identified unambiguously by mass spectrometry as HbG-Philadelphia. Nine patient samples were found by mass spectrometry to contain additional Hb variants, which could not be identified using the existing hospital method.

Conclusion : The top down approach was found to provide results comparable with other mass spectrometry approaches and has advantages over the existing clinical approach in terms of cost, and improved diagnostic information.

Topic 12

Integrative proteomics and systems biology

OP046 - DEEP PROTEOMICS, TRANSCRIPTOMICS AND SYSTEMS BIOLOGY ANALYSIS OF VASCULAR SMOOTH MUSCLE CELLS REVEALS NOVEL ANGIOTENSIN II –REGULATED PATHWAYS UNDERLYING VASCULAR WALL THICKENING

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[1]Cnic

Introduction

Mature vascular smooth muscle cells (VSMC) are able to switch from a quiescent contractile to a proliferating secretory phenotype upon activation, turning on and off a myriad of signaling pathways. This phenotypic switch is the basis of many cardiovascular diseases. Angiotensin II (AngII) promotes VSMC activation by still unknown mechanisms. Here we present an in-depth characterization of dynamic protein abundance and mRNA expression changes induced by AngII in VSMC, and identify novel AngII-regulated pathways by a novel systems biology approach (SanXoT).

Methods

Proteins were extracted from VSMC after 0, 2, 4, 6, 8 and 10-hours of AngII treatment and digested. The resulting peptides were labelled with iTRAQ 8-plex, separated into 6 fractions by cation exchange chromatography and analysed by LC-MS on a Q-Exactive instrument. Transcriptomics data from 4-hours vehicle or AngII-treated VSMC were also obtained and analysed by the GCOS software (Affymetrix). Protein or gene quantification, protein integration into DAVID&Ingenuity functional categories and statistical analysis were performed by an in-house developed algorithm (SanXoT).

Results and Discussion

We quantified more than 10,000 proteins in VSMC, constituting the deepest proteomics analysis to date of these cells. More than 100 proteins changed their abundance along the time course of AngII treatment. These changes are consistent with increased contractile capacity and reduced secretory functions of VSMC. Further analysis by systems biology revealed that AngII modulates by transcriptional or post-transcriptional mechanisms the abundance of proteins belonging to key routes, which are up- or down-regulated in a tightly coordinated manner. Among others, we found increased muscle protein synthesis and oxidative stress response, and decreased homocysteine biosynthesis and DNA replication.

Conclusions

Using a novel statistical model for systems biology analysis, we identified new pathways of AngII-induced VSMC hypertrophy, shedding some light on the molecular mechanisms underlying vascular wall thickening.

OP047 - OSTEOGENESIS IMPERFECTA: AFFECTED PATHWAYS IN SKIN, BONE, AND LUNG FROM THE BRTLIV MURINE MODEL

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Introduction and objectives: Osteogenesis imperfecta (OI) is a heritable collagen-related disorder mainly caused by mutations in one of the two genes coding for type I collagen. OI is characterized by a wide phenotypic variability spanning from mild bone defects to lethality. Such phenotypic extremes are resembled in the OI murine model BrtlIV that we used in our investigations.

Even if the skeletal system is primarily affected by the disease, OI is a systemic disorder and also not skeletal tissues can have a pathological outcome. In spite of this, the understanding of molecular and biochemical bases of extra skeletal manifestations are still in their infancy and only few researches attempted OI bone and not skeletal tissues' data correlation. Here we investigated phenotypic variability in bone, skin and lung from BrtlIV, with moderate and lethal outcomes, and then compared, combined, and cross-linked the results performing pathway analysis.

Methods: OI heterogeneity was investigated by transcriptomic and/or proteomic techniques. Differentially expressed proteins were processed applying MetaCore pathway analysis and the reliability of generated networks was proved by immunohistochemistry and immunoblotting.

Results and Discussion: Our results corroborated the concept that OI pathoetiology is not limited to an abnormal extracellular matrix deposition but rather it is related to a general cellular machinery malfunction with altered cellular homeostasis. In particular, mutant animals with worst outcome showed aberrant expression of some proteins correlated to cytoskeleton rearrangement and TGF-β signalling, all them well integrated into the built hybrid nets. Based on functional analysis results, we experimentally proved elevated cytoskeleton aberrancies and TGF-β/Smad2-Smad3 altered concentrations or activation in lethal animals.

Conclusions: A broad biomolecular overview on OI pathomechanisms was provided and some common pathways, despite tissue specificity, were highlighted as affected by the disease in Brtl bone, skin, and lung. This may contribute in defining novel therapeutic approach to OI.

OP048 - PROTEOMIC CHARACTERISATION OF TIO2 NANOPARTICLE-PROTEIN CORONA FORMATION IN VITRO

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Nanotechnology offers great promise in the emerging field of nanomedicine. Nevertheless, nanoparticles (NPs) are coated with proteins (or other biomolecules) once in contact with biological media leading to the formation of a so-called "corona". At physiological conditions, it is therefore the NP-corona complex rather than the "naked" NP that it is crucial for cellular interactions.

This has implications for future applications in nanomedicine, raising also concerns related with toxicity. A systematic characterization of the NP-protein corona (PC) could thus contribute for a rational "safe by design", based on the NP interactions with extracellular and/or intracellular proteins. A label-free shotgun proteomics analysis was performed to characterize the PC formed by titanium dioxide (TiO2) NPs in contact with proteins either from cell culture media (e.g., DMEM supplemented with 10% FBS) and/or 2 human cell lines (HaCat and HMEC-1).

Sample proteins were prepared according to FASP (filter aided sample preparation) method (Wiśniewski et al., 2009). By using high resolution mass spectrometry (LTQ Orbitrap Velos Pro-ETD), we characterised the PC formed according to different NP sizes (10 and 30 nm) and concentration (25 and 100 µg/mL). About 500 proteins were reproducibly identified either in the corona of NPs incubated solely with cell culture media supplemented with 10% FBS (CCMs) or in the corona of NPs pre-incubated with CCMs plus protein cell lysates. Our results show that NP size imparts more differences in the identity of the PC than concentration.

The identified proteins were mainly involved in cellular and metabolic processes, but also in response to stimulus, developmental process, localization, immune system and reproduction. The major cellular components attributed by GO were cytoplasm, organelles, nucleus, membranes, cytoskeleton and extracellular region. The nature of TiO2 PC can thus help to predict main targets at cellular level and therefore be used as an early warning of toxicity.

OP049 - PROTEOMICS MOVES FROM EXPRESSION TO TURNOVER: UNDERSTANDING THE DYNAMIC PROTEOME

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Introduction

Proteomics has rapidly developed into a sophisticated discipline, which seeks to provide a functional link between expressed genes and phenotypic outcomes. Moving from a static "snap-shot" of a system to dynamic processes presents a considerable challenge and requires the use of sophisticated analytical and bioinformatic strategies. We have developed a robust stable isotope labelling methodology combined with high resolution mass spectrometric analysis to determine the synthesis and degradation rates of hundreds of proteins simultaneously from human cell lines and complex organisms.

Methods

Cells are cultured in media where a specific amino acid is replaced with a stable isotope labelled analogue and the incorporation (or removal in a 'pulse-chase' experiment) is monitored by LC-MS/MS. Mathematical modelling of these data permit the first order rate constants to be derived allowing an analysis of the proposed promulgators of protein stability. In animal models, the stable isotope is administered through the diet.

Results and Discussion

The rates of degradation of over 900 proteins from human cell lines were determined and the data used to probe the relationship between protein stability and physiochemical properties of the proteins. Similarly, protein synthesis rates from hundreds of proteins from zebrafish muscle have been calculated. These data are now informing comparative studies investigating the effect of diet composition and stress.

Conclusion

We have demonstrated the feasibility of our approach in human cells and have extended this work to the zebrafish, a key model organism for human health and disease. Our data have allowed a direct comparison of protein stability between tissue types and experimental conditions. The approach we have developed is applicable to a range of biological systems and is therefore a critical tool in the integration of systems-level data.

P-502.00
A SYSTEMS BIOLOGY APPROACH UNRAVELS THE ROLE OF MIR-17-92 IN FINE-TUNING MYC-CENTERED REGULATORY NETWORKS TO SUSTAIN CANCER PROGRESSION

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Background: Tumorigenesis is characterized by changes in the mRNA landscape, including 3' UTR shortening, which affect the outcome of miRNA action. Although, the synergism between c-MYC and miR-17-19b, a truncated version of the miR-17-92 cluster, has been demonstrated in tumor initiation, little is known about the effect of these miRNAs in established malignancies. In this study we address this question and, more in general, we investigate the functional relationship between MYC-regulated transcription and miR-17-19b-controlled post-transcriptional regulatory mechanisms
Methods: We investigate the role of miR-17-19b in a highly aggressive model of c-MYC driven B cell lymphomas through an integrated, systems biology approach centered on SILAC (Stable Isotope Labeling by Amino acids in Cell culture)-based quantitative proteomics, transcriptomics and 3' UTR analysis to comprehensively dissect the molecular outcome of miR-17-19b induction in established B cell lymphoma.

Results: We identified more than one hundred novel miR-17-19b targets, of which about 40% are potentially regulated by MYC, indicating the role of the cluster in dampening MYC transcriptional activity. Interestingly, our data supports the role of MYC in protecting 3' UTRs from shortening for a set of targets in common with miR-17-19b, thus allowing miRNA-mediated fine-tuning of these transcripts. We also revealed that miR-17-19b indirectly down-regulates translation of MYC, possibly through the novel target Chek2. Down-regulation of Chek2 leads to increased recruitment of HuR/RISC to c-MYC-mRNA, which inhibits c-MYC translation. In line with these results, we observed that a subtle increase in miR-17-19b levels in lymphoma cells reduces tumor aggressiveness.

Conclusion: Differently from tumor initiation, in established tumors miR-17-19b fine-tunes c-MYC oncogenic activity by exerting a tight control over its expression and functions, ultimately ensuring cancer cell homeostasis.

Our results help a deeper understanding of the complex regulatory networks centred on the key cancer gene c-MYC in the maintenance of the malignant cell phenotype

P-503.00

PROTEOMIC AND TRANSCRIPTOMIC ANALYSIS OF LIQUID NON-SPORULATING STREPTOMYCES COELICOLOR CULTURES DEMONSTRATES THE EXISTENCE OF A COMPLEX DIFFERENTIATION COMPARABLE TO THAT OCCURRING IN SOLID SPORULATING CULTURES

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Introduction and objectives

Streptomyces species produce many clinically relevant secondary metabolites and exhibit a complex development that includes hyphal differentiation and sporulation in solid cultures. Industrial fermentations are usually performed in liquid cultures, conditions in which Streptomyces strains generally do not sporulate, and it was traditionally assumed that no differentiation took place. The aim of this work was to compare the proteomes and transcriptomes of S. coelicolor growing in liquid and solid cultures, deepening the knowledge of Streptomyces differentiation.

Methods

iTRAQ was used for quantitative proteomics. Microarrays were from Oxford Gene Technology (Agilent ink-jet technology).

Results and discussion

Gene and protein expression in liquid and solid cultures were comparable and data indicated that physiological differentiation was similar for both conditions. Eighty-six percent of all transcripts and proteins showed similar abundances in liquid and solid cultures, such as those involved in the biosynthesis of actinorhodin (actVA, actII-4) and undecylprodigiosin (redF); activation of secondary metabolism (absR1, ndsA); genes regulating hydrophobic cover formation (aerial mycelium) (bldB, bldC, bldM, bldN, sapA, chpC, chpD, chpE, chpH, ramA, ramC, ramS); and even some genes regulating early stages of sporulation (wblA, whiG, whiH, whiJ). The two most important differences between liquid and solid cultures were: first, genes and proteins related to secondary metabolite biosynthesis (CDA, CPK, coelichelin, desferrioxamine clusters) were highly up-regulated in liquid but not in solid cultures; and second, genes and proteins involved in the final stages of hydrophobic cover/spore maturation (chpF, rdlA, whiE, sfr) were up-regulated in solid but not in liquid cultures. New information was also provided for several non-characterized genes and proteins differentially expressed in liquid and solid cultures which might be regulating, at least in part, the metabolic and developmental differences observed between liquid and solid cultures.

P-504.00

QUANTITATIVE PROTEOMIC ANALYSIS OF RAT MESENCHYMAL STEM CELLS AT DIFFERENT AGES

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Introduction and objectives: Mesenchymal Stem Cells (MSCs) are highly relevant for regeneration of mesenchymal tissues such as bone and cartilage. The promising role of MSCs in cell-based therapies and tissue engineering appears to be limited due to a decline of their regenerative potential with increasing donor age. To establish a framework for future comparative and functional studies, we have analyzed by iTRAQ the proteins differentially expressed in MSCs of rat bone marrow from animals of different ages.

Methods: Quantitative proteomic assay was performanced by iTRAQ using an 8-plex iTRAQ workflow. In solution tryptic digestions of protein extracts of BM-MSCs- from six aging groups of Wistar rat -neonate, infant, young, pre-pubertal, pubertal and adult- were fractionated by basic reversed phase extraction in a 1200 HPLC system (Agilent) and by acidic reversed phase in a nano Tempo system (Eksigent). Eluting peptides were deposited on a MALDI LC-plate using a SunCollect (SunChrom) spotter and injected in a 4800 MALDI-TOF/TOF platform (ABSciex). Protein identification and relative quantification were done using ProteinPilot 4.0 software (ABSciex).

Results and discussion: 57 differentially expressed proteins where determined in our quantitative proteomic assay. All of them were included into three physiology groups, proliferation, pluripotency and metabolism using the String 9.0 Software. Our results indicated that BM-MSCs from infant over-express proteins involved into proliferation and pluripotency when compared to BM-MSCs from newborn, indicating that infant BM-MSCs could be an adequate group to try cell-based tissue engineering therapies. On the other side, BM-MSCs from adult group over-express proteins involved into mitochondrial metabolism when compared to BM-MSCs from the others group, these results suggest that adult group could be adequate to study senescence.

P-505.00

COMPREHENSIVE CHARACTERIZATION OF A LIVER TISSUE AND HEPG2 CELLS TRANSCTIPROTEOME FOR HUMAN CHROMOSOME 18

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The goal of our "transcriptoproteome" analysis was to analyze and compare the results of transcriptome profiling and proteome mapping for the genes on Chr 18 in liver tissue and the HepG2 cell line. The transcripts in the tissue samples were independently measured using two RNAseq platforms, SOLiD and Illumina. Measurements with qRT-PCR and ddPCR were also conducted to perform cross-comparisons for selecting the optimal RNAseq platform for future experiments. The proteome profiling was conducted through targeted mass-spectrometry using selected reaction monitoring (SRM) with sensitivity up to 10-18 M. To compare the transcriptome versus the proteome, all measurements were expressed in numbers of mRNA/protein copies per HepG2/liver cell.

In total, protein copy numbers were estimated for 267 master-proteins, including quantitative data on 252 in HepG2 cell line and 259 in liver tissue. Most proteins were present in HepG2 cells and liver tissue at 105 copies per 1 cell. Summarizing the transcriptome section, there were 224 transcripts jointly found in liver and HepG2 cells, of which 11 transcripts were only found in the liver, while four transcripts were only observed in HepG2 cells. The correlation between RNAseq and RT-PCR data enabled us to use the dependency as the regression model for translation of the RPKM values to the transcript copy-numbers.

The transcriptome data for the HepG2 cell line and liver tissue was expressed as a number of transcript copies per cell and correlated to protein abundance, approximated as protein copy numbers attributed to Chr 18 genes in HepG2 and liver cells, respectively. We observed a moderate degree of relationship between the proxies for transcript and protein copy numbers ($R^2 \sim 0.3$) for high- and medium copied protein and no transcript-to-protein correlation for ultra-low copied proteins as for HepG2 cell line as for liver tissue.

P-506.00
DIGESTOMICS OF HUMAN MILK DURING LACTATION AND MASTITIS

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Introduction and objectives:
Milk is a self-digesting biofluid rich in proteases and a cohort of activators and inhibitors. Previous results have shown that milk protein digestion releases peptides with beneficial functions beyond the infant nourishment. However, as these results were based on in vitro digestion, the biological relevance of these findings is unknown. The aims of this work are the characterization of the in vivo proteolysis of milk, the identification of naturally occurring bioactive peptides and their potential role on the progress of mastitis.

Methods:
Milk samples were collected at different moments of lactation from healthy individuals and mothers diagnosed with mastitis. Individual protein content and endogenous peptides were determined for each sample by mass spectrometry. Enzymatic activity was determined by incubation with chromophoric peptides and spectroscopy. Milk endogenous peptides were synthesized and tested for different bioactivities.

Results and discussion:
The proteolytic pattern observed in milk was similar for all the mothers and is characterized by the presence of abundant peptides from specific regions of some proteins. Our results suggest that the presence of specific proteases, the position of cleavage sites and the intrinsic disorder of segments of the protein drive this proteolytic specificity. A number of the more abundant endogenous peptides found in these samples have been described to have beneficial activities, even more, peptides never described before have been tested and proved to have antibacterial, immunomodulatory and anti-inflammatory properties. Our preliminary results show that both peptide and protein content slightly decrease as lactation advances. During mastitis, there is a significant change on the formation of endogenous peptides but not in the protein content suggesting that this variation is driven by the activity of the proteolytic machinery.

Conclusions:
Our results probed the in vivo specific formation of bioactive peptide sequences that may have implications on the progress of mastitis.

P-507.00

THE HUMAN KIDNEY SPECIFIC PROTEOME DEFINED BY TRANSCRIPTOMICS AND ANTIBODY-BASED PROFILING

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Introduction and Objectives: The kidney is a specialized tissue and plays a vital role in maintaining body homeostasis. To understand renal biology and disease, it is important to define the molecular constituents of the various subcompartments of the kidney, including glomerus and tubule.

Methods: we have used an integrated omics approach involving genome wide transcriptomics analysis using deep RNA sequencing across 27 different human tissue types combined with antibody-based immunohistochemistry in 48 different tissues to identify the genes overrepresented in the kidney and to further map the localization of the corresponding protein. All human genes were classified into categories of expression patterns with regards to expression pattern in the kidney as compared to the other anlyzed tissues.

Result and Discussion: Altogether 387 genes were found to be elevated in kidney as compared to the other analyzed tissues and 64 genes were found to have a significant higher expression in kidney and these genes are designated as highly and moderately enriched. In-depth analysis of all the elevated genes using antibody-based allowed us to create a nephron and collecting duct segment-specific map, including 149 proteins with a unique localization, including 12 proteins in glomeruli, 120 in proximal tubuli, 9 in distal tubuli and 8 in collecting duct. An analysis of the identified genes supports their role in the function of each segment. Among the gene products identified as kidney-enriched, we found several proteins hitherto not previously described in the context of the kidney.

Conclusion: In summary, we have identified and mapped proteins with elevated expression in kidney through an integrated omics approach and these proteins are important starting points for further functional studies. The identified protein targets can also serve as the vantage point for identification of early kidney specific biomarkers of disease and injury before clinical symptoms arise.

P-508.00

IDENTIFICATION OF ENRICHED PTM CROSSTALK MOTIFS FROM LARGE-SCALE EXPERIMENTAL DATASETS

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Post-translational modifications (PTMs) play an important role in the regulation of protein function. Mass spectrometry based proteomics experiments nowadays identify tens of thousands of PTMs in a single experiment. A wealth of data has therefore become publicly available. Evidently the biological function of each PTM is the key question to be addressed, however, such analyses focus primarily on single PTM events. This ignores the fact that PTMs may act in concert in the regulation of protein function, a process termed PTM crosstalk.

Relatively little is known on the frequency and functional relevance of crosstalk between PTM sites. In a bioinformatics approach we extracted PTMs occurring in proximity in the protein sequence from publically available databases. These PTMs and their flanking sequences were subjected to stringent motif searches, including a scoring for evolutionary conservation.

Our unprejudiced approach was able to detect a respectable set of motifs, of which about half were described previously. Amongst these we could add many new proteins harboring these motifs. We extracted also several novel motifs, which through their widespread appearance and high conservation may pinpoint at previously non-annotated concerted PTM actions. By employing network analyses on these proteins we propose putative functional roles for these novel motifs with two PTM sites in close proximity.

P-509.00
CONSERVED AND DIVERSE ASPECT OF PROTEOME PROFILE ACROSS MULTIPLE YEAST SPECIES

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Omics analysis is versatile approach for understanding conservation and diversity of molecular systems across multiple organisms. In this study we compared the proteome expression profile obtained by non-label based quantitative mass spectrometric analysis across four yeast species, including Saccharomyces and Kluyveromyces genus, grown on glucose or glycerol media.

Inter-species correlation of protein abundance was lower than intra-species correlation between different carbon sources, suggesting that adoptive response to nutrient alteration is accomplished via minor change of proteome as keeping inter-species diversity of proteome profile. In particular, protein with specific function, including mitochondrial activity, response to oxidative stress, and fatty acid oxidation, showed high abundance in Kluyveromyces genus compared to Saccharomyces one. These types of function were considered to essential for high growth rate of Kluyveromyces genus on glycerol media, where energy production require respiration chain through which reactive oxygen species are produced.

On the other hand, up-regulation of some groups of proteins when grown on glycerol, involved in glycerol catabolism, gluconeogenesis, tricarboxylic aid cycle, and lipid biosynthesis, was conserved in all yeast species. It is plausible that these alterations of protein abundance are critical event for growth on and utilization of glycerol. Saccharomyces genus has about 900 paralog genes, which duplicated and retained via whole genome duplication. Intriguingly, only in the case of duplicated genes with high sequence intra- and inter- similarity, comparable level was sum of the protein abundances from paralog gene pair of Saccharomyces genus and those from non-duplicated gene orthologue of Kluyveromyces genus. This provoked a question why such type of duplicated genes has been retained during evolutionary process. Thus comparative proteomics across multiple species will provide valuable insight into biological processes, such as essential adoptive response to environmental conditions, and role for gene duplication and protein dosage on evolutionary history.

P-510.00
SANXOT: A NOVEL FUNCTIONAL CLASS-SCORING ALGORITHM FOR SYSTEMS BIOLOGY AND ANALYSIS OF COORDINATED PROTEIN BEHAVIOR

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Introduction:
The analysis of high-throughput proteomics data presents the challenge of extracting biological meaning from a wealth of protein quantifications. In a previous work we presented the WSPP statistical model for the analysis of quantitative proteomics experiments using stable isotope labelling approaches. Here we further developed this model in form of a functional class-scoring algorithm that captures the coordinated behavior of proteins belonging to the same functional categories, and present its related software package (SanXoT).

Methods:
A large number of high-throughput quantitative proteomics experiments have been analyzed, using yeast, human, pig, rat and mouse models. Quantified proteins were integrated into ontological categories from DAVID, Ingenuity, and custom databases. A pseudo-null hypothesis (PNH) data set was prepared by shuffling the protein-category relations while preserving the overall protein-category network structure. In all cases the distributions describing the intra- and inter-category variability were analysed. The validity of the model was assessed analysing a real null-hypothesis (NH) experiment from yeast.

Results and discussion:
In the real NH yeast experiment, as in all PNH experiments, no significant category deviations were detected, validating the model. In contrast, numerous categories were found to deviate in the treated samples. Moreover, analysis of protein distribution within categories revealed a significantly coordinated protein behavior in all cases, meaning that category changes were a result of coordinated increase or decrease of the proteins, rather than due to individual protein deviations. The degree of coordination was evaluated in all studied proteomes, whereas the NH and the PNH were found not to be coordinated.

Conclusions:
SanXoT provides an innovative approach to the systems biology analysis of quantitative high-throughput proteomics, allowing the global interpretation of biological results in terms of ontological alterations as well as the analysis of coordinated protein behavior.

P-511.00

THE PROTEOMIC POINT OF VIEW ON HUMAN TEETH AND SALIVA SAMPLES

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Introduction and objectives: The teeth are the hardest part of human body composed of enamel, dentin, cementum, and tooth pulp. They are (together with the saliva) the center of interest, due to medical problems with them. The proteomic research of human teeth and saliva is now accelerating [1]. The research can help stomatology in early diagnosis, identification of risk factors, and systematic control. The aim of our work was to investigate the proteomic profile of human dentine, tooth pulp and saliva from adult people and compare them mutually.

Methods: Sound human teeth extracted for clinical reasons from adult patients (aged 17-40) together with the saliva collection were divided into various groups with respect to patient sex, age status and number of dental caries (caries-susceptible vs. caries-resistant people). The proteins from individual dentine, pulp and saliva samples were extracted and processed by method described previously [1]. The proteins were separated by two-dimensional electrophoresis, digested by trypsin, and analyzed by nano-liquid chromatography coupled to a maXis Q-TOF mass spectrometer with ultrahigh resolution.

Results and Discussion: We detected more than several hundreds of proteins in each kind of sample (pulp, dentin, saliva). Individual proteome maps were compared and changes in the protein abundances were identified among the individuals. The observed differences were statistically evaluated to find the significant changes. Special emphasis was put on looking for the abundance changes of proteins that may be involved in formation of dental caries due to their biological functions.

Conclusion: The differences in the whole proteomes of teeth and saliva samples from various groups of people were determined.

P-512.00

ANALYSIS OF CHRONIC LUNG ALLOGRAFT DYSFUNCTION (CLAD) AFTER LUNG TRANSPLANTATION BY PROTEOMIC APPROACHES OF BRONCHOALVEOLAR LAVAGE AND PLASMA FLUIDS

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With 39,000 procedures performed worldwide in the least 30 years, lung transplantation (LT) has become the standard treatment for patients with advanced lung diseases. Unfortunately, about 50% of LT recipients (LTR) will develop within 5 years a chronic lung allograft dysfunction (CLAD), a generic term regrouping two clinical pictures named bronchiolitis obliterans syndrome (BOS) and restrictive allograft syndrome (RAS). Moreover, CLAD diagnosis based of the decline of respiratory function is often late when lung damages are irreversible and no early symptoms of this pathology are currently known. Thereby, identification of biomarkers at early stage would allow the use of adequate treatments for patients that will develop BOS or RAS syndromes.

The goal of our study, which is part of an EU-funded FP7 project named SysCLAD, was to identify at 6 and 12 months after LT biomarkers that will predict CLAD occurrence 3 years later. We used for this purpose patient samples from a European prospective cohort of 500 LTR, whom 100 reached 3 years follow up at the time of the study (Cohort Of Lung Transplantation (COLT)). Proteome of plasma and bronchoalveolar lavages withdrawn at 6 and 12 months post-transplantation were analyzed by two approaches. SELDI-TOF profiles of LTR developing a BOS or RAS phenotype were compared to those of stable patients, allowing characterization of several proteins differentially expressed between each group.

Then, identification of these candidate biomarkers will be performed by a bottom-up approach. Secondly, samples from the same group (BOS, RAS and stable LT) were pooled and analyzed by iTRAQ quantitative proteomic approach using 2D fractionation (OFFGEL, nanoHPLC) and MALDI-TOF/TOF mass spectrometer. All these results will be integrated into a prediction computational model of CLAD based on principles of systems medicine developed from all the clinical and experimental data (environment, phenotype, microbiome, biology, omics) of donors and recipients.

P-513.00
BUILDING MODELS OF SIGNALLING NETWORKS WITH UNTARGETED MASS SPECTROMETRY: APPLICATION TO GROWTH PATHWAYS IN CANCER

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Introduction and objectives: Studies that have attempted to characterise kinase-substrate networks in cells have revealed extensive indirect and compensatory effects. Because kinases are common targets for cancer therapies, understanding kinases and kinase inhibitors specificity and underlying networks is of paramount importance. Untargeted quantitative proteomics data can be instrumental in this process, and mass spectrometry (MS) is a widespread method to acquire it. However, analysis of such data is challenging because it has high-content (but low throughput), non-negligible noise and missing data issues, and frequently samples poorly characterised areas of signalling. Hence, most MS studies result in a list of modulated abundances, underutilising the scope and unbiased character of this data and failing to produce networks-oriented functional insights.

Methods: Here, we present PHONEMeS (PHOsphorylation NEtworks for MS), a method to build predictive and explanatory models of signalling by training networks to untargeted MS phosphoproteomic data upon perturbation. Our method combines a rigorous statistical framework for data processing and a Boolean logic formalism. A heuristic iterative scheme is used for model training.

Results and discussion:Using our newly developed method, we analyse a dataset with multiple small molecule inhibitors targeting canonical growth pathways kinases, applied to a breast cancer cell line. We show the potential of our method to understand drug specificity and investigate unexpected effects of AKT, PI3K and MTOR inhibitions that are potentially of clinical relevance.

Conclusions: Our unbiased and exploratory approach generates deep insights into the function of the phosphoproteome by organizing and visualizing shotgun MS data in the context of reconstructed pathways, and predicts unexpected effects of drugs and previously unknown signalling paths.

P-514.00

AN EXTENSIVE PROTEOME PROFILING OF VISCERAL ADIPOSE TISSUES FOR UNDERSTANDING THE RELATION WITH EARLY PATHOGENESIS OF TYPE 2 DIABETES MELLITUS

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Introduction and objectives

Adipose tissue is a complex endocrine organ which is known to play important pathophysiological roles relating metabolic abnormalities, such as obesity, cardiovascular disease and type 2 diabetes mellitus (T2DM). Especially alterations in visceral adipose tissue (VAT) have been reported closely related to the pathogenesis of T2DM. However, studies systematically exploring the molecular signature of VATs relating to early pathogenesis of T2DM have not yet been proposed. Here we present an extensive proteome profiling of VATs obtained from drug-nave early T2DM patients and subjects with normal glucose tolerance (NGT).

Methods

Two independent set of VATs from five T2DM patients and six subjects with NGT were collected for proteome profiling. Tissues were homogenized using bead beating and resultant protein extracts were enzymatically digested thorough filter-aided sample preparation method. For individual samples, triplicate LC-MS/MS analyses were performed (totally 33 runs) not only for profiling proteome but for comparing abundance through label-free approach. Furthermore, extensive proteome profiling was facilitated by introducing 26 offgel fractionation after pooling tryptic peptides of five NGT and five T2DM samples. Identified differentially expressed proteins were analyzed with GO biological processes and network modeling was constructed for selecting proteins relating pathogenesis of early T2DM.

Results and discussion

An extensive profiling generated comprehensive VAT proteome information including 22,250 peptides of 4,707 proteins. Label free quantification between T2DM and NGT showed 772 differentially expressed proteins with 444 up-regulated in T2DM and 328 down-regulated. Finally 11 proteins were selected through functional enrichment analysis and network modeling and subjected to validation with Western blotting. Considering the consistency of abundance change with LC-MS/MS results, six proteins (FABP4, C1QA, S100A8, SORBS1, ACADL and PLIN4) were presented as bimolecular signature for distinguishing T2DM patients.

Conclusions

Our extensive proteome profiling data provide comprehensive information indicative of T2DM for the classification, therapy, and pathogenesis of T2DM.

P-515.00

INVESTIGATION OF DRUG-INDUCED STEATOSIS IN HEPARG CELLS AFTER LONG-TERM TREATMENT WITH VALPROIC ACID USING LC/MS-BASED QUANTITATIVE PROTEOMICS

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The European NOTOX project (notox-sb.eu) develops systems biology approaches for in vitro toxicity studies and in vitro-in vivo extrapolation. Here, we present the results of a case study on drug-induced steatosis in the hepatic cell line HepaRG® after long-term treatment (two weeks) with valproic acid. We investigated both the intracellular and extracellular proteome ("Secretome") with the latter being of special interest for finding biomarker candidates for drug-induced liver toxicity.

After liquid digestion, tryptic peptides of intracellular and extracellular proteins were analysed on an Impact HD QToF equipped with a captive spray source and nanoBooster (BRUKER DALTONICS). Proteins were identified using Mascot and quantified based on extracted ion-chromatograms from MS1-spectra using Skyline. Furthermore, we assessed LC/MS platform stability during the experiment by spiking all samples with reference peptides (iRT, Biognosys AG) and by regularly injecting a pool of all samples. More than 2000 intracellular proteins and more than 200 extracellular proteins were identified (FDR

In conclusion, we show that the applied workflow and instrumentation are well suited for robust differential label-free proteomics. Quality controls (e.g. spiked-in reference peptides) allowed real-time monitoring of the instrument status. Moreover, the detected differences are in concordance with the expected steatotic effects of valproic acid, i.e. changes in lipid homeostasis.

P-516.00

DYNAMIC ASSESSMENT OF FIN REGENERATION IN ZEBRAFISH USING A PULSED SILAC APPROACH

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Introduction
The zebrafish owns remarkable regenerative capacities allowing regeneration of several tissues, including heart, liver and brain. To identify protein dynamics during fin regeneration we used a pulsed SILAC (stable isotope labeling of amino acids in cell culture) approach.

Methods
The SILAC zebrafish diet, which contains a mixture of Lys-6, labeled mouse tissue, Lys-6 mouse chow, Lys-6 yeast, and Lys-6 bacteria enabled us to detect the incorporation of 13C6-lysine (Lys-6) into newly synthesized proteins. At four different time points samples were taken from non-injured (background) and regrowing fins. Incorporation rates were monitored using a combination of single-shot 4 h gradients and high-resolution tandem mass spectrometry (Q Exactive Plus).

Results and Discussion
We found more than 5000 labeled proteins during the first two weeks of fin regeneration indicating high coverage of the proteome. We were able to monitor proteins, which are responsible to initialize and restore the shape of these complex appendages and cluster analysis revealed dynamic Lys-6 incorporation rates of proteins involved in various biological processes like cell proliferation, cell migration, and differentiation. Moreover, the differential comparison of lys-6 incorporation rates between non-injured and regrowing fins enabled us to identify proteins which are directly involved in regeneration compared to background protein labeling. For example, we observed increased incorporation rates of two actinodin family members in regrowing fins, which are essential components of elastoidin a collagen-like structure. Additionally increased mRNA levels of actinodin 1-4 were confirmed by in-situ hybridization.

Conclusion
Our zebrafish diet can be used as a versatile tool to monitor newly synthesized proteins in a pulsed SILAC manner and will help to characterise cellular dynamics during regenerative processes in zebrafish beyond fin regeneration.

P-517.00

AN ENHANCED INDUSTRIAL-RELEVANT PRODUCTS PORTFOLIO FOR CORYNEBACTERIUM GLUTAMICUM BY MEANS OF PROTEOMIC APPROACHES.

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Corynebacteria are Gram-positive Actinobacteria inhabitants of diverse ecological niches such as human skin, soil or cheese. Among the 35 species considered of medical relevance in man (pathogens and/or commensals), Corynebacterium diphtheria stands out. By contrast, the most industrial-interesting species is Corynebacterium glutamicum, which is a GRAS bacterium involved in amino acids production as food/feed enhancers or pharmaceutical products.

Thus, this bacterium produces per year not only more than 2.1 million tons of L-glutamic acid and 1.4 of L-lysine, but also 230 thousand tons of threonine and 4.8 of tryptophan. However, the coryneform bacteria entered the 21st century with a refocusing of their industrial point of view. Consequently, relevant bioproducts such as chemicals and biodegradable polymers as well as ingredients or additives in food, feed, cosmetics and pharmaceuticals have extended the portfolio of Corynebacterium [e. g.: cadaverine, human heterologous proteins, dicarboxylic acids, valine, putrescine, isobutanol, pantothenic acid, pyruvate, D-lactic acid, alanine or ethanol]. Routinely, the studies improve C. glutamicum metabolism to obtain the best yields, but no attention is paid to the effect produced by these new products on the growth or how to address it. Proteomics allows regarding the skyline of those proteins involved in stress resistance, new compounds production and strain selection as better producers or processes improvement to support the final application of the Synthetic Biology tools.

Thus, the 2D-DIGE technology combined with MS protein identification is allowing identifying target proteins involved in the enhancement of the new trending products portfolio of C. glutamicum. Hence, we are facing up: i) the effect of accumulation of relevant products at industrial scale; ii) the influence of increasing bioreactor inhomogeneity occurring at industrial bioreactors; and iii) the screening of different C. glutamicum variants for valuable products production. The solution to these questions is helping to obtain better industrial strains.

P-518.00

PROTEOMIC ANALYSIS OF THE ENZYMATIC EXTRACT OF THE FERULOYL ESTERASE PRODUCER PENICILLIUM CHRYSOGENUM B13

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Filamentous fungi are known to be the major producers of extracellular enzymes, mainly enzymes for plant cell wall degradation, including cellulases, xylanases, pectinases and feruloylesterases (FAEs). The joint action of all of these enzymes is known to strengthen the complete degradation of the plant cell wall structure.

For this reason and because of their multiple biotechnological applications, these enzymes have experienced a rising and widespread interest in recent years. Further higher efficient degradation of the plant cell wall will be achieved thanks to the use of these enzymatic extracts in most common biotechnological industrial processes (food, paper, biofuel, pharmaceuticals, brewing, textiles, detergents, etc). P. chrysogenum B13 strain is a FAEs overproducer able to hydrolyze the ester bonds among the hydroxycinnamic acids (mainly ferulic acid) and the plant cell wall polysaccharides. As a result of FAEs activity, the polysaccharides become more accessible, allowing for a complete digestion by the rest of the enzymes involved in plant cell wall degradation.

P. chrysogenumB13 strain was grown in presence of sugar beet pulp and the obtained enzymatic extract secreted to the culture medium correlated with the greatest FAE activity time. Two-dimensional gel electrophoresis and DIGE of proteins was performed. Proteins were identified by means of a MALDI TOF/TOF mass spectrophotometer system.

The analysis of the results showed a 9,27% increase in FAE activity with respect to the wild type strain P. chrysogenum Wisconsin 54-1255 and that 42,22% of the secreted proteins were enzymes associated with plant cell wall degradation, as compared with a quantity of 11,59% when the culture medium contained no plant substrates.

P-519.00

PROTEOMIC ANALYSIS OF A MUTANT OF TRICHODERMA ARUNDINACEUM IMPAIRED IN TRICHOTHECENE BIOSYNTHESIS REVEALS A SYSTEMIC FUNCTION OF THESE COMPOUNDS IN FUNGAL PHYSIOLOGY

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Trichothecenes are sesquiterpene mycotoxins produced by several fungal genera including Fusarium, Trichothecium, Myrothecium, Stachybotrys, and Trichoderma. These toxins have attracted great attention because they are frequent contaminants of food and animal feed, and can be easily absorbed by animals and human beings via the integumentary and gastrointestinal systems. Exposure to these toxins can cause feed refusal, immunological problems, vomiting, skin dermatitis, and hemorrhagic lesions.

Trichoderma trichothecenes have been recently studied as model compounds since they are relatively structurally simpler compounds, and their biosynthesis requires relatively fewer genes, in comparison with trichothecenes produced by other genera. Most of the Trichoderma trichothecene genes are within a cluster but the sesquiterpene cyclase gene tri5 is located in a different genomic region. Tri5 controls the first specific step in trichothecene biosynthesis, cyclization of farnesyl diphosphate (FPP) to trichodiene. In the present work we analyzed the proteome changes produced in TaΔTri5, a trichothecene non-producing tri5 mutant, in comparison with the wild-type strain, T. arundinaceum IBT 40837. Sixty differentially expressed spots were detected with 2D-DIGE analysis and identified with MALDI TOF-TOF.

Among them, 21 spots were differentially overproduced in TaΔTri5 and the other 39 were differentially overproduced in the T. arundinaceum wild-type strain. The differences suggest that genetic disruption of tri5 had a systemic effect of the fungal physiology, including changes in regulation of intracellular FPP and acetyl CoA levels.

P-520.00
SERUM PROTEOMICS IN MELANOMA BIOMARKER DISCOVERY
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Introduction and objectives
Patients with early-stage melanoma and no lymph node involvement have a high likelihood of cure after complete excision of the tumor by surgery. However, there is no effective treatment for metastatic melanoma, which makes it a potentially lethal form of cancer. The aim of our work is to identify a set of markers of malignant progression that could be used in the prognosis of malignant melanoma.

Methods
Proteomic profiles of sera obtained from melanoma patients with good prognosis (ten years disease-free after surgery of the primary tumor) and patients who developed metastasis during the first two years follow up were compared. Serum samples were obtained from peripheral blood of patients at the moment of diagnosis and stored at -80ºC until use. We applied 2D electrophoresis analysis, after enrichment of low-abundance serum proteins by ProteoMiner kit. Gels, stained with SYPRO were analyzed with Progenesis SameSpots software. The spots of interest were excised, digested by trypsin, and subjected to LC-MS/MS analysis.

Results and Discussion
Seven of the forty-four identified proteins were selected for validation by ELISA assay in 100 controls and 348 melanoma patients in early stages (I and II according AJCC). These proteins were Serum amyloid A4, Clusterin, Apolipoprotein A1, Ficolin 2, Vitronectin, Junction Plakoglobin and Dermcidin. Melanoma patients presented higher serum levels of all these proteins compared to healthy controls. Multivariate analysis of serum values of these proteins and clinical data showed that levels of Apolipoprotein A1, Vitronectin and Dermcidin are related with metastatic progression of melanoma. Moreover, REPTree algorithms revealed that patients with a melanoma in stage II and serum levels of Dermcidin .

Conclusions
Serum proteomic and data mining methods could be valuable tools to identify new potential prognosis serum biomarkers for melanoma progression.

P-521.00

AN INTEGRATED PROTEOMICS AND METABOLOMICS APPROACH FOR MOLECULAR CHARACTERIZATION OF ESOPHAGEAL SQUAMOUS CELL CARCINOMA

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Introduction and objectives

Esophageal squamous cell carcinoma (ESCC) is among the most common malignancies worldwide and is particularly prevalent in India and China. We employed an integrated analysis by combining mass spectrometry-based quantitative proteomics, phosphoproteomics and metabolomics to investigate ESCC. The primary objective of these studies was molecular characterization of ESCC and identification of potential biomarkers and therapeutic targets.

Methods

We employed SILAC and iTRAQ-based quantitative proteomics methods to investigate aberrant protein expression pattern in ESCC using cell lines as well as surgically resected primary tumors. In order to characterize aberrantly regulated kinase signaling pathways, we carried out phosphoproteomic analysis using TiO2 and anti-phosphotyrosine antibody-based phosphopeptide enrichment. Mass spectrometry based untargeted metabolomic profiling was also carried out to characterize metabolic reprogramming in ESCC.

Results and Discussion

Quantitative proteomics and phosphoproteomics studies resulted in identification of aberrantly regulated kinase driven signaling pathways in ESCC. Cell based functional assays (proliferation, invasion and cell migration) by inhibiting activated kinase signaling pathways using pharmacological inhibitors and/or RNAi based approach demonstrated potential therapeutic utility of these targets in ESCC. In addition, several secreted and plasma membrane-bound biomarkers including protein disulfide isomerase A3 (PDIA3), transforming growth factor beta induced (TGFBI), anoctamin 1 (ANO1), galectin 3 binding protein (LGALS3BP) and osteopontin (OPN) were identified as overexpressed molecules that were further verified using immunohistochemical labeling. Untargeted metabolomics studies revealed dysregulation of phosphatidylcholines and ether phospholipids and provided significant insights into metabolic reprogramming in ESCC.

Conclusions

We present a comprehensive molecular characterization of ESCC using mass spectrometry based proteomics, phosphoproteomics and metabolomics. This multi-pronged approach resulted in identification of several biomarkers and therapeutic targets of ESCC that can be further explored to determine their potential clinical utility.

P-522.00
UTILIZING A QUANTITATIVE PROTEOMIC APPROACH TO DISCOVER NOVEL SUBSTRATES OF PROPROTEIN CONVERTASE 7

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Introduction and Objective:
Proprotein subtilisin/kexin like convertases (PCSKs or PCs) are a family of nine serine proteases known to cleave and activate many secretory precursor proteins such as growth factors, hormones and important enzymes. PCSK7/PC7, the seventh member of the PC family is a basic amino acid specific protease which cleaves its substrates after arginine residues in the [R/K]-X0,2,4-R↓ consensus motif. Although PC7 is believed to have some functional redundancy with other family members, it has been found that knocking out PC7 in mice results in an anxious phenotype with learning and memory impairments suggesting that PC7 plays an important role in brain function. So far, the only known substrates for PC7 are transferrin receptor, which is involved in the regulation of iron metabolism and epidermal growth factor precursor. In order to further elucidate the function of PC7 as a protease and its effect on metabolic pathways, it is of great importance to identify additional substrates.

Methods:
In this study, we utilized quantitative proteomics to discover novel potential substrates of PC7. Human embryonic kidney (HEK) 293 cells were transiently transfected with an expression vector of human PC7 or an empty vector control. The secretomes of both sets of cells were collected 48 hrs post transfection. SILAC (stable isotope labeled amino acid in cell culture) spike-in based quantitative proteomics was used to analyze the differential secretomes. Samples were analyzed with an Orbitrap-Elite mass spectrometer and data processed using Maxquant.

Results, Discussion and Conclusion:
Among the 500 proteins identified, we detected significantly increased levels of shed transferrin receptor from the secretome of PC7 transfected cells. These preliminary results suggest that this is a promising approach to find new substrates of PC7, gain further insights into its biological activity and unravel its role in brain physiology.

P-523.00

PROTEOMICS AND BIOMARKERS IN OSTEOARTHRITIS

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The application of modern proteomic techniques to disease states affords the opportunity to identify deregulated pathways that contribute to pathogenesis. These discoveries in turn may lead to new therapeutic targets, as well as "wet" biomarkers that aid in diagnosis, prognosis and the prediction of treatment responses. Building on the concept that osteoarthritis (OA) is a disease of all joint structures (synovium, cartilage and bone), and that synovial fluid (SF) may represent of synthesis of inputs from these structures, we compared the proteomic profile of knee joint SF from patients with early and late stage OA to unaffected controls by 2-dimensional gel electrophoresis and mass spectrometry.

In our recent publication, using this relatively unbiased approach, 66 proteins were reported as differentially represented in healthy vs. OA SF (1). Pathway analysis identified three biologic processes among these proteins: the complement and coagulation systems and the acute phase response. Interestingly, early and late OA manifested a very similar proteomic profile. Together, these findings suggest the osteoarthritic disease processes involves activation of inflammatory pathways that are well-established by the time patients are diagnosed.

This presentation will explore 1) the relative contribution of joint tissues to the SF OA proteome, including cartilage and synovium, 2) how proteomics can illuminate the pathogenesis of OA to identify therapeutic targets, 3) validation of proteomic discovery findings using multiplexed selected reaction monitoring (SRM) mass spectrometry peptide assays and 4) translation of SF protein biomarkers to quantitative serum based assays to predict disease progression in OA patients. At the end of this presentation, attendees should understand some of the major protein constituents of OA SF and how knowledge of this proteome may inform pathogenesis and biomarker development for this difficult disease.

P-524.00

ITRAQ-BASED PROTEOME PROFILING OF THREE HUMAN LIVER-DERIVED CELL LINES: COMPARISON WITH PRIMARY HEPATOCTYES AS MODELS FOR DRUG SAFETY ASSESSMENT

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Introduction and objectives

Drug-induced liver injury (DILI) is a significant health problem and a major challenge for new drug development in the pharmaceutical industry. DILI is the 2nd most common cause of attrition during clinical trials. Late stage failure of drug candidates indicates that current preclinical tests to identify DILI hazard are not fit for purpose. Whilst primary human hepatocytes remain the gold standard model for DILI screening, their low availability, high variability and propensity to lose metabolic capability has prompted the use of alternative models, particularly hepatoma-derived cell lines. However, these cells do not show comparable functionality in all cases. As part of an EU Innovative Medicines Initiative (IMI) to improve preclinical DILI prediction (MIP-DILI), we have conducted a proteomic comparison of primary and immortalised liver cells, with a particular emphasis on drug metabolizing enzymes (DMEs) and transporters.

Methods

HepG2, HepaRG and UpCyte cell lysates were compared with cryopreserved human hepatocytes for protein expression using iTRAQ-based quantitative analysis.

Results and Discussion

4794 unique proteins were identified across the cell samples, with 2722 quantified in every sample. Excellent representation of critical DMEs and transporters was obtained, with 15 CYPs and 27 drug transporters measured. Relatively low expression of CYPs was seen across all cell lines, with the exception of CYP3A4 in HepaRG cells, which was overexpressed compared with primary cells. In contrast, many transporters were expressed at similar levels in primary and immortalised cell lines.

Conclusions

Primary human hepatocytes cannot be exclusively used for drug toxicity testing because of their cost, availability and instability in culture. Alternative models are required for preclinical safety testing, but they must be fit for purpose. Using iTRAQ-based expression profiling, we have generated a database of phenotypic features for three different potential cell models, which can be used to assess their suitability for toxicity screening.

P-525.00

THE METACASPASE MCA1P HAS A DUAL ROLE IN FARNESOL-INDUCED APOPTOSIS IN CANDIDA ALBICANS

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Manipulating the apoptotic response of Candida albicans may help in the control of this opportunistic pathogen. The metacaspase Mca1p has been described as a key protease for apoptosis in C. albicans but little is known about its cleavage specificity and substrates. We therefore initiated a series of studies to describe its function. We used a strain disrupted for the MCA1 gene and compared its proteome to that of a wild-type isogenic strain, in the presence and absence of a known inducer of apoptosis, the quorum-sensing molecule farnesol.

Label-free and TMT labeling quantitative proteomic analyses showed that both mca1 disruption and farnesol treatment significantly affected the proteome of the cells. The combination of both conditions led to an unexpected biological response: the strong overexpression of proteins implicated in the general stress. We studied sites cleaved by Mca1p using native peptidomic techniques, and a bottom-up approach involving GluC endoprotease: there appeared to be a "K/R" substrate specificity in P1 and a "D/E" specificity in P2. We also found 80 potential substrates of Mca1p, implicated in protein folding, protein aggregate resolubilization, glycolysis and a number of mitochondrial functions. These various results indicate that Mca1p is involved in a limited and specific proteolysis program triggered by apoptosis.

One of the main functions of Mca1p appears to be the degradation of several major Heat Shock Proteins, thereby contributing to weakening cellular defenses and amplifying the cell death process. Finally, Mca1p appears to contribute significantly to the control of mitochondria biogenesis and degradation. Consequently, Mca1p may be a link between the extrinsic and the intrinsic programmed cell death pathways in C. albicans.

P-526.00

A SENTINEL PROTEIN ASSAY FOR THE QUANTIFICATION OF CELLULAR PROCESS ACTIVITIES USING PRM AND DIA

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We developed a novel proteomic screening approach that provides a system-wide, quantitative snapshot of the activity status of a variety of cellular processes, simultaneously. The approach is based on the concept of sentinels which are biological markers whose change in abundance characterizes the activation state of a given pathway or functional module in a cell. Sentinels can be specific proteins, phosphorylation sites or degradation products and an assembled panel of these sentinels can be targeted in a single LC-MS run. The strategy breaks with the current trend of constantly increasing the number of proteins measured.

Yeast cells were sampled under normal conditions and various conditions. Extracted protein lysates were digested and analyzed by nanoLC-Q Exactive Plus MS using Parallel reaction monitoring (PRM) and data independent acquisition (DIA).

Based on literature evidence and computational prediction, we selected a panel of 309 sentinels that covers 182 different cellular processes to probe the physiology of yeast cells under different conditions. Here, we tested different PRM and DIA acquisition methods for the measurement of the sentinel assay.
In a single PRM LC-MS run, we could detect more than 90% of the protein sentinels in at least one condition. Due to the lower sensitivity and specificity of the DIA method some of the lower abundant (phospho-)peptides could not be detected or quantified in DIA. However, we could extend the list of sentinel proteins and extract their abundances post-acquisition.

The sentinel assay precisely quantified the activity of many different cellular processes in a single LC-MS run, thereby providing a rapid, system-wide snapshot of the physiology if yeast cells across the different conditions. Rather than obtaining a large amount of complex and redundant data as when striving for the highest numbers of identifications, the sentinel assay provides a condensed and information-rich snapshot.

P-527.00

ABSOLUTE QUANTIFICATION OF PROTEINS AND PROTEIN MODIFICATIONS ON THE SINGLE-CELL LEVEL BY COMBINING MASS CYTOMETRY AND TARGETED PROTEOMICS

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Analyzing the content of individual cells permits to unravel cell-to-cell variability. Single-cell analyses also play important roles in systems biology, supporting our understanding of regulatory networks. The complexity of signaling pathways and their interplays requires high-throughput, quantitative and multiparameter single-cell technologies. Mass cytometry, based on atomic mass spectrometry, allows for single-cell network analysis using antibodies. However, due to unknown antigen-binding site occupancies, molecules absolute copy numbers cannot be directly determined, losing information about networks structure, reaction rates and signaling thresholds that commit cells into defined disease states.

To enable the absolute quantification on the single-cell level in mass cytometry, we combine it with targeted proteomics, aiming to study in a quantitative and temporal fashion relevant signaling pathways, including MAPK, AKT and JAK/STAT.

Cells of interest are grown and split. Half of the cells are labeled with metal-tagged antibodies and analysed by mass cytometry (CyTOF). Here, mass and abundance are determined of the metals bound to the cells.

The other half, after digestion, is quantitatively measured by targeted proteomics (SRM). Aqua peptides are used for their absolute copy numbers determination. This value is used to calibrate the CyTOF signal, determining copy numbers per single cell.

Important features to study complex signaling pathways and their perturbation\change upon stimuli are: single-cell analyses in a multiplexed manner; absolute quantification of molecule copy numbers. After selecting proteins and phosphorylation sites covering multiple signaling pathways, we designed a panel of antibodies for CyTOF analysis. Together, SRM assays were performed for the absolute quantification of the same proteins/phosphorylation sites and to calculate the average absolute copy number of each target per cell population. This value is computed to extrapolate single-cell copy numbers on CyTOF data. This approach provides absolute information to study regulatory signaling networks in cancer.

This integrated CyTOF-SRM approach will significantly advance quantitative single-cell analyses.

P-528.00

SWATH-MS FOR QUANTITATIVE PROTEOMICS OF NUCLEIC ACID BINDING AND REGULATORY PROTEINS

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The development of SWATH-MS (1) has provided a powerful platform for label-free proteomics. However, the transparent analysis of data generated by SWATH-MS has been a challenge that, until recently (2), has not been documented in detail, in which we describe a straightforward methodology for generation of a spectral library enriched for nucleic acid binding and regulatory proteins, data transformation and normalization, statistical analysis, and bioinformatic analysis.

We have since expanded this methodology to investigate the global proteomic alterations of macrophages infected with HIV-1 and also exposed to methamphetamine (METH) for 24 hours. In this investigation, we sought out to identify altered proteins involved in host response to both HIV-1 infection and to METH exposure. This 2 x 2 experimental design (HIV-1 +/-, METH +/-) required expansion of the macrophage spectral library as well as adaptation of additional statistical methods and bioinformatic platforms. Using two-way repeated-measures ANOVA, we were able to identify 135 proteins that were significantly altered ($p < 0.05$) in the interaction of HIV-1 and METH.

Using a variety of bioinformatic tools, including DAVID, PANTHER, KEGG, and Ingenuity Pathway Analysis, we have novel evidence to suggest a role for the mitochondria in both innate immunity as well as response to drugs of abuse.

P-529.00

WEAK LINEAR MOTIF-MEDIATED INTERACTIONS CONTRIBUTE TO THE REARRANGEMENT OF MODULAR ARCHITECTURE IN COMPLEX PROTEIN INTERACTION NETWORKS

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Biological modules are groups of genes that cooperatively carry out cellular functions and determines phenotypic consequences. However, the molecular components that lead to the rearrangement of modular architecture in protein interaction networks have not been clearly identified.

Here we propose that domain-linear motif interaction (DLIs) is a key component to rewire different functional modules due to its physical nature: weak and transient. We show that weak DLIs are more likely to connect different biological modules than strong domain-domain interactions (DDIs), investigating functional groups, protein complexes, and subcellular localizations. Moreover, the change of linear motifs works as ¡°evolutionary interaction switches¡± because subtle amino acid changes can cause the short sequences in linear motifs to appear and disappear.

We also show that DLI/DDI information can improve module identification processes, which often rely on network topology lacking the molecular characteristics of PPIs. Our findings could improve the functional annotation process by identifying biologically relevant modules in various biological networks

P-530.00

INTEGRATIVE MAPPING OF THE ZEBRAFISH EMBRYO PROTEOME AND TRANSCRIPTOME

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Background: The Zebrafish Danio rerio, whose genes are highly conserved with those of higher eukaryotes including human, is a popular system for studying vertebrate development and disease mechanisms. To understand the mechanisms deployed during ontogeny, we performed comparative analysis of whole proteome and transcriptome at early-stage (24h) zebrafish embryo.

Methods: We used deyoked protein samples from 24h zebrafish embyos for the proteome analysis. Trypsin digested samples were fractionated at protein and peptide level followed by liquid chromatography Mass Spectrometry (MS) analysis. MS data were processed using Trans-Proteomic Pipeline. RNA was sequenced in a SOLiD3 (ABI) platform generating 50-bp single-ended readings. Bioinformatics analysis was performed using integrated standard programs.

Results: We quantified 8363 proteins through a combination of thorough deyolking and extensive fractionation procedures. Bioinformatics analysis revealed that the expressed proteome and transcriptome display a moderate correlation for the majority of cellular processes. Integrative functional mapping of the quantified genes demonstrate that embryonic developmental systems differentially exploit transcriptional and post-transcriptional regulatory mechanisms to modulate protein amounts. Network mapping identified proteins belonging to various signal transduction pathways that may be regulated at the post-transcriptional level and important in embryonic development. Proteogenomics analysis of the MS data allowed identification of novel proteins/exons.

Conclusions: This study revealed differential deployment of molecular regulatory mechanisms that underlie zebrafish embryonic development. Similar analysis over the entire time course of development will unravel many novel mechanisms and various levels of gene expression control during embryogenesis. The novel proteins/exons identified by our proteogenomics analysis will be a valuable resource for zebrafish research as well as useful to probe for human homologs of such novel proteins. Furthermore, these data will be helpful to improve and evaluate gene annotation tools.

P-531.00

GENE ESSENTIALITY ON MODEL ORGANISM EXPLAINS CLINICAL SEVERITY OF HUMAN GENETIC DISORDERS

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Genotype-phenotype mapping in model organisms is crucial for understanding human disease phenotypes that emerge from genetic variations. However, it is under debate whether or not essential genes, indispensable genes for the survival of organisms, are associated with human disease genes. The perturbations of the essential genes have been suggested to show discernible phenotypic symptoms of human diseases. In contrast, mutations in nonessential genes have also been postulated to be associated with human disease genes, since mutations in essential genes likely result in lethal phenotype during reproductive ages rather than demonstrate disease phenotypes in adult stage.

Here, we found that both essential and nonessential genes are associated with diseases genes. However, essential and nonessential genes are associated with different human diseases classes. In particular, essential genes are enriched in cancer, cardiovascular, endocrine, developmental, respiratory and gastrointestinal disease. Nonessential genes are enriched in ear-nose-throat, connective tissue, ophthalmological, psychiatric and immunological diseases. We also found that the disease classes enriched with essential genes tend to be clinically more severe than those enriched with nonessential genes and show higher mortality in patient population.

Our results suggest that genotype-phenotype mapping in model organisms can predict human disease progression and help identifying patients who need intensive clinical care.

P-532.00

USING AN ITRAQ APPROACH TO INVESTIGATE THE EFFECT OF CIGARETTE SMOKE EXPOSURE IN A 90-DAYS INHALATION STUDY FOLLOWED BY 42-DAYS RECOVERY PERIOD ON SPRAGUE-DAWLEY RATS LUNG TISSUE.

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In order to update and advance our knowledge regarding the impact of cigarette smoke on the lungs of Sprague-Dawley rats, a system biology approach that combines state-of-the-art proteomics with transcriptomics and toxicological endpoints, was developed. A 90-day inhalation study followed by recovery period of 42 days was conducted as described in the Organization for Economic Co-operation and Development (OECD) Testing Guideline 413. A quantitative proteomics approach using isobaric tags for absolute and relative quantification (iTRAQ®) was performed on the lung tissues of Sprague-Dawley rats to detect changes in protein expression levels between control rats (exposed to air), and rats exposed to mainstream smoke (MS) of the Reference Cigarette 3R4F (2 exposure concentrations of 8, and 23 mg/L nicotine).

For the recovery period, the same treatment and control groups were used. Six biological replicates were analyzed to assess reproducibility within each group. The obtained mass-spectrometry data was searched against the Uniprot rat reference proteome database. Exposure of Sprague-Dawley rats to MS induced inflammatory changes in the lungs as detected by an increase in number of differentially expressed proteins and networks perturbed (as determined by network enrichment approaches) compared to the control sample. At the end of the recovery period, the protein expression profiles of MS-exposed rats showed a decreased amount of differentially expressed proteins indicating reverting back to normal levels compared to the control rats; that was shown by monitoring at least10 of the highly regulated proteins.

The obtained proteomics results complemented those obtained from other endpoints such as transcriptomics, lipidomics as well as lung histopathology, thereby providing a unique opportunity to reveal underlying molecular mechanisms of MS exposure that were captured within this study that will further establish the foundation of PMI's systems biology approach to assess the impact of modified risk tobacco products (MRTPs) on biological systems.

P-533.00

DYNAMICS PERSONAL OMICS PROFILES DURING PERIODS OF HEALTH, DISEASE, WEIGHT GAIN AND LOSS

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To understand how human physiology changes during periods of health and disease/stress we have performed integrated Personal Omics Profiling (iPOP), combining genomic, DNA methylomic, transcriptomic, proteomic, metabolomic, and microbiomic (gut, nasal and other) information, in a cohort of 50 individuals during healthy and aberrant periods.

The individuals are sampled on a regular basis, as well as frequently during times of respiratory illness and twenty of the subjects have experienced a high caloric diet (and weight gain) for thirty 30 days followed by a low caloric diet (and weight loss) for 60 days. Our iPOP analysis of blood, urine and microbiome components revealed extensive, dynamic and physiologically-relevant changes in diverse molecular components and biological pathways across healthy and disease conditions as well as during weight gain and loss.

Our study describes the epigenetic, biochemical and omic pathways associated with respiratory and weight gain stresses at a systems-wide level and we describe similarities and differences in these responses across individuals and different stressors.

P-534.00

THE GLOBAL LANDSCAPE OF HEMATOPOIETIC STEM CELLS AND THEIR IMMEDIATE PROGENY

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Introduction: Hematopoietic stem cells (HSC) harbor the highest self-renewal capacity and generate a series of multipotent progenitors (MPP) that differentiate into lineage-committed progenitors and subsequently mature cells. Despite intense research over the last decades the molecular basis of essential HSC features such as self-renewal and quiescence remains poorly understood.

Methods: To determine the molecular programs employed by HSCs and MPPs, we performed an extensive global analysis combining quantitative proteome, transcriptome (RNA-seq) and DNA-methylome analyses on five FACS-sorted HSC and MPP populations - HSC (Linneg Sca-1+ cKit+ ,LSK, CD34- Flt3- CD150+ CD48-), MPP1 (LSK CD34+ Flt3- CD150+ CD48-), MPP2 (LSK CD34+ Flt3- CD150+ CD48+), MPP3 (LSK CD34+ Flt3- CD150- CD48+) and MPP4 (LSK CD34+ Flt3+ CD150+ CD48+) - as previously described in our laboratory (Wilson et al., Cell, 2008).

Results: Integration of Proteomics and RNA-seq analyses identified more than 6,000 proteins and 27,000 genes and revealed striking consistency between RNA and protein levels, arguing for limited but potentially important post-transcriptional regulation at the transition from HSCs to MPPs. Further, the presented first proteome analysis of refined HSC-MPPs revealed potential novel HSC markers. HSCs are defined by stage-specific expression clusters including Wnt and Lin28-Hmga signaling, the imprinted-gene-network, Hox genes, retinoic acid metabolism.

Conclusion: This study provides a comprehensive genome-wide resource for functional exploration of the molecular, cellular and epigenetic processes operational at the pinnacle of the hematopoietic hierarchy.

P-535.00

COMMUNITY PROTEOMIC ANALYSIS OF ARCHAEAL COMMUNITY OF CELLULOSE-ENRICHED MUD AND WATER SLURRY FROM MT. MAKILING MUD SPRING, LOS BAÑOS, PHILIPPINES

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Analysis of microbial community composition is usually done by DNA-based approaches. In this study, the Archaeal community of cellulose-enriched mud and water slurry from Mt. Makiling Mud Spring was characterized using the community proteomics method. Total protein was extracted from the concentrated cell pellets obtained from the carboxymethyl cellulose-enriched set-ups using NaOH and phenol extraction method with visualization through SDS-PAGE. The peptides obtained from in-solution digestion of the protein extracts using trypsin were analyzed by tandem mass spectrometry (nanoLC-ESI-MS/MS).

Mass spectrometry analysis resulted in a total of 183 proteins, each represented by at least two tryptic peptides. Most of the proteins identified were from Sulfolobus solfataricus, S. shibatae, S. tokodaii, S. acidocaldarius, and Sulfolobus sp.. Furthermore, proteins from Caldivirga maquilingensis and Metallosphaera sedula and from other Archaea species, namely, Desulforococcus mobilis, Ignicoccus hospitalis, Methanobacterium thermoautotrophicum, Pyrobaculum arsenaticum, Pyrobaculum islandicum, Pyrococcus furiosus, Pyrococcus abyssi, and Pyrodictium occultum were also detected.

Compared to DNA-based community analysis previously conducted on Mud Spring samples, this study showed that community proteomics is capable of detecting more microorganisms derived from protein information extracted directly from the environment. This is the first reported use of community proteomics approach in analyzing a microbial community in the Philippines.

P-536.00

STRUCTURE-BASED PREDICTION AND BIOINFORMATICS ANALYSIS OF THE THIAMIN-DEPENDENT PROTEOME: IMPLICATIONS FOR THE THIAMIN DEFICIENCY STATES AND MODELS.

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Thiamin (vitamin B1) deficiency leads to neurological disorders corrected by thiamin administration, with high doses of thiamin improving cognition in patients with neurodegenerative diseases. In addition to thiamin diphosphate, the well-known coenzyme of central metabolism, living systems synthesize the non-coenzyme derivatives, such as thiamin triphosphate and adenylated thiamine triphosphate, suggested to be involved in neurotransmission and/or stress response. However, poor identification of biosynthetic enzymes and molecular targets of the non-coenzyme derivatives impedes understanding molecular mechanisms of action and pharmacological potential of these compounds.

Our work aims at molecular identification of proteins and pathways mediating the non-coenzyme action of thiamin. To address this goal, we defined the thiamin-binding structural motifs by using resolved structures of protein complexes with thiamin, its natural derivatives or biosynthetic precursors, and performed the PROSITE scan of the protein/genome databases against the 21 motifs created. Conservation of a motif within homologous proteins and/or co-occurrence of different thiamin-binding motifs within one protein were considered to heighten functional significance of the motif in the PROSITE hits. Examination of available 3D structures addressed the question if spatial configuration of the thiamin-binding motif in a predicted protein enables the binding function. Human proteome comprising proteins possessing the thiamin-binding motifs was analyzed by DAVID. The most enriched functional annotation terms and protein clusters of the proteome were related to extracellular matrix, signaling and post-translational modifications, supporting experimental data on the thiamin deficiency affecting cell junctions and signal-related protein modifications.

Comparison of the predicted and experimental thiamin-binding proteomes pointed to enzymes of central metabolism possessing the functionally competent thiamin-binding motifs near the active and/or regulatory sites. Kinetic assays confirmed allosteric (non-coenzyme) regulation of the enzymes by thiamin and/or derivatives, providing proof-of-concept for the bioinformatics-guided identification of the thiamin-dependent proteins. Thus, our structure-based predictions help identification of pharmacological targets of thiamin and derivatives.

P-537.00

TARGET IDENTIFICATION OF NON-TAGGED SMALL MOLECULES WITH DARTS AND LC/MS/MS

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Bioactive small molecules exert their activity by binding with their cellular target proteins. Therefore, it is critical to identify the target proteins to reveal the mechanism of bioactive small molecules. Target identification methods usually consist of probes linked to small molecules (tagged small molecules) to effectively pull-down target proteins by with high affinity. However, the drawback to this conventional method is that probe synthesis step is required, and the tagged small molecule may lose its functionality. Here, we propose DARTS (drug affinity response target stability) combined with LC/MS/MS method (DARTS-MS) to resolve these drawbacks. DARTS is a target identification method that depends on the target protein stability when bound to a small molecule.

The small molecule is bound to the entire cell lysate, a proteome pool. Then the possible target proteins of the small molecule increases in stability, and makes it resistant to protease treatment. The other proteins with no or weak binding with small molecule are degraded and only the target protein with high affinity binding is left, ready for identification through LC/MS/MS analysis. Herein, we used the entire cell lysate of HeLa cells to identify the target proteins of APZ, an autophagy inducer with unknown mechanism. Through mass spectrometry analysis, the entire proteome was identified, which resulted in 72 proteins resistant to protease with APZ treatment. Through molecular and cellular validations, the target candidates for APZ were found as -tubulin, Hsp70, and HLA-A. Among these candidates, -tubulin takes part in the localizing the autophagosome and the lysosome together so that fusion takes place for autophagolysosome formation.

Collectively, we present that DARTS-MS based target identification method is an effective platform to identify target proteins of non-tagged small molecules.

P-538.00

PROTEOMIC COMPARATIVE STUDY OF SMOOTH MUSCLE CELLS ISOLATED FROM SMALL PULMONARY AND SYSTEMIC ARTERIES

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Introduction and objectives: The cellular compositions of the systemic and pulmonary arterial walls are very similar. However the functional characteristics of these vessels show many differences. One of the most noticeable is in their reaction to hypoxia. We assume this difference can be consequence of the smooth muscle protein composition of the peripheral arteries. Thus we carried out the proteomic analysis of the smooth muscle cells obtained from the peripheral systemic and pulmonary vessels.

Methods: We used 14 adult male Wistar rats (200-250g). Animals lived in normoxia (n=9) or were exposed to isobaric hypoxia (FiO2=10%) in hypoxic chamber for 4 days (n=5). The rats were euthanased by the intraperitoneal injection of thiopental. Peripheral pulmonary and mesenteric arteries were dissected under the microscopic control. Smooth muscle cells were obtained by the enzymatic digestion of the vascular fragments and the isolated material was subjected to sonication in lysis buffer. Protein mixture was analyzed by one-dimensional gel SDS-polyacrylamide electrophoresis followed by two-dimensional gel SDS-polyacrylamide electrophoresis and identified using nLC-MS/MS. Proteins were identified by correlating tandem mass spectra to IPI and SwissProt databases.

Results and discussion: We detected the significant differences in the protein composition between the samples separated from the pulmonary and systemic smooth muscle cells and also between the pulmonary smooth muscle isolated from the rats living in normoxia and exposed to 4 days hypoxia.

Conclusions: We detected the significant increase of collagen VI quantity in the normoxic smooth muscle cells in comparison with animals exposed to hypoxia. This result brings new finding about extracellular matrix remodeling during hypoxia.

P-539.00

IDENTIFICATION OF SMALL OPEN READING FRAMES WITH HIGH CODING POTENTIAL IN MOSS PHYSCOMITRELLA PATENS

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Introduction and objectives: It has been revealed that small open reading frames (sORFs, up to 100 codons) have the potential to encode biologically active peptides that have regulatory roles in eukaryotic cells. However, most ab initio gene prediction programs are not well suited for identifying sORFs with coding potential. Moreover, existing standard proteomic approaches poorly suited for the identification of proteins less than 10 kDa.

Methods: We used prediction program sORFfinder (Hanada et al., 2012) to find intergenic regions with high coding potential in the genome of the model object moss Physcomitrella patens. High-throughput RNA-Seq by SOLiD 4 genetic analyzer (Life Technologies, Applied Biosystems) and identification of native peptides by TripleTOF 5600 LC-MS/MS (ABSciex) has been carried out on gametophore, protonema and protoplast cells of Physcomitrella patens. Optimal procedure for endogenous peptide extraction and identification has been worked out to demonstrate translation of sORFs.

Results and discussion: Using sORFfinder we distinguished 241,228 sORFs within intergenic region with high coding potential. RNA-Seq confirmed transcription of 8,450 sORFs from intergenic region and 16,928 previously known genes of Physcomitrella patens. Tandem mass-spectrometry analysis resulted in identification of 18 peptides derived from 12 sORFs within intergenic region, 52 peptides derived from 42 sORFs that were previously thought to be untranslated region of mRNAs and more than 100 peptides from about 100 alternative sORFs within previously known ORFs. Comparative analyses of sORFs sequences distinguished in Physcomitrella patens with genomes of other plant species revealed high conservation in terms of synonymous/nonsynonymous substitutions. The report will be discussed further steps to validate the results: overexpression and knockout mutants of coding sORFs, functional categorization and expression under stress.

Conclusions: Systems biology approaches based on high-throughput proteomics and transcriptomics led to identification of short peptides derived from sORFs. Carried out steps on validation confirmed the accuracy of most identifications.

P-540.00

ADVANCED TI4+-IMAC (PHOSPHO)PROTEOMICS TO IDENTIFY NOVEL MELANOMA COMPANION DRUG TARGETS AND UNCOVER PHOSPHORYLATION DYNAMICS AND PATHWAY DEPENDENCE IN SENESCENCE SIGNALING

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Introduction and objectives

Malignant melanoma is mainly fuelled through the ERK pathway, mainly due to oncogenic BRAFV600E. Development of targeted therapy gives unprecedented clinical benefits, however, eventually all melanomas become resistant to inhibition of the ERK pathway, and patients succumb to the disease. Therefore, there is a dire need to find alternative (combination) drug targets or activate preventative mechanisms as oncogene-induced senescence (OIS). Here we use MS-based (phospho)proteomics, using Ti4+-IMAC, and targeted phospho-SRM approaches, to describe dynamics of several of the pathways involved and report a novel combination target, additive to directed therapy against melanoma.

Methods

After lysis, proteins were digested by Lys-C and Trypsin, dimethyl labeled, fractionated by SCX, and for the phosphoproteomics enriched by Ti4+-IMAC. Samples were analysed directly using nanoRP-UHPLC coupled to an Orbitrap Elite or Q-Exactive. Phospho-SRM measurements were performed using a TSQ Vantage triple quadrupole MS.

Results and Discussion

We identified ~13,000 phosphosites and ~5,700 proteins in our drug sensitizer screen. Overall, 588 proteins were significantly changing between control, 1 day, and 3 days of treatment, with the most dramatic change in protein receptors after 3 days. We were able to identify several proteins previously reported to be involved in melanoma drug resistance. Using our integrated proteomics and kinome shRNA genomics screen we identified a novel target additive to vemurafenib melanoma therapy. Next, to gain more insight into the vital tumour suppression mechanism OIS, we performed an MS-based screening in cycling and OIS cells as well as cells that have abrogated senescence (OISb), followed by further investigation of one the regulated pathways by targeted phospho-SRM assays.

Conclusions

Comprehensive global and PTM-targeted screening of BRAFV600E mutant cells reveals novel drug combination targets and phosphorylation controlled pathway dependence.

Topic 13

Inflammatory and rheumatic diseases

OP066 - AUTOANTIBODIES IN THE SERA OF PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Introduction and objectives

Amyotrophic lateral sclerosis (ALS), also known as Lou Gehrig's disease, is a progressive neurodegenerative disorder. The pathological hallmark of ALS is the degeneration of motor neurons in the spinal cord, brain stem, and motor cortex. ALS is generally a sporadic disease, but a genetic component has been found in 5-10% of ALS patients. Diagnostic criteria have been developed to improve ALS disease classification. Diagnosis of this disorder is based on clinical assessment, but early classification is not likely to be reliable. The average survival time is less than 3 years.

Methods

Injections of IgG from SALS patients into mice revealed the specific labeling of murine motor neurons 2,3. Additionally, IgG has been found in upper and lower motor neurons in ALS patients. To identify potential autoantibodies, earlier studies used hypothesis-driven approaches. In contrast to such ELISAs, we set out to utilize protein microarrays in a case-control study.

Results and Discussion

We identified 20 candidate proteins that could differentiate between the study groups with 100% specificity and 99.9% sensitivity1. Total immunoglobulin levels assessed using routine laboratory variables were not different between cases and controls.

Conclusions

The identified panel may be a potential biomarker to use in ALS diagnostics and disease progression monitoring. However, validation in a prospective study with ALS patients is important.

1.May C, Nordhoff E, Casjens S, et al. Highly Immunoreactive IgG Antibodies Directed against a Set of Twenty Human Proteins in the Sera of Patients with Amyotrophic Lateral Sclerosis Identified by Protein Array. PLoS One 2014;9:e89596.

2. Engelhardt JI, Soos J, Obal I, Vigh L, Siklos L. Subcellular localization of IgG from the sera of ALS patients in the nervous system. Acta Neurol Scand 2005;112:126-33.

3. Pagani MR, Gonzalez LE, Uchitel OD. Autoimmunity in amyotrophic lateral sclerosis: past and present. Neurol Res Int 2011;2011:497080.

OP067 - 2D-DIGE/MS ANALYSIS ALLOWS THE IDENTIFICATION OF A DISTINCT SERUM PROTEIN PROFILE IN CD38-DEFICIENT MICE ASSOCIATED WITH EITHER COLLAGEN-INDUCED ARTHRITIS OR INFLAMMATION.

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Introduction and objectives: Collagen type II-induced arthritis (CIA) is an autoimmune disease that is accompanied by a complex host systemic response, which includes inflammatory and autoimmune reactions. Since to develop CIA is required the injection of antigen in complete Freund's adjuvant (CFA), which is a water-in-mineral-oil emulsion containing killed Mycobacteria, systemic response proteins related with inflammation can confound the detection or diagnosis of arthritis. CIA in CD38 deficient mice (CD38 KO) is milder than that in C57BL/6 (B6 WT) mice. The purpose of this study was to identify CD38-dependent changes in serum protein abundance in mice with CIA versus mice with overt inflammation caused by CFA injection alone.

Methods: Blood serum samples were treated with ProteoMiner beads to equalize protein concentrations and subjected to 2D-DiGE and MS-MALDI-TOF/TOF analysis to identify proteins that were differentially expressed in CD38 KO and B6 WT mice with arthritis or with inflammation. Differential protein expression was validated by ELISA, or Western-blotting.

Results and Discussion: Altered proteins included those involved in acute phase response (SAA1), inflammation (B2m), complement activation (Ficolins, C4-B fragments, C1qb, C3 beta chain) and lipid metabolism (ApoE, ApoAI, ApoAII and ApoJ/Clusterin). Multivariate analyses of serum protein profiles between the autoimmune and inflammation models revealed a set of proteins that were distinct to arthritis-bearing mice, whereas other protein changes were clearly part of the non-specific host inflammatory response. Moreover, the proteins that were differentially expressed were useful to discriminate between CD38 KO and B6 WT mice in their response to either collagen immunization or to CFA injection.

Conclusions: This proteomic approach provides a basis for distinguishing between protein changes in serum or plasma that are arthritis-related and those that are part of a non-specific host inflammatory response.

OP068 - PROTEOMIC STUDIES OF HMGB1 INVOLVEMENT IN LUNG INFLAMMATION

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Introduction and Objectives: High mobility group proteins (HMG) are a family of proteins initially identified as nonhistone nuclear proteins that bind to DNA and are involved in regulating gene transcription. We have focused on one member of the family, HMGB1, which has been described to have cytokine-like proinflammatory activity and shown to be a late mediator of endotoxin lethality. The multifunctional features of HMGB1 in part stem from its translocation out of the nucleus to the cytoplasm due to posttranslational modifications and actions of various stimuli. HMGB1 has been implicated in many disease scenarios involving inflammation. Our proteomic studies were established to investigate the release of HMGB1 from lung epithelial cells and leukocytes in response to various cytokines and respiratory viruses especially involved in the pathogenicity of lung inflammation focusing on asthma and viral infections.

Methods: Human lung epithelial cells and isolated purified quiescent leukocytes were exposed to various cytokine stimuli implicated in lung inflammation as well as to respiratory syncytial virus (RSV). Protein expression was analyzed by a combination of analytical methods including 2-dimensional gel electrophoresis, mass spectrometry, flow cytometry, Western blot analysis, immunohistochemistry, and electron microscopy.

Conclusions: We have shown that many cytokines known to play a role in lung inflammation are able to cause the release of HMGB1 (sHMGB1). sHMGB1 was investigated for posttranslational modifications and functional activity. Our results show that sHMGB1 induces phosphorylation of p38, MAPK, and NF-êB p65 in human monocytes causing proinflammatory mediator release. Moreover, sHMGB1 caused the release of VEGF from bronchial epithelial cells, a feature of asthma pathogenesis. Additionally, we have found that RSV infection of bronchial epithelial cells releases sHMGB1. Finally and importantly, we have established that much of sHMGB1 release is mediated via an exosomal mechanism.

OP069 - MOLECULAR PROFILING OF AUTOIMMUNE DISEASE FOR IMPROVED DIAGNOSIS, PROGNOSIS, AND CLASSIFICATION

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Introduction and objectives: Current diagnostic and prognostic procedures for autoimmune diseases, illustrated by Systemic Lupus Erythematosus (SLE), have major caveats, and no single blood- and urine-based tests are pathognomonic. Instead, diagnostic, prognostic, and treatment related decisions rely on combinations of clinical, laboratory, and/or imaging features, resulting in costly delay of optimal care and impaired well-being of the patients. To resolve these unmet clinical needs, we will explore the use of affinity proteomics to decipher disease-associated molecular serum (urine) profiles of SLE, as well as other autoimmune conditions (e.g. SSc, RA, pSS), for improved, early differential diagnosis, prognosis, and classification in a manner hitherto not yet accomplished.

Methods: We have used a set of in-house designed recombinant antibody micro- and nano-array technology platforms for miniaturized, multiplexed protein expression profiling of multiple cohorts of urine and/or serum samples. Targeting mainly immunoregulatory proteins, we have harvested the immune system as an early, specific, and sensitive sensor for disease.

Results and discussion: The results showed that we have successfully de-convoluted several SLE-associated serum and urine biomarkers. In more detail, we have pre-validated a serum biomarker panel (< 25 markers) for diagnosis with high specificity and sensitivity. Furthermore, we have also delineated the first condensed serological and/or urological biomarker panels reflecting both prognosis, i.e. disease activity, as well as phenotype, i.e. disease severity. In addition, we have taken the first steps towards deciphering serum biomarker panel(s) for differential diagnosis of autoimmune diseases, e.g. SLE vs SSc.

Conclusions: Hence, we have shown that affinity proteomics could be used to define crude, non-fractionated serum and urine proteomes, extracting molecular portraits of SLE and other autoimmune conditions, paving the way for improved (differential) diagnosis, prognosis, and classification based on a simple blood (urine) test, as well as for an enhanced understanding of the underlying disease biology.

OP070 - DETECTION OF BACTERIAL AND HOST PROTEINS IN PEDIATRIC ULCERATIVE COLITIS FROM A SINGLE COLONIC BIOPSY SAMPLE BY SWATH-MS

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Introduction
The Data independent acquisition (DIA) strategy termed SWATH-MS combines the accurate quantification of targeted proteomics with protein identification capability of data dependent acquisition (DDA). In this study SWATH-MS was used to analyze biopsies from patients suffering from ulcerative colitis. Ulcerative colitis is a chronic inflammation of the gastrointestinal track confined to the colon and rectum only. The aim of the study was to classify the tissues' clinical status based on the bacterial and human protein information. Due to the mixture of the human and bacterial proteins in a sample, human gut biopsy as a proteomic sample represents extreme complexity.

Methods
Samples were collected from patients during the diagnostic colonoscopy (median age 15 years). Directly after the sample collection the enzymes were heat inactivated and tissues were processed and analyzed in MS by using previously established method (PCT-SWATH, Guo, et al. submitted).

Results and discussion
From the 18 UC-patient samples (9 patients, inflamed and non-inflamed sample per patient) we were able to identify 5707 proteins (226 bacterial proteins) and quantify 4569 proteins (149 bacterial proteins) across all patients. Human calprotectin, together with myxobacterial proteins were observed to be over expressed in inflamed tissue where as human proteins involved in cell adhesion and cell junctions were observed to be down regulated. A defective mucosal barrier function has been reported as one of the conditions in UC. Fecal calprotectin is used in clinic as biomarker for inflamed tissue and overexpressed myxobacterial proteins are involved in bacterial secretion system and transport system. Identified bacteria are known to produce cytotoxic metabolites, which induces the inflammation.

Conclusions
We were able to identify bacterial and human proteins from the same clinical tissue (1-2mg) by using PCT-SWATH. From the acquired information we could classify the inflamed and non-inflamed tissue samples based on human and bacterial proteins.

P-541.00

DISCOVERY OF SERUM BIOMARKERS FOR PREDICTION OF RESPONSE TO ADALIMUMAB IN RHEUMATOID ARTHRITIS TREATMENT

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Introduction/objectives:

Biologic drugs such as Adalimumab, an anti-TNF monoclonal antibody, have been a great advance in Rheumatoid Arthritis (RA) treatment. However, for unknown reasons, a significant fraction of RA patients do not respond to specific drugs of this type, whereas they respond to different biologics. The current approach for selecting the biologic to administer is trial-and-error, checking after six months of treatment whether it's working. This approach is inefficient for both —patient and healthcare system. Thus, biomarkers for prediction of treatment response at an early stage are needed. The aim of this study is the discovery of predictive protein biomarkers for Adalimumab response in serum samples taken before treatment.

Methods:

Serum was collected from RA patients (according to ACR criteria) before beginning treatment. Donors were treated with Adalimumab and clinical response (EULAR criteria) was determined six months later. Eight samples from patients with good clinical response (n=4) and with no response (n=4) were selected. After depletion of high-abundance proteins and trypsin digestion, samples were differentially labeled with the iTRAQ 8-plex labels. Peptides were fractionated by SCX-LC and analyzed by LC-MS (Orbitrap Velos, Thermo). ProteinPilot v4.0 was used for identification and relative quantification of proteins.

Results/discussion:

349 proteins were identified in all samples, most of them proteins of medium and low abundance in serum. 333 proteins could be quantified using iTRAQ labeling. Nine proteins presented concentration differences between responders and non-responders (p

Conclusions:

A new panel of serum protein biomarkers for the prediction of response to Adalimumab was identified by a quantitative proteomics approach using isotopic labeling. The predictive value of these proteins should be validated on a larger sample. These markers would be useful for the classification of RA patients before starting treatment, so that non-responders could be treated with a different drug.

P-542.00

DIFFERENTIALLY EXPRESSED PROTEINS IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

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Lupus nephritis (LN) happens when lupus involves kidneys and it can lead to kidney failure and even death. In this study, we aimed to uncover proteins differentially expressed and excreted in plasma and urine of systemic lupus erythematosus (SLE) patients, as well as those that were specific to each class of LN patients to facilitate the diagnostic procedures in the future. Plasma and urine samples were recruited from SLE patients without kidney involvement, LN class II-V patients, LN class IV remission patients, and healthy individuals from University of Malaya Medical Centre (UMMC), Kuala Lumpur.

Two-dimensional difference gel electrophoresis (2D-DIGE) and liquid chromatography tandem mass spectrometry (LC-MS/MS) approaches were used for protein comparison and identification. A total of 33 plasma proteins were found to be correlated with SLE regardless of clinical manifestations. Serum constitutional and transport proteins, including haptoglobin and histidine-rich glycoprotein, occupied the largest portion among plasma proteins (~28%).

The other differentially expressed plasma proteins included complement fractions, metal binding proteins, proteases and inhibitors. Several plasma proteins were newly discovered in the present study, such as afamin, hemopexin, retinol-binding protein 4, and vitamin D-binding protein. Class-specific proteins involved up-regulation of Ig kappa chain C region which was specific to LN class IV and down-regulation of histidine-rich glycoprotein which was unique to LN class V. In the case of urine, 38 urine proteins were found to be associated with SLE regardless of clinical manifestations. Most proteins were serum constitutional and transport proteins, and proteases and inhibitors (23.7% each).

Majority of the urine proteins was newly reported to be associated with SLE in this study, for instance tetranectin and zinc-alpha-2-glycoprotein. Class-specific urine proteins were identified as mannan-binding lectin serine protease 2, Ig kappa chain C region, and kininogen-1 for LN class III, retinol-binding protein 4 and alpha-1-antichymotrypsin for LN class IV, etc.

P-543.00

PROTEOMIC ANALYSIS OF THE MESENCHYMAL STEM CELLS SECRETOME USING A LABEL-FREE APPROACH

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Introduction and objectives

Mesenchymal stem cell (MSCs) have been shown to have a pivotal role in tissue repair and in local control of inflammation. In the last few years there has been a growing interest in using them to treat human inflammatory diseases, including severe steroid resistant acute graft versus host disease (aGVHD). Although there is evidence from in vivo studies that MSCs are able to reduce inflammatory damage, it is not clear whether their immunomodulatory effects rely on soluble factors or cell-cell contacts. It has been reported that the encapsulated MSCs are extremely efficient in down-modulating in vivo immune responses, such as antigen-specific T cell responses and acute GVHD, and that this activity is based on soluble factors released by MSC into the blood stream. Based on these evidences, in collaboration with Humanitas Clinical and Research Center, we compared the secretome of MSC, isolated from murine bone marrow cells, before & after treatment with pro-inflammatory cytokines (TNFs, IL1b, and IL6).

Methods

To achieve this goal, we employed a label-free quantitative approach that allows us to examine differences in global protein expression between samples. Samples have been derivatized, digested and analyzed by LC-nanoESI-LTQ Orbitrap Velos equipped with a RPC18 column prior to MS/MS analysis. For protein identification and quantification, raw data files were processed using MaxQuant 1.3.0.5.

Results and discussion

Two biological replicates with five technical replicates were analyzed. The results of label-free quantification allowed to identify 1839 and 2005 proteins in the two replicates, respectively. Statistical analysis (Student's t-test, $p < 0.01$) performed on each sample revealed that 422 and 522 proteins were significantly changed between cell lines. Among these, 144 proteins were differentially expressed in both replicates; 91 were down-regulated and 53 up-regulated. The main categories enriched are those related to signal, secretion, innate immunity and inflammation.

P-544.00

POTENTIAL PROTEOMIC BIOMARKERS ASSOCIATED TO MUCOSAL HEALING IN CROHN'S DISEASE

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Introduction and objectives: In Crohn's disease (CD), there is a discrepancy between clinical activity of the disease (symptoms) and intestinal healing. However absence of tissue healing is associated with the risk of relapse and tissue damage progression. Endoscopy is costly and invasive. Hence biomarkers correlating with intestinal healing could improve disease management. We aimed to identify potential biomarkers associated to CD mucosal healing by a shotgun proteomics label free study.

Methods: We used the STORI clinical trial cohort (n=103) aiming at identifying markers associated to relapse prediction after Infliximab treatment withdrawals. We used serum samples of patients in clinical remission, grouped according to the degree of intestinal healing seen at endoscopy. We performed depletion of the 20 most abundant plasma proteins on each serum pools and ran a proteomics label free differential analysis using 2D-nanoUPLC-MSE HDMS Synapt G1 for data acquisition and Protein Lynks Global Server vs 2.4 for data analysis (Waters, Corp., Milford, USA).

Results and Discussion: We obtained potential biomarkers and designed a multiplexed - selected reaction monitoring (SRM) method for validation of these candidates in each individual patient. The method may also be tested in an independent set of IBD patients with and without mucosal healing.

Conclusions: This research strategy and results of SRM validation of potential biomarkers associated to mucosal healing in this cohort of CD patients as well as the tests done on other CD patients, may provide new opportunities for CD follow-up tests development.

P-545.00
PROTEOMIC ANALYSIS OF CLUBFEET

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Introduction and objectives: Congenital talipes equinovarus (CETV), also referred as clubfeet too, occurs in 1 to 2 in 1000 live births, and is one of the most common birth defects involving the musculoskeletal system [1]. It is recognizable at birth and is readily distinguishable from positional foot anomalies because the foot is rigid and does not correct with passive movement. The pathogenesis of this disease is unknown despite numerous hypothesis.

Methods: We studied the capsule specimen of talo-navicular joint on medial side of the foot of six patients with CTEV. The extracted peptides (trytic digestion) were analyzed, and detected by nano-liquid chromatography coupled to a maXis Q-TOF (quadrupole – time of flight) mass spectrometer with ultrahigh resolution.

Results and discussion: In total we detected 19 extracellular matrix proteins in affected tissue of clubfeet disease. Most of these proteins are incorporated into tissue remodelling and/or fibrosis. We detected in total 14 fibrosis-related proteins for the first time: six leucine-rich repeat proteoglycans, three extracellular matrix glycoproteins, three collagens, periostin, and TGFIp.
In addition to these findings, we indirectly confirmed the presence of TGF. This protein has been observed in higher concentration in connection with this disease before [2].

Conclusions: This study opens new view to clubfeet disease by complex proteomic analysis and brings new alternative targets proteins, which are closely connected to fibrosis processes.

P-546.00

MOLECULAR PROFILING OF PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMCS) FOR THE IDENTIFICATION OF CELLULAR RESPONSES TO DEXAMETHASONE

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Introduction
The intake of drugs over a long time period can lead to a loss of drug efficiency. This may occur not because the active component is old or loses its activity, but rather because cells are able to change their responses to drugs. It seems that they adapt their phenotypes to varying surrounding conditions via different intracellular mechanisms, which are not yet fully understood. Comprehensive proteome profiles of cells in different functional states could be helpful in identifying such complex mechanisms in in vitro model systems.

Methods
To reflect an inflammatory state in vivo, we activated PBMCs by stimulation with Lipopolysaccharide (LPS) and phytoheamagglutinin (PHA). Afterwards, the activated cells were treated with dexamethasone, which allowed identifying possible cellular processes occurring in response to drug application. In depth proteome profiling was achieved by using a nanoLC system coupled to a QExactive orbitrap. Furthermore, to determine abundance of proteins we applied the MaxQuant software.

Results and Discussion
After a successful inflammatory activation of PBMCs, treatment of the cells with dexamethasone led again to a down-regulation of NFκB target genes such as interleukin (IL)-1α, IL-1β, IL-6 and IL-8, at least in the secretome. In contrast, the expected down-regulation of interferon family members did not occur. Furthermore, we observed the accumulation of inflammatory molecules such as IL-1α within the cell´s cytoplasm, possibly reflecting regulatory effects caused by suppression of secretion. After an initially promising therapy, secondary necrosis may lead to the release of such cytoplasmic cytokines, and in consequence to the reoccurrence of inflammation.

Conclusion
The understanding of such intracellular mechanisms could help improving routinely used therapies, as well as finding new strategies for treatment options.

P-547.00

PROTEOMIC ANALYSIS USING NUCLEID ACID PROGRAMMABLE PROTEIN ARRAYS FOR SERUM AUTOANTIBODY PROFILING IN RHEUMATIC DISEASES

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INTRODUCTION AND OBJECTIVES: The progressive release of proteins from the cartilage and an abnormal metabolic activity can be specifically detected by the immune system, leading to a humoral immune response producing immunoglobulins against these proteins. The aim of this study was to detect the presence of autoantibodies in osteoarthritis (OA) serum samples, and to compare these results with those obtained from healthy (CTRL) and rheumatoid arthritis (RA) sera using a specific Nucleic Acid Protein Programmable Array (NAPPA).

METHODS: NAPPA was designed and constructed as previously described by Ramachandran et al. 2008, containing 80 sequence-verified full-length human selected genes obtained from the Center for Personalized Diagnostics at the Arizona State University. Once proteins were displayed by in situ cell-free protein expression system, NAPPA arrays were incubated in optimized conditions with 20 OA, 20 RA and 18 CTRL serum samples. The autoantibodies were detected by anti-human IgG-HRP and Cy3 dye. For the data analysis, fluorescent intensities were converted to define reactivity according to a specific threshold. The statistical analysis was performed by Wilcoxon signed-rank test.

RESULTS AND DISCUSSION: The levels of 4 autoantibodies were observed to be significantly (p-value

CONCLUSIONS: We used NAPPA to identify different serum autoantibodies profiles, which allowed distinguishing between OA and CTRL patients and most interestingly, between OA and RA patients. These autoantibodies released to the serum might have biomarker value for a more accurate early diagnosis and prognosis of OA patients in clinical routines.

P-548.00

PROFILING OF CARTILAGE-CHARACTERISTIC PROTEINS TO IDENTIFY A NOVEL PANEL OF OSTEOARTHRITIS BIOMARKER CANDIDATES.

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INTRODUCTION AND OBJECTIVES: The aim of this study was to perform a quantitative proteomics approach to identify and quantify proteins released from normal (N) and different zones of osteoarthritis (OA) human articular cartilages to provide a background for a better understanding of OA onset and progression, and to detect novel OA protein biomarker candidates.

METHODS: Cartilage discs obtained from femoral heads and tibial condyles of 4 N and 4 OA human cartilages were placed into 96-well plates and incubated during 6 days. Among OA samples, we differentiated the wounded zones (WZ) from those corresponding to the area adjacent to the lesion, or unwounded zones (UZ). Proteins released were collected, digested with trypsin and labelled with iTRAQ reagents (ABSciex). Then, labelled peptides of the different conditions were mixed and separated by liquid chromatography (LC) followed by reversed-phase nano-LC coupled to MALDI mass spectrometry (MS). The identification and relative quantification of the proteins was carried out with Protein Pilot 3.0 software.

RESULTS AND DISCUSSION: After statistical analysis, we found 104 secreted proteins showing significant differences in abundance between the different OA zones (WZ and UZ) and N samples. We classified them into 3 sets of proteins: a first group of proteins modulated specifically in UZ samples; a second group of proteins altered only in WZ samples, and finally a third group modified in both OA zones. Many of these proteins are novel candidates of the disease onset (the first group) and progression (the second and third groups). We also observed different protein profiles between hips and femoral condyles.

CONCLUSIONS: We describe a novel panel of cartilage-secreted proteins with potential biomarker value characteristic of the affected joint. This panel is now being explored in biological fluids (synovial fluid and serum) for the development of early diagnosis and anti-OA therapy monitoring strategies.

P-549.00

SILAC-BASED PROTEOMIC ANALYSIS REVEALED DIFFERENTIALLY EXTRACELLULAR PROTEIN PROFILES OF OSTEOARTHRITIC AND NORMAL MESENCHYMAL STEM CELLS UNDERGOING CHONDROGENESIS.

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Introduction and objectives: A major challenge for the osteoarthitic (OA) cartilage reparation by stem cell-based approaches is the understanding of chondrogenesis or cartilage formation. In this work, we have analyzed the extracellular protein expression profile of human bone marrow MSCs (hBMSCs) of OA patients and controls undergoing chondrogenesis, in order to compare the mechanisms involved in the cartilage extracellular matrix (ECM) remodeling that occurs during this process.

Methods: hBMSCs isolated from 3 OA patients and 3 healthy controls were grown with different isotope variants of lysine and arginine (Arg6, Lys4 for the control population and Arg10, Lys8 for the OA population) during 4-6 weeks, until achievement of full protein labeling. The labeled populations were then subjected to differentiation in 3D cultures (micromasses) supplemented with chondrogenic inducers for 14 days. Proteins in the conditioned media from the two cell populations were combined, separated by 1D-SDS-PAGE and subjected to in-gel trypsin digestion using an automatic digestor. The resulting peptide mixtures were analyzed by nanoLC coupled on-line to an LTQ-Orbitrap XL mass spectrometer and quantified using the MaxQuant software and the Perseus tool.

Results: A high content of collagen type II and different proteoglycans were found in normal donors compared to the OA patients. SILAC labeling revealed 56 differentially expressed extracellular proteins between the two conditions. Several of these proteins increased in OA were cartilage specific proteoglycans (hyaluronan and proteoglycan link protein 1, aggrecan core protein or lumican) as well as proteins with a well-known role in the pathogenesis of OA like COMP or MMP3. Interestingly, we detected several proteins decreased in OA which belong to the tenascin protein family and WISP2.

Conclusions: Using double-SILAC strategy we were able to identify extracellular chondrogenic markers that could be useful for the molecular monitorization of this process in cell therapy-based approaches for cartilage repair.

P-550.00

SERUM PEPTIDE PROFILES OF OSTEOARTHRITIS AND RHEUMATIC DISORDERS BY MALDI-TOF MASS SPECTROMETRY

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Introduction and objectives

Osteoarthritis (OA) is the most common articular disease, however the diagnostic methods currently available are limited and lack sensitivity. The aim of this work was to search serum peptide profiles of OA and two different rheumatic diseases using an easy and fast proteomic approach.

Methods

A total of 80 serum samples, 20 donors with OA, 20 with rheumatic arthritis (AR), 20 with psoriatic arthritis (APS) and 20 healthy donors, were subjected to a protocol previously described by our group. Briefly, the samples were depleted with the chemical sequential depletion protocol involving two precipitation steps. Then, the samples were digested with trypsin accelerated with ultrasounds. The resulting peptides were retained in a commercial tip with a C18 chromatographic media, and were eluted in four different amounts of acetonitrile (ACN). Each eluted fraction was analysed by quintuplicate using MALDI-TOF/TOF spectrometry. The reproducible peptides masses of each condition were used to obtain peptide profiles that discriminate between the four conditions with statistical significance. PCA analyses were performed with these peptides in order to validate their ability to differentiate the serum samples.

Results and discussion

One hundred and seven discriminatory peptides were obtained from the reproducible peptides masses of each condition with a p-value < 0.05. These peptides were distributed in two groups, 51 peptides more expressed in one condition versus the other conditions (13 for OA, 13 for AR, 12 for APS and 13 for control) and 56 peptides more expressed in two or more conditions at the same time. The PCA performed with the selected OA and control peaks showed the separation of the OA and healthy serum samples in at least two planes.

Conclusions

The serum peptide profiles obtained can discriminate patients with OA from healthy donors, and from donors with another rheumatic disease like AR or APS.

P-551.00

CHARACTERIZATIONS OF HEPARIN-BINDING PROTEINS IN HUMAN URINE AND IDENTIFICATION OF "L-X(2,3)-A-X(0,1)-L" AS A NOVEL HEPARIN-BINDING MOTIF.

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Introduction and Objectives: Heparin-binding proteins (HBPs) are considered as potential modulators of kidney stone formation. Previously, HBPs had been identified mainly in human plasma, seminal fluid and neutrophils, but not in urine. The present study thus aimed to characterize HBPs in the urine.

Methods: Cellufine sulphate column chromatography was employed to isolate HBPs from normal human urine and the recovered proteins were identified by liquid chromatography coupled to quadrupole time-of-flight tandem mass spectrometry (LC-Q-TOF MS/MS). Finally, heparin-binding motifs were analyzed.

Results: A total of 83 urinary proteins were identified as potential HBPs. The identified proteins included those involved in metabolic processes, cellular processes, immune system, developmental processes, response to stimuli, cell communications, transport, cell adhesion and others. From these, 59, 55 and 51 had the known heparin-binding motifs "XBBXnBX", "XBXnBBX" and "XBBBnX", respectively (B=basic amino acids). In addition, a novel heparin-binding motif "L-x(2,3)-A-x(0,1)-L" was found in 58 identified HBPs using PRATT search tool. The sensitivity and specificity of this novel motif were 85% and 100%, respectively, in validation using 20 known HBPs and 11 non-HBPs, respectively.

Conclusions: We report herein for the first time a large number of HBPs in normal human urine and identified "L-x(2,3)-A-x(0,1)-L" as a novel heparin-binding motif. These findings will be useful to further understanding of renal physiology and may also lead to identification of novel modulators of kidney stone formation.

P-552.00

PROTEOMICS REVEALED AN IMPORTANT ROLE OF LAMIN A/C IN KIDNEY STONE DISEASE.

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Because of ineffective prevention of kidney stone disease, which reflects poor understanding of the disease, we employed proteomics to explore mechanisms of kidney stone formation. Expression proteomics using 2-D PAGE revealed changes in levels of 20 proteins (15 increased 5 decreased) in renal tubular cells after exposure to calcium oxalate monohydrate (COM) crystals, which are the major crystalline composition found in kidney stones. One among the up-regulated proteins was identified as lamin A/C (LMNA).

The increased levels of LMNA and its partner, nesprin-1, in MDCK cells upon COM crystal adhesion were confirmed by Western blotting and immunofluorescence staining. LMNA was then knocked-down by small interfering RNA (siRNA), whereas si-Control served as the controlled condition of the siRNA transfection. Immunofluorescence staining confirmed the efficiency of si-LMNA, which also reduced expression of its partner, nesprin-1. Scratch assay and total cell count revealed defects in tissue repair and cell proliferation, respectively, whereas cell death quantitation showed no cytotoxicity in si-LMNA-transfected cells. Crystal-binding assay highlighted the role of LMNA in crystal adhesion as demonstrated by a dramatic reduction of number of COM crystals adhered on the si-LMNA-transfected cells. Protein network analysis revealed interactions between LMNA and potential COM crystal receptors. Their associations were confirmed by reduced levels of these proteins, including vimentin, tubulin, enolase, S100 and annexin A2, in the si-LMNA-transfected cells.

These data have demonstrated for the first time that LMNA in renal tubular cells is important for COM crystal adhesion, tissue repair and cell proliferation, and is associated with potential receptors of COM crystals. Therefore, LMNA may serve as a potential target for prevention of kidney stone disease and its recurrence.

P-553.00

DYNAMIC PROFILING OF CHROMATIN PROTEOME AT ENHANCERS OF INFLAMMATORY GENES

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Introduction and objectives:

Inflammatory stimulus drives a fine rearrangement of cell-specific chromatin determinants at cis-regulatory regions of inflammatory genes. Although few determinants have been characterized (like H3K4me1 and Pu.1), a global picture on enhancers' molecular signature and how specific determinants dynamically synergize during inflammation remain still incomplete.

Mass Spectrometry (MS) is a potent tool to study comprehensively histone modifications (hPTMs) and chromatin-binding proteins (chromatome). Recently, we established a proteomic approach (ChroP), combining chromatin immunoprecipitation (ChIP) and MS-proteomics, to characterize the proteome at specific chromatin territories. Here, we employ the ChroP approach to enrich enhancer regions of macrophages inflammatory genes and to profile their chromatome composition both at the steady state and in a dynamic fashion upon inflammation.

Methods:

The analysis was carried out in resting macrophages and upon LPS. Native chromatin was used to profile hPTM patterns (N-ChroP) while formaldehyde-fixed chromatin was employed with SILAC and competition assay to identify specific chromatin factors (X-ChroP) and with triple-SILAC setup to profile their plasticity during inflammation (tc-ChroP). Antibodies against H3K4me1 and Pu.1 were used in ChIP to specifically enrich enhancers. Samples were analyzed by UHPLC coupled with Q-Exactive. hPTMs were quantified by a label free approach while specific interacting proteins were defined based on their SILAC ratios.

Results and Discussion:

Preliminary data indicate, at basal condition, the enrichment of hPTMs associated with euchromatin and the appearance, upon LPS, of hPTMs typically associated with transcribed gene bodies.

X-ChroP at basal condition identifies a set of potential novel enhancers' determinants; among them we focused on Mpeg1 and MafG.

The time-course experiment reveals a subset of proteins either recruited, evicted or stably associated to enhancers during inflammation. Some of the proteins enriched upon inflammation are under investigation, like Ifi204.

Conclusions:

ChroP enables to discover novel enhancers' determinants and to profile their plasticity upon transcriptional activation.

P-554.00

QUANTITATIVE PROTEOMICS (ITRAQ) REVEALS PUTATIVE BIOMARKERS IN PRE-RADIOLOGICAL OSTEOARTHRITIS

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Introduction:

We have identified proteins differentially abundant in the serum of Osteoarthritis (OA) patients comparing four different progressive pathological grades using mass spectrometry and iTRAQ technique for relative quantification. Our final aim is to establish a panel of biomarkers useful to predict the pathology in pre-radiological stages, but also for its handling and the developing of trials of treatment in radiological stages.

Methods:

15 individual samples for each condition (OA Grade 0, pre-radiological stage Grade I, and radiological stage grades II-III and IV) were pooled in three groups. After immunodepletion, the pooled samples were subjected to in-solution digestion, followed by iTRAQ labelling following manufacturer instructions and Reverse Phase peptide separation in a LC system. Fractions were again separated in a nanoLC system, automatically deposited on a MALDI plate and analyzed in a 4800 MALDI-TOF/TOF system. Relative quantitative analysis was done using ProteinPilot software.

Results and discussion:

We have detected two big sets of serum proteins modulated in the early pre-radiological OA process. Apolipoproteins serum levels are altered when comparing Grade I vs. Grade 0. Specifically, Apolipoprotein E and apolipoprotein B-100, that mediates the binding, internalization, and catabolism of lipoprotein particles are accumulated in Grade I samples. Furthermore, up to six components of the complement are decreased in serum in early OA grades. Among them, complement component 5 -C5-, that have been recently identified as key player of the OA process, is much less abundant in any OA grade in comparison to Grade 0.

Conclusion:

Our results indicate that early pathological grades of the OA process are linked to an imbalance in the metabolism and, specifically, in the lipid metabolism. Altered serum levels of apo-lipoproteins could be used, in combination with other "dry" biomarkers, as an indicator for early OA process and to detect the pathology in pre-radiological stages.

P-555.00

SECRETED PEPTIDOME OF HUMAN CARTILAGE: A NOVEL SOURCE FOR EARLY OA BIOMARKER DISCOVERY

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Introduction and objectives: The aim of this study is to investigate the cartilage secretome by means of peptidomic analysis, providing a novel source for osteoarthritis (OA) peptide biomarker discovery.

Methods: OA cartilage shavings from the unwounded zones (UZ) and the wounded zones (WZ) of the tissue were cut into 6 mm discs, placed into 96-well plates, and cultured in serum-free DMEM up to 6 days.
Conditioned media were collected and the selective extraction of endogenous peptides was accomplished by combining ultrafiltration and solid phase extraction. Then, peptides were analyzed by nano reversed phase liquid chromatography coupled to an LTQ Orbitrap Velos Pro. Peptide/protein identifications were searched against human_fasta databases using the SEQUEST algorithm (Thermo).

Results and Discussion: This study led to the identification of a panel of 262 peptides corresponding to 36 proteins that were differentially released from the UZ and WZ in osteoarthritic cartilage. 60 peptides (belonging to 20 proteins) were detected only in the UZ (early biomarkers), while 18 peptides (belonging to 5 proteins) only in the WZ (late biomarkers). Finally, 11 proteins were detected in both zones; in this case the number of peptides was also higher in the UZ than in the WZ. These results suggest that cartilage from the UZ displays a higher protein turnover rate than cartilage from the WZ, thus facilitating the discovery of novel OA biomarkers in an early stage of the disease, when the tissue is macroscopically normal and radiographic signs are not yet detectable.

Conclusions: Cartilage peptidome profiling represents a promising alternative in the field of OA biomarker discovery both for early disease detection (early biomarkers) and for monitoring disease progression (late biomarkers). A search of these specific peptides in proximal body fluids (such as synovial fluid) by targeted proteomics approaches will be performed to evaluate their putative biomarker value.

P-556.00

EVALUATION OF ADHERENCE TO "GLOBAL INITIATIVE FOR CHRONIC OBSTRUCTIVE LUNG DISEASE" GUIDELINES FOR MANAGEMENT OF COPD EXACERBATION IN FAGHIHI HOSPITAL, SHIRAZ, IRAN 2012-13

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Aim: To obtain the adherence to the Global initiative for Chronic Obstructive Lung Disease (GOLD) guidelines, we compare our inpatient management of Chronic Obstructive Pulmonary Disease (COPD) exacerbation with GOLD guidelines.

Method: This prospective cross-sectional study was conducted from January 2012 to April 2013, in a 360-bed teaching hospital, Shiraz, Iran. We recorded the management data of 96 consecutive patients with COPD exacerbation. To evaluate the adherence to GOLD guidelines, we compared their managements with those extracted from GOLD guidelines. SPSS 11.5 software (SPSS Inc., Chicago, IL, USA) was used for statistical analyses.

Results: The mean (SD) age of patients were 67.3(14.1) years, and more than 75% of them were men. The adherence for starting antibiotics was 84.4%. Totally 82.3% received O2 with 66.7% adherence to the GOLD. Nearly ninety five percent of them received short-acting bronchodilator and 87.5% received long-acting bronchodilator. The adherence to the guidelines was 19.8% and 61.5% for oral and inhaled steroids. The adherence to the guidelines for starting N-acetyl cysteine, anti-tussive, and Xanthine derivatives (Aminophylline and Theophylline) were 49%, 77.1%, and 13.5% respectively. The overall adherence to the GOLD was 65.4% in our hospital.

Conclusion: The level of adherence to the GOLD guidelines for management of COPD exacerbation was suboptimal in our teaching hospital. Further improvements in adherence to guidelines are needed.

P-557.00
CHARACTERIZATION OF THE HOST AND MICROBIOTA PROTEOME IN PEDIATRIC IBD

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Introduction and objectives
Inflammatory bowel disease (IBD) is a chronic inflammatory disorder, consisting of two predominant forms, ulcerative colitis (UC) and Crohn's disease (CD). The exact mechanism by which this disease arises is unknown, however it is believed to emanate through a combination of an exaggerated immune response to the commensal gut flora, in a genetically susceptible host, triggered by environmental factors. There is an urgent need of biomarkers capable of distinguishing the subtypes of IBD, as well as biomarkers adept in classifying the activity of disease.

Methods
In order to address this inadequacy in IBD diagnosis, our study is applying a shotgun metaproteomic approach to mucosal-luminal interface gastric lavage samples of 350 patients, which includes non-IBD controls, UC and CD patients with varying degrees of the disease. Within the gastric lavage samples, we can detect the presence of both human and bacterial components.

Results and Discussion
Preliminary proteomic analysis of the human component has revealed an enrichment of proteins involved in remodeling of epithelial adherent junctions, clathrin-mediated endocytosis signaling and mitochondria dysfunction, for proteins whose expression is significantly different between IBD patients and non-IBD controls. Preliminary hierarchial clustering analysis, groups non-IBD controls alongside inactive CD and inactive UC, with the greatest distance in branching seen with increasing severity in active CD and active UC. Our preliminary proteomic data is also consistent with the literature; we observe a significant increase in the expression of S100A12 and M2 isoform of pyruvate kinase, in IBD gastric lavage samples compared to non-IBD controls. Both proteins expression have been shown to be significantly higher in IBD patient fecal samples.

Conclusions
In addition to identifying biomarkers, our study aims at providing insight into the molecular pathogenesis of IBD.

P-558.00

ITRAQ APPROACH TO INVESTIGATE THE IMPACT OF CIGARETTE SMOKE, SMOKING CESSATION AND SWITCHING TO A PROTOTYPIC MODIFIED RISK TOBACCO PRODUCT ON THE LUNG PROTEOME OF C57BL/6 MICE

Thomas Schneider[1], Sophie Dijon[1], Ashraf Elamin[1], Blaine Phillips[2], Emilija Veljkovic[1], Bjoern Titz[1], Florian Martin[1], Nikolai Ivanov[1] Julia Hoeng[1] Manuel Peitsch[1]

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In this study, the impact on the development of emphysema / chronic obstructive pulmonary disease (COPD) following inhalation of aerosol from two tobacco products, a reference cigarette (3R4F) and a prototypic modified risk tobacco product (pMRTP), was evaluated in C57Bl/6 mice. The mice were exposed to an aerosol from 3R4F (750 µg/l TP M, 34.4 µg/l nicotine), pMRTP (nicotine concentration-matched) or filtered air (sham) for 4 hours per day, 5 days per week, up to 7 months.

After 2 months of exposure to 3R4F, switching and cessation groups were exposed to pMRTP or filtered air, respectively. A quantitative proteomics approach using isobaric tags for absolute and relative quantification (iTRAQ®) was performed on lung tissues of mice to detect changes in protein expression levels between control, 3R4F, pMRTP, cessation and switching group samples. Exposure of C57BL/6 mice to mainstream CS induced up-regulation of biological functions such as xenobiotic metabolism, macrophage/neutrophil-related proteins, surfactants, and other metabolic processes including the pentose-phosphate pathway in a time dependent manor. Animals exposed to pMRTP exhibited negligible changes.

Cessation following 2 months of cigarette smoke exposure resulted in a reduction of changes close to control group levels. Switching to a pMRTP aerosol after 2 months cigarette smoke exposure resulted in similar trends as in the cessation group. These data demonstrate that exposure to pMRTP for up to 7 months resulted in a response similar to fresh air-exposed animals. Furthermore, following a 2 month 3R4F cigarette smoke exposure period, both cessation and switching to a pMRTP aerosol resulted in the reversal or stabilization of parameters assessed.

The results complemented those obtained from other endpoints such as transcriptomics, lipidomics as well as lung histopathology. This further establishes the foundation of our systems biology approach to assess the impact of conventional and modified risk tobacco products on biological systems.

P-559.00
THE EFFECT OF HMGB1 A BOX ON ACUTE LUNG INJURY

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Acute lung injury accompanied a significant elevation in the levels of pro-inflammatory cytokines and leukocyte infiltration in the lungs. Also, Acute lung injury is a severe illness with high rate mortality.

Therefore, we used HMGB1A and Heparin which have anti-inflammatory activities. High mobility group box 1 (HMGB1) is an abundant nuclear and cytoplasmic protein. Besides, HMGB1 is released from necrotic cells as well as macrophages and pro-inflammatory cytokine. Here, we used HMGB1 A box (HMGB1A) as an antagonist fragment of HMGB1. Also, HMGB1A have anti-inflammatory activity and heparin binding site (Amino acid 6~12).

Together with HMGB1A, we used heparin, which possesses anti-inflammatory activity, and inhibits NF-¥êB activation. For this reason, we examined HMGB1A and Heparin would reduce cytokine in in vitro and in vivo in mice. HMGB1A bound to heparin strongly by charge interaction. The HMGB1A and Heparin complex was non-toxic and decreases cytokine level, such as tumor necrosis factor-¥á (TNF-¥á) in lipopolysaccharide(LPS)-induced acute lung injury in mice.

In conclusion, HMGB1A and Heparin complex would be better than only HMGB1A and only Heparin for anti-inflammatory activity to treat acute lung injury.

P-560.00

MOLECULAR PORTRAITS OF SYSTEMIC LUPUS ERYTHEMATOSUS USING AFFINITY PROTEOMICS FOR IMPROVED DIAGNOSIS BASED ON A BLOOD TEST

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Introduction and objectives:
Systemic Lupus Erythematosus (SLE) is a severe chronic autoimmune connective tissue disease. The course of the disease is unpredictable, with periods of flares alternating with remission, and its symptoms vary so much it often mimics or is mistaken for other illnesses. In fact, SLE is often called "the invisible disease". The lack of validated biomarkers enabling diagnosis represent a key unmet clinical need.
The aim of this cross-disciplinary project is to study SLE on a detailed molecular level using affinity proteomics. We will perform translational research, going from the bed-to-bench and back again, delivering biomarker panels, setting a novel standard for diagnosis based on a simple blood test.

Methods: We have used an in-house designed recombinant antibody microarray technology platform for miniaturized, multiplexed serum protein profiling of SLE, targeting mainly immunoregulatory proteins. Several cohorts of well-characterized serum samples have been analyzed, and the array data was correlated to various clinical parameters using state-of-the-art bioinformatics.

Results and discussion: The results showed that we have successfully deciphered several SLE-associated serum biomarkers. In more detail, we have defined a condensed (< 25), pre-validated serum biomarker signature differentiating SLE vs. healthy controls with high specificity and sensitivity ($AUC > 0.90$). Further, the observed serological differences were found to increase with increasing disease severity. In addition, the biomarkers could provide an enhanced understanding of the underlying disease biology.

Conclusions: Hence, we have shown that affinity proteomics could be used to de-convolute crude, non-fractionated serum proteomes, extracting molecular portraits of SLE, paving the way for improved diagnosis based on a simple blood test. In addition, it will also enhance our fundamental understanding of this complex autoimmune condition.

P-561.00

A MECHANISTIC STUDY USING 2D-PAGE PROTEOMIC APPROACH TO INVESTIGATE THE EFFECT OF CIGARETTE SMOKE-INDUCED COPD, CESSATION AND SWITCHING TO MODIFIED RISK TOBACCO PRODUCT IN THE LUNGS OF C57BL/6 MICE

Sophie Dijon[1], Thomas Schneider[1], Ashraf Elamin[1], Emilija Veljkovic[1], Blaine Phillips[2], Bjoern Titz[1], Florian Martin[1], Nikolai Ivanov[1] Julia Hoeng[1] Manuel Peitsch[1]
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In this study, the impact on the development of emphysema/COPD following inhalation of aerosol from two tobacco products, a reference cigarette (3R4F) and a prototypic modified risk tobacco product (pMRTP), was evaluated in C57Bl/6 mice.

The mice were exposed to an aerosol from 3R4F (750 µg/l TPM, 34.4 µg/l nicotine), pMRTP (nicotine concentration-matched) or filtered air (sham) for 4 hours per day, 5 days per week, up to 7 months. After 2 months of exposure to 3R4F, switching and cessation groups were exposed to pMRTP or filtered air, respectively. To analyze the progression of emphysema at molecular level, quantitative proteomics approach using 2D-PAGE was performed on lung tissues at months 1, 3, 5 and 7. Six biological replicates were analyzed to assess repeatability and reproducibility across measurements. The gel images were aligned and analyzed using imaging software (Samespots; Totalab). The differentially expressed proteins/spots were excised, digested and analyzed using MALDI-TOF/TOF for protein identification by conducting the searches against the mouse Uniprot/Swissprot reference database.

The protein profiles showed minimal changes associated with pMRTP exposure, and recovery near to sham-exposed levels following either switching or cessation. The generated proteomics datasets of the differentially expressed proteins will be presented (e.g. Ctsd, Fabp5; Sftpa). Exposure of mice to mainstream CS induced acute inflammatory changes in the lung and airways in a time-dependent manner across.

The results complement the data from iTRAQ LC MS/MS approach on lung tissues, verification by reverse-phase microarray and the analysis of the other endpoints (transcriptomics) within the study. The generated output will further establish the foundation of PMI's systems biology approach to assess the impact of pMRTP on biological systems.

P-562.00

PROTEOMIC ANALYSIS OF ULCERATIVE COLITIS (UC): REDUCED MUCOSAL LEVEL OF INSOLUBLE KERATIN 8 PROTEOFORMS IN ACTIVE UC RELATIVE TO PROXIMAL UNINVOLVED COLONIC MUCOSA.

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Introduction and Objectives: Intermediate filaments (IF) are one of the main components of the human cell cytoskeleton. K8, K18 and K19 are the principle IF proteins in colonic epithelial cells. Alterations in keratin function are implicated in development of ulcerative colitis (UC). For example, K8 -/- mice develop chronic colitis. The aim of this study was to use mass spectrometry (MS)-based relative quantification of IF proteins from human colorectal samples to assess their role in active versus inactive ulcerative colitis.

Methods: IF proteins were extracted from rectal biopsies in patients with active colitis (n=10) and compared to uninvolved proximal colonic mucosa i.e. paired samples from the same individuals. Protein profiling of pooled samples was performed using an iTRAQ workflow, with follow up antibody based quantification analysis using K8-specific antibodies, informing analysis of K8 proteoforms by high definition MS (HDMS) with ion mobility.

Results and Discussion: Reduced levels of K8 in the insoluble IF fraction of active UC relative to un-inflamed proximal colonic mucosa were observed using both label free (HDMS) and iTRAQ MS workflows. Multiple K8 immunoreactive bands were observed in immunoblot analysis of uninflamed tissue compared to a single K8 band in inflamed tissue.The K8 proteoforms were characterised by HDMS, findings include potentially novel acetylation sites in addition to previously characterised acetylation (Lys100, Lys482) and phosphorylation (Ser23, Ser34) sites.

Conclusion: This study indicates reduced expression of insoluble keratins in colonic epithelial cells from inflamed mucosa relative to the un-inflamed proximal colonic mucosa and identifies K8 and post-translationally modified peptides as potential biomarkers of disease status. The study demonstrates the value of integrated approaches of MS (iTRAQ, label free) to the study of disease mechanism.

P-563.00

ON THE WAY TOWARD A THERAPEUTIC DECISION-MAKING TOOL FOR A BETTER MANAGEMENT OF PATIENTS WITH RHEUMATOID ARTHRITIS

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P-564.00

SHARED IMMUNOLOGICAL TARGETS IN THE LUNGS AND JOINTS OF PATIENTS WITH RHEUMATOID ARTHRITIS: IDENTIFICATION AND VALIDATION.

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Introduction and objectives

Rheumatoid arthritis (RA) is a chronic, inflammatory, systematic autoimmune disorder, characterized by joint inflammation and subsequent cartilage and bone destruction. About 1% of the world's population is affected, prevalently women. Two thirds of the patients exhibit antibodies against citrullinated proteins (so-called ACPA). It has still not been established how or where these antibodies are first generated. Here, we investigate if a link between the lung and joints can be found in patients with RA.

Methods

Proteins extracted from bronchial (n=6) and synovial (n=7) biopsy specimens from patients with RA were investigated by mass spectrometry-based proteomics using LTQ Velos Orbitrap MS. Citrullinated peptides were identified by Mascot and validated manually. Relative amounts were calculated using peptide intensities, and in one case, using stable isotope labeling and AQUA. One candidate peptide was synthesized and used to investigate by ELISA the presence of antibodies in patients with RA (n=393), healthy controls (n=152) and disease controls (n=236).

Results and Discussion

In total, 5,322 peptides corresponding to 1,200 proteins were identified. Out of these, ten citrullinated peptides belonging to seven proteins could be validated. Accurate mass and retention time, enabled detection of eight of these in synovial biopsies and seven in bronchial biopsies, with five peptides shared between the synovial and bronchial biopsy specimens. The high false positive rate in identifying citrullinated peptides made manual validation crucial. Antibodies to a synthesized citrullinated vimentin peptide candidate were present in 1.8% of healthy controls, 15% of patients with RA, and 3.4% of disease controls.

Conclusions

Identical citrullinated peptides are present in bronchial and synovial tissues, which may be used as immunological targets for antibodies of patients with RA. The data provide further support for a link between lungs and joints in RA and identify potential targets for immunity that may mediate this link.

Topic **14**

Glycomics in biology and diseases

OP062 - GLYCOPROTEOMIC IDENTIFICATION OF CELL SURFACE ATTACHMENT MOLECULES FOR ENTEROVIRUS 71

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Introduction and objectives: Discovering the factors which mediate the attachment and entry of enterovirus 71 (EV71) to host cells is an important issue for uncovering the mechanisms of virus infection and pathogenesis. Although several receptors/adhesion molecules for EV71 have been reported, the attachment, entry and infection mechanisms of EV71 are still unclear. This project aims to identify novel cellular attachment molecules for EV71 by glycoproteomic approaches.

Methods: Glycoproteins purified by lectin chromatography from the cell membrane extraction were treated with neuraminidase followed by immunoprecipitated with EV71 particles. The identified proteins were then characterized and evaluated by biochemical and virological examinations.

Results and discussion: Several proteins were identified, and cell-surface nucleolin showed significant binding signal with EV71 in virus-overlay protein binding assay (VOPBA). We found that EV71 interacted with nucleolin directly and anti-nucleolin antibody could reduce the binding of EV71 to host cells. In addition, knockdown of nucleolin decreased the attachment of all tested EV71 strains to RD cells. The binding of all tested EV71 strains to nucleolin expressed EV71 non-susceptible cells were also significantly elevated. Furthermore, virus induced cytopathic effect could be observed in human nucleolin overexpressed mouse cells.

Conclusions: These results strongly suggested that nucleolin could mediate the early phase of EV71 infection to host cells by itself. Our findings also demonstrated that glycoproteomic approaches is a reliable methodology for the discovery of novel receptors for pathogens.

OP063 - PRONOUNCED DIFFERENCES IN GLYCOSYLATION PROFILES OF HUMAN PANCREATIC ADENOCARCINOMA CELL LINES AFFECT THEIR RECOGNITION BY LECTIN RECEPTORS

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Among malignancies, pancreatic ductal adenocarcinoma (PDAC) has one of the worst prognoses [1]. With recent technologies, diagnosis is often only made in advanced states of the disease, making therapy very difficult [2], and patient-survival after 5 years less than 6% [3]. Therefore, new insight into the biology of pancreatic cancer progression and metastasis is urgently needed to improve current screening and treatment methods.

Recent data on two closely related pancreatic cancer cell lines PaTu8988S (PaTu-S) and PaTu8988T (PaTu-T) show major differences regarding cell growth characteristics, but also metastatic capacity. While PaTu-S features a tumor-like behavior, PaTu-T shows mesenchymal-like growth and high metastatic potential [4]. Here, we investigate the glycosylation of the two cancer cell lines compared with a non-cancerous pancreatic cell line (hTERT-HPNE) to reveal differences and investigate possible mechanism of metastasis.

In depth analysis of the N-glycosylation was performed by MALDI-TOF(/TOF)-MS after membrane-based enzymatic glycan release. Derivatization by ethyl esterification allowed discrimination of $\alpha2,3$ and $\alpha2,6$ N-acetylneuraminic acid linkages [5]. This new workflow was performed in 96-well-plate format with small sample amounts (0.5x10E6 cells), making it well suited for high-throughput analysis.

We found increased expression of high-mannose type N-glycans as well as $\alpha2,3$-sialylation in the metastatic PaTu-T cells, whereas PaTu-S shows higher $\alpha2,6$-sialylation and fucosylation, especially on antennae, as well as terminal N-acetylhexosamines indicating a reduced galactosylation. Corresponding trends were observed in FACS assays with glycan-binding proteins and expression levels of glycosyltransferases from glycan gene arrays. Interestingly, both cell lines are recognized by immature dendritic cells, but clear differences in interaction with various C-type lectins were observed.

The changes of glycosylation result in a differential interaction with cells and lectins, which may contribute to different behavior of PaTu-S and PaTu-T. Based on these findings, further investigations can aid to identify new strategies to target tumor cells and suppress metastasis.

OP064 - CHARACTERIZATION OF THE COMPETITION BETWEEN ASPIRIN-ACETYLATION AND GLYCATION ON HUMAN PLASMA PROTEINS.

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Introduction and Objectives.

Aspirin plays a key role in the prevention of ischemic events in high risk cardiovascular patients. Of note, the beneficial effect of aspirin seems to be reduced in patients with diabetes mellitus, suggesting that protein glycation may impair the acetylation process of aspirin. A qualitative and quantitative analysis is here performed in order 1) to identify acetylated and glycated proteins, 2) to quantify the level of acetylation and glycation and 3) to elucidate the common modification sites.

Methods.

Plasma of healthy volunteers was incubated with, 30 mM glucose for 24h followed by 500 μM aspirin for 30 min. Qualitative and quantitative analysis were carried out by Western blot and tandem mass spectrometry (MS) to assess the acetylation and glycation levels.

Results and Discussion.

Label free MS evidenced a decrease of the glycation level after 30 min of aspirin incubation for the majority of the identified plasma proteins. Interestingly, an increase in the acetylation level after glucose incubation was observed by Western blot and confirmed by MS for specific plasma proteins. This could be possibly due to conformational changes exerted by glucose, but this requires further investigations. The common amino-acid sites of several plasma proteins where both acetylation and glycation took place, were also identified.

Conclusions.

The present study assessed the extent of aspirin-acetylation and glycation of several plasma proteins. This strategy will be applied to other blood compartments and to diabetic patients in order to better understand the interplay between these two post-translational modifications.

OP065 - COMPREHENSIVE N-GLYCOME PROFILING OF CULTURED HUMAN EPITHELIAL BREAST CELLS IDENTIFIES UNIQUE SECRETOME N-GLYCOSYLATION SIGNATURES ENABLING TUMORIGENIC SUB-TYPE CLASSIFICATION

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The secreted cellular sub-proteome is a rich source of biologically active glycoproteins. N-glycan profiling of secretomes of cultured cancer cells provides an opportunity to investigate the link between protein N-glycosylation and tumorigenesis. Utilizing carbon-LC-ESI-CID-MS/MS of protein released native N-glycans, we accurately profiled the secretome N-glycosylation of six human epithelial breast cells including normal mammary epithelial cells (HMEC), two luminal (MCF7, SKBR3) and three triple-negative breast cancer cells (MDA-MB157, MDA-MB231, HS578T).

Based on intact molecular mass, LC retention time and fragmentation pattern, a total of 74 N-glycans were confidently identified and quantified. The secretomes comprised significant levels of sialylated and fucosylated complex type N-glycans, which were highly expressed in all cancer cells relative to HMEC (57.6-98.4% and 26-78%, respectively). The triple-negative breast cancer cells expressed preferentially N-glycans with α2,3-sialylation. Similarly, other glycan features were found to be altered in breast cancer secretomes including expression of paucimannose N-glycans, complex type N-glycans containing bisecting β1,4-GlcNAc and LacdiNAc determinants.

Pathway analysis indicated that the regulated N-glycans were closely related in the biosynthetic machinery. Tight clustering of the breast cancer sub-types based on the obtained N-glycome profiles supported the involvement of N-glycosylation in cancer. In conclusion, we are the first to report on the detailed N-glycosylation profiles of the secretome of a panel of related breast epithelial cell lines.

Complementing proteome and lipid profiling, N-glycome mapping yields important pieces of structural information to understand the biomolecular de-regulation in breast cancer development and progression – knowledge which may facilitate the discovery of candidate cancer markers.

P-565.00

NOVEL PROTOCOL FOR PREPARATION AND NLC-MS/MS CHARACTERIZATION OF PROTEOGLYCAN LINKAGE REGION GLYCOPEPTIDES OF HUMAN PLASMA, URINE AND CEREBROSPINAL FLUID SAMPLES

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Introduction and Objectives: The structural and functional characterization of proteoglycans (i.e. proteins carrying sulfated glycosaminoglycan chains (GAGs) eg. heparan, chondroitin, dermatan or keratan) has for long been focused on their very long and complex glycan chains. This is adequate since most of the biological effects (e.g. extracellular matrix support, growth factor signaling, differentiation, neurogenesis, morphogenesis etc.) are related to differences in the GAGs. However, there is presently a profound lack of knowledge of the structural variability of the GAG core hexasaccharide called the "linkage region" and the exact attachment site(s) of these GAGs to various amino acids along the proteoglycan peptide chains. Our aims were to develop new tools for structural characterization and quantification of proteoglycans in clinical samples.

Methods: Using a combination of anion exchange chromatography, trypsin digestion and glycan hydrolysis using Chondroitinase ABC, core glycopeptides of bikunin (protein AMBP; inter-alfa-trypsin-inhibitor light chain), the simplest chondroitin sulfate proteoglycan (CSPG) of human urine, were enriched and analyzed by reversed phase nLC-MS/MS in positive mode using CID or HCD Orbitrap instrumentation. The protocol was optimized for speed and simplicity and employed for analyses of 1-2 mL of urine, cerebrospinal fluid and plasma samples where various CSPGs were identified, CS attachment sites defined and their CS linkage regions characterized.

Results: Fourteen proteins, earlier known as extracellular and cell surface CSPGs were identified, but also some new CSPGs were identified. The bikunin peptide 206AVLPQEEEGSGGGQLVTEVTK226 of human urine was characterized in detail and shown to have at least 15 different glycoforms, all carrying a linkage region hexasaccharide, with different substitutions of sulfate/s, phosphate and sialic acid, bound to the Ser215 and in half of the isoforms also carrying a mucin type sialylated O-glycan on a Thr residue.

Conclusion: Our methodology and findings holds promise to become advanced clinical analyses of CSPGs of human samples.

P-566.00

EVALUATION OF SERUM CARBOHYDRATE-DEFICIENT TRANSFERRIN BY HPLC AND MALDI-TOF MS

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The percentage of carbohydrate-deficient transferrin (%CDT) in serum is used as a marker of habitual alcohol intake in Western countries. Determination of %CDT can be achieved using antibody detection of abnormal transferrin such as disialo sugar chains at D432 and D630 of transferrin. However, %CDT is not widely used as a marker in Japan because of a lack of specificity for alcoholic liver disease. To decrease the false positive rate in patients with non-alcoholic liver diseases, we developed a screening method using the disialo sugar chain at D630 alone.

Serum samples were obtained from 12 patients with alcoholic liver disease, 12 patients with type C chronic liver disease, 6 patients with NASH, and 12 healthy subjects (non-alcohol drinkers). Transferrin with two sialic acids (disialotransferrin) was fractionated from serum using HPLC, digested with trypsin, and evaluated using MALDI-TOF MS. An abnormal disialo sugar chain at D630 of transferrin was not detected in serum samples of the 12 healthy subjects, but was detected in 9 patients (75%) with alcoholic liver disease. An abnormal sugar chain at D630 was detected in 3 samples negative for CDT using an N-Latex CDT kit and in one sample negative for CDT and with normal GGT levels. An abnormal sugar at D630 was not detected in patients with non-alcoholic chronic liver diseases regardless of the severity of hepatopathy.

Carbohydrate-deficient transferrin that cannot be detected using conventional methods was detectable by HPLC / MALDI-TOF MS based on an abnormal disialo sugar chain at D630. This approach may permit identification of habitual alcohol drinkers when used in combination with current markers.

P-567.00

A MULTIPLE REACTION MONITORING METHOD TO SPECIFICALLY CHARACTERIZE AND RELATIVELY QUANTIFY THE O-GLYCANS OF THE POTENTIAL BIOLOGIC LUBRICIN

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The common degenerative joint disease, osteoarthritis, disrupts the synovial joint surface that is maintained by lubricin, the boundary lubricating, heavily O-glycosylated, synovial protein. The glycan component of lubricin creates its lubricating properties. Recent studies showed that the reintroduction of lubricin into joints may aid in osteoarthritis symptoms and preventing disease progression. For lubricin to be a safe and effective osteoarthritis biologic, the appropriate O-glycans for lubrication must be present and none with potentially unwanted immunological effects.

Therefore a multiple reaction monitoring (MRM) method was created to sensitively annotate and relatively quantitate the entire detectable repertoire of O-glycans on native lubricin, to understand their alterations during disease, and to assess a potential biologic. The reductive β-elimination released O-glycans from a pooled lubricin sample were used for MRM development. LC-MRM analysis was performed using a porous graphitized carbon (PGC) column and a triple quadrupole/linear ion trap hybrid mass spectrometer in high mass negative ion mode. MRM transitions were created for sensitivity, as well as specificity to differentiate isomers, aided by the PGC chromatography. This approach created an effective method to differentiate, and where possible, specifically characterize sets of isomers. The method focused on the core 1 (Galβ1-3GalNAc) and core 2 (Galβ1-3(GlcNAcβ1-6)GalNAc) families. Their galactose extended, monosulfated, monosialylated and disialylated structures and their combinations and isomers were also included.

The method was successfully tested with a study including eight normal and eight osteoarthritic human synovial fluid lubricin samples. This study was able to create an MRM relative quantitative method for the complete O-glycan profile of a single heavily glycosylated protein. The glycans analyzed here are common to many glycoproteins, and the success of this approach and the minimal glycan specialized skill required to use it, is encouraging for the creation of an expanded "targeted discovery" method broadly applicable to O-glycosylated proteins.

P-569.00

USE OF HYDRAZINONICOTINIC ACID AS DERIVATIZATION REAGENT FOR ENHANCED DETECTION OF OLIGOSACCHARIDES BY MALDI-MS

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The study of oligosaccharides experienced a rapid growth due to the awareness of the important roles that oligosaccharides play in so many important biological processes, such as cell division, cellular localization, tumor immunology and inflammation.

Nevertheless, due to the inherent low abundances and poor ionization efficiencies of oligosaccharides it is still a hard work to analyze them directly by MALDI-MS. In this article, we report a novel derivatization reagent, hydrazinonicotinic acid (HYNIC), for improved and selective detection of oligosaccharides. The derivatization strategy was realized through tagging the reducing terminus of oligosaccharide with diazanyl group of HYNIC by dehydration. After derivatization, the ionization efficiencies of oligosaccharides could be enhanced. HYNIC derivatization also allowed sensitive detection of sialylated glycan under negative mode. It is noteworthy that HYNIC reagent not only effectively labeled the reducing end of glycans in the presence of tryptic peptides, but also suppressed the detection of peptides.

Therefore, with derivatization, analysis of glycans became much easier due to the omission of laborious separation steps. At last, more than 50 N-glycans were detected in 10 μL human serum using this method.

P-570.00

N-GLYCOMIC AND N-GLYCOPROTEOMIC ANALYSIS OF THREE SNAKE VENOMS

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Snake venom is a complex cocktail including a variety of biological active proteins and proteinaceous components, which have considerable medical and pharmacological importance. N-glycosylation is widely implicated as a common modification on numerous venom proteins and impacts the in vivo venomic functions.

However, systematic N-glycome and N-glycoproteome survey on snake venoms has not been undertaken. In this study, employing combination of N-glycomics and N-glycoproteomics strategies, we for the first time explored the N-glycosylation including both N-glycoproteins and N-glyco-chains in three venoms, Agkistrodon blomhoffii, Naja naja atra Cantor and Vipera russelii siamensis Smith which are amongst the most abundant venomous snakes in Asia. As a result, 9, 8 and 18 N-glycoproteins, 115,100 and 95 N-glycans were respectively identified in Agkistrodon blomhoffii, Naja naja atra Cantor and Vipera russelii siamensis Smith venoms.

However, the overlaps of N-glycoproteins and N-glycans among the three venoms were small. Thus, the N-glycome and N-glycoproteome exploration results indicate that N-glycosylation increases the complexity and variety of the three venoms. Our research provided us with new horizons for the comprehensive understanding of venoms variation, which is helpful for both basic venom research as well as the management of snake envenomation.

P-571.00

CAPILLARY ELECTROPHORESIS FOR MONITORING GLYCOPROTEIN CHANGES ASSOCIATED TO VASCULAR DISEASES AND PROSTATE CANCER

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Introduction: Glycoproteins may present variations in the peptidic chain, glycosylation, or other post-translational modifications (PTMs). As a consequence, forms of a glycoprotein differing in charge and/or size are produced.

PTMs, and namely glycosylation, of proteins depend, among other factors, on the pathophysiological conditions of the individual. Glycosylation changes in alpha 1-acid glycoprotein (AGP) have been associated to vascular and prostate pathologies, among others. Alterations in prostate-specific antigen (PSA) glycosylation related to prostate cancer have been described.

Capillary electrophoresis (CE) in the zone mode (CZE) is a high-resolution technique that separates molecules depending on their charge/size ratio.

The objective of this work is to explore CE as a tool to show changes in intact (non-hydrolyzed) glycoproteins related to diseases. The CE profile of AGP isoforms as a potential biomarker of vascular diseases is studied. In addition, the first steps for studying the CE profile of PSA isoforms as prostate cancer marker are taken.

Methods: Purification of AGP and PSA from biological fluids is performed by immunoaffinity chromatography (IAC) in HPLC format. Separation of isoforms of each glycoprotein is carried out by CZE. A pilot study to evaluate the role of AGP isoforms as vascular disease marker is conducted with a small cohort of individuals including healthy people and patients with abdominal aortic aneurism (AAA) and carotid atherosclerosis (CTA); linear discriminant analysis (LDA) is performed using the proportion of each AGP isoform.

Results and discussion: High-purity AGP from serum or plasma and high-purity PSA from seminal plasma are obtained with the home-made anti-AGP and anti-PSA columns. The CE methods developed separate isoforms of AGP and PSA. LDA allows 92% correct classification for the healthy, AAA, and CTA groups.

Conclusion: Capillary electrophoresis combined with immunochromatographic purification can be adequate tools to study the role of changes in intact glycoproteins as disease biomarkers.

P-572.00

PREPARATION OF A NOVEL SENSITIVITY LECTIN SUSPENSION MICROARRAY SYSTEM AND APPLICATIONS TO SERUM PEPTIDOME GLYCAN PROFILING

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Glycomics is the study of comprehensive structural elucidation and characterization of all glycoforms found in nature and their dynamic spatiotemporal changes that are associated with biological processes. However, the immense diversity and complexity of glycan structures and their multiple modes of interactions with proteins pose great challenges for development of analytical tools for delineating structure function relationships and understanding glycocode. Several tools are being developed for glycan profiling based on chromatography and mass spectrometry, which was limited in high-throughput methodologies to study the structural and functional aspects of glycan structures. Lectins, which have long been used in glyco-immunology, printed on a microarray provide a versatile platform for rapid high throughput analysis of glycoforms of biological samples.

In this study, a novel sensitivity lectin suspension microarray and a suspension array assay was prepared with the assistant of PEG-diglycolic acid and dendrimer signal amplification. The biocompatible polymer material serves as the molecular spacers to maximize the lectin immobilization capacity and minimize the unspecific binding on metal spheres surface. In addition to, the PEG-diglycolic acid made contributes to the good dispersible of magnetic spheres in aqueous solution. Furthermore, the signal was amplified with the assistance of dendrimer. Then, the rapid, sensitivity and high-throughput lectin suspension microarray was prepared and applied for glycan profiling analyses. The peptidome and peptidomics as logical extension to proteome and proteomics were caught more attention by scientists and provided diagnostic and prognostic information on cancer and other diseases. However, few reports on glycan profiling of peptidome can be found.

Therefore, the novel sensitivity, high-throughput and fast lectin suspension microarray system was applied for overall and real-time glycan profiling analyses of serum peptidome. The serum peptidome glycan profiling of intrahepatic cholangiocarcinoma was established and had great potential in biomarker discovery.

P-573.00

N-LINKED GLYCOPROTEOMIC ANALYSIS OF ESOPHAGEAL CELLS STIMULATED BY PRO-INFLAMMATORY CYTOKINES

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Introduction: Esophageal cancer remains as the leading cause of cancer related death because it is usually asymptomatic at early stages. Esophageal carcinogenesis has been closely linked with inflammation and pro-inflammatory cytokines, while abnormal glycosylation has been envisioned as a hallmark of inflammation and cancer. It is of biological interest to investigate the modulation of protein glycosylation in esophageal cells by pro-inflammatory cytokines.

Methods: We selected the immortalized HET-1A normal esophageal cell line as our model system. High doses of five different pro-inflammatory cytokines were used to stimulate these cells, respectively. Four different lectins were selected to capture N-linked glycoproteins based on their corresponding sugar chains. These concentrated glycoproteins were further digested by trypsin/PNGase F and the obtained peptides were analyzed by UPLC system interfaced to a Q Exactive Mass Spectrometer. MS/MS spectra were searched against the SwissProt protein database using the MASCOT search engine. The resulting spectral matches were assembled and analyzed by Scaffold software, using DTA files generated by MASCOT.

Results and Discussion: Using LC-MS/MS, 661 unique glycoproteins were identified and quantified, among which 416 were enriched by the AAL lectin, 363 were enriched by ConA, 184 were enriched by PHA-L, and 338 were enriched by WGA. We further found that 39 N-linked proteins were consistently deglycosylated and another 5 proteins were newly glycosylated by all 5 cytokines, including ERO1-like protein alpha. In total, 55 unique glycoproteins have been significantly modulated by at least 3 cytokines, and 43 of these contain at least one predicted N-linked glycosylation site. Other identified glycoproteins contain consensus O-linked glycosylation sites. About 80% of these proteins have evidenced interactions, either in direct or indirect fashion.

Conclusions: Although pro-inflammatory cytokines have diversified impact on cellular protein glycosylation, these discovered candidate glycoproteins are promising biomarkers for mechanism research of inflammation and tumor progression.

P-574.00

GLYCOPATTERNS OF HUMAN SALIVARY GLYCOPROTEINS AND THEIR ROLES AGAINST INFLUENZA A VIRUS

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Recent studies have elucidated that expression of certain glycoproteins in human saliva are increased or decreased according to age, meanwhile, human saliva may inhibit viral infection and prevent viral transmission. However, little is known about the age- and sex-associated differences in the glycopatterns of human salivary glycoproteins and their significant roles against influenza A virus (IVA).

Here we investigate the glycopatterns of human salivary glycoproteins with 180 healthy saliva samples divided into six age/sex groups using lectin microarrays and fabricate saliva microarrays to validate the terminal carbohydrate moieties of glycoproteins in individual saliva samples, and assess the inhibiting and neutralizing activity of saliva against two strains of influenza A (H9N2) virus. Furthermore, the MAL-II and SNA magnetic particle conjugates were utilized to selectively isolate the glycoproteins and their glycomes from the elderly saliva samples, and the isolated glycoproteins and their N-linked glycans were identified by the Orbitrap LC/MS and MALDI-TOF/TOF-MS, respectively.

We find that seven lectins (e.g., MAL-II and SNA) show significant age differences in both females and males, and seven lectins (e.g., WFA and STL) show significant sex differences in children, adults and elderly people. Interestingly, we observe that elderly individuals have the strongest resistance to IVA mainly by presenting more terminal Á2-3/6-linked sialic acid residues in their saliva, which bind with the influenza viral hemagglutinations. A total of 19 and 18 glycoproteins as well as 31 and 43 N-glycans from the elderly females, 13 and 19 glycoproteins as well as 31 and 35 N-glycans from the elderly males are identified with MAL-II and SNA magnetic particle conjugate-based methods, respectively.

We conclude that age- and sex- associated differences in the glycopatterns of human salivary glycoproteins may provide pivotal information to help understand some age related diseases and physiological phenomenon, and develop an oral or nasal spray against IVA.

P-575.00

CARBOHYDRATES MODIFICATIONS IN GRASS POLLEN GROUP 13 ALLERGENS

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Introduction and objectives

Polygalacturonases, pectin degrading enzymes, are already reported as Group 13 of grass pollen allergens and known to be cross reactive allergens.

Our study aims at characterizing carbohydrates modifications that may be responsible for IgE reactivity in humans.

Methods

Trypsin digested allergens Zea m 13, Phl p 13 and Dac g 13 were analysed by nanoLC-MS/MS using Qexactive before and after endoglycosidase A treatment. Glycans were analysed by MALDI-LTQ Orbitrap Data were managed by using Proteome discoverer 1.4, Mascot 2.3 and Peaks studio.

Results and discussion

The released oligosaccharides were structurally characterized by multi-stage mass spectrometry on MALDI-LTQ Orbitrap, revealing the presence of the typical vegetal paucimannosidic type structures containing β1,2-xylose and α1,3-fucose. High mannose structures were detected for the allergen Zea m 13 and were completely absent in the others. The N-glycosylation microheterogeneity and the site occupancy were investigated by the interpretation of HCD spectra of the analyses performed before the glycosidase treatment. During HCD glycopeptides give rise to the occurrence of oxonium ions at m/z 204.09 (N-acetylglucosamine) and m/z 366.14 (N-acetylglucosamine+galactose) that can be detected in the FT-Orbitrap in order to assign the glycosylation site with high confidence. Furthermore the presence of intense Y1 ions corresponding to the peptide backbone+N-Acetylglucosamine confirmed the univocal attribution of the N-glycosylation site.

Conclusion

The characterization of proteins glycosylation requires the integration of multiple analytical approaches. Here we used the traditional deglycosylation protocol to fully characterize the glycan structures. The microheterogeneity was analyzed by HCD fragmentation of the entire glycopeptides in order to obtain Y1 ions, allowing the univocal attribution of the N-glycosylation sites. To the best of our knowledge this was the first time that allergens belonging to this group were investigated to find carbohydrates modifications.

P-576.00

GLYCAN CHANGES OF PLASMA TRANSFERRIN IN ELASTASE-INDUCED EMPHYSEMA AND LPS-INDUCED ACUTE LUNG INJURY MICE MODELS

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Introduction and Objectives: Glycans play a key role in the onset, diagnosis and treatment of various diseases. In order to know the changes of glycans as biomarker candidates for acute respiratory distress syndrome (ARDS) and chronic obstructive pulmonary disease (COPD) in humans, the present study aims to see whether or not plasma transferrin undergoes specific glycan changes in the lipopolysaccharide (LPS)-induced acute lung injury model for ARDS, and the elastase-induced emphysema model for COPD.

Methods: All animal procedures were approved by the animal Ethics Committee of RIKEN. We analyzed glycan structures on plasma glycoproteins in elastase-induced emphysema model mice and LPS-induced acute injury model mice to find these markers. Mice were divided into three groups. Group 1 was the elastase-induced emphysema model mice. Group 2 was the LPS-induced acute lung injury model mice. Group 3 was the control group. PBS, elastase and LPS were all administered via trachea by using a syringe. Plasma from each mouse was subjected to SDS-PAGE, and the transferrin band was collected. Transferrin was in-gel digested with trypsin after reduction and alkylation. Tryptic peptides and glycopeptides were separated on an ODS column, and the eluate introduced into an electrospray ionization–mass spectrometry system to characterize the glycan structures of transferrin in each mouse.

Results and Discussion: In the LPS-induced acute injury model group, increases in trisialo-biantennary glycans (TBGs) and decreases in fucosylated glycans (FGs) were observed as compared with those in the control group whereas no significant changes were found in elastase-induced emphysema model group. These glycan changes were well correlated with the number of inflammatory cells (neutrophils, macrophages and lymphocytes) in the bronchoalveolar lavage fluid of each model mice. Conclusions: Changes of TBGs and FGs of transferrin could be a possible clinical biomarkers for the ARDS in humans.

P-577.00

GRIP-GUI: GRAPHIC USER INTERFACE FOR GLYCOPEPTIDE REVEAL & INTERPRETATION PLATFORM.

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Glycosylation is one of the most important post-translational modifications (PTM), however, software developed for correctly assignment glycan structure to MS spectrum remains on the initial stage, let alone the interpretation for the fragment of glycopeptides.

Here, we use network-centric method to develop an intelligent software named GRIP-GUI, for rapidly searching the glycopeptides from a large number of spectra in an ease way. In the test of standard glycoprotein HRP, GRIP-GUI can correctly identify the glycan structures on all the glycosylation sites, and GRIP-GUI have obtained 745 glycopeptides of human serum in one run.

Moreover, the design of the software is aiming to suit for the spectra from all different kinds of MS spectrum.

P-578.00

THE SEPARATION AND IDENTIFICATION OF GLYCANS USING AN ULTRA HIGH RESOLUTION COLUMN AND AN ULTRA HIGH RESOLUTION MASS SPECTROMETER

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Understanding, measuring, and controlling glycosylation in glycoprotein-based drugs and thorough characterization of biosimilars has become increasingly important. Liquid chromatography (LC) coupled to mass spectrometry (MS) has emerged as one of the most powerful tools for the structural elucidation of glycans.

Here we present a novel stationary phase that provides superior selectivity and ultra fast resolution, with isomeric separation, as compared to commercially available columns. The column is based on novel mixed-mode column chemistry, combining both weak-anion exchange (WAX) and reversed-phase (RP) retention mechanisms. The WAX functionality provides retention and selectivity for negatively charged glycans, while RP mode facilitates the separation of the same charge according to their isomeric structure, polarity, and size. As a result this column provides resolution with more than 4 times the glycan structures identified compared to existing commercial columns, for bovine fetuin N- glycans. The ability to separate isomers reveals a greater complexity of the glycan population from a given glycoprotein. Namely, far more MS/MS spectra need to be triggered in a single analysis. Additionally, wider dynamic range and sensitivity are needed to detect and generate good quality MS2 spectra not only for the most abundant glycans but the low abundant species as well. Orbitrap Fusion with it's wide dynamic range and ultrahigh mass resolution was selected for looking deeper into the glycome and confidently identifying low-abundance glycans.

Overall, 135 unique glycan structures were identified using the mixed-mode column and Orbitrap Fusion. To our knowledge this is the largest number of glycans identified for bovine fetuin in a single analysis. Similar column performance was demonstrated for 2-AA labeled N-glycans from antibodies.

P-579.00

PROTEOMIC ANALYSIS OF THE EFFECTS OF ALOE VERA IN KIDNEY OF STREPTOZOTOCIN-INDUCED DIABETIC RATS

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Diabetes is a chronic disorder with high prevalence in the global population that causes several complications in different organs and may lead to the development of nephropathy. It is known that Aloe vera has hypoglycemic effects; however the mechanisms of its action have not been fully established. The proteomic analysis allows quantitative and qualitative proteins evaluations of cellular metabolism, contributing to characterization of cellular responses to certain drugs.

This project aims evaluating the effect of Aloe v. buthanolic fraction (BF) in kidneys of diabetic rats, identifying sub or overexpressed proteins by proteomic analysis. Thirty male Wistar rats were divided into 5 groups: 1) control, 2) untreated and treated diabetics with 3) glibenclamide (5 mg/Kg), 4) BF (50 mg/Kg) and 5) insulin (0,1 U.I). The diabetes was induced by streptozotocin (55 mg/Kg). Blood glucose was measured before treatment and after 3 and 6 hours. Kidney samples were analyzed by quadrupole-orbitrap mass spectrometer. Only BF treatment was capable to maintain the body weight after 25 days. All treatments showed significant reduction in blood glucose compared to baseline levels. The effect caused by BF was similar to glibenclamide, however it was more long-lasting, remaining at least 6 hours after. In proteomics analysis none of the treated groups showed stress response proteins, differently of the untreated group. Glibenclamide-treated group presented the transaldolase protein, which might contribute indirectly to control hyperglycemia because it participates in an alternative route for glucose oxidation. BF-treated group presented the UDP-glucose:glycoprotein glucosyltransferase protein that plays an important role in regulation of the endoplasmic reticulum operation, preventing the death of pancreatic β-cells in Type 1 diabetes; and the aldose reductase, responsible for generating toxic products during diabetes.

Thus, Aloe v. buthanolic fraction showed more efficiency in the control of diabetes compared with glibenclamide; however, high concentration dose may generate toxic effects.

P-580.00

GLYCOFOREST - DISTILLING GLYCOMIC MS/MS DATA

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Liquid-chromatography combined with tandem mass-spectrometry (LC-MS/MS) is a popular technique to study released glycans. The analysis workflow[1] shares similarities with those used for proteomics. However the data analyses differ greatly as it is not possible to construct a theoretical database containing only those glycans that occur in one organism. As a consequence glycomics has limited tools for automated data analysis. Spectra are generally solved manually leaving many glycans unidentified.

We are developing Glycoforest, a software suit for the high-throughput processing of glycans. Glycoforest distils large MS/MS datasets into a graph data structure that represents the relationship between the spectra. The nodes in the graph represent unique glycan structures and edges represent the structural similarities of the glycans. Graph nodes are generated using an algorithm that first clusters within a LC-MS/MS run and then between runs. This produces a unique consensus spectrum for each glycan. Within run clustering uses a similarity function based on spectra similarity, precursor m/z and retention time whereas the between run similarity function only uses spectra similarity and precursor m/z. The graph edges are generated by calculated the similarities between nodes using a function that takes the addition and removal of monomers and substituents into account[3]

We have used Glycoforest to processed 4,000,000 MS/MS spectra resulting in a graph containing 598 nodes and 1,558 edges. The structural similarity was validated by mapping previously identified spectra from Unicarb-DB[4] on to the graph resulting in the structural annotation of 47 nodes. Inspecting the identified nodes and their edges has shown that the Glycoforest graph contains meaningful structural relationships that can be used to develop algorithms to infer glycan structure.

P-581.00

SITE SPECIFIC GLYCOSYLATION OF SERUM PROTEINS IN CANCER DISEASES – FROM DISCOVERY TO QUANTIFICATION

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Introduction and Objectives

Altered glycosylation of proteins has been associated with a variety of diseases. Detection and quantification of site specific glycosylation abnormalities could assist in the development of biomarkers for diagnostic purposes. We have attempted the identification and quantification of glycopeptides that seem to differentiate among patients with different types of cancer.

Methods

UHPLC coupled to high resolution mass spectrometry (HRMS) (Q Exactive Plus, Thermo Fisher Scientific) was used for the analysis of complex enzymatic digests of albumin depleted patients' sera. Differential analysis software helped pinpoint statistically significant changes among the sample groups. Selected candidates were further identified via enhanced database search providing protein identification as well as the assignment of specific glycosyl moiety and its localization within the peptide sequence. A targeted quantitation method was designed for the characterized glycopeptide markers and used in analysis of another sample set.

Results and discussion

The HRMS fast scanning instrument with advanced software for identification of glycopeptides allowed for the comprehensive analysis of glycopeptides from crude protein mixtures. We have found several serum glycoproteins, including haptoglobin, hemopexin, etc. The neuraminidase treatment seemed to enhance MS signals of glycopeptides and improved visualization of low abundant fucosylated glycoforms. The relative quantification of selected glycopeptides using targeted method uncovered the major changes in expression of fucosylated glycoforms.

Conclusions

We have identified glycopeptides from complex enzymatic digests of samples prepared from patients' sera that showed quantitative changes in different patient groups. We designed a qualitative and quantitative methodology based on HRMS system with a high potential for clinical applications.

P-582.00

N-GLYCOSYLATION ANALYSIS OF TRASTUZUMAB BIOSIMILAR CANDIDATES BY LIQUID CHROMATOGRAPHY AND MASS SPECTROMETRY STRATEGIES

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The biotechnological production of protein-based therapeutics is one of the fastest growing sectors of the pharmaceutical industry1. Biosimilars, also known as follow-on biologics, constitute subsequent "copies" of innovator biopharmaceutical products made by a different sponsor following patent and exclusivity expiry on the innovator product. Differences in impurities and/or breakdown products can have serious health implications and therefore it´s of high importance to deliver biosimilars with the same or the nearest structural properties than the patented product.

Trastuzumab (Herceptin), a commercially available monoclonal antibody (IgG1) employed to block the ErbB2 overexpression in breast cancer, it´s comprised of a tetramer of two heavy and two light chains with one N-glycosylation in each heavy chain. Several factors, such as growing conditions or cell types can determine the final structure of the glycans, significantly affecting the properties of the generated antibodies. Therefore, the production of antibodies with the appropriate N-Glycosylation is a critical step and one of the most time consuming parts of the developing process for those companies actually producing Herceptin biosimilars (or any other glycosylated drug).

In the present study, we describe two different but complementary strategies to characterize the N-glycosylation of Trastuzumab biosimilars currently in process. Both methodologies include a first step of enzymatic N-glycan releasing from the protein and a second step of glycan characterization. In the first case, N-glycans are fluorescently labeled with 2-aminobenzamide (2-AB) and separated by Normal phase HPLC (NP-HPLC). Different sugars will elute at different times and can be detected and identified employing specific sugar standards. In the second approach, released glycans are permethylated and analysed by MALDI-TOF/TOF, being able to determine the structure because of the differential sugar masses.

Herein, the N-glycosylation structures of two different Trastuzumab biosimilars will be compared, and both approaches, HPLC and MALDI analysis, will be described.

P-583.00

INFORMATICS SUPPORT FOR ISOMERIC SEPARATION AND THE STRUCTURAL IDENTIFICATION OF LABELED N-GLYCANS FROM PROTEINS

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The separation of glycans by chromatography prior to MS analysis can provide number of benefits. Primarily, separation can reduce sample complexity, minimize ion suppression, increase dynamic range of analysis and provide separation of structural isomers. Recent developments in mixed –mode column chemistries and faster scanning mass spectrometers have increased the number of glycans resolved and identified by LC-MS workflows. However, the increase in peaks resolution and detected compounds also leads to large data sets.

Additionally, chromotograms of isomeric glycans are complex with some isomers co-eluting under a single peak. Manual deconvolution of such complex chromatograms, identification of isotopic peaks components, identifying MS/MS scans for detected compounds and selection of correct precursor m/z values from the isotope cluster for MS/MS data analysis is time consuming task. Therefore, we have developed a software tool (SimGlycan) to streamline this process.

Software modules were developed for automatic detection of compounds, deconvolution of chromatograms to separate glycan isomers, identification of isotope clusters and MS/MS scans corresponding to detected compounds and precursor m/z selection. 2AB-labeled glycans from various proteins were separated and analyzed on a Thermo Scientific GlycanPac AXR-1 column coupled to Orbitrap Fusion Tribrid mass spectrometer. LC-MS and MS/MS data was subjected to the program. The accuracy of the results were also tested on Q Exactive mass spectrometer.

All isomeric glycans correctly detected, separated and identified by the program were manually validated for these experiments.

P-584.00

IMPROVED GLYCOPEPTIDE ANALYSIS USING ACETONITRILE ENRICHED SHEATH GAS AND OXONIUM ION DEPENDENT ETD

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Introduction and objectives

Mass spectrometric analysis of protein glycosylation is a major topic. The analysis of glycopeptides in complex biological samples is due to their low ionization efficiency and high heterogeneity challenging. This study uses solvent-enriched sheath gas to boost glycopeptide signal intensities for improved detection. Site-specific glycosylation profiling is based on the most efficient combination of CID and fragment triggered ETD.

Methods

A tryptic digest of Universal Proteomics Standard (UPS1-Sigma) was separated on an UltiMateTM 3000 nanoRSLC system. A quadrupole ion trap MS (amaZon speed ETD, Bruker Daltonics) equipped with a CaptiveSpray nanoBooster source was used for fragment triggered ETD experiments. Acetonitrile-enriched nitrogen was used as sheath gas to enhance glycopeptide intensities and increase charge states. Data processing was performed using ProteinScape 3.1 software (glycopeptide characterization and identification).

Results and discussion

The main challenges when analyzing glycopeptides in complex mixtures are the low concentrations combined with their low ionization efficiency compared to non-glycosylated peptides. A significant improvement of detection sensitivity can be achieved by acetonitrile-enriched sheath gas.

Low energy CID was used for both, the identification of non-glycosylated peptides and the characterization of the glycan moiety of glycopeptides. Mascot was used for protein identifications, and the glycopeptide CID spectra were searched against CarbBank using GlycoQuest in order to identify the glycan moiety.

As ETD is mandatory for sequencing of the glycopeptide backbone, we used an advanced acquisition strategy in which the ETD acquisition is only triggered if oxonium ions have been observed in the former CID spectrum ("Fragment Triggered ETD"). This acquisition method allows for an efficient analysis of glycopeptides in complex mixtures. Moreover, ETD spectra quality is significantly improved by the acetonitrile-enriched sheath gas, as it does not only increase the signal intensity but also the charge state of glycopeptides.

P-585.00

DEVELOPMENT AND APPLICATION OF A METHOD FOR THE GLYCOPEPTIDE-BASED SITE-SPECIFIC GLYCOMIC ANALYSIS

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Introduction and objectives: □gGlycoproteomics□h is generally a term for an approach of large-scale identification of de-glycosylated (formerly glycosylated) peptides. However, a single glycosylation site has multiple different glycans without exception, and the glycomic changes associated with diseases such as carcinogenesis and fibril formation are shown to be useful for indicator (biomarker) of the diseases. Therefore, we developed a method to assign site-specific glycome on glycosylated peptides which had been identified as de-glycosylated peptides by the existing glycoproteomic technology, IGOT-LC/MS (Kaji et al. 2003, Nat Biotechnol.).

Methods: Glycopeptides were enriched by HILIC from a protease digest of glycoprotein(s). An aliquot was subjected to the IGOT method to identify a series of glycopeptide core sequences. Another aliquot was analyzed by LC/MS using a high accuracy LTQ-Orbitrap spectrometer for acquisition of the masses (m/z) of glycopeptides. According to the chromatographic features of glycopeptides, signals of the glycopeptides were selected from all the detected signals as clusters using in-house software. Then, by matching the theoretical masses of core peptides and the observed masses of glycopeptides considering the masses of glycan portion, the core sequences and their glycan compositions were assigned.

Results and Discussion: Detailed site-specific glycomes of a model glycoprotein were revealed and large numbers of glycopeptides carrying a specific glycan motif were assigned from a complex protein mixture of a mouse tissue.

Conclusions: The technology we have developed will serve as a tool for comprehensive elucidation of site-specific glycan heterogeneity and for the glycan structural verification of disease glycobiomarker molecules. The acquired list of large numbers of glycopeptides will be a useful resource for a glycoprotein database being expanded based on GlycoProtDB.

P-586.00

HIGH-SENSITIVE DIFFERENTIAL GLYCAN PROFILING OF SERUM MUC1 TOWARD THE DEVELOPMENT OF A GLYCO-DIAGNOSTIC AGENT FOR CHOLANGIOCARCINOMA

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Introduction and objectives: Glycoform on MUC1 in cancer cells is altered dramatically along with their differentiation, and thus qualitative detection and verification of the alteration of glycosylation on MUC1 have potential diagnostic value. However, there are no conventional methods for differential glycan analysis of multiple glycan changes on MUC1 in a slight amount of clinical specimens. We describe here a methodology for high throughput glycan profiling of serum MUC1 utilizing the antibody-overlay lectin microarray. Our goal in this study is the development of a novel sero-diagnostic agent for cholangiocarcinoma by the differential glycan profiling targeting to serum MUC1.

Methods: An antibody-overlay lectin microarray was optimized focusing on the glycan profiling of sialyl-MUC1 using sialyl-MUC1 specific monoclonal antibody, MY.1E12, and culture supernatants of intrahepatic cholangiocarcinoma cell, KMC-1. Differential glycan profiling for discovery of cholangiocarcinoma-specific glycan alteration on MUC1 was performed using 121 serum specimens (including cholangiocarcinoma, benign bile duct disease patients, and normal controls).

Results and Discussion: We successfully obtained the glycan profiling of MUC1 in as sparse as 5 µL of serum from normal controls. Differential glycan profiling identified some lectins with significant differences between cholangiocarcinoma and controls (benign diseases and normal), and we finally found that the combinational use of certain lectins clearly distinguished between cholangiocarcinoma and others with the best accuracy for cholangiocarcinoma diagnosis (AUC = 0.829).

Conclusion: An ultra-sensitive detection system for glycosylation alteration on sialyl-MUC1 was constructed. This system enabled us to elucidate the qualitative glycan changes of a sparse amount of serum MUC1 using multiple lectins. Our methodology for differential glycan profiling is also applicable to other mucin producing diseases. This work was supported in part by a grant on "Medical Glycomocs Project" from New Energy and Industrial Technology Development Organization (NEDO).

P-587.00

COMPREHENSIVE IDENTIFICATION OF GLYCOPROTEINS CARRYING LEWIS X AND SITE-SPECIFIC GLYCAN ALTERATION IN FUT9 KNOCKOUT MICE

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Introduction and objectives

f¿1,3-Fucosyltransferase 9 (Fut9) is a key enzyme to synthesize the Lewis x structure (Gal$f$À1-4(Fuc$f$¿1-3)GlcNAc-R) on glycoproteins, proteoglycans, and glycolipids. Lewis x is an epitope of stage specific embryonic antigen-1 (SSEA-1) and known as a neural stem cell marker. The Fut9 gene is expressed in the stomach, colon, kidney, nerves, and other organs; however, Lewis x-carrying glycoproteins have not been revealed. To identify the Lewis x-carrying proteins comprehensively, site-specific glycomes on glycopeptides were analyzed in mouse kidney. Furthermore, to analyze the glycan alteration associated with the Fut9 gene knockout, comparative analyses of glycopeptide-based site-specific glycomes were performed in the knockout (Fut9-/-) and wild-type (wt) mice.

Method: Glycopeptides were collected from the tryptic digests of wt and Fut9-/- kidneys by HILIC, and N-linked glycans were released by PNGase and analyzed by MALDI-TOF MS. Then, glycopeptides carrying fucosylated glycans were isolated by AAL affinity chromatography and identified by IGOT-LC/MS method. To distinguish the Lewis x-carrying glycoproteins from those carrying core fucose only, we analyzed site-specific glycome on the identified glycopeptides of wt by our novel glycoproteomic technology (Available as a poster by Kaji et al.). Furthermore, glycan alterations associated with Fut9 knockout were analyzed site-specifically.

Results: The wt profile showed complex-type glycans containing fucose (dHex) extensively at both core and terminal GlcNAc, but relatively low content of sialic acids. The terminal fucoses were presumed Lewis x, as the multiple fucosylations were disappeared into single fucosylation, probably core fucose, by Fut9 knockout. We identified about 1,200 AAL-captured glycopeptides as fucose-carrying glycopeptides from both wt and Fut9-/-. The new method for site-specific glycome analysis identified many Lewis x-carrying glycopeptides. Comparison of site-specific glycomes elucidated the glycan alterations by Fut9 knockout.

Conclusion: The new glycopeptide-based glycome analysis method could identify large numbers of Lewis x-carrying glycoproteins from mouse kidney.

P-588.00

ASSIGNMENT OF SACCHARIDE IDENTITIES THROUGH ANALYSIS OF OXONIUM ION FRAGMENTATION PATHWAYS IN LC-MS/MS OF GLYCOPEPTIDES

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Protein glycosylation play critical roles for the regulation of diverse biological processes, and determination of glycan structure-function relationships is important to better understand these events. However, characterization of glycan and glycopeptide structural isomers remains challenging and often rely on that biosynthetic pathways are conserved. In glycoproteomics analysis using liquid chromatography – tandem mass spectrometry (LC-MS/MS), saccharide oxonium ions containing N-acetylhexosamine (HexNAc) residues are very prominent.

Through analysis of higher-energy collisional dissociation (HCD) and collision induced dissociation (CID) spectra of synthetic, and natively derived, N- and O-glycopeptides originating from human cerebrospinal fluid and urine samples, we found that the glycan fragmentation patterns characteristically differ between GalNAcα1-O-, GlcNAcβ1-O-, Galβ3GalNAcα1-O-, Galβ4GlcNAcβ-O-, and Galβ3GlcNAcβ-O- terminated peptides. Specifically, we found that GalNAcα1-O- and Galβ3GalNAcα1-O- substituted peptides resulted in particularly more prominent m/z 126 and m/z 144 ions whereas Galβ4GlcNAcβ terminated N- and O-glycopeptides resulted in prominent m/z 138 and m/z 168 ions.

The difference in oxonium ion fragmentation signatures for such glycopeptides may thus be used to distinguish between the glycan structures, and this knowledge will be of importance in LC-MS/MS based glycoproteomics studies.

P-589.00

A GLOBAL BOTTOM-UP STRATEGY FOR THE ANALYSIS OF GLYCOPROTEINS

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Glycosylation is the most common posttranslational modification in proteins. O- and N-glycan structures are altered in many diseases such as congenital disorders of glycosylation or cancer. Moreover, certain glycan structures elicit immunogenic responses in humans, being necessary an exhaustive quality control of recombinant glycoproteins marketed as biopharmaceuticals. High performance separation techniques coupled to mass spectrometry have become indispensable in glycoproteomics. Bottom-up strategies to analyze specific low molecular mass glycosylation markers originated after glycoprotein digestion is a spreading alternative to the top-down approach, where the complexity and the lower sensitivity for intact glycoproteins hinders a detailed characterization.

The aim of this work is to describe different bottom-up methodologies for the accurate characterization of the oligosaccharide structures in glycoproteins. In this regard, glycosylation may be analysed through the glycans released from the protein or the glycopeptides. In the first case, glycans released with PNGase-F are derivatized, separated and identified by zwitterionic-hydrophilic interaction liquid chromatography coupled to mass spectrometry (ZIC-HILIC-MS). This method provides information about the structure and composition of the oligossacharides, but not about the glycosylation sites or their degree of occupancy. In the second case, glycopeptides are obtained from the glycoprotein by tryptic digestion and then analysed by capillary electrophoresis and capillary liquid chromatography coupled to mass spectrometry (CE-MS and µLC-MS). While CE allows for separation of glycopeptide glycoforms according to their charge-to-mass ratios, µLC offers the possibility of using different stationary phases such as C-18, ZIC-HILIC or porous graphitic carbon (PGC) to separate glycopeptides from more hydrophobic non-glycosylated peptides, or even isomeric glycoforms.

Both bottom-up strategies are applied to different glycoproteins (e.g. erythropoietin, apolipoprotein C-III, transferrin and alpha-acid glycoprotein), resulting in an excellent characterization thanks to the valuable complementary information. While more glycan diversity is found with the first approach, the second provides more information about the glycosylation sites.

P-590.00

CONSTRUCTION OF THE STANDARD METHOD FOR MOUSE TISSUE GLYCOME MAPPING IN HUPO BD-GPP INITIATIVE

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Introduction and objective: Lectin microarray we have developed is the most sensitive and reliable tool for glycome analysis, which acquires glycan profiles of a tiny amount of biological specimens. Recently we efficiently sophisticated it optimizing for tissue glycome mapping in combination with laser microdissection (LMD). However, there have been no universal methods for continuous accumulation of a vast array of data toward the ⬜gglycoproteome atlas⬜h. In this study, we aimed to standardize the method for mouse tissue glycome mapping.

Methods: For reproducible sample pre-treatment and glycan analysis, LMD was adopted for tissue dissection. Formalin-fixed paraffin-embedded tissue sections (5-micrometer thickness) of 5 organs originated from C57BL/6J mice were mounted on glass slides with polyethylene naphthalate foil membrane, and 0.5 mm2 of tissue sections were collected. Protein extraction and Cy3-labelling, lectin microarray analysis, and data standardization were basically performed as described by Matsuda et al. BBRC (2008).

Results and discussion⬜FThree lots of each organ section were prepared from three individuals. Two sets of them were used for optimization of isolation protocol with LMD. After confirming the reduction of the lot-to-lot variation, we obtained about 100 glycan profiles and compared with 40 glycan profiles of another set handled by a different operator. As a result, operator-to-operator variation was not significant.

Conclusions: We constructed a reproducible method for mouse tissue glycome mapping. Now three research institutes have started to perform the mapping using mouse sections from a single supplier in accordance with the standardized method.

P-591.00

GLYCOPROTEOMIC CHARACTERIZATION OF TRICHOMONAS VAGINALIS

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Proteomics of human microbiome and infectious diseases

OP071 - AN OPTIMIZED METAPROTEOGENOMIC PIPELINE FOR IN-DEPTH CHARACTERIZATION OF THE MOUSE GUT MICROBIOME

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Introduction and objectives
The recent effort aimed at characterizing the gut microbiome (GM) has revealed its relevance in the development of several diseases, for most of which mouse is used as animal model. Metaproteomics enables to actually measuring the functional expression of a microbiome, but methods still need improvement. Therefore, we aimed to optimize a metaproteogenomic pipeline suitable to study the GM of a mouse model of type-1 diabetes (T1D).

Methods
Feces were collected from 6 NOD mice. Samples were subjected to direct extraction (DE) and to extraction after differential centrifugation (DC). Bead-beating was used for protein extraction, followed by FASP and single-run LC coupled to LTQ-Orbitrap Velos MS. Illumina DNA sequencing was also performed to generate metagenomic databases. Bioinformatic analyses were performed using Sequest-HT/ Percolator (FDR

Results and Discussion
DC yielded no increase in microbial identifications but significant differences were seen in taxonomic distribution when compared to DE. The number of host proteins identified were also different. An iterative strategy combining public and metagenomic databases led up to 50% increase in identifications compared to a standard approach. The optimized pipeline allowed the identification of up to 18,000 microbial proteins and 23,000 non-redundant peptides per sample (15% from non-prokaryotic microorganisms), belonging to 2000 different microbial species and hundreds of metabolic pathways. The analysis of a T1D mouse model consistently revealed several taxonomic and functional differential features.

Conclusions
The metaprotegenomic pipeline enabled the generation of the largest gut metaproteome dataset to date. For the first time the impact of fecal sample pre-processing on the metaproteomic results was evaluated, and an iterative bioinformatic approach was established. Microbes and proteins associated with T1D development were also found in a proof-of-principle study.

OP072 - HIGH-RESOLUTION LC-MS ANALYSIS OF TARGET CELLS TREATED WITH CLOSTRIDIUM DIFFICILE TOXINS.

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Introduction and objectives

The anaerobic bacterium Clostridium difficile is one of the most common nosocomial pathogens that induces gastrointestinal infections ranging from mild diarrhea to life-threatening pseudomembranous colitis. Toxins TcdA and TcdB are the two major virulence factors that specifically glucosylate and inactivate small GTPases. The consequences are reorganization of the cytoskeleton, loss of cell-cell contacts, and finally cell death.

Methods

A comprehensive proteome analysis was conducted using Caco-2 cells that were treated for different time periods with wild type TcdA or mutant TcdA. Short (5 h) and long term (24 h) effects on the colonic proteome were analyzed using SILAC and ICPL techniques. Results were verified by western blot and MRM analysis. The activity of clostridial glycosylating toxins was evaluated in vivo by identification of their target GTPases and determination of their glucosylation rate.

Results and Discussion

Wild type TcdA induced considerable changes in the protein profile of colon cells. More than 800 of the over 7600 identified protein groups were altered in their abundance. Higher abundant proteins were involved in regulation, metabolic processes, respiratory chain complexes, endocytosis, and organelle function. Less abundant proteins participate in cell-cycle, translation, cytoskeleton organisation, and RNA binding. Glucosyltransferase deficient TcdA induces only changes after short incubation periods. Regulation of several proteins was confirmed by western blot and MRM analysis. Besides the known TcdA targets RhoA, RhoC, and RhoG, glucosylation was also identified in Rap1(A/B), Rap2(A/B/C), Ral(A/B), and (H/K/N)Ras which had not been considered as TcdA targets before. A SILAC based quantification method of GTPase glucosylation was established and kinetic and dose response correlations will be determined to analyze glucosylation pattern in higher detail.

Conclusions

This proteome analysis demonstrates that TcdA affects several cellular processes that have not been considered before to be affected by clostridial glycosylating toxins.

OP073 - HIGH-THROUGHPUT TARGETED SWATH-MS PROTEOMICS OF A HUMAN PATHOGEN IMPLICATES NOVEL PROTEIN QUANTITATIVE TRAIT LOCI (PQTL) IN THE REGULATION OF KEY VIRULENCE FACTORS

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Background: The human pathogen Streptococcus pyogenes causes over 700 million infections per year, but the molecular mechanisms underlying its virulence remain poorly understood. We used exhaustive SWATH-MS proteomics and OpenSWATH, a novel targeted analysis software, to analyze the proteome of 64 sequenced clinical isolates and establish a direct link between specific mutations, protein expression and phenotype.

Method: We exhaustively measured the proteomes of 64 paired M3 strains (32 invasive / 32 noninvasive) by SWATH-MS. For this analysis, we developed OpenSWATH, which provides automated targeted data extraction, feature scoring and FDR-estimation. Our novel multi-run nonlinear retention time alignment algorithm allowed for contextual and consistent quantification of all detected peptides across all samples by using intense signals from aligned measurements.

Results: Using OpenSWATH analysis and alignment, we quantified over 80% of the expressed proteome in all 64 strains (1085 quantified proteins). Correlating the 793 distinct SNPs with the protein expression identified over 75 proteins whose differential abundance could be directly linked to specific genetic changes ($p < 1e-7$). Multiple proteins were found to be both differentially expressed between the invasive and noninvasive group ($p < 1e-14$) and significantly associated with specific genetic loci.

Conclusion: Using SWATH-MS and OpenSWATH allowed us to exhaustively quantify a microbial proteome for a large number of genetically different S. pyogenes strains with unprecedented proteomic coverage. Correlating genetic mutation data with protein expression and clinical outcome provides novel insights into how the genome shapes the proteome and ultimately the emergence of microbial virulence in S. pyogenes.

OP074 - PROTEOMICS UNRAVELS EXTRACELLULAR VESICLES AS CARRIERS OF MOONLIGHTING PROTEINS IN CANDIDA ALBICANS

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Introduction and Objectives

Candida albicans is a commensal fungus in healthy humans and may cause different type of infection mainly in immunocompromised patients. C. albicans secretes a considerable number of proteins involved in different processes. However, predicted cytosolic proteins have been detected in the extracellular medium of C. albicans. These proteins must use alternative routes of exportation. In this way, C. albicans and other fungi extracellular vesicles have been described by transmission electron microscopy (TEM). In this work, we made for the first time a comparative proteomic study of C. albicans soluble secreted proteins and extracellular vesicles.

Methods

C. albicans SC5314 was grown in defined medium to late exponential phase. The cell-free culture supernatant was harvested by centrifugation and separated in vesicles sample (visualized by TEM), according to Rodriguez et al.2007, and vesicle-free secretome that was concentrated using a 10 kDa cut-off filter. Three biological replicates of both samples were obtained, digested and analyzed by LC-MS/MS (Orbitrap).

Results and Discussion

A total of 75 and 61 proteins with two or more peptides were identified in extracellular vesicle and vesicle-free secretome, respectively. Out of them, 40 were common in both samples. Differential proteins identified from free-vesicles were enriched in cell wall related and secreted proteins whereas metabolism and exocytosis proteins were abundant in vesicles sample. Bioinformatic analysis showed that 89% of vesicle-free proteins were predicted as secreted whereas only 60% of the vesicles proteins had signal peptide.

Conclusions

This first comparative proteomic study of soluble secreted proteins and extracellular vesicles in C. albicans revealed that this fungus is able to use different mechanisms to secrete different types of proteins which could be crucial for virulence, host immune response and/or intercellular communication.

P-592.00

LIQUID CHROMATOGRAPHY AND MASS SPECTROMETRY FOR COMPARING CLINICAL ISOLATES OF MRSA AND MSSA

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Introduction and objectives

Staphylococcus aureus (S. aureus) is a highly successful human opportunistic pathogen which causes severe infections. The aim of this study to discover the differential virulence controversy between methicillin resistant S. aureus (MRSA) and methicillin susceptible S. aureus (MSSA) and the impact of their produced toxins on the clinical course of disease.

Methods

Two isolates of MSSA and MRSA were subjected to comparative proteomic analysis of their exoproteins using 1D-SDS-PAGE- LC-MS/MS. They were cultured to stationary phase of growth. Exoproteins were precipitated, washed, and dissolved in SDS sample buffer. Proteins were separated on a SDS-PAGE then reduced, alkylated, and digested with trypsin. The tryptic digests were analyzed by nanoflow LC-linear iontrap-TOF MS. Data processing and databank searching were performed with Mascot software against NCBInr database.

Results and Discussion

Number of proteins identified from MSSA and MRSA were 168 and 262; respectively, from them 118 was shared. The MRSA unique proteins were 144. They are classified according to their function and location. Around 41.7% were virulence determinants, 10% involved in carbohydrate metabolism, 15.7% in protein synthesis, 9.4% in cell division, 5.8% in transcription and replication, and 17.4% were miscellaneous. MRSA unique proteins location was predicted using Psortb2 software, 48.7% were extracellular, 16.7% cytoplasmic, 3.5% cell wall, and 31.1% had unknown localization. Characteristics determinants for MRSA were identified like pyruvate dehydrogenase (lipoamide) subunit E1beta, cysteine protease, molybdopterin biosynthesis protein, and transcriptional repressor CodY.

Conclusions

MRSA unique proteins are mainly virulence determinants. MSSA & MRSA shared extracellular products are highly significant in MRSA than in MSSA with more protein score, high number of peptide matches, and increased exponentially modified protein abundance index which affirm that these proteins are secreted relatively in higher abundant in MRSA than in MSSA. Our results support the highly invasiveness of MRSA over MSSA in the pathogenesis.

P-593.00

DISCOVERY AND VALIDATION OF BIOMARKERS TO GUIDE CLINICAL MANAGEMENT OF PNEUMONIA IN AFRICAN CHILDREN

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Background

Pneumonia is the leading cause of death in children globally. Clinical algorithms remain suboptimal for distinguishing between severe pneumonia from other causes of respiratory distress like malaria, and between bacterial pneumonia and pneumonia from others causes such as viruses. Molecular tools could improve diagnosis and management.

Methods

We conducted a mass spectrometry-based proteomic study to identify and validate markers of severity in 390 Gambian children with pneumonia (N=204) and age, sex and neighborhood-matched controls (N=186). Independent validation was conducted on 293 Kenyan children with respiratory distress (238 with pneumonia, 41 with P. falciparum malaria and 14 with both). Predictive value was estimated by the area under the receiver operating characteristic curve (AUROC).

Results

Lipocalin-2 (Lpc-2) was the best protein biomarker of severe pneumonia (AUROC: 0.71 [95% CI, 0.64-0.79]) and highly predictive of bacteremia (AUROC 78% [95% CI, 64-92%]); pneumococcal bacteremia (AUROC 84% [95% CI, 71-98%]); and 'probable bacterial etiology' (AUROC: 91% [95%CI 84-98]). These results were validated in Kenyan children with severe malaria and respiratory distress who also met the WHO definition of pneumonia. The combination of Lpc-2 and haptoglobin distinguished bacterial versus malaria origin of respiratory distress with high sensitivity and specificity in Gambian children (AUROC: 99% [95%CI 99-100%]) and in Kenyan children (AUROC: 82% [95% CI, 74-91%]).

Conclusions

Lpc-2 and haptoglobin can help discriminate the etiology of clinically defined pneumonia, and could be used to improve clinical management. These biomarkers should be further evaluated in prospective clinical studies.

P-594.00

APPLICATIONS OF PROTEOMIC METHODS IN SEVERE SEPSIS RESEARCH

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Introduction and objectives

Severe sepsis (SS) is defined as the host inflammatory response to microbial infection with the presence of hypoperfusion or organ dysfunction. This life-threatening complication is a Public Health challenge due to high prevalence and mortality. Objective: To investigate proteome profiles in SS patients and healthy individuals for detecting different protein expression patterns.

Methods

Serum samples were obtained from 5 patients (media age 57.4) during the first 48 hours after SS diagnosis, and 5 healthy controls (media age 51.6). The most abundant serum proteins were depleted from samples prior to 2DE analysis by using Proteominer Protein Enrichment Large-capacity Kit. Soluble protein samples containing 100μg of protein were mixed with rehydration buffer and loaded in 11cm IPG-strips (pH range 4-7). Strips were subjected to isoelectric focusing (IEF) using Protean-IEF-Cell (Bio-Rad) and were equilibrated before loading to gels for electrophoresis. Gels were stained with Sypro-Ruby, and the differential analysis was performed with LUDESI-software. The spots of interest were excised, digested by trypsin, and subjected to MALDI-TOF/TOF and/or nLC-MS/MS analysis. Research was approved by the Ethics Committee.

Results and Discussion

All patients are alive and were treated in the Intensive Care Unit with supportive measurements and antibiotics. Clinical features were: affected organs (heart 5/5, kidney 1/5, lung 2/5, cytopenia 1/5), 2 positive blood cultures (E.coli and S.Aureus). Seven proteins were differentially expressed in patients and controls: Serum amyloid, Clusterin, Antithrombin III, Apolipoprotein E, Hemoglobin Beta, Filaggrin, Complement factor H-related protein 1. The validation by ELISA is currently ongoing in a larger sample.

Conclusions

Proteomic analysis has shown to be a feasible tool in SS study, and has allowed us to find seven proteins of potential interest. Further studies are needed to assess the potential roles of the identified proteins as diagnostic tools and prognostic biomarkers.

P-595.00

ANTIMICROBIAL ACTIVITIES OF SEVEN ESSENTIAL OILS FROM IRANIAN AROMATIC PLANTS AGAINST COMMON CAUSES OF ORAL INFECTIONS

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Background: Over the past two decades, there has been a growing trend in using oral hygienic products originating from natural resources such as essential oils (EOs) and plant extracts. Seven aromatic plants used in this study are among popular traditional Iranian medicinal plants with potential application in modern medicine as anti-oral infectious diseases.

Objectives: This study was conducted to determine the chemical composition and antimicrobial activities of essential oils from seven medicinal plants against pathogens causing oral infections.

Materials and methods: The chemical compositions of EOs distilled from seven plants were analyzed by gas chromatography/mass spectrometry (GC/MS). These plants included Satureja khuzestaniea, Satureja bachtiarica, Ocimum sanctum, Artemisia sieberi, Zataria multiflora, Carum copticum and Olveria decumbens. The antimicrobial activities of the essential oils were evaluated by broth micro-dilution in 96 well plates as recommended by the Clinical and Laboratory Standards Institute (CLSI) methods.

Results: The tested EOs inhibited the growth of the examined oral pathogens at concentrations of 0.015-16 µL/mL. Among the examined oral pathogens, E. faecalis had the highest MICs and MMCs. Of the examined EOs, Satureja khuzestaniea, Zataria multiflora, and Satureja bachtiarica respectively showed the highest antimicrobial activities, while Artemisia sieberi exhibited the lowest antimicrobial properties. Conclusion: Based on these results, the EOs of the above mentioned plants can possibly be used as an antimicrobial agent in treatment and control of the oral pathogens.

P-596.00

HYDATID CYST DISEASE OF THE SPINE: A CASE REPORT

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Introduction: Hydatid cyst disease is a significant clinical problem in endemic regions.

Case report: Herein we report a 55-year-old man who referred to our outpatient clinic due to back pain and progressive numbness and weakness of both lower extremities and disability in walking. In imaging work ups Magnetic resonance imaging (MRI) revealed an epidural cystic lesion extending from T6 to T7. Laboratory analyses were performed. With impression of hydatid cyst of spine, Daily doses of albendazole 400 mg (twice per day) were used for 2 weeks and then the patient underwent surgical intervention. He was symptom-free after operation in three years follow up.

Discussion: Hydatid disease of the spine is rare and has poor prognosis in such condition; the severity of disease is related to the neurological complications. Paraplegia is the most serious complication which is caused by compression of the spinal cord by the cysts. The treatment relies on the actual surgical removal of cysts although the bone involvement is quite challenging. The poor outcome of posterior decompression and laminectomy for intraosseous spinal hydatid disease were reported by several authors .In endemic countries, prevention and health education the best measures

P-597.00

CEREBROSPINAL FLUID BIOMARKER CANDIDATES ASSOCIATED WITH HUMAN WNV NEUROINVASIVE DISEASE

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Introduction and objectives

During the last decade, the epidemiology of WNV in humans has changed in the southern regions of Europe, with high incidence of West Nile fever cases, but also of West Nile neuroinvasive disease (WNND). The lack of human vaccine or specific treatment against WNV infection imparts a pressing need to characterize indicators associated with neurological involvement. By its intimacy with central nervous system (CNS) structures, modifications in the cerebrospinal fluid (CSF) composition could accurately reflect CNS pathological process

Methods

The aim of the present study was to apply the iTRAQ technology in order to identify the CSF proteins whose abundances are modified in patients with WNND

Results and Discussion

Forty-seven proteins were found modified in the CSF of WNND patients as compared to control groups, and most of them are reported for the first time in the context of WNND. On the basis of their known biological functions, several of these proteins were associated with inflammatory response

Conclusions

The present study provided the first insight into the potential CSF biomarkers associated with WNV neuroinvasion

P-598.00

DEVELOPMENT OF A RAPID MICROBIAL PREPARATION SYSTEM FROM A BLOOD CULTURE BOTTLE USING CATIONIC PARTICLES FOR BACTERIAL IDENTIFICATION WITH MATRIX-ASSISTED LASER DESORPTION IONIZATION TIME-OF-FLIGHT MASS SPECTROMETRY.

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Introduction
Generally, the microbes require preparation prior to MALDI-TOF MS analysis. Although many conventional protocols were published, they are not used widely in clinical field due to complicated operation, involving significant hands-on time and interference of residual blood component debris (Especially hemoglobin-related proteins).
We develop a new preparation method with our proprietary technology of cationic polymer. The method could simply and rapidly yield pretreated microbes for the MS identification without interference of hemoglobin-related proteins.

Methods
The method is composed of the following three steps
STEP1: An aliquot of cationic particles was added into suspension of microbes, and the resulting aggregates was separated by a handy desk-top centrifuge (-6000rpm).
STEP2: The precipitate was re-suspended with acetonitrile and formic acid, and the supernatant was separated by the desk-top centrifuge.
STEP3: The obtained supernatant was mixed with acetonitrile including CHCA, then applied to MALDI plate.
E.coli (gram negative bacterium) and lactococcus (gram positive bacterium) were used as model samples for MALDI Biotyper analysis.

Results and discussion
The method gave rise an equivalent identification score to the conventional method for both microbes tested in this study (Score of > 2.0), demonstrating sufficient recovery performance of the method in routine testing in spite of the use of a handy desk-top centrifuge (Recovery was 80- 90%). The most importantly, no interference of hemoglobin (up to 500 mg/dL, interference check A plus) was observed in MS fingerprints. The results indicated cationic particles had specific association with microbes, but not with hemoglobin-related proteins. Finally, the processing time of the method was significantly shorter (10-20 minutes) than that of one of several conventional methods used in the conventional methods.

Conclusions
We here conclude that the method could be routinely used as a pretreatment method for the MS identification of microbes, and make improvement of the efficacy in clinical laboratories.

P-599.00

QUANTIFICATION OF BEXSERO® OMV PROTEINS BY A LABEL FREE LC MS/MS APPROACH

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Meningococcal disease is a major cause of morbidity and mortality worldwide. Its epidemiol-ogy is currently dominated by five capsular serogroups (A, B, C, W-135, and Y). While effec-tive vaccines already exist for serogroups A, C, W-135 and Y, no vaccine was available against serogroup B. Recently, a four component vaccine, Bexsero®, designed to prevent in-fection caused by this serogroup, has been approved by the European Commission for use in individuals from two months of age and older.

The active components of this vaccine are three recombinant proteins identified by reverse vaccinology combined with detergent ex-tracted outer membrane vesicles (dOMV) prepared from a New Zealand epidemic strain. Considering their intrinsic complexity, we performed additional characterization of dOMVs on top of the standard quality control testing carried for batch release. We applied the Hi3 la-bel-free LCMSE methodology to qualitatively and quantitatively characterize the dOMV pro-tein content. We first, successfully investigated the robustness and the accuracy of the meth-odology for the dOMV characterization and we then applied it to compare six dOMV produc-tion lots. Around 100 proteins were quantified from each preparation. When classified accord-ing to their predicted cellular localization, about 90% of the total protein amount belongs con-sistently to the outer membrane compartment.

Using nonparametric hypothesis testing and complementary log-log linear regression, the quantifications of a subset of 21 proteins com-mon to all lots and including approximately 90 % (85-92%) of the total protein amount quan-tified in any dOMV lot were found consistent across lots. The relevance of these results is two-fold, showing that the Hi3 quantification methodology is robust for a broad range of pro-teins and indicating that the manufacturing process currently used for the production of the Bexsero® dOMV components is highly reproducible and consistent.

P-600.00

IDENTIFICATION OF NATURALLY PROCESSED MHC CLASS I-RESTRICTED HIV EPITOPES PRESENTED BY HUMAN HIV-INFECTED CELLS

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HIV-specific cytotoxic T-lymphocytes play a critical role in containing HIV infection. Yet correlates of immune protection against HIV are not defined. Identification of peptides endogenously processed and uniquely presented by HIV-infected cells remains essential to identify potent immune responses against HIV and for vaccine design.

Human 293T cells expressing HLA-A02/B07 were transfected with, HIV-derived lentiviral vector producing GFP and pseudotyped with VSV-G envelope: LV-GFP-VSVg (293T-HIV-transfected). This LV-GFP-VSVg was used to infect HLA-A03/A11/B35/B51 and HLA-A01/A32/B27/B57 EBV-transformed human B cell lines (B-HIV-infected). Peptides displayed by 293T-HIV-transfected, B-HIV-infected cells and control-uninfected cells were eluted by mild acid treatment. Eluted peptide pools were enriched by size fractionation and analyzed by LC-MS/MS. We identified 43 HIV-derived peptides eluted from 293T-HIV-transfected cells and 16 peptides from each of B-HIV-infected cell lines. HIV peptides identified were derived primarily from the most abundantly expressed Gag protein and secondary from Pol protein. 80% of identified peptides come from epitope rich areas. 80% were 8-12aa long peptides suitable for MHC-I loading and 20% were >12aa, indicating fairly frequent presentation of longer HIV peptides by MHC-I. 35%(293T-HIV-transfected) and 56%(B-HIV-infected) of 8-12aa peptides contained anchor residues corresponding to HLA-I expressed by the cells. 52%(293T-HIV-transfected) and 44%(B-HIV-infected) represented potential extended HLA-I restricted binders. 13%(293T-HIV-transfected) were non-HLA-I restricted. 10% of identified peptides corresponded to optimal HIV epitopes reported to elicit immune responses in HIV-infected individuals.

T cell epitope screening with identified HIV peptides revealed 30% of the previously reported immune responses in HIV-infected individuals. 40% of the responses were observed with identified HIV peptides containing reported HIV epitopes and 30% responses were revealed with yet unknown epitopes. This first report of peptides displayed by HIV-infected cells provides fundamental insight into antigen presentation during HIV infection and may lead to unbiased identification of novel immune responses important to control HIV infection and for immunogen design.

P-601.00

WHY DATABASE MATTERS: IMPACT OF SEQUENCE DATABASES ON METAPROTEOME ANALYSIS OF A MOCK MICROBIAL MIXTURE

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Introduction and objectives:
Metaproteomics allows the investigation of the protein repertoire expressed by complex microbial communities. To exploit its full potential, improvement in bioinformatic approaches for data analysis are still needed. In this context, sequence database selection represents a key challenge. This work evaluated the impact of different databases in metaproteomic investigations by using a mock microbial mixture.

Methods:
The microbial mixture was assembled including seven prokaryotes and two eukaryotes. Genomes extracted from the 9 individual strains and from the mock mixture were subjected to Illumina next-generation sequencing, in order to generate genome- and metagenome-derived protein databases, which were used along with public databases (namely, NCBI, UniProtKB/SwissProt and UniProtKB/TrEMBL, parsed at different taxonomic levels) to analyze the metaproteomic dataset achieved by shotgun LTQ-Orbitrap MS analysis. The information obtained was comparatively evaluated in respect to number and overlap of peptide identifications, FDR behavior and peptide degeneracy, and reliability of taxonomic attribution according to the lowest common ancestor approach (using MEGAN and Unipept software).

Results and Discussion:
The quantitative comparison demonstrated that only 35% of peptides identified were common to all database classes; moreover, genus/species-specific databases provided up to 17% more identifications compared to databases with generic taxonomy, while the metagenomic database enabled a minor increment in respect to public databases. In addition, public databases with generic taxonomy exhibited a markedly different behavior in terms of false discovery rate and peptide degeneracy compared to the counterparts. Furthermore, the level of taxonomic attribution misassignments varied among the different databases, and specific thresholds based on the number of taxon-specific peptides were established to minimize false positives.

Conclusions:
This study reveals that database selection plays a pivotal role in metaproteomics, and suggests the use of iterative searches and suitable filters for taxonomic assignments to improve depth and trustworthiness of metaproteomic results.

P-602.00

WHOLE PROTEOME AND MAIN POST-TRANSLATIONAL MODIFICATIONS (PTMS) ANALYSIS OF K. PNEUMONIAE CLINICAL ISOLATES

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A shotgun proteomic approach, able to identify a large number of global proteins was performed on three K. pneumoniae clinical isolates. Since PTMs (post-translational modifications) are involved in physiological conditions as well as in pathogenicity and virulence of the bacterial cell, affecting its protein functions, we focused our attention on some of them, specifically on Phosphorylation and S-thiolation, the latter consisting of Glutathionylation and S-Cysteinylation. Our goal was to create a rich dataset of proteins which undergo these modifications and can potentially influence bacterial signaling.

Clinical isolates, collected from different sites of isolation and harboring different antibiotic susceptibility, were grown on agar plates and incubated at 37 °C and 10% CO_2. Bacterial pellets were digested by trypsin and purified to remove salts which could interfere with the LC-MS/MS analysis. Peptide mixture was enriched for phosphopeptides by TiO_2 beads. In order to investigate S-thiolation, protein extracts were digested with trypsin without any reduction and alkylation step, purified and separated into six fractions by SCX. Samples were analyzed using a Q-Exactive mass spectrometer.

By means of LC-MS/MS analysis we identified 41 and 16 sites of glutathionylation and cysteinylation for one of the two clinical isolate, whereas 18 and 12 sites respectively for the other strain. 110 sites of phosphorylation on different a.a. (C,D,H,R,S,T,Y) were identified on 104 peptides corresponding to 72 proteins.

S-thiolation may serve an antioxidant role in the protection of protein SH groups against irreversible oxidation, or alternatively under basal (physiological) conditions may serve a regulatory role analogous to other post-translational modifications such as protein phosphorylation. This work represents the first global study regarding these PTMs performed on K. pneumoniae bacterium. The dataset of PTMs generated will be used to select and perform a targeted quantitative analysis (SRM) on interesting peptides identified during the preliminary study.

P-603.00

IDENTIFICATION OF API88 BINDING PARTNERS IN ESCHERICHIA COLI USING PHOTOAFFINITY-CROSSLINK STRATEGIES AND LABEL FREE QUANTIFICATION

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The steadily increasing number of multi-drug resistant bacterial pathogens represents a major health threat all over the world. Besides the development of new antibiotics, it is also important to reveal their mode of action and bacterial targets, which would allow optimizing their structures for improved target binding. Here, we studied the proteome of E. coli BL21AI for possible interaction partners of Api88, which is an optimized version of the proline-rich antimicrobial peptide apidaecin 1b expressed in honeybees (Apis mellifera).

A Tyr-7-para benzoylphenylalanine analogue of Api88 (L-Api88-PC) was synthesized, which binds to the suggested target DnaK as efficient as Api88 and thus should be able to crosslink the peptide to its binding partners in vivo after UV irradiation. L-Api88-PC contained additionally biotin to enrich cross-linked products by affinity chromatography. After incubation of a bacterial cell culture with L-Api88-PC at 37°C for 2 h, the cells were irradiated with UV light (4500 mJ/cm²), harvested, washed, and lysed. Proteins were extracted, enriched with streptavidin magnetic beads and separated by SDS-PAGE. The gel lane was cut into 24 pieces of equal size and each gel piece was incubated with trypsin. Tryptic peptides were separated by reversed-phase chromatography on a nanoUPLC and identified on-line on an LTQ-Orbitrap XL mass spectrometer using data dependent acquisition for the six most abundant peptides.

Peptides were quantified with the skyline software relative to controls, where the bacteria were grown without peptide or in the presences of the inverse peptide analogue D-Api88-PC. In total 33 proteins were identified at significantly higher quantities in the presence of L-Api88-PC than in the controls indicating specific Api88-protein interactions. Most proteins were involved in pathways related to gene expression and protein biosynthesis explaining the mode of action. This was further confirmed by functional studies.

P-604.00

AFFINITY PROTEOMICS REVEALS ELEVATED MUSCLE PROTEINS IN PLASMA OF CHILDREN WITH CEREBRAL MALARIA

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Systemic inflammation and sequestration of parasitized erythrocytes are central processes in the pathophysiology of severe Plasmodium falciparum childhood malaria. However, it is still not understood why some children are more at risks to develop malaria complications than others.

To identify human proteins in plasma related to childhood malaria syndromes, multiplex antibody suspension bead arrays were employed. Out of the 1,015 proteins analyzed in plasma from more than 700 children, 41 differed between malaria infected children and community controls, whereas 13 discriminated uncomplicated malaria from severe malaria syndromes. Markers of oxidative stress were found related to severe malaria anemia while markers of endothelial activation, platelet adhesion and muscular damage were identified in relation to children with cerebral malaria.

These findings suggest the presence of generalized vascular inflammation, vascular wall modulations, activation of endothelium and unbalanced glucose metabolism in severe malaria. The increased levels of specific muscle proteins in plasma implicate potential muscle damage and microvasculature lesions during the course of cerebral malaria.

P-605.00

COMPARATIVE 2D-DIGE ANALYSIS OF DEPLETED SERUM SAMPLES OF PATIENTS WITH 2009 INFLUENZA A (H1N1).

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Introduction and objectives

The Influenza virus is a member of the Orthomyxoviridae family. There are three types of influenza viruses: A, B and C. In the spring of 2009, a new influenza A (H1N1) virus emerged to cause illness in people. This virus was very different from regular human influenza A (H1N1) viruses and the new virus caused the first influenza pandemic in more than 40 years. Using a proteomic approach the principal objective was to provide an experimental evidences of a differential expression pattern using depleted samples of patients with a confirmatory probe for pandemic influenza A (H1N1) and control samples.

Methods

Serum samples were quantified, the integrity determined by SDS-PAGE and depleted using a column Agilent Human 6 Multiple Affinity Removal System. The depleted serum samples were separated using 2D-DIGE gels, the first dimension using 24- cm IPG strips (pH 4-7 or 4.5-5.5), and the second dimension using 12 % polyacrylamide gels, the spot detection and relative quantification of proteins was done using the DeCyder software.

Results and Discussion

In the narrow range of pH (4-7), 94 % of the proteins had the same expression level, however 32 proteins were over or under-expressed. On the other hand, using a micro-range pH 4.4-5.5, 34 spots had a differential expression; all the analysis was using a threshold of $\pm$ 2 fold change.

Conclusions

Using the IPG strips with a micro-range of 4.5 to 5.5 a better resolution was obtained. Several proteins are in the MS analysis and will be validated using WB.

P-606.00

A COMPARISON OF SEVERE AND NON-SEVERE VIVAX MALARIA PATIENTS FOR THE IDENTIFICATION OF DISEASE MONITORING MARKERS USING QUANTITATIVE PROTEOMICS

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Introduction and Objectives: In spite of the overall global burden, economic impact and severity of the disease, vivax malaria is largely neglected in terms of research concern and financial investments. The present study has been conducted to perform an in-depth comparative analysis of serum proteome profiles of severe and non-sever vivax malaria patients for the identification of prognostic and disease monitoring markers.

Methods: In this study serum samples from severe (n = 42) and non-severe (n = 118) vivax malaria patients confirmed through microscopic examination of a thin peripheral blood smear followed by rapid diagnostic test (RDT), along with community controls (n = 92) and febrile diseased controls (n = 28) from three different endemic regions of India were investigated. 2D-DIGE and iTRAQ-based quantitative proteomics approaches were used in the discovery phase of the study and interesting targets were validated by ELISA. Efficiency of 7 classifier proteins; serum amyloid A, hemopexin, apolipoprotein E, haptoglobin, ceruloplasmin, retinol-binding protein and apolipoprotein A-I for discrimination of severe and non-severe vivax malaria was analyzed by ROC curves.

Results and Discussion: Functional pathways analysis involving the identified proteins revealed the modulation of different vital physiological pathways, including acute phase response, chemokine and cytokine signaling, complement cascades and blood coagulation in vivax malaria. 39 proteins exhibited differential expression between severe and non-severe Plasmodium vivax infections. ROC curve analysis demonstrated SAA, Apo E and haptoglobin as efficient predictor proteins (AUC > 0.90) for vivax malaria. Interestingly, haptoglobin exhibited sequential alteration in expression level with respect to the disease severity.

Conclusion: Investigation of parasite induced alterations in host proteome and modulations of vital physiological processes in different severity levels of the infection have immense clinical relevance and can enhance our understanding regarding disease pathogenesis of malaria and aid in pattern-recognition of different severe malaria associated syndromes.

P-607.00

THE STAPHYLOCOCCUS AUREUS HG001 PROTEOTYPE RESOURCE

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Introduction and objectives: Staphylococcus aureus is on the one side a colonizer of humans but also a pathogen, which displays resistances against most antibiotics and can thus cause life-threatening infections. Proteome analyses of host-pathogen-interactions provide important insight into molecular processes and the pathophysiology of this pathogen and thus make contributions to the development of new antibacterial intervention
 strategies.

Methods: In this work we present a Q Exactive nanoLC-tandem mass spectrometry (MS) driven proteome-wide characterization of the strain S. aureus HG001. Therefore, different preparation methods were applied namely standard workflow, off-gel fractionation, salt-step fractionation.

Results and Discussion: Combining 144 high precision MS data sets, we identified 19,200 unique peptides from 2,088 S. aureus HG001 distinct proteins, which are roughly 70% of the predicted ORFs. This comprehensive and unique peptide set was further characterized concerning pI, GRAVY, and detectability scores in order to conceive the low peptide coverage of only 8.7%.

The high quality and well characterized peptide-centric spectra were further converted to a SpectraST database and used for the fast and reliably identification of S. aureus-typic peptides from a highly complex host pathogen experiment. As a result, the library search significantly improved the number of identified proteins (~50%) compared to MASCOT-based identifications.

Conclusion: The proteomics workflow applied will now allow access to new, thus far unexplored facets of the S. aureus host interplay. The high quality spectra resource introduced here also represents an important repository for deciphering the S. aureus proteome using selected reaction monitoring in order to quantify a specific subset of proteins.

P-608.00

SURFACOME CHANGES OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS IN BACTERIAL GROWTH UNDERGOING USNIC TREATMENT BY PROTEOMIC APPROACH Antonella Riviello[1], Arianna Pomilio[1], Fabrizio Di Giuseppe[1,2], Gianni Di Bonaventura[1], Marilisa Sulpizio[1,2], Stefania Angelucci [1,2], Carmine Di Ilio[1,2], Enrica Eleuterio[1,2]

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Introduction and Objectives: bacteria surfacome contains proteins from all membrane compartments exhibiting pivotal role in host colonization. Methicillin-resistant Staphylococcus Aureus (MRSA), a gram + bacteria resistant to a large variety of antibiotics is considered one of the emerging pathogens in pulmonary failure in cystic fibrosis (CF)(1). Nowadays, treatment for lung infections is focusing on the use a novel biomolecule, usnic acid with antimicrobial activity, arising from Lichens as Secondary Metabolite (SML) (2). In our previous investigation regarding antibacterial action of SMLs against MRSA in CF patients, we demonstrated that usnic acid is active at subinhibitory concentration (MIC), lower than other hepatotoxic SLMs (3).
No data literature for proteomic, key to understanding the molecular mechanism that drives multiple effects of usnic acid on virulence and chemo resistance of MRSA have been published. So the aim of the present work is to analyze surfacome changes in membrane fraction from usnic acid-treated MRSA.

Methods: we performed a comparative proteomic analysis between membrane protein fractions from untreated (CTRL) and usnic acid-treated (USNIC) samples using 2-DE combined with MALDI-TOF MS/MS experiment. For each condition all samples were electrophoretically run three times as 3 technical and 2 biological replicates.

Results and Discussion: 23 differentially expressed proteins were uniquely MS-identified. Noteworthy was the usnic acid treatment which induced the synthesis of 2 isoforms of Acyl- CoA esterase family protein which is involved in the synthesis of fatty acids within the bacterial cell wall biogenesis, ensuring survival and virulence for MRSA.

Conclusions: The discovery of the enzymes Acyl-CoA esterase could represent a milestone for the development of novel and efficient antibacterial drugs.

P-609.00

QUANTITATIVE ANALYSIS OF THE SALIVARY PROTEOME IN HUMAN PAPILLOMAVIRUS (HPV) PATIENTS

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Introduction: Studies have suggested that salivary proteins may be important to establish the diagnosis and prognosis of oral and systemic diseases. Thus, the study of saliva became more attractive than other body fluids because it is a non invasive process and easy to collect. The comparative analysis of the salivary proteome is a promising strategy to identify biomarkers that can be used for detection and diagnosis. In the case of human papillomavirus (HPV) infection early diagnosis and prevention are the best alternatives. Since the early stages of the disease can be treated successfully in about 90% of cases. In the present study, we analyzed differences in the protein profiles of whole saliva from patients with HPV and healthy controls.

Methods: Our purpose is to detect changes in the level of expression of salivary proteins and the repression/ induction of new proteins. The samples were individually analyzed by mass spectrometry based shotgun proteomic approach, which detects all protein from complex mixture without gel separation technique. The samples were ultracentrifugated and separated using Microcon devices (cut off 10 kDa and 3 kDa). So, all the proteins present in each fraction were hydrolyzed with trypsin. The peptides that resulted from tryptic digestion were analyzed by nano Liquid Chromatography connected online to a linear quadrupole ion trap-Orbitrap (LTQ-Orbitrap Velos Pro) mass spectrometer.

Results: The majority of the detected peptides in all of the samples correspond to alpha-amylase, keratins or serum albumin. 33 proteins were identified exclusively in the HPV patients. The majority corresponds to peptides associated with inflammation (immunoglobulins). Keratin 1 and collagen type VII were present in 4 patients.

Conclusion: This approach shows the protein profile during the course of the disease, which may contribute to an improvement in diagnosis.

P-610.00

A HUMAN PROTEOME MICROARRAY IDENTIFIES THAT THE HETEROGENEOUS NUCLEAR RIBONUCLEOPROTEIN K (HNRNP K) RECOGNIZES THE 5' TERMINAL SEQUENCE OF THE HEPATITIS C VIRUS RNA

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Stem-loop I (SL1) located in the 5' untranslated region of the hepatitis C virus (HCV) genome initiates binding to miR-122, a microRNA required for hepatitis HCV replication. However, proteins that bind SL1 remain elusive. In this study, we employed a human proteome microarray, comprised of ~17,000 individually purified human proteins in full-length, and identified 313 proteins that recognize HCV SL1.

Eighty-three of the identified proteins were annotated as liver-expressing proteins, and twelve of which were known to be associated with hepatitis virus. siRNA-induced silencing of eight out of 12 candidate genes led to at least 25% decrease in HCV replication efficiency. In particular, knockdown of heterogeneous nuclear ribonucleoprotein K (hnRNP K) reduced HCV replication in a concentration-dependent manner. Ultra-violet-crosslinking assay also showed that hnRNP K, which functions in pre-mRNA processing and transport, showed the strongest binding to the HCV SL1. We observed that hnRNP K, a nuclear protein, is relocated in the cytoplasm in HCV-expressing cells.

Immunoprecipitation of the hnRNP K from Huh7.5 cells stably expressing HCV replicon resulted in the co-immunoprecipitation of SL1. This work identifies a cellular protein that could have an important role in the regulation of HCV RNA gene expression and metabolism.

P-611.00
COMPARATIVE SALIVA PROTEOME OF HEPATITIS B AND C-INFECTED SUBJECTS

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Introduction: Hepatitis B and C virus (HBV and HCV) infections are an important cause of cirrhosis and hepatocellular carcinoma. The natural history has a prominent latent phase and infected patients may remain undiagnosed. This situation may continue to spread these infections in the community. This problem can be circumvented by the use of alternative specimens like saliva for the detection of HCV and HBV infections. Compelling reasons exist to use saliva as a diagnostic fluid. It meets the demands for inexpensive, noninvasive and easy-to-use diagnostic methods. Comparative analysis of salivary proteomic using mass spectrometry is a promising new strategy for identifying biomarkers. Our purpose is to apply Orbitrap-based quantitative approach to explore the salivary proteome profile in HBV and HCV infected patients.

Methods: In the present study whole saliva was obtained from 20 healthy adults (control), 20 HBV-infected and 20 HCV-infected subjects. Two distinct pools containing saliva from 10 subjects of each group were obtained. The samples were ultracentrifuged and separated using Microcon devices (cut off 10 kDa and 3 kDa), all fractions were hydrolyzed (trypsin) and injected in LTQ-VELOS ORBITRAP. The identification and analyses of peptides were performed by Proteome Discoverer1.3 and ScaffoldQ+v.3.3.1.

Results And Discussion- From a total of 362 distinct proteins identified, 344 proteins were identified in HB, 326 proteins were identified in HC and 303 in control group. Some blood proteins like flavin reductase (wich converts biliverdin to bilirubin) were detected in in HCV group. The data showed a higher presence of several types of immunoglobulin fragments in HBV-patients and ceruloplasmin in control group. Peptides of serotransferrin were less detected in HCV group.

Conclusion- This study provides an integrated perspective of salivary proteome that should be further explored in future studies targeting specific disease markers for HBV and HCV infection.

P-612.00

A NOVEL STRATEGY FOR TYPING MYCOPLASMA PNEUMONIAE USING MALDI-TOF MS-CLINPROTOOLS

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Introduction and objectives The typing of Mycoplasma pneumoniae (M. pneumoniae) mainly relies on the detection of nucleic acid, which is limited by the use of only a single-gene target, complex operation procedures and lengthy assay time. Here, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) was used to type M.pneumoniae.

Methods MALDI-TOF MS coupled to ClinProTools was used to discover MALDI-TOF MS biomarker peaks and generate a classification model based on a genetic algorithm (GA) to differentiate between type 1 and type 2 M. pneumoniae. Twenty-five M. pneumoniae strains were used to construct an analysis model, and 43 Mycoplasma genus strains were used for validation.

Results and Discussion For the GA typing model, the cross validation values, which reflect the ability of the model to handle variability among the test spectra and the recognition capability value, which reflects the model¡¯s ability to correctly identify its component spectra, were all 100%. This model contained 7 biomarker peaks (m/z 3318.8, 3215.0, 5091.8, 5766.8, 6337.1, 6431.1, 6979.9) used to correctly identify 31 type 1 and 7 type 2 M. pneumoniae isolates from 43 Mycoplasmae strains with a sensitivity and specificity of 100%. The strain distribution map and principle component analysis based on the GA classification model also clearly showed that the type 1 and type 2 M.pneumoniae can be divided into two categories based on their peptide mass fingerprints.

Conclusions With the obvious advantages of being rapid, highly accurate, highly sensitive, low cost and high-throughput, MALDI-TOF MS ClinProTools is a powerful and reliable tool for M. pneumoniae typing.

P-613.00

LABEL-FREE PROTEOMIC ANALYSIS OF ENVIRONMENTAL ACIDIFICATION-INFLUENCED STREPTOCOCCUS PYOGENES SECRETOME REVEALS A NOVEL ACID-INDUCED PROTEIN HISTIDINE TRIAD PROTEIN A (HTPA) INVOLVED IN NECROTIZING FASCIITIS

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Introduction and objectives

Streptococcus pyogenes is a human pathogen responsible for various diseases. To establish infection, S. pyogenes must adapt to adverse environments, such as the acidic environment of a wound. Acidic stimuli may stimulate S. pyogenes to invade into deeper tissue. However, how this acidic stimulus causes S. pyogenes to manipulate its secretome for the facilitation of invasive infection remains unclear.

Methods

To investigate temporal secretome changes under acidic environment, a comparative secretomics approach using label-free LC-MS/MS was undertaken to analyze the secretome in acidic and neutral conditions. The acid-influenced proteins were selected to investigate their functions by using virulence tests and functional assays.

Results and discussion

The dynamic label-free LC-MS/MS profiling identified 97 proteins, which are influenced by environmental acidification. Among these, 33 (34%) of the identified proteins were predicted to be extracellular proteins. Regulon analysis showed that Mga and RofA/Nra may be associated with environmental acidification and growth-phase cues. Interestingly, classical secretory proteins comprise approximately 90% of protein abundance of the secretome in acidic condition at the stationary phase. In contrast, these proteins only comprise 30% of the abundance observed in neutral conditions. Among 33 acid-influenced secreted proteins, HtpA, was selected to investigate its role in invasive streptococcal infection. The mouse infected by the htpA deficient mutant showed lower virulence and smaller lesion area than the wild-type strain. The mutant strain was more efficiently cleared at infected skin than the wild-type strain. Besides, the relative phagocytosis resistance is lower in the mutant strain than in the wild-type strain.

Conclusion

These data indicate that a novel acid-induced virulence factor, HtpA, which improves anti-phagocytosis ability for causing necrotizing fasciitis. Our investigation provides vital information for documenting the broad influences and mechanisms underlying the invasive behavior of S. pyogenes in an acidified environment.

P-614.00

THIORIDAZINE INDUCES ALTERATIONS IN THE MYCOBACTERIUM TUBERCULOSIS CELL WALL PERMEABILITY BARRIER

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The increasing occurrence of multidrug resistant tuberculosis (MDR-TB) exerts a major burden on treatment of this major infectious disease. Previously, several neuroleptics like thioridazine revealed activity against Mycobacterium tuberculosis both in vitro and in vivo. Thioridazine even revealed a high activity against XDR-TB when added to other second and third line drugs.

The synergistic effects between thioridazine and other antibiotics has long been thought to be caused by the inhibition of efflux pumps. Using an unbiased proteomic approach, we set out to unravel the mechanism of this potential new anti-tuberculosis component, by examining the impact of continuous drug exposure on the proteome of M. tuberculosis. We discovered differential expression of several proteins that are involved in maintaining the cell wall permeability barrier, but not any of the known mycobacterial efflux pumps. By assessing the accumulation of both hydrophilic and hydrophobic fluorescent dyes in the bacterial cells over time, we demonstrate that long term drug exposure of M. tuberculosis indeed lowered the cell wall permeability. The thioridazine induced decrease in cell wall permeability, and thereby the enhanced uptake of compounds, could explain the previously reported synergistic effects between thioridazine and other anti-tuberculosis drugs.

Thereby our study provides an alternative mechanism for thioridazine next to the previously suggested role in efflux pump inhibition. This new insight in the working mechanism of this anti-tuberculosis compound in M. tuberculosis could open novel perspectives of the future drug administration in combinational therapy.

P-615.00

DIFFERENTIAL PROTEOMICS OF ANTIBIOTIC RESISTANCE PROFILING OF ESCHERICHIA COLI FROM CLINICAL SOURCE

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Introduction and objectives. Humans are colonized by an enormous variety of bacteria which make up a part of the human microbiome. Normally these bacteria are commensal, but there is more and more evidence that they are developing antibiotic resistance. Proteomics is a valuable tool to understand the mechanisms involved in antibiotic resistance by accurately characterizing which proteins are expressed and when, and the place of those proteins in specific metabolic pathways. The purpose of this work was to identify and characterize proteins related to antibiotic resistance of E. coli from humans and correlate this information with the metabolic pathways involved.

Methods. The genotypes of Escherichia coli from clinical sources were analyzed and antibiotic resistance genes were detected. Proteins were then extracted and separated by 2-DE, with the use of fractionated proteome extraction protocols followed by protein spot detection and excision. MALDI/TOF-MS/MS was performed in order to identify the proteins from the 2-DE gels using bioinformatics tools and web database questioning.

Results and Discussion. Protein spots were identified in extracellular, periplasmic, cytoplasmic and the external membrane sub-proteomes following a previous detection of 164 proteins from the total proteome. Proteins were identified and characterized and thus associate with specific metabolic pathways and biological mechanisms. Proteins related to antibiotic resistance and biological functions responsible for the survival of bacteria in adverse environments, such as stress responses, were found. Several proteins were found to be related to virulent or pathogenic serotypes and possible explanations for their expression in clinical bacteria are given.

Conclusions. Clinical bacteria can be accurately analyzed by proteomics providing an important source of information on the study of antibiotic resistance in humans. Knowledge of the differential proteomes and expression patterns of proteins of interest will be useful in understanding the mechanisms which allow a bacterium to survive in the presence of antimicrobials.

P-616.00

SEROLOGIC PROFILING TO THE CANDIDA ALBICANS INTRACELLULAR PROTEOME FOR THE DIAGNOSIS OF INVASIVE CANDIDIASIS IN NON-NEUTROPENIC PATIENTS

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Introduction and Objectives: The clinical management of invasive candidiasis (IC) remains unclear in non-neutropenic patients. It often leads to delayed initiation of appropriate antifungal therapy and poor clinical outcomes in this high-risk population. Early IC diagnosis could improve this clinical setting. The goal of this study was to identify and validate serologic biomarkers that alone or in combination could differentiate early and accurately between IC and non-IC patients from this population.

Methods: Serological proteome analysis and bioinformatics were used to profile serum IgG antibody-reactivity levels to the Candida albicans intracellular proteome in non-neutropenic patients with and without IC. We then developed prototype assays to validate the diagnostic usefulness of candidate IgG-antibody biomarkers in a different set of non-neutropenic patients.

Results and Discussion: Serum anti-C. albicans IgG antibody-reactivity profiles from non-neutropenic patients were heterogeneous. Despite this variability, unsupervised clustering analysis showed that IgG antibody-reactivity patterns to a panel of 22 C. albicans antigens accurately discriminated between IC and non-IC patients from this high-risk population. Supervised classification analysis identified a 2-IgG antibody-reactivity signature for early diagnosis of IC, from which a diagnostic score was derived. We then confirmed its discriminative power using analytically-validated ELISA prototype assays in an independent group of IC and non-IC patients. Multivariate logistic-regression models demonstrated that the two signature biomarkers predicted IC in a different way in non-neutropenic patients. Receiver-operating-characteristic curve plots highlighted that this 2-IgG antibody-reactivity signature was more accurate in distinguishing IC from non-IC patients than the individual biomarkers alone, and had good ability to IC detection in non-neutropenic patients.

Conclusions: We conclude that if confirmed in prospective cohort studies, our serum 2-IgG antibody-reactivity signature may accurately identify IC in non-neutropenic patients. Our results further provide new insights into serologic response to the C. albicans intracellular proteome in this high-risk population.

P-617.00

COMBINING NEUCODE WITH LYSC AND LYSN DIGESTION FOR DE NOVO SEQUENCING APPLICATIONS

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Introduction

De novo sequencing is used to determine peptide sequence information without database searching. Although there are some software tools which aid in de novo sequencing, distinguishing b- and y-type ions can still be difficult. We used the NeuCode method (Hebert et al, 2013) to incorporate heavy isotopologues of L-lysine into recombinant proteins or cellular proteins. After protein digestion using LysC or LysN, the incorporated Neucode amino acids generated unique b- and y-type ions with doublet patterns for de novo assignment.

Methods

The Thermo Scientific™ Pierce™ Heavy in vitro translation Kit was used to incorporate 13C615N2 L-lysine or D8 L-lysine isotopologues into recombinant proteins. SILAC DMEM media supplemented with dialyzed FBS was used to incorporate heavy amino acids into A549 cells. After stable isotope incorporation, protein samples were mixed 1:1, reduced/alkylated and digested with LysN for 2 hours at 50oC or LysC for 16 hours at 37oC. Peptides were analyzed using nano-LC coupled to a Thermo Scientific™ Orbitrap Fusion™ instrument.

Results and Discussion

In order to improve b- and y-ion assignment for de novo sequencing, we used Neucode heavy amino acids to label proteins prior to digestion with LysC or LysN. During MS/MS, b- and y-ions are generated from peptide fragments to provide sequence information. We used 13C615N2 L-lysine or D8 L-lysine isotopologues to label recombinant proteins expressed using a mammalian cell-free system or cultured cell proteins using SILAC. For LysC digested samples, we were able to observe a unique y-ion series pattern containing doublet peaks at high resolutions > 100K at 400 m/z using FT-CID or FT-HCD. For LysN digested samples, we observed a similar doublet pattern for the b-ion series using FT-CID.

Conclusions

The combination of Neucode labeling with digestion with LysN or LysC digestion provides a unique MS/MS signature that aids in de novo sequencing assignment.

P-618.00

FAST AND EFFICIENT CHARACTERIZATION OF BRACHYSPIRA´S PROTEOMA BY ONE-SHOT LC/MS ANALYSIS

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Introduction and objectives

Spirochetes of the genus Brachyspira are anaerobic bacteria that colonize the large intestine of animals and birds. Brachyspira hyodysenteriae is one of the most pathogenic species, being the etiological agent of swine dysentery, a very expensive disease of the growing pig [1]. Despite the importance of these species, few proteins are annotated as evidence at protein level. This is further complicated by the high genomic variability in Brachyspira strains. In order to characterize these proteomes, fast and efficient identification methods are required. Here, we describe the use of 60 cm column to obtain around 60-70% coverage of the bacterial proteome.

Methods

Proteome analyses of Brachyspira were carried out on a liquid chromatography-tandem mass spectrometry system using LC-MS without prefractionation. Tryptic peptides were directly injected onto a 60 cm long, 100 μm i.d. C18 column and an 8-h gradient was applied at 0.25 µL/min. Also, fractionation alternatives have been compared, using techniques based on cell compartment or isoelectric point fractionation previously to the analysis in the mass spectrometer.

Results and Discussion

We identified more than 11000 unique tryptic peptides from approximately 1300 proteins. Our results indicate that this simplified one-shot proteomics approach with long columns is advantageous for rapid, deep, sensitive, and reproducible proteome analysis.

Conclusions

In this study we present the first complete proteome study for Brachyspira specie, obtaining a coverage of 60-70% using one-shot proteomics, demonstrating to be a rapid and fast method that will allow the comparison of several strains avoiding sample manipulation in a reasonable time.

P-619.00

SEMIQUANTITATIVE PROTEOMIC ANALYSIS OF THE INTERACTION BETWEEN CANDIDA ALBICANS AND ACINETOBACTER BAUMANNII

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Introduction: We describe the pathogenic interaction between two ecologically related and clinically troublesome important opportunistic pathogens, the prokaryote, Acinetobacter baumannii, and the eukaryote, Candida albicans. The objectives of this study were to investigate the extracellular proteome and its effect on survival of these microorganisms.

Methods: The protein profiles of the secretomes of single and mixed planktonic grown cells produced by both microorganisms grown in sabouroud culture media, was explored using label-free quantitation technique using LC-MS/MS. We also evaluated the effect of the secretomes on survival of both microorganisms by plating.

Results : We found that C. albicans caused total killing of A. baumannii over a 2 days period and also a significant C. albicans growth inhibition was observed when both microorganisms grown together.
Proteomic analysis resulted in the identification of 179 and 100 non-redundant proteins in A. baumannii and C. albicans respectively. This analysis revealed that the amount of 30/78 proteins was increased while the amount of 54/20 proteins was reduced in C. albicans/A. baumannii respectively.
The up-regulated proteins, in the case of C. albicans, include proteins involved in the: (i) Virulence (SAP5, SAP4); (ii) oxidative stress response (HYR1, TRX1); (iii) adhesion (ALS3); and (iv) chaperones (SSA2, HSP70). And in A. baumannii involved in the: (i) Virulence (PPIase, ferric siderophore receptor proteína, OmpA family protein, pilus assembly protein); (ii) oxidative stress response (peroxiredoxin, chloroperoxidase precursor, superoxide dismutase); (iii) toxin (RTX toxin).
We observed that secretome from C. albicans caused almost a total kill of A. baumannii with the degree of killing being mostly or totally dependent on the pH and on the concentration of farnesol.

Conclusions:
The combination of the pH and the farnesol caused almost a total kill of A. baumannii. This is the first time that a proteomics approach has been used to study C. albicans-A. baumannii interaction.

P-620.00

SOLUBLE HLA ISOTYPES AND PEPTIDOME ASSOCIATED WITH HIV PATHOGENESIS

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HIV infection is associated with strong immune activation and immune cells dysfunction. Human Leukocyte Antigen (HLA) molecules classified in haplotypes are part of surface MHC-I or MHC-II complexes that present self- or pathogen-derived peptides to T cells as a part of immunosurveillance system. Several HLA haplotypes are associated with either HIV control or disease progression. HLA also exists as soluble forms (sHLA) in plasma and their levels are increased with HIV progression. However, the relationship between sHLA haplotyes, their peptide repertoire and immune dysfunctions in HIV infection have not been characterized.

We recently established an approach for efficient isolation of membrane-bound HLA molecules, and the LC-MS/MS-based identification of their specific isotypes and of the peptides they display. We identified potentially new HIV-derived epitopes uniquely presented by HLA molecules of HIV-infected cells surface, and demonstrated their immunogenicity in HIV-infected patients. The present study was initiated to identify sHLA haplotypes secreted by human primary cells and to characterize their peptide repertoire in the context of HIV infection. We identified by Western blot sHLA molecules released in the serum-free media by human primary peripheral blood mononuclear cells (PBMCs) from healthy donnors and HIV-infected patients. Western blot analysis indicated higher levels of sHLA from HIV+ PBMCs than from control PBMCs.

The sHLA isotyping by established LC-MS/MS approach revealed the first identification of non-classical sHLA-E molecules in HIV-infected samples, in addition to classical sHLA-A and sHLA-B molecules. sHLA were isolated by immunoaffinity from serum-free media of HIV+ and control PBMCs and, their bound peptide repertoire is currently been identified and compared to that of membrane-bound HLA. The impact of specific sHLA-peptide on the functions of HIV-specific T cells isolated from HIV-infected donors of our Institute's cohort will provide further insight into the role of specific sHLA-peptide to immune dysfunction in HIV infection.

P-621.00
SUBCELLULAR FRACTIONATION AND SEVERAL GROWING CONDITIONS TO INCREASE PROTEOME COVERAGE IN CANDIDA ALBICANS PEPTIDEATLAS

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Introduction
The current existing build of the Candida albicans PeptideAtlas accounts for 22000 distinct peptides that belong to 2562 proteins, representing approximately 41% of the estimated proteome. While this represented an unprecedented proteome coverage for this fungus and was the first human fungal pathogen present in PeptideAtlas, it is still far from other organisms' PeptideAtlas, such as, Saccharomyces cerevisiae or Schizosaccharomyces pombe . To overtake such gap we have acquired new MS data from a wide array of experiments including an extensive subcellular fractionation and different growing conditions aimed at the maximization of the variability of the detectable proteome.

Methods
The extensive subcellular fractionation was performed by differential sequential centrifugations and filtrations,that selectively enrich in different types of membranes, organelles and secreted vesicles. These subcellular fractions were trypsinized and the proteins corresponding to cell surface were obtained by trypsin treatment on live cells. For the different growing conditions, a pool of extracts of cells from cultures in temperature stress, oxidative stress, hypha-inducing medium and minimal medium was trypsin digested into peptides and separated on an off-gel system. Samples, distributed into four different datasets, were in all cases subject to LC-MS/MS on Orbitrap instruments.

Results and discussion
In overall, a total of 149 high resolution MS output spectra files were obtained from the four different datasets and were used to create this new build of the C.albicans PeptideAtlas. This nearly doubles the number of MS runs that were used to assemble the first build and, in addition, unlike the first build, they all come from high-resolution Orbitrap instruments.

Conclusion
Since this new C. albicans PeptideAtlas build has been assembled from a wide array of experiments concerning extensive subcellular fractionations and different growing conditions, it completes the first build rendering a much more complete view of the C. albicans proteome.

P-622.00

EFFECT OF THE GLOBAL REGULATOR CRC IN VESICLE AND VESICLE-FREE EXTRACELLULAR PROTEOME OF PSEUDOMONAS AERUGINOSA

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Introduction

Pseudomonas aeruginosa is an opportunistic human pathogen that causes acute or chronic infections. P. aeruginosa secretes different virulence determinants, including proteases and toxins as well as small molecules siderophores, such as pyocyanin and pyoverdine and as many other Gram-negative bacteria constitutively produce outer membrane vesicles (OMVs) that contain proteins and other factors, some of them associated with virulence. The global regulator Crc modulates the expression of several P. aeruginosa proteins, virulence factors included. In the aim of understanding the effect of Crc on P. aeruginosa exoproteome, we present a comparative proteome profile of P. aeruginosa secretome of soluble and OMVs proteins of a crc mutant and wild type strain.

Methods

Vesicle and vesicle-free extracellular proteome were isolated from culture supernatants as previously described. Three biological replicates of PAO1 and Äcrc vesicle and vesicle-free extracellular proteome were digested and labeled using iTRAQ. The obtained spectra were searched against the P. aeruginosa PAO1 database.

Results and Discusion

Proteomic analysis of vesicle and vesicle-free secretome were performed by MS/MS. In total, 839 and 1058 proteins were identified in vesicle and vesicle-free extracellular proteome, respectively. Out of them, 489 and 644 proteins were quantified in vesicle and vesicle-free samples, 70% of these proteins were identified and quantified in more than 2 replicates. Regarding to the proteins secreted via vesicles, we identified 38 more abundant proteins secreted by P. aeruginosa Äcrc and 10 less secreted comparing with PAO1. On the other hand, P. aeruginosa Äcrc secretome allows us to discover 31 proteins increased and 15 decreased in vesicle-free supernatant of the bacteria. The potential role of these proteins in P. aeruginosa virulence will be discussed.

Conclusion

The global regulator Crc has a role in the secretion of a higher number of proteins including virulence factors that were not previously identified.

P-623.00

A COMPREHENSIVE PROTEOMIC STUDY OF CANDIDA ALBICANS SURFACE AFTER INTERACTION WITH HUMAN SERUM

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Introduction: The yeast Candida albicans is the most important opportunistic fungi causing a wide variety of infections ranging from superficial to invasive (IC). IC produces high morbidity and mortality in intensive care, post-surgery and cancer patients. The high mortality outcome of these infections is determined by the scarcity of antifungal agents, the emergence of resistance to them and the diagnostic limitations. The aim of this study is know which human serum proteins interact with C. albicans surface and which Candida proteins could be candidates for diagnostic markers, potential vaccines or therapeutic targets.

Methods: Cells were grown on Lee media pH 6.7 with 10% normal human serum (NHS) or heat inactivated serum (HIS), at 37ºC for 6 hours, followed for a brief trypsin treatment directly over the surface of intact cells. These peptides were properly clean-up and analyzed by a LTQ Orbitrap XL. The databases CGD (http://www.candidagenome.org/) and Swiss-Prot (http://www.uniprot.org/) were consulted to identify proteins of Candida and human respectively.

Results: Surfome analysis allows the identification of 173 proteins from NHS sample and 144 proteins from HIS sample in at least two of the three replicates. There are 133 proteins on common between all of the samples, 57 only on NHS and 67 only on HIS. Among them, the most relevant protein groups identified are from complement pathway, apolipoproteins and immunoglobulins. Also, 61 proteins of C. albicans were identified in three biological replicates. These include proteins related with cell wall organization (Phr1p, Ecm33p, Rbt5p, Rbt1p, Bgl2p, Ssr1p, Als3p, Pir1p, Mp65p, Ywp1p) and several ones described as immunogenic cell surface proteins (Eno1p, Pgk1p, Met6p).

Conclusion: This proteomic approach to study the C. albicans surface after interacting with human serum renders the identification of several relevant human proteins attached to the fungi cell surface.

P-624.00

UNRAVELLING THE PATHOGENESIS PROCESSES OF STAPHYLOCOCCUS PSEUDINTERMEDIUS TOWARD NEW THERAPEUTIC STRATEGIES

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Introduction and objectives

Pyoderma caused by Staphylococcus pseudintermedius is one of the most common skin infections in small-animals veterinary practice worldwide. This gram-positive bacterium is an opportunistic pathogen. The recent emergence of methicillin-resistant strains has renewed interest in the pathogenesis and virulence of this species, some already isolated from human samples. The characterization of membrane, cell wall and biofilm proteomes can help to identify new potential antigens, in order to develop new therapeutic strategies. Together with cytoplasm proteome information are useful to deepen the knowledge on the infection process developed by these bacteria.

Methods

The studied strain of S. pseudintermedius (5819/10) was isolated from a dog with deep pyoderma at FCM-UL. Optimized protocols were used to obtain four cellular protein fractionations. Their proteome characterization was done combining, 2-DE MALDI-TOF/TOF, 1-DE-LC-MALDI-TOF/TOF and GeLC-MSMS approaches. Three biological replicates were used for each assay.

Results and Discussion

By 2-DE MALDI-TOF/TOF a total of 1317 protein gel spots were detected. From those 378 unique proteins were identified as cytoplasmatic (33%), membrane (8%), extracellular (1%), cell wall (1%), ribosomal (8%) or with unknown location (49%). However, 8% of the total proteins are referred as uncharacterized, suggesting that further investigations are needed to determine their functions. Identification data obtained by 1-DE-LC-MALDI-TOF/TOF and GeLC-MSMS approaches are being processed for posterior global integration and functional analysis.

Conclusions

The optimized protocols for cellular fractionation and protein extraction have allowed the enrichment in diverse protein types, as shown by the 2DE gel separation partners and nanoLC separation profiles of tryptic peptides. The use for three proteomic strategies as allowed extending the number of identified proteins, in particular of those localized in cell membrane and cell wall.

P-625.00

PROTEOMIC CHARACTERIZATION OF HUMAN PROINFLAMMATORY M1 AND ANTI-INFLAMMATORY M2 MACROPHAGES AND THEIR RESPONSE TO CANDIDA ALBICANS.

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Introduction

In response to different stimuli, macrophages can differentiate into either a pro-inflammatory subtype (M1, classically activated macrophages) or acquire an anti-inflammatory phenotype (M2, alternatively activated macrophages). Candida albicans is the most important opportunistic fungus in nosocomial infections, and it is contended by neutrophils and macrophages during the first steps of the invasive infection. Murine macrophages responses to C. albicans have been widely studied, whereas the responses of human polarized macrophages remain less characterized. In this study we have characterized the proteomic differences between human M1- and M2-polarized macrophages, both in basal conditions and in response to C. albicans, by quantitative proteomics (2-Dimensional In Gel Electrophoresis).

Methods

In the present work, we have analysed for the first time the differential protein expression profile between M1 and M2 macrophages by 2D-DIGE technology, and determined their differential response to C. albicans.

Results and Discussion

This proteomic approach allowed us to identify metabolic routes and cytoskeletal rearrangement components that are the most relevant differences between M1 and M2 macrophages. The analysis has revealed Fructose 1,6-bisphosphatase (Fbp1), a critical enzyme in gluconeogenesis, up-regulated in M1, as a novel protein marker for macrophage polarization. Regarding the response to C. albicans, an M1-to-M2 switch in polarization was observed. This M1-to-M2 switch might contribute to Candida pathogenicity by decreasing the generation of specific immune responses, thus enhancing fungal survival and colonization, or instead, may be part of the host attempt to reduce the inflammation and limit the damage of the infection.

Conclusion

The most relevant pathways significantly modulated by macrophage polarization are metabolic routes, stress and immune responses, as well as cytoskeleton rearrangement. And we propose Fructose 1,6-bisphosphatase (Fbp1) as a novel protein marker for macrophage polarization.

Topic 16

Standardisation in proteomics

OP075 - SCRIPT-MAP, A DATABASE OF "BEST-RESPONDERS", PUSHES ACCURACY IN MS QUANTIFICATION

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Mass spectrometry based protein quantification has being become a conventional choice in biological researches, as it features high throughput and sensitivity in protein quantification across different pathology/physiology conditions.

It is well accepted that the key in MS1 and MS2 quantification is the selection of suitable peptides and transitions that have highest MS response intensity and widest dynamic range. Those "suitable" peptides and transitions are called "best-responders". Recently, we have built up SCRIPT-MAP, an experimental database of linear MS response curve of global peptide-transition ions in mammalian proteome, which includes intensity and dynamic range of over 1,000,000 transitions from 90,000 peptides (represent 9,500 gene products).

MS quantification based on "best-responders" in SCRIPT-MAP is more accurate than traditional iBAQ algorithm, which counts all identified peptide without quality filtration. By using SCRIPT-MAP, quantification concatamer (QconCAT) internal standards were designed accordingly to dissect stoichiometry of typical metabolism pathways, such as lipid metabolism, carbohydrate metabolism, and TCA cycle, across different conditions. In summary, we provided a useful database for MS quantification, rendering quantitative proteomics a more convenient and accurate approach in biological researches.

OP076 - THE MZTAB DATA STANDARD FORMAT FOR REPORTING MS-BASED PEPTIDE, PROTEIN AND SMALL MOLECULE IDENTIFICATION AND QUANTIFICATION RESULTS

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Introduction and objectives: To facilitate data sharing in mass spectrometry (MS)-based proteomics, the HUPO Proteomics Standards Initiative (PSI) has developed several standard data formats; most of them XML-based. The main ones are: mzML (to store the mass spectra and chromatograms), mzIdentML (to report peptide and protein identifications), and mzQuantML (to store quantitative information). All three formats resulted in relatively complex schemas to be able to adequately represent the inherent data complexity.
The new mzTab file format aims to describe the qualitative and quantitative results for MS-based proteomics and metabolomics experiments in a simpler tabular format.

Methods: The development of mzTab was influenced by existing text-based output formats from several search engines and analysis pipelines. The model was developed as a tab-delimited file, accompanied by controlled vocabulary (CV) terms.

Results and Discussion: The complete mzTab specification document (version 1.0) can be found at http://mztab.googlecode.com. The data is stored in five different sections (in italics): metadata, protein, peptide, PSM (peptide spectrum match) and small molecule. With mzTab's flexible design it is possible to report results at different levels: ranging from a simple summary or subset of the complete information (e.g., the final results) up to fairly comprehensive representation of the results including the experimental design. There are already several existing software implementations for mzTab. The jmzTab Java API (available at https://code.google.com/p/mztab/downloads/list, Xu et al., Proteomics, 2014) is the current reference implementation.

Conclusions: Many downstream analysis use cases are only concerned with the final results of an experiment in an easily accessible format that is compatible with tools like Microsoft Excel® or R. Therefore, mzTab is ideally suited to make MS proteomics and metabolomics results available to the wider biological community.

OP077 - EXPEDIENTS IN PROTEOMIC RESEARCH – STANDARDISATION AND OPTIMIZED PROTOCOLS

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Introduction

Proteomics has become an emerging technology in biological, pharmaceutical and clinical research. There is an ever-present need for tailored standards and protocols which allow assessing the efficiency, quality and reproducibility of ideally all steps of the analytical workflow – from sample preparation to protein quantitation.

Methods

We use different strategies to quality control (QC) and standardize important steps of the analytical workflow. We control efficiency and reproducibility of every proteolytic digestion as well as the LC-MS performance using a tailored stable isotope labeled (SIL) peptide standard, which can be spiked into any sample to QC label free quantification on-the-fly. We furthermore extensively exploited the unique possibilities of the Archaea bacterium Pyrococcus furiosus (Pfu) as a universal standard in proteomics, owing to its huge evolutionary distance from humans and mammals. We designed a unique phosphopeptide reference standard that for the first time allows evaluating phosphorylation site localizations algorithms in a realistic environment.

Results

The presented strategies allow us to systematically monitor and optimize proteomic workflows and LC-MS performance in a routine and straightforward fashion. This helps to control, achieve and maintain reproducibility, robustness and throughput required for e.g. large-scale studies of clinical samples. All our QC strategies can be easily established in any lab. We furthermore specifically designed a novel (phospho-)peptide set that is based on Pfu sequences that (i) can be spiked into human and mammal cells without interference and (ii) cover the entire retention time window of a typical LC-MS experiment.

Conclusion

Based on our results we conclude that the presented standards and strategies enable the optimization of dedicated proteomic workflows with regard to reproducibility and reliability. The systematic evaluation holds impact that proteomics is ready for fundamental research and biomedical research such as the identification and validation of potential biomarkers and clinical compounds.

OP078 - AN AUTOMATED QUALITY CONTROL PLATFORM FOR NANOLC-MS BASED PROTEOMICS

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Mass spectrometry based proteomics is becoming a common tool in biomedical and biological research. The number of proteomic studies has been increasing dramatically both in frequency and scale. Current proteomic datasets range from a handful of LC-MS runs up to several hundreds. Validation of the quality of the acquired datasets and proper diagnosis of the instrument performance is becoming a prerequisite for every LC-MS proteomic experiment.

Due to this overwhelming amount of data, manual validation, even when performed by an experienced user, is practically infeasible. To ensure proper quality control and quality assurance for every proteomics experiment, we have developed a framework based on dozens of different proven proteomics metrics spanning sample preparation, chromatography, ionization, ion transmission, dynamic sampling, and peptide identifications as well as a specific set of highly validated proteomic workflows to provide consistency.

By continually monitoring these metrics and applying statistical process control, we have engineered a general purpose and consistent framework for monitoring and improving the quality of the proteomics experiments. The framework is managed by automated software that inspects system performance and stability, triggers an alert upon deviations from expected values, and guides users and service engineers in case of system failure or performance loss.

The software has been evaluated over hundreds of LC-MS runs and on several instruments.

Overall, this automated quality control platform will greatly improve the quality, traceability, and performance as well as drive the continuous improvement of proteomic experiments.

P-626.00

IMPACT OF GLYCOSYLATION ON ASSIGNING PROTEIN MASS VALUES AND THEIR TRACEABILITY

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The accuracy of clinical measurement results is essential not only for reliable diagnosis and treatment of disease but also for identification of new clinical markers. The use of certified reference materials (CRMs), i.e. materials for which one or more of their property values are certified with a reliably estimated uncertainty and an assured metrological traceability, should ensure the necessary accuracy and comparability of the data.

The use of these CRMs in the quantification of targeted biomarkers would reinforce the discrimination between the healthy and the disease status of the patient and therefore the decision making. In this context a number of protein-based CRMs have been successfully produced.

Most proteins have post-translational modifications (PTMs) of different clinical relevance. However, the impact of these PTMs on the protein value assignment has been so far hardly or just very punctually addressed (glycosylated haemoglobin). Glycosylation is a ubiquitous PTM of high clinical relevance due to its central role in almost all biological processes: protein activity, folding, interaction, stability, signalisation and mobility.

Therefore, we investigated the variability of glycosylation in IgG, IgM and IgA and analysed its impact on the value assignment. Samples were digested with N-glycanase and the released glycans were purified and labelled with fluorescence groups prior to their separation using liquid chromatography (LC) and capillary electrophoresis (CE). At least 14 different glycosylations, Ig type-dependent, were observed using these complementary separation techniques.

The glycan structural characterisation and location within the protein sequence were determined using LC-mass spectrometry. Finally the amount of protein was defined as a function of the ratio of glycosylations, observed by fluorescence after CE and LC separation, relative to total Ig. It is demonstrated that for relating the measured amount of target biomarker to the protein mass, it is necessary to consider the presence of PTMs and their relative abundance.

P-627.00

HIGH-ACCURACY, LABEL-FREE QUANTITATION OF HUMAN PLASMA PROTEINS FOR ESTIMATION OF BLOOD-DERIVED PROTEINS INCLUDED IN CLINICAL TISSUE SPECIMENS

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[INTRODUCTION] The amount of blood remained in clinical samples is usually variable and could interfere with accurate quantitation of proteins in clinical proteomics. This problem will be resolved if we can estimate quantities of plasma proteins identified in clinical samples before quantitative comparison.
We developed simultaneous quantitation procedure of major plasma proteins by label-free approach.

[METHODS] Plasma samples were taken from 3 healthy male volunteers at ages of 28-33 years. All the samples were desalted by gel filtration spin columns, and were subjected to OFFGEL fractionation using pH 3-10 IPG strips (24 fractions). All the fractions were acetone-precipitated, DTT-reduced, iocoacetamide-alkylated in 8 M urea, and trypsin-digested. Trypsin digests were purified with C18-spin columns and quantified by a modified BCA method specifically developed for peptide quantification. Two LC-MS runs using 0.38 µg peptide were conducted for each fraction using a conventional nanoLC-tandem MS spectrometer. Proteins identified under stringent criteria were quantified with Normalized Spectral Index (SIN), a spectral counting method utilizing spectral counting and total ion intensity of MS/MS spectra of matched peptides.

[RESULTS] We could estimate absolute quantities (microgram/microliter of plasma) of around 100 major plasma proteins which were identified in all the samples. Correlation between different samples and same samples were excellent with correlation coefficient of 0.97 to 0.99 and p < 0.0001.

[CONCLUSIONS] Our estimation of absolute amounts of major plasma proteins could be used as a reference data for estimation of proteins derived from plasma and improving quantitative, comparative comparison of proteomes between samples without interference of blood-derived proteins.

P-628.00

COMPARISON OF METHODS FOR THE QUANTIFICATION OF STABLE ISOTOPE LABELLED PROTEIN AND PEPTIDE STANDARDS FOR QUANTITATIVE PROTEOMICS

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Quantitative analysis of biomarker proteins for proteome based diagnostics requires sensitive reproducible methods, ideally with multiplexing capabilities. Whereas multiplexed immunoassays are well established, LC-MS based protein quantification from biofluids, tissues and cells is becoming increasingly popular in research and clinical diagnostics. The precision of such assays is, amongst other things, dependent on the quality of the isotope labelled peptides or proteins employed as internal standards as well as on their accurate quantity. We compared different methods for absolute peptide and protein standard quantification with regard to ease of use, throughput and accuracy.

Quantification of purified standard proteins and peptides was performed by (AAA) amino acid analysis (OPA), UV absorption at 280 nm, BCA assay and infrared spectroscopy of amide bonds (Direct Detect™, Merck Millipore). Furthermore, online purification (size exclusion and reverse phase (C4,C8)) and subsequent UV and ESI-MS detection was evaluated. The GELFREE™ (Expedeon Ltd) system was also assessed as an offline alternative for protein purification. Results were compared based on accuracy, cost and throughput.

BCA, UV280nm, IR and AAA based quantifications rely on protein and peptide samples of high purity. AAA showed the highest accuracy for all types of samples, whereas IR and UV280nm are less costly, easily executed and could therefore be useful for regular quality control of the protein and peptide standards. While online purification methods coupled to MS afford high-throughput, they are limited in their separation capacity for complex samples mixtures. Nevertheless they can be useful for the characterization of crude expression mixtures regarding identity and quantity of the respective protein standards. Although currently only available for offline analysis, the GELFREE system represents a useful tool for universal protein purification.

For isotope labelled proteins and peptides different quantification methods should be used depending on the experimental objective. A workflow will be presented to illustrate this point.

P-629.00

COMPARISON OF DESALTING METHODS FOR PROTEOMIC ANALYSIS OF WHOLE SALIVA AS AN EXAMPLE FOR HUMAN BIOFLUIDS

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Introduction:
Accurate protein identification and quantitation are dependent on manifold factors including sample purity. Therefore, negatively interfering substances (salts, specific detergents and buffers) need to be removed during sample preparation to ensure a high sensitivity and dynamic range of the LC-MS analysis. In this study, we compared four single-step desalting devices in terms of reproducibility and recovery of tryptic peptides prior to LC-MS/MS measurements.

Material and Methods:
Whole saliva of healthy volunteers was used to test four different methods for peptide purification (µC18 ZipTip® pipette tips, C18 ZipTip® pipette tips, TopTip C-18 and Oasis® HLB µElution Plate) at different loading amounts.

Results:
About 350 proteins could be identified within each purification method, 84% of these were commonly identified with all devices which shows method-depending variations in protein composition. Nevertheless, the inter-approach Pearson correlation coefficients of >0.95 for each measured protein profile indicate high reproducibility and recovery of proteins.

Conclusion:
Our results show that the applied, commercially available devices performed well in removing undesired contaminants and compounds from samples prior LC-MS/MS measurement providing high quality data for quantitative proteome analyses. Hence, the device selection should be based on the available amount of peptide extract and the sample number.

P-630.00

PROTEOSUITE - STANDARD SUPPORTING SOFTWARE FOR QUANTITATIVE ANALYSIS

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Different techniques have been devised for quantifying proteins by mass spectrometry (MS), including metabolic labelling (e.g. SILAC, 18O, etc.), isobaric tagging reagents (e.g. iTRAQ, TMT) or label-free methods (e.g. intensity-based or spectral counting). At present, there is an extensive variety of both commercial and freeware/open-source software for analysing such data; however, most packages are designed for a single experimental method, a single instrument vendor's file format, or lack an intuitive graphical interface, suited for bench scientists. To support a range of different techniques and file formats, we have developed ProteoSuite
(http://www.proteosuite.org), an open-source graphical software suite for quantitative proteomics which complies with all relevant Human Proteome Organization - Proteomic Standards Initiative (HUPO-PSI) standard formats: mzML, mzIdentML and mzQuantML.

ProteoSuite is a Java-based software package, integrating external libraries or packages, such as x-Tracker (http://www.x-tracker.info), MSGF+
(http://proteomics.ucsd.edu/Software/MSGFPlus.html), and application programming interfaces, such as jmzML, jmzIdentML and jmzQuantML. ProteoSuite is released under permissive licence, free for academic or commercial users.

In the version 1.0 release, we have included quantitation routines for isobaric tagging and label-free analysis by both spectral counting and intensity-based methods. We have also built-in support for visualisation of raw data from any instrument (via conversion to mzML format), identification data from any search engine (via conversion to mzIdentML format) and quantitative data in the mzQuantML standard. For performing identifications, we have integrated different open source search engines and post-processing routines directly into ProteoSuite, with an intuitive graphical interface.
Various benchmarking data sets have been used to test the performance of the software against other packages – both commercial and open source, demonstrating high-quality performance across a range of different methods.

We have developed new software for quantitative proteomics, supporting current community data standards and providing an intuitive graphical interface.

P-631.00
EVALUATION OF STAIN-FREE SDS-PAGE FOR GELC-MS APPLICATIONS

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GeLC-MS is a widely-used method for shotgun proteomics analyses of complex protein mixtures (samples).

In this method, samples are initially separated using 1-D SDS-PAGE and stained for visualization of protein lanes. Subsequently, the entire lane is 'fractionated' into individual slices along the length of the gel and each fraction processed for in-gel protein (tryptic) digestion, peptide purification, and LC-MS/MS analysis. Several reports have demonstrated that compared to 'in-solution' protein digestion, GeLC-MS enables higher protein identifications, with particular benefits for membrane/hydrophobic proteins (Fang et al., 2010 J Proteome Res, Piersma et al. 2010 J Proteome Res). In spite of the benefits of traditional GeLC-MS, improvements in the traditional workflow (eg: in terms of speed and ease of processing) would enable its wider adoption for deeper investigations of complex proteomes.

Here, we investigated the suitability of stain-free SDS-PAGE for streamlining GeLC-MS analysis of a complex proteome.

Mouse 3T3 whole cell lysates were resolved either using TGX stain free SDS-PAGE(4-15%) versus traditional Tris-HCl gels (4-15%) and processed via otherwise standard GeLC-MS workflow described above. The potential benefit of stain-free SDS-PAGE to GeLCMS comes from faster electrophoresis times and eliminating the need for manual protein staining/destaining post-electrophoresis. Fractions were collected across each gel lane (either Tris-HCL or stain-free SDS-PAGE) and proteins identified via analysis of purified, fractionated tryptic peptides on a Velos-Orbitrap mass spectrometer. Generated spectra were processed for standard protein identification using SEQUEST and incorporating a 58Da mass shift for modified tryptophans induced by stain-free chemistry.

Near identical number of proteins, peptides, spectra were identified at identical spectral identification rates using either Tris-HCl or TGX Stain free SDS-PAGE gels. However, using Stain-free SDS-PAGE substantially simplified the geLC-MS proteomics workflow. Details of the improved GeLC-MS workflow will be presented.

P-632.00

ELIMINATING WESTERN BLOT VARIABILITY WITH SALLY SUE

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Traditional Western blots were developed over thirty years ago, but standardization of the technique has been unachievable due to a high degree of method and data inconsistencies. Though it is of the most commonly used techniques in research labs today, traditional Western blots require a lot of manual steps each of which introduce variables to your experiment. While portions of the technique have been automated to improve precision, there hasn't been a major leap in the technology that transforms the traditional Western blot into a reliable, reproducible, and quantitative proteomic research method.

Until now! Simple Western eliminates all this variability by automating the entire process for you delivering superior data reproducibility. You can now identify your favorite protein and achieve reliable quantitation quickly and with very little sample.

We present results demonstrating the data precision offered by Sally Sue, the size-based high throughput member of the Simple Western family who can generate 96 data points from 5uL of sample in a single experiment. A number of protein targets were screened against control and treated cell lysates in triplicate on three different Sally Sue's to evaluate the inter- and intra-assay variability and the low CVs obtained clearly demonstrates how Sally Sue can transform your immunoassay into a truly quantitative assay. This means you can now monitor either a specific class of proteins or a particular signal transduction pathway with improved data day-to-day, lab-to-lab, and instrument-to-instrument reproducibility so you can make better, faster decisions.

P-633.00

INTEGRATED WORKFLOW FOR PROTEIN PURIFICATION/FRACTIONATION BY NGC SYSTEM AND VISUALIZATION BY STAIN-FREE SDS-PAGE

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For top-down proteomics/protein-focused applications, fractionation of complex biological mixtures is a frequent starting point with chromatographic approaches being the standard workhorse tool. This requires considerable investment of time for development of an optimal purification or fractionation workflow.

Workflow optimization involves systematic evaluation of various 'influencing' parameters (eg: column/media, pH/salt concentration of load material, elution buffer gradients, complexity of the biological sample) and visual confirmation of their effect using orthogonal means (eg: SDS-PAGE and protein staining). To address these requirements, we evaluated an integrated workflow that combined Bio-Rad's NGC chromatography system (that automates optimization of various 'influencing' parameters), and stain-free SDS-PAGE (that enables quick & easy orthogonal visual confirmation of the protein purification/fractionation process without manual staining).

Here, we present results from this integrated workflow to automate protein purification and biochemical fractionation schemes by focusing on E. coli lysates expressing targeted proteins of interest. As proof of principle, we initially utilized NGC's automated column switching functions to evaluate three different anion exchange columns for their ability to efficiently bind and recover two different E. coli proteins – Prancer Purple and Oxidoreductase and separate them from other sub-cellular fractions containing distinct proteins of interest for downstream mass spectrometric and western blotting analysis. Further, we demonstrate how the ability to automate selection of optimal pH or binding/elution conditions on the NGC system simplifies method development.

Finally, we illustrate the value of visually confirming all purifications/biochemical fractionations by stain-free SDS-PAGE which eliminated the need for manual handling associated with protein staining/destaining. Details of our integrated workflow and how it compares to the traditional approaches for protein purification or fractionation for proteomic analyses will be presented here.

P-634.00

THE EFFECTS OF PREANALYTICAL VARIABLES ON SERUM PEPTIDOME PROFILING BY MATRIX-ASSISTED LASER DESORPTION/IONIZATION TIME-OF-FLIGHT MASS SPECTROMETRY

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Human serum and plasma are the most favored clinical specimens in proteomics. Peptidome profiling of human serum is a promising tool to identify novel disease-associated biomarkers. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is widely used for peptidomic biomarker discovery. Cares should be taken, however, for sample collection and handling procedures which could have profound impact on serum peptidome patterns.

The effects of preanalytical variables on serum peptidome profiles have not been fully clarified yet. In the present study, we tested effects of preanalytical variables including the time intervals between venipuncture and serum separation (clotting time), freezing methods and the ways how to thaw the specimens on MALDI-TOF MS based serum peptidome patterns.

Three different freezing methods, freezing in liquid nitrogen, freezing directly in freezer (-20C, -80C) were compared. Frozen specimens were thawed in the two different ways. Effects of the temperature on long-term storage up to 6 months (as of March, 2013) were also assessed. Aliquots of serum samples were pretreated with magnetic beads with weak cation exchanger (Bruker Daltonics, Germany) followed by MALDI-TOF MS by the ClinProtTM (Bruker Daltonics, Germany) system automatically with ClinProtRobot. A number of significant differences in peak intensities were observed depending on the variables for sample processing conditions.

We are currently identifying the peaks in which significant differences of intensities were observed. These peaks should be sample quality markers. Furthermore, essentially the same experimental protocols as indicated above have been applied to serum proteome analysis by SDS-PAGE with densitometry. The effects of preanalytical variables on both gel-based and gel-free serum proteome and peptidome patterns as evaluated in detail in this study will allow us to standardize blood sampling and storage conditions for interlaboratory and multi-center studies for validation of biomarker candidates.

P-635.00

SYSTEMATIC COMPARISON OF SDS-PAGE AND HIGH-PH RPLC FRACTIONATION METHODS

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Two dimensional separation are widely used in in-depth high through-put proteome studies. SDS-PAGE and high pH RPLC are two common methods used as first dimensional separation in protein level and peptide level respectively. In this study, we systematic compared the two commonly employed methods via 7 workflows. Totally 9793 proteins were identified. Among them, 8581 and 7933 proteins were identified by high pH RPLC and SDS-PAGE fractionation method, respectively.

According to the physicochemical properties of the identified proteins and unique peptides, SDS-PAGE fractionation method benefits for the neutral PI peptides but it cause miss-cleavage in arginine. In the modification analysis, SDS-PAGE fractionation methods have more Oxidation modification and Propionamide modification. While the high pH RPLC fractionation method have relatively more abundance in Carbamidomethyl and Carbamylation.

Among the four SDS-PAGE run, pooling the gel pieces according the grey value and increasing the fraction number facilitate high efficient identification. Among the three high pH RPLC runs, shorter column with more fractions also give better results.

P-636.00

A SIMPLE PROTOCOL TO ROUTINELY ASSESS THE UNIFORMITY OF PROTEOMICS ANALYSES

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Mass spectrometry-based proteomic approaches are increasingly applied to biological and clinical studies. Initially used by specialized laboratories, the technology has matured and gained acceptance by the community, using various analytical processes and platforms. To facilitate data comparison and integration across laboratories there is a need to harmonize analytical processes. In a first step towards the harmonization of proteomics datasets, a quality control procedure was developed to routinely assess the uniformity of proteomics analyses.

The reference material was constituted of three yeast proteins. For each of the proteins, two tryptic peptides and their corresponding synthetic isotopically labeled analogs (in two isotopic forms) were selected and added to the sample at two distinct stages of the sample preparation process (prior to digestion and prior to analysis). The test mixture was analyzed by LC-MS(/MS) on triple quadrupole and quadrupole-orbitrap instruments to determine the signal intensity of the peptides generated by tryptic digestion, which were then compared to the corresponding isotopically labeled counterparts.

Following the double addition of internal standards, the LC-MS analysis allowed, through the measurement of the signals triplets of the different isotopic variants, a straightforward read-out of the analytical process uniformity. The relative intensities of the signals within the triplets enabled the immediate assessment of the digestion efficiency, and the overall recovery of the full sample preparation, and in turn the reproducibility of the experiment. The main chromatographic and MS attributes of the reference peptides were routinely monitored to assess the performance of the LC-MS system, including the retention time and chromatographic peak shape, the mass accuracy, the signal intensity, and the signal-to-noise ratio.

This simple protocol proposed enables the assessment of both sample preparation and LC-MS performance in one single analysis. The procedure is simple and easy to implement into routine workflows typically employed by the proteomics community.

P-637.00

NORMALIZATION OF CAPTURE PROBE CONCENTRATION USING A FLUORESCENT CALIBRANT IMPROVES ANTIBODY MICROARRAY REPRODUCIBILITY

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Antibody microarray printing with quill pins affords excellent spot alignment but is subject to greater spot-to-spot variation due to evaporation in the pins channels and other unknown factors. We hypothesize that the movement of liquid following acceleration of the printing head affects printing reproducibility, and we propose a normalization method to counteract this effect, which consists in adding a calibrant molecule to the spotting buffer when printing capture antibodies.

We first printed the calibrant alone, AF546-labeled goat anti-rabbit (GAR) IgG, while varying four printing parameters that affect the printing head movement: printing order, z-depth, contact time, and z-speed. We then added different concentrations of the calibrant to a printing buffer containing an unlabeled capture antibody, followed by blocking and incubation with an AF647 labeled analyte. Whole slides were printed with a single pin loading, and were scanned in two color channels simultaneously. The normalized signal was calculated by dividing the analyte by the calibrant signals. GAR-AF546 is easily bound to the slide surface and AF546 is stable in the presence of oxygen and light. Among the four printing parameters studied, we found contact time and block printing order to affect spotting variability. Increasing contact time and minimizing the printing head displacement during printing led to a decrease in variability from 60% to 35%. To further decrease variability due to the printing process, we added the calibrant to a capture antibody, and found that 5ug/mL of GAR-AF546 led to a sufficient calibrant signal without decreasing the analyte signal.

Normalizing signals led to a further decrease in variability down to less than 20%. Normalizing the analyte signal with the calibrant signal gives a measure of the density of bound antibody, which is less subject to variations from the printing process. Adjusting printing parameters and normalizing significantly improved the reproducibility of printing.

P-638.00

COMPARATIVE PROTEOMIC ANALYSIS OF AQUEOUS HUMOR FROM HUMAN EYES AFTER CATARACT SURGERY AND FROM POST-MORTEM EYES WITHOUT OPHTHALMIC DISEASES

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Aqueous humour (AH) is an intraocular fluid which is needed to supply the nutrients and to remove the metabolic waste from the avascular tissues of the eye. It contains the proteins secreted from anterior eye segment tissues which could play a role in the pathogenesis of various eye diseases. Traditionally the AH of patients with cataract is used as a control group in studies of ocular diseases, but it remains possible that cataract affect AH protein composition AH by increasing the abundance of certain proteins. Samples from normal eyes would be better controls, but samples of this type cannot be obtained, for ethical reasons. In this study the AH from middle-aged cadaver without any ocular and systemic pathologies was used as control. The goal of the study was to analyze the protein composition of normal and pathological AH and to find potential biomarker for cataract.

Samples were obtained by paracentesis from twelve human eyes at elective cataract surgery, and from six age-matched post-mortem eyes. Samples were resuspended in urea and submitted to reduction and alkylation. Proteins were precipitated and digested (trypsin) for further HPLC MSMS analysis (Agilent LTQ FT Ultra). Proteins were identified against human IPI protein sequence database using Mascot Server (Matrix Science, UK). Bioinformatics analysis including GO annotation, proteins quantification was carried out using Scaffold 4.0 (Proteome Software Inc., Portland, OR).

More than 300 proteins were identified in AH in normal and disease states. Some of these proteins were previously described while others were identified for the first time in AH. The protein composition was found to be different in AH of cataract patients and control group. Some of the proteins were found only in the cataract AH. These proteins could be a potential biomarker for cataract and may play a role in the mechanisms of cataract development.

P-639.00

THE NASAL FLUID PROTEOME IN HEALTHY CHILDREN

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Introduction and Objectives. Diseases affecting the upper airways in children are important cause of morbidity worldwide. To date however, establishing the nasal proteome in children remains to be shown. The aim of this study is to investigate the proteome in the nasal lavage fluid (NLF) derived from 6 – 9 year old children.

Methods. 14 samples nasal aspirates derived from healthy children were analyzed using ultraperformance liquid chromatography coupled with electrospray ionization quadrupole time-of-flight tandem mass spectrometry (The Xevo Q tof, Waters Corporation). Data was collected using MassLynxTM 4.1 and processed using ProteinLynxTM. Protein quantification was performed with ISOQuant 1.5

Results. We identified 1758 peptides which correspond to 904 proteins. Ontology analysis demonstrated enrichment of several biological processes in the nose reflecting its expected role in metabolic process, cellular process and developmental process. Protein molecular functions included binding, catalytic activity, structural molecular activity and translational regulator activity. Pathway enrichment analysis showed that proteins were associated mostly to cytoskeletal regulation by Rho GTPase, cytokine signaling- and Integrin signalling pathway.

Conclusions. This study determined the proteome in NLF in children and has provided a comprehensive insight into the nasal protein composition which may have therapeutic and preventive medical implications in disease.

P-640.00

QUICK AND CLEAN PEPTIDE MAPPING USING ULTRAFAST LC-MS SAMPLE DELIVERY SYSTEM

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With the growing number of protein based drugs, obtaining complete sequence coverage of a protein of interest is requisite for full characterization of its post translational modifications, especially in the case biopharmaceuticals. This challenge requires careful selection of the appropriate digestion enzyme or use of multiple enzymes to obtain 100% coverage1.

This task is made more difficult by the need to analyze in a high throughput fashion many different sample preparations and digestions with associated sample clean-up. This entire process can be streamlined and simplified making use of a StageTip based LC system which performs online sample clean-up followed a short gradient. In this study, we demonstrate that this system coupled to a benchtop mass spectrometer can provide fast and reproducible optimization of sample preparation and peptide mapping. Following reduction and alkylation monoclonal antibody was digested using MS-sequencing grade AspN, trypsin, chymotrypsin, LysN, and GluC.

Afterwards all samples were loaded onto C18 StageTips, online cleaned/desalted, and eluted with a short 9 minute gradient. LC/MS data were collected on a QExactive mass spectrometer using a MS1 resolution of 140k/70k followed by HCD fragmentation of the top 10 precursor ions and FTMS detection at 30k/15k resolution. Data were searched to evaluate peptide coverage and quality of sample preparation using Proteome Discoverer 2.0 with Byonic and Preview nodes.

Each digestion condition for monoclonal antibody was run in triplicate and the amount of carryover which was found to be extremely low. The digestion parameters most optimal for antibody digestion were determined to be a complementary digest of GluC and LysN with consistent and reproducibly high peptide coverage. In total, nearly 150 distinct samples/digestion conditions were able to be assayed in a single day.

P-641.00
PROCESS – PROTEOMICS DATA COLLECTION, SOFTWARE AND STANDARDS TO SUPPORT OPEN ACCESS AND LONG TERM MANAGEMENT OF DATA.
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Introduction:
The rise of high throughput instrumentation for 'omics has brought increasing sizes of datasets, which, coupled with requirements from research councils for data archival/sharing, has led to vastly increased costs. The Proteomics Standards Initiative, charged with developing the next generation of open data standards and supporting software, has previously developed mzML for raw data, mzIdentML for identification data, mzQuantML for quantitative data, and mzTab as a simple summary of "final" results.

Methods:
The development of PSI standards occurs in an open process coordinated through the PSI website (http://www.psidev.info/), with regular conference calls and an annual meeting each year to which any interested party can join.

Results:
We are working on the maintenance of existing PSI standards and supporting software, and developing new features required to cope with new experimental techniques and increased volumes of data. New features include improved support for protein grouping/inference, expression of ambiguity in pinpointing PTMs in mzIdentML, and support for DIA, IMMS and top-down in mzML.
We are planning to build a universal converter enabling bench scientists to easily convert their identification and quantification data to any PSI standard, with individual software projects already implementing support for vendor and open raw formats. A major thread of development in PROCESS is building data compression into mzML. It has been observed that mzML files can be an order of magnitude larger than vendor raw files. As instruments continue to produce larger volumes of data, effective compression techniques could have enormous value. Evaluation is underway to determine the accuracy of database searches and quantitative routines after 'near-lossless' compression to ensure that the fidelity of the data is maintained; near-lossless has been added to the jmzML library already.

Conclusion:
Reducing the sizes of data sets expressed in standard formats should benefit the wider community in the long term.

P-642.00

A HIGH-THROUGHPUT AND REPRODUCIBLE WORKFLOW TO PREPARE HUMAN PLASMA SAMPLES FOR PROTEOMIC ANALYSIS

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Introduction and objectives

Biomarker discovery projects typically lead to the identification of a multiplicity of candidates that must then be verified through quantitative assays of a statistically significant number of clinical samples. Selective Reaction Monitoring (SRM) and SWATH provide avenues to simultaneously quantify multiple analytes in a small sample volume without the need to generate and characterize antibody pairs. Sample preparation represents a major bottleneck for these peptide-based mass spectrometry techniques, as there are numerous processing steps susceptible to technical variation.

Methods

To increase throughput and reduce technical variation, we have implemented automated peptide preparation on a liquid handling workstation (Biomek NXP) coupled with an MRM workflow using a dual harmonized reverse-phase LC system (MPX™-2) linked to a triple quadruple mass spectrometer (QTRAP® 5500 or 6500 system). Human plasma samples arrayed in a 96-well format were denatured, reduced, alkylated, and digested with trypsin in a total time of less than two and half hours. For quality control, each sample was spiked with beta-galactosidase. Samples were desalted online using post-column diversion of the flow-through to waste prior to peptide elution.

Results and Discussion

The complete analysis had a coefficient of variation of less than 10% based on assessment of the internal beta-galactosidase standard. The coefficient of variation between the two LC columns was less than 5%. Online desalting as a substitute for solid-phase extraction provided the largest contribution to reduced technical variation.

Conclusions

We present an automated high-throughput workflow with sample processing robotics and multiplexed LC/MS/MS for accurate and reproducible large-scale analysis of biological/clinical samples.

P-643.00

AN AUTOMATED WORKFLOW SOLUTION FOR IDENTIFICATION AND QUANTIFICATION OF PROTEOMES

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Efforts during the past 15 years to characterize protein dynamics using proteomics have led to a better understanding of molecular drivers for complex biological processes and diseases. However, our ability to translate proteomic results to clinical success has been remarkably low. One big reason is the quality of the proteomics data, mostly due to the technical difficulties during the validation phase. This is a consequence of the steep learning curve of the technology, the lack of standardized methods, and the complexity of the experiments.

By developing a total workflow automation solution based on Q-Exactive technology, we aim to overcome these challenges, and facilitate access of the technology to non-expert users. Researchers are provided with standard workflows, kits, protocols, methods, and reports for all steps of a proteomic study and specific software that guides through the experiment. This solution offers several proteomic workflows ranging from shotgun analysis to differential expression analysis using the SILAC method.

In a first set of experiments, we analyzed the yeast proteome using the one-shot nanoLC-MS/MS analysis workflow. Within 4 hours, ~26,000 peptides and over 3,600 proteins were identified. Their cellular abundance ranged across 6 orders of magnitude, and many transcription factors were observed. In a second set, the fractionation workflow was tested. Hela cells, were harvested, digested, and fractionated following the established protocols. Over 7,000 protein groups were observed within one day of MS analysis time. Finally, we performed a quantitative proteomic experiment where A549 cells were labeled in light/medium/heavy SILAC medium. Harvested cells were mixed to certain ratios before lysis and digestion.

Overall, our results show that the complete workflow solution described here not only produces high quality data comparable to those published by key opinion leaders in proteomics, but guarantees the robustness and reproducibility, making the workflows accessible to proteomic users with no experience.

P-644.00

THE VALUE OF SUBCELLULAR FRACTIONATION IN PROTEOMIC STUDIES

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After having deciphered the human genome, the characterization of the whole proteome is the next main challenge. Proteins vary between samples, cellular compartments or mutational stages, making difficult its study. Furthermore, post-translational modifications, such as oxidations, acetylations or methylations, change the properties, not being able to be considered as the same protein.

Traditionally, 2-DE has been the most common proteomic strategy employed to deal with these drawbacks. Nevertheless, distinguishing proteins with high hydrophobicity or pI as well as low abundance proteins constitute a limit in the field. The solution is focused on using pre-fractionation techniques prior to the analysis and mass spectrometry-based approaches. Here, we perform six protein extraction procedures including sub-fractionation and total extraction protocols. The main goal was the reduction of the complexity and enrichment of the sample to improve the analysis of the whole proteome. A human Burkit's lymphoma cell line was selected to perform the assays.

Briefly, protein fractions from cytoplasmic, nucleus, membrane, and mitochondrial compartments were obtained and ran in SDS-PAGE gels. Each lane was cut into 15 parts and individually analysed by LC-MS/MS. Our studies provided information about which total extraction procedure reported the highest number of identified proteins, being more efficient in the extraction, and which sub-cellular fractionation separated them in a better way, reducing the cross-contamination.

We compared the given strategies and concluded that the four-fraction procedure extracts up to 6,700 proteins, a 2-fold increase in comparison with the rest of approaches. In addition, this protocol correctly separated the proteins in function of their expected sub-cellular compartment. Verification with UniProtKB/Swis-Prot database revealed up to 73% of identified proteins in the appropriate fraction. These results show the improvement in protein analysis through sub-fractionation steps combined with LC-MS/MS strategies that may lead to a protein characterization in a reliable and fast way.

P-645.00

COMBINED PROTEOMIC AND METABOLOMIC ANALYSIS APPROACHES TO INVESTIGATE THE MODIFICATION IN THE PROTEOME AND METABOLOME OF IN VITRO MODELS TREATED WITH GOLD NANOPARTICLES (AUNPS)

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Introduction and objectives:
Emerging approaches in the area of exposure to nanomaterials and assessment of human health effects combine the use of in vitro systems and advanced analytical techniques to study the perturbation of the proteome and/or the metabolome. We investigated the modification in the cytoplasmic compartment of the Balb/3T3 mouse fibroblast cell line after exposure to 5 and 15 nm gold nanoparticles for 72 h.

On one hand, the proteomic approach is quite standardised even if it requires particular precautions when dealing with in vitro systems. On the other hand, metabolomic analysis is challenging due to the chemical diversity of cellular metabolites that complicate data elaboration and interpretation. Our work aims at bringing metabolomics to the front by working on the data processing workflow.

Methods:
The proteomic approach combines protein separation by two-dimensional gel electrophoresis with identification of de-regulated protein by mass spectrometry. Proteome Discoverer with the Sequest workflow was used in combination with Uniprot databank.

The untargeted metabolomic approach is based on liquid chromatography coupled to mass spectrometry. We designed a complete infrastructure of the metabolomics data processing server called "Metabostudio". This server allows the data processing with the R/XCMS package that is increasingly used worldwide by metabolomics scientists.

Results and discussion:
By investigating the differentially expressed proteins triggered by AuNPs exposure, it was possible to identify several pathways. Differentially expressed proteins were found to cover a range of functions including stress response, cell metabolism, cell growth and cytoskeleton organization. In addition, a certain number of de-regulated metabolites were highlighted and tentatively annotated using HMDB.

Conclusions:
The "omics" fields hold huge promises in the interaction of nanoparticles with biological systems. This study aims to improve existing knowledge, necessary for a correct assessment of AuNPs potential adverse effects. The combination of proteomics and metabolomics data is possible however challenging.

P-646.00
VARIABILITY OF 15N METABOLIC LABELING-BASED PROTEOMIC WORKFLOWS IN MOUSE SPECIMENS.

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Introduction and objectives
In vivo 15N metabolic labeling is a valuable tool for quantitative proteomics comparisons and hypothesis-free identification of molecular biosignatures in mouse models. However, the repeatability of 15N metabolic labeling proteomics workflows has not been addressed to date in mice. In this study, technical and sample preparation variability was assessed in mouse plasma and brain.

Methods
Plasma and brain specimens from 15N-labeled and unlabeled 8 week old male mice were mixed at a 1:1 (w/w) ratio and technical variability was assessed by measuring the 15N/14N plasma and the 15N/14N brain samples three times each by nanoLC-ESI-MS/MS (technical replicates). Sample preparation variability was assessed by measuring three different brain and plasma samples derived from the same 15N/14N protein extract but processed independently for MS analysis (sample preparation replicates).

Results and Discussion
LC variability was evaluated by comparing peptide RTs across brain and plasma technical replicates revealing an average RT correlation coefficient of 0.96 for plasma and 0.98 for brain. In sample preparation replicates of brain and plasma the average RT correlation coefficient were 0.95 and 0.93, respectively. The technical variability of log2(15N/14N) ratios for replicate pairs in plasma and brain revealed correlation coefficients of 0.91 and 0.78, respectively. The average log2(15N/14N) ratio correlation coefficient in brain sample preparation was 0.76, markedly lower than the corresponding plasma sample preparation coefficient which reached 0.84.

Conclusions
Comparison of plasma and brain data revealed that specimen type (i.e. plasma or brain) largely affects the variability of the method in an LC-independent manner.

P-647.00

EVALUATION OF MASS SPECTROMETER PERFORMANCES USED IN LARGE-SCALE PROTEOMICS

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The renewal of instruments on proteomic platforms is an important issue. Considering that proteomics is a quickly developing field and that mass spectrometers sensitivity, resolution and speed are continually progressing, the choice of novel instruments is not always easy. In order to compare mass spectrometers, several tests can be realized.

Here we present the evaluation of a number of mass spectrometers in their ability to identify and quantify large numbers of proteins without prefractionation steps before nanoLC and to correctly detect known variant proteins in complex samples. To do so, either a well-known bacterial lysate or a yeast protein lysate spiked with 48 human proteins (UPS1) at different concentrations was used. The yeast proteome is uniquely attractive as a performance standard because it is the most extensively characterized complex biological proteome and the only one associated with several large scale studies estimating the abundance of proteins (Ghaemmaghami et al., 2003).

Samples were run on ThermoFisher Q-Exactive, Q-Exactive Plus and on AB Sciex TripleTOF mass spectrometers. To eliminate any variation inherent to the chromatographic fractionation step, all analyses were performed using rigorously the same nanoLC system and set-up. Data-dependent acquisition methods were chosen for our comparisons. Rigorous bioinformatics and statistical workflows were then applied.

This experimental design allowed us to assess the performances of the tested instruments in terms of sensitivity (reaching the best coverage of our sample) and accuracy for the detection of variant proteins, by measuring the number of true and false-positives (respectively UPS1 or yeast background proteins found as differential).

Results highlighted the specificity and complementarity of each instrument. Setting-up such experiments would also be very important to establish the performances of spectrometers in time. Those results could provide a basis for laboratories to benchmark their own performances, to improve current methods, and to evaluate new technologies.

P-648.00

SUCCESS VIA STANDARDISATION – BRINGING RELIABILITY TO CLINICAL PROTEOMIC BIOMARKER DISCOVERY

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Introduction:
Translation of putative disease biomarkers from proteomic discovery projects to clinical practice has historically proved an unreliable process. Many candidate molecules which initially appear to have great potential fail to demonstrate adequate levels of performance and clinical utility during subsequent validation studies. A likely cause of many failures is the unintentional introduction of technical noise into discovery data sets, the magnitude of which masks the underlying biological signal. To overcome this issue, Biosignatures Ltd. has developed and implemented a set of highly standardised processes for best-in-class study design, execution and quality-control, which enable collection of extremely homogenous proteomic data with a very low and well-characterised technical noise profile.

In this presentation we will discuss how this approach, applied to large numbers of samples and followed by data processing using the Tiger computational analysis system has enabled the reliable discovery of diagnostic proteomic biomarkers for prostate cancer which successfully validated in a prospective blinded clinical study.

Methods:
Study participants were recruited prospectively at specialist hospital urology clinics. EDTA-anticoagulated plasma samples were prepared from each participant and stored at -80°C.

Proteomic processing was performed using 2DE, with pre-separation minimal covalent fluorescent protein labelling. No pre-separation depletion was performed.

Data derived from scanned gel images was subjected to online quality control, based on SPC methodologies, before processing by the Tiger system to identify candidate biomarkers.

Results:
Having discovered a panel of candidate biomarkers, a large prospective blind validation study was performed, in which the ability of these biomarkers to accurately predict detection of prostate cancer by subsequent prostate biopsy was demonstrated. This result demonstrates the validity of this approach to clinical proteomics.

Conclusions:
Identifying and controlling technical variation has enabled the problems which have historically frustrated clinical proteomics to be overcome; this is a robust methodology which facilitates reliable proteomic biomarker discovery.

P-649.00
CONFIRMING THE REMARKABLE IMPROVEMENT IN LC-MS SENSITIVITY FOR PROTEOMICS BESTOWED BY DIMETHYLSULFOXIDE AS A LC SOLVENT ADDITIVE

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Introduction

Hahne et al (Nature Methods 2013 10:989-992) recently reported on the remarkable increase in sensitivity achieved by adding 3-5% dimethylsulfoxide (DMSO) into the LC-MS solvents. To determine if we should adopt the method we also investigated the use of DMSO additives, to evaluate the sensitivity, consistency and robustness in analysis, and the ease of technical implementation. We also sought to add to the body of evidence surrounding the method, since if such advances can be confidently and repeatedly reproduced by the proteomics community, they can be more rapidly adopted into standardised techniques, and better facilitate delivery particularly for translational and clinical applications.

Methods

Tryptic human lysate and phosphopeptides, and a 6 protein mixture dilution series were analysed by LC-MS/MS using an Agilent 1200nano LC coupled to an Thermo Orbitrap Velos mass spectrometer. Data was interrogated against Swissprot databases using ProteomeDiscoverer v1.4 and Mascot v2.3. Sensitivity differences were assessed by comparison of the total ion currents, the peak areas of assigned peptide-spectrum matches, and the number of unique proteins and peptides identified, and the Mascot ion scores returned.

Results

We easily observed at least a 2-fold increased intensity of peptide ions during LC-MS using 3% DMSO, with concomitant increases in unique peptide and protein identifications. As previously reported, background noise was significantly reduced. In all, we found analyses to be cleaner, more pure and robust for even low attomole level analytes.

We also found the 401.922718 lock mass ion to be inconsistent, varying from an intense and reliable lock mass to a weak and inconsistent signature. Attempts at using the weaker signal resulted in mass errors up to 15 ppm, when the lock mass could not be reliably found.

Conclusions

We recommend the additive for LC-MS proteomics and have adopted a 3% DMSO LC solvent additive in our standard workflows.

P-650.00

MZQ LIBRARY -- AN OPEN SOURCE JAVA LIBRARY FOR SUPPORTING QUANTITATIVE PROTEOMICS STANDARD

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The mzQuantML standard (version 1.0, http://www.psidev.info/mzquantml) developed by the Proteomics Standards Initiative (PSI) is becoming the de facto standard for capturing, archiving and exchanging quantitative proteomic data, derived from mass spectrometry (MS). Following its release, a Java API (Application Programming Interface) for mzQuantML, called jmzQuantML, has been developed, providing a bidirectional mapping from XML to Java objects, with methods for reading and writing valid files (http://code.google.com/p/jmzquantml/).

Using jmzQuantML, we have developed an open source Java library, mzq library (http://code.google.com/p/mzq-lib/), which includes a set of applications/modules to facilitate use of mzQuantML within analysis pipelines. The mzq library provides common routines for post-processing quantitative proteomics data via the command line or graphic user interface (GUI). There are currently three categories in the library: i) converter ii) viewer and iii) downstream processor.

In the convertor category, the mzq library provides various format converters from mzQuantML files to CSV, mzTab, HTML, or XLS format. The options of each convertor allow users to specify the section of interest from mzQuantML to be extracted. The downstream processor contains many useful tools which in the current version includes i) the identification mapper - which takes peptide identification evidence from mzIdentML files and maps the identification to quantified peptides/features in the mzQuantML file; ii) protein group inference which groups proteins according to the peptide assigned, and performs protein group-level abundance calculation from peptide-level values; iii) normalisation at both the peptide and protein level. The library is extensible and more routines can be added as required to support new features or techniques.

P-651.00

MASS SPECTROMETRY-BASED PROTEOMICS AS TOOL FOR THE QUALITY CONTROL OF INTRAVENOUS IMMUNOGLOBULIN.

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Topic **17**

Cardiovascular and haematological proteomics

OP079 - QUANTITATIVE MEMBRANE PROTEOME PROFILING TO DISCOVER THERAPEUTIC TARGETS FOR HTLV-I ASSOCIATED DISEASES

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Introduction
Human T-cell leukemia virus type 1 (HTLV-1) is a human retrovirus, which is an etiological agent of adult T-cell leukemia (ATL) and HTLV-I Associated Myelopathy / Tropical Spastic Paraparesis (HAM/TSP) due to its infection of CD4+ T-cells. HTLV-1 is prevalent in Japan, Caribbean islands, South America and Africa with 20-30 million infected individuals worldwide. Although both ATL and HAM/TSP are fatal rare diseases, there is no effective therapy. Here we performed quantitative membrane proteome profiling of HTLV-1 infected CD4+ T-cells to discover novel therapeutic targets for ATL and HAM/TSP.

Methods
CD4+ T-cells were isolated by flow cytometry from normal donors (n = 14), asymptomatic careers (n = 21), ATL (n = 13), and HAM/TSP patients (n = 21). To focus on membrane proteome, glycopeptides were enriched by a ConA-lectin affinity chromatography plate and analyzed by LC/MS/MS. Raw data were transferred to Expressionist Proteome Server to conduct label free quantitative analysis.

Results
Welch's t-test using 56,705 detected peptides identified 26 or 7 peptides as potential therapeutic targets for ATL or HAM/TSP, respectively ($p < 0.05$, fold change > 2). Protein identification of those were achieved by 2D-LC/MS/MS analysis, in which 946 N-glycoproteins (6,791 N-glycopeptides) were identified.
 Eventually 8 or 4 cell surface proteins were defined as therapeutic targets for ATL or HAM/TSP, respectively. Importantly, two of them were targets of already FDA-approved drugs.

Conclusions
Our glycoproteomic quantification approach provided an effective way to identify disease-specific cell surface antigens. Making antibody drugs and antagonists against these targets are in progress.

OP080 - A PROTEOMIC INSIGHT TO A NOVEL THERAPEUTIC TARGET FOR MYOCARDIAL INFARCTION

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Cathepsin A (Ctsa) is a multi-functional serine protease involved in the regulation of hemodynamic functions by processing hormone peptides. Ctsa has been recently identified as a novel target in the treatment of cardiac diseases. A new inhibitor against Ctsa was developed and is undergoing phase I clinical trials. In a mouse model of cardiac hypertrophy and a rat model of ischemia/reperfusion-induced atrial fibrillation, the inhibitor has been shown to have greater beneficial effect than the existing standard care for heart failure. However, the precise molecular mechanism underlying the curative effects of Ctsa inhibition has yet to be elucidated.

In the present study, we investigate the effect of Ctsa inhibition on both proteome and functional levels. We first profiled the alterations in the cardiac proteome upon myocardial infarction in a mouse model of chronic heart failure. We identified 271 proteins which are altered upon infarction, and interestingly, 83% being up-regulated (n=4). Our proteome profiling shows an overall increase in proteasome activity and mitochondrial dysfunctions. After treatment with Ctsa inhibitor, the severity of the disease, measured by number of differentially regulated proteins, is decreased by approximately one third. Proteomic analyses indicate that Ctsa inhibitor dampens the up-regulation of proteins involved in inflammatory pathways and in fibrosis, such as periostin.

In addition, we have successfully established a model of ischemia in vitro using the rat cardiomyocytes cell line H9C2. Here, we show the protective effect of the compound in different stress conditions, such as ischemia and oxidative burst. Quantitative proteomics and activity based assays revealed lower induction of apoptosis upon treatment with the inhibitor, in both stress conditions.

Taken together, these findings provide new insights into the cardio-protective effect of a novel and promising Ctsa inhibitor.

OP081 - PROTEOMIC ANALYSES OF ARCHIVAL FORMALIN-FIXED PARAFFIN-EMBEDDED HUMAN AORTAS STORED FOR 25 YEARS

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Introduction and objectives: Formaldehyde-fixed, paraffin-embedded (FFPE) aorta repositories are a valuable resource for studying atherosclerosis. It is challenging to extract proteins from FFPE aorta tissues stored for long time. In order to further investigate proteins implicated by genomic studies and network analysis as important in the early stages of atherosclerosis, we have developed procedures to extract proteins from archival FFPE aorta tissues, some of which were collected 27 years ago.

Methods: Paraffin was removed from FFPE aorta tissues using xylene and the tissues were rehydrated with gradient ethanol and water. The tissues were then ground in liquid nitrogen and homogenized. Proteins were extracted using various buffers and digested under various conditions. Subsequently, peptides were analyzed on an Orbitrap Elite mass spectrometer. An SRM assay was developed to quantify target proteins selected though a combination of spectral counting and genomics. Freshly-frozen aortas were also investigated to compare with FFPE tissues.

Results and Discussion: With 306 core proteins identified in every sample, an average of 430 proteins was identified from each trypsin digest of 2.5 μg protein extracted from an FFPE sample. Meanwhile, we have developed an SRM assay for 10 target proteins: Apo A-1, Apo A-2, Apo A-4, Apo B-100, Apo C, Apo E, Periostin, Lactadherin, Mat 2β, and Scube 3. Those proteins, which have different abundance in aortic tissue, can all be detected and quantified in FFPE samples. Preliminary results suggest an increase in the amounts of Apo A-1 and Apo B-100 in atherosclerotic lesions. More samples and target proteins are under investigation.

Conclusions: Archival FFPE aortic tissues stored for more than 25 years can be used to obtain stable protein extracts for proteomic analysis. This makes it possible to use a large archive of rare FFPE aortas for biomarker discovery and quantification.

P-652.00
RELATIOSHIP OF OSTEONECTIN LEVEL WITH INFLAMMATORY, OXIDATIVE AND LIPID BIOMARKERS IN BLOOD IN CORONARY ATHEROSCLEROSIS AND ITS COMPLICATION

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The aim of the study was to investigate the concentrations of stem cells proteins-markers CD34 and osteonectin and to evaluate its relationship with key atherosclerosis biomarkers (inflammatory, oxidative, lipid, metabolic) in coronary atherosclerosis and its complication – myocardial infarction (MI).

Concentrations of stem cells proteins-markers CD34 and osteonectin were studied with help of proteome technology «PureProteome Protein A and Protein G Magnetic Beads» by direct method of biomagnetic protein separation with magnetic microspheres in 40 men with angiographically proved stenotic atherosclerosis (SA) and calcinosis of coronary arteries and coronary heart disease (CHD), 20 men with MI and in 40 age control men without CHD. Key atherosclerosis biomarkers – inflammatory (high sensitive C-reactive protein, hsCRP, tumor necrotic factor alpha, TNF-alpha, interleukins, IL-1-beta, IL 6 and 8), oxidative (lipid peroxidation product in low density lipoproteins, LPO product in LDL, LDL resistance to oxidation), lipid (triglycerides, TG, high density lipoprotein cholesterol, HDL-CH, total CH, LDL-CH), metabolic (c-peptide, glucose) were estimated in blood of men.

Increased concentration of blood osteonectin in men with SA and calcinosis of coronary arteries (up to 2,7-fold, p
These results indicate that osteonectin as marker of stromal stem cells with osteogenous potential, probably, plays important role in atherogenesis and is one of the new biomarkers of SA and calcinosis of coronary arteries.

P-653.00

HALLMARKS OF INFLAMMATION, PROTEOLYSIS AND OXIDATIVE STRESS IN THE HDL PARTICLES FROM HUMAN ABDOMINAL AORTIC ANEURYSM PATIENTS

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Introduction and Objectives: High- density lipoprotein (HDL) cholesterol levels have been inversely associated with abdominal aortic aneurysm (AAA). Only recently, however, has protein composition rather than HDL concentration been suggested to be involved in the protective mechanisms of HDL in atherothrombosis. The objective of this work is to detect protein alterations in HDL particles from AAA patients.

Methods: HDLs were isolated from plasma of AAA patients (n=14) and controls (n=7) by ultracentrifugation. To assess quantitative changes in the HDL proteome, proteins were subjected to relative quantification in multiplexed mode based on iTRAQ labelling followed by LC-MS/MS analysis using a QExactive. Quantitative data were analysed by means of a home-made workflow built around the statistical model recently developed in our laboratory that allows integration and comparison of data from biological replicates, detailed analysis of variance at the protein level, and systems biology analysis of protein functionality.

Results and Discussion: We quantified up to 535 proteins in the HDL proteomes, and detected 15 proteins whose abundance consistently changed in patients with AAA relative to controls. Among others, serum paraoxonase/arylesterase 1, peroxiredoxin-6 and HLA class I histocompatibility antigen were found increased, whereas complement C3, alpha-2-macroglobulin and alpha-1-antitrypsin were decreased in HDL from AAA patients. Further analysis by western-blot validated the differences observed in the proteomic studies. Quantitative systems biology analysis revealed that the abundance of proteins involved in antigen processing/presentation process and acute-phase response was consistently increased, whereas those associated with regulation of proteolysis, inflammatory response and complement activation were decreased in HDL from AAA patients as compared to controls.

Conclusions: Proteins involved in redox balance, immuno-inflammation and proteolysis are altered in HDL from AAA patients. This study identifies novel markers potentially underlying the beneficial effects of HDL in AAA.

P-654.00

THE SEARCH FOR HEART SECRETORY PROTEINS AS HEART DISEASE BIOMARKERS

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Introduction :

Heart secretory protein (HSP) are expected as biomarker candidates for heart disease pathology, because their expression varies with the state of the heart. However, the search of HSP have not been progressed enough, so that it is expected the presence of unknown HSP. In this study, we performed a proteomic analysis of sera obtained from the inlet and outlet of the coronary vessel to identify novel HSP.

Material and Method :

Sera were obtained from atrial fibrillation patient☐fs femoral artery (FA) and coronary sinus (CS), as the inlet and outlet of the coronary vessel, respectively, at the time of catheter ablation treatment. Highly abundant proteins, which interfere serum proteomics, were depleted from each sera by the antibody column, and proteins from flow-through fraction were evaluated by SDS-PAGE. Proteome of CS and FA sera were analyzed by LC-MS/MS utilizing iTRAQ system.

Results and Discussion :

The SDS-PAGE analysis showed that highly abundant proteins were depleted as much as from each sera. A total of 668 proteins were identified and quantified by proteomic analysis, and 4 proteins were increased more than 2-fold in the sera of CS compared with FA. It was reported that these increased proteins were related to antibacterial activity, hydrolytic degradation and angiogenesis inhibition, whereas they have not been studied about the relation to the heart. For this reason, these 4 proteins have the potential to be novel HSP which may be heart disease biomarkers, and further validation of quantitative blood levels are on the way.

Conclusion :

In order to search novel HSP, we performed quantitative proteomic analysis focusing on the coronary vessel which HSP are concentrated in. Then, we identified 4 candidates of novel HSP.

P-655.00

HIGH-THROUGHPUT LC-MS MRM ANALYSIS OF CALCYCLIN IN PRE-ECLAMPSIA PATIENT SERA

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Pre-Eclampsia (PE) is a pregnancy-specific disease that complicates 2-8% of all pregnancies and is associated with perinatal and maternal morbidity and mortality. We previously showed increased calcyclin expression in placental tissue. A microfludic LC-MS based assay was developed to evaluate this observation by MRM in tryptic digested SCX fractionated serum.

Measurements were applied for the quantitative analysis of calcyclin using 2 isotopically labeled peptides. The linear correlation coefficients were ≥ 0.99 and LODs equal to 0.36 and 0.70 ng/ml in serum. The correlation between both peptides was 0.997 and endogenous calcyclin was quantified in all samples.

The peptide concentrations were significantly different between pregnant and non-pregnant sera but preterm pregnant control and PE samples could not be distinguished. Compared with nanoscale LC, a throughput increase of about 4 fold was achieved.

P-656.00

URINARY QUANTITATIVE PROTEOMIC REVEALS NEPHRIN 1 AS A MARKER OF SALT SENSITIVE HYPERTENSION IN HUMANS

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Introduction

One third of the population is affected by hypertension and half of patients is salt sensitive as increases blood pressure upon dietary salt intake. Up to now the mechanisms leading to salt sensitive hypertension remain poorly understood. Furthermore, there is not a well-accepted gold standard test for salt sensitivity. Aim of this work is the discovery of urinary markers of salt sensitivity that would be extremely beneficial both for the diagnosis and for the understanding of the pathophysiology of this disease.

Methods

We used a label free quantitative proteomic approach to analyze urine from hypertensive patients. We enrolled in the study a cohort of salt resistant and salt sensitive patients at early stage of hypertension, just diagnosed and never treated before. We also added a control of healthy normotensive donors. To confirm the proteomic data, the most representative proteins were validated by western blot experiments on a larger cohort of patients thus assessing biomarkers sensitivity and specificity.

Results and Discussion

We identified 812 protein groups, 247 of which resulted to be differently modulated among the three groups of subjects. We confirmed uromodulin up-regulated in all hypertensive patients vs. healthy donors with an AUC of 0.80, suggesting its key role in the pathogenesis of hypertension and nephrin 1 more abundant exclusively in the urine of salt sensitive patients with an AUC of 0.77. In these patients we also found an increased albumin to creatinine ratio that strongly correlates with nephrin 1 excretion.

Conclusion

This study identifies nephrin1 as a novel marker for salt sensitive hypertension, highlighting early sign of glomerular damage that might justify the appearance of micro-albuminuria often associated to the progression of this disease. Moreover, the availability of such marker in clinical practice might help in tailoring a more appropriate personalized therapy.

P-657.00

DIFFERENTIAL PROTEOMIC SIGNATURE IN CHRONIC CORONARY ARTERY DISEASE PATIENTS WITH AND WITHOUT A RECURRENT ACUTE EVENT

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Background: Identifying patients with coronary artery disease (CAD) who are at risk of developing acute ischemic events remains one of the major challenges of cardiovascular medicine. In this study we sought to investigate the plasma proteomic profile in a group of CAD-patients who developed an acute ischemic event (any acute coronary syndrome (ACS) or stroke) in comparison with those remaining stable at follow-up.

Methods: The study included chronic CAD-patients that had suffered an ACS 0.6 ± 0.04 years before sample collection and were followed-up for 2.3 ± 0.3 years afterwards. Plasma samples were obtained from two groups of patients: those who suffered an acute ischemic event at follow-up (E;N=6) and those who did not had acute ischemic events (NoE;N=6). Samples were prefractionated by the depletion of the 14 most abundant plasma proteins and the differential proteomic profile of both groups was analyzed by 2-DE and MALDI-TOF-TOF. Ingenuity-pathway-analysis (IPA) system was used to identify the significantly modified functional networks.

Results: Among the identified proteins, haemostasis-related proteins represented 22% of the observed changes. IPA analysis confirmed the modification of coagulation/fibrinolysis-related proteins (P

Conclusions: In CAD-patients the imbalance between tetranectin and vitronectin could lead to a decrease in the fibrinolytic activity that together with a coordinated change in the coagulation pathway could induce a pro-coagulant phenotype predisposing those patients to develop acute ischemic events.

P-658.00

ELUCIDATING THE RELATIONSHIP BETWEEN CALCIFIC AORTIC STENOSIS AND ATHEROSCLEROSIS: A COMPREHENSIVE PROTEOMIC STUDY IN AORTIC VALVE TISSUE

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Introduction

Calcific aortic stenosis (AS) is characterized by the thickening of the aortic valve leaflets followed by progressive calcification, which produces a blockage of the blood flow from the left ventricle into the aorta. AS lesion and atherome plaque share histopathological behaviors at initiation, as well as risk factors. Previous studies in our lab have compared stenotic with healthy valves from transplants or necropsies, valves which do not suffer the functional alteration related to calcification. In this context, the use of non calcified valves from aortic insufficiency (AI) seems valuable, since these patients develop ventricular hypertrophy and cardiac insufficiency in a similar way to AS.

Methods

In a first experiment, 2D-DIGE was used to analyze differences between AS and AI patients. Additionally, a second experiment was performed using 6 different groups of valves: transplant, AI and AS (with and without atherosclerosis in every case). After statistical analysis, spots of interest were identified using MALDI-TOF/TOF analysis.

Results and discussion

In the first study, 26 spots were found differentially expressed (1.5-fold and p-value ≤0.05). After PCA and hierarchical clustering, AI valves were found to separate into two different groups according to atherosclerosis incidence. Taking into account these results, a new study was design. As expected, after statistical analysis, the greater differences were found when comparing the different diseases of the valve (AS and AI) with control valves. Nevertheless, different patterns of expression exist between valves with and without atherosclerosis. Significantly altered proteins were analyzed with DAVID Bioinformatics Resources (NIH), in order to characterize the pathways in which these proteins are involved.

Conclusions

The present study enables us to deepen in the association of pathological mechanisms of AS and atherosclerosis, providing useful information for developing new prognostic, diagnostic and therapeutic tools in the near future.

P-659.00
PROTEOMIC CHARACTERIZATION OF HUMAN CORONARY THROMBUS IN PATIENTS WITH ST-SEGMENT ELEVATION ACUTE MYOCARDIAL INFARCTION: RIGHT TO THE EPICENTRE OF THE PATHOGENESIS

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Introduction and objectives:
Acute Myocardial Infarction with ST-segment elevation (STEMI) initiates with intraluminal thrombosis and results in total occlusion of the coronary artery. Although proteomic studies have been performed both in the atherosclerotic plaque and in the different cell types involved in the thrombotic process, coronary thrombus protein characterization studies in STEMI patients have not been performed to date. Methods In the present study we aimed to describe the proteome of the coronary thrombus of STEMI patients employing three proteomic approaches: 2-DE followed by mass spectrometry (MALDI MS/MS), 1-DE combined either with liquid chromatography coupled to mass spectrometry in a MALDI TOF/TOF (LC-MALDI-MS/MS), or in a LTQ-Orbitrap (LC-ESI-MS/MS). A subset of 16 proteins which have not been previously associated with coronary thrombosis were analyzed by western blot (WB) and selected reaction monitoring (SRM) in thrombi from STEMI patients, and the correlation of their abundance in the thrombus with ischemia time was analyzed by means of fibrin and cell specific markers quantification.

Results and discussion
With this approach, we were able to identify a total of 708 proteins in the thrombus. Energetic metabolism and cytoskeleton proteins (implicated in focal adhesion and intracellular signalling) are enriched in the coronary thrombus proteome. Sixteen proteins were detected in thrombus by an orthogonal technique (WB and SRM) therefore reinforcing the proteomic characterization performed and highlighting their implication in human coronary thrombus development. Proteome of coronary thrombus is affected by ischemia time.

Conclusions
The human coronary thrombus presents a complex and dynamic proteome, which may constitute a novel source of thrombotic biomarkers shed to the blood. Blood levels of these proteins in STEMI patients are at this moment being analyzed in order to evaluate their utility as prognostic biomarkers.

P-660.00

ITRAQ: A STRATEGY FOR BIOMARKER PROFILING IN AORTIC STENOSIS DISEASE

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Introduction and objectives:
Degenerative aortic stenosis is the most common worldwide cause of valve replacement. Defining the proteome of human aortic valves is an important and challenging task that may be fundamental to understand the mechanisms and physiological/pathological processes that lead to the development of AS. For this reason, here we have implemented our previously results in the lab, with iTRAQ quantitative proteomics, a non-gel approach that uses liquid chromatography (LC) separation, tandem mass spectrometry and database searching.

Methods
Aortic valves samples were collected from 20 patients that underwent aortic valve replacement (55 % males, mean age of 74years) and 20 normal control valves were obtained from necropsies (40% males, mean age of 69years). Protein content was solubilized, digested with trypsin, and the peptides were labeled with 8-plex iTRAQ reagents. The iTRAQ mixtures were analyzed in an LTQ-Orbitrap XL ETD. All identifications were performed by Proteome Discoverer 1.0 software (Thermo Fisher).

Results and discussion
We observed that 101 proteins were found altered in aortic stenotic valves (72 upregulated and 29 downregulated). Six proteins were detected in aortic stenotic valves by an orthogonal technique (WB and SRM) therefore reinforcing the proteomic results performed.
More than 50% of the differential proteins identified in our study were involved in cytoskeleton processes. This fact could be due to a loss of remodeling tissue happened on the stenosis aortic due to the left ventricle progressively increases in diameter, stiffens, and gradually loses its ability to generate enough contractile force to compensate for aortic stenosis.

Conclusions
These results provide a novel and characteristic profile associated with the development of aortic stenosis disease, which may be of great utility by themselves, or supplemented with previous results in the lab using other proteomic techniques.

P-661.00

A CHARACTERISTIC PROTEOMIC AND METABOLOMIC PROFILE IN BLOOD PLASMA WITH UTILITY IN PREDICTING EVOLUTION OF PATIENTS WITH ARTERIAL HYPERTENSION TO ALBUMINURIA

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Introduction and objectives

Albuminuria is defined as a urinary excretion of albumin above normal levels, as a consequence of renal damage in patients with arterial hypertension (AHT). The presence of albuminuria has been established as a potent predictor for future development of cardiovascular and renal events in the general population. Chronic RAS suppression is accompanied by sustained and de novo developed albuminuria in a certain subset of patients, therefore pointing to an inappropriate response to treatment. We investigated patients in both situations together with a control group with sustained normoalbuminuria. The aim of this study was indeed to compare proteins and metabolites from plasma from albuminuric patients with respect to patients with arterial hypertension without albuminuria, in order to identify novel biomarkers with potential prognostic value.

Methods

For protein analysis, plasma samples were depleted of the 14 most-abundant proteins and analyzed by 2D-DIGE, as well as by iTRAQ in an LTQ Orbitrap XL ETD. Metabolomic analysis was performed by GC-MS/MS using a gas chromatograph 6890N coupled to a single quadrupole mass detector 5975C (Agilent technologies).

Results and discussion

With this approach, we found 25 spots by 2D-DIGE and 50 proteins differentially expressed by iTRAQ. In addition, 24 metabolites with differential expression were identified in albuminuric patients.

The combination of a proteomic and metabolomic analysis of plasma from these patients provided an overview of the altered metabolic pathways and gave us a better understanding of the mechanisms associated to the development of albuminuria. These results allowed us to efficiently classify patients with chronic albuminuria, patients that have developed albuminuria in the last years and normoalbuminuric patients.

Conclusions

These results provide a novel and characteristic profile associated with the development of albuminuria, which may be of great utility for prognostic purposes in the follow-up of AHT treatment.

P-662.00
PROTEIN CONSTITUENCY OF LIPOPROTEINS DETERMINED BY RECONSTRUCTION OF SIZE FRACTION PROFILES COMBINED WITH SHOTGUN PROTEOMICS

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Introduction:
Globally, coronary artery disease (CAD) continues to be a major cause of morbidity and mortality. Lipoproteins are complex, heterogeneous macromolecules that are vital historical biomarkers revealing cardiovascular health. Accurately delineating the proteome of lipoproteins in various stages of cardiovascular health could provide a greater ability to predict adverse coronary outcomes and expand avenues of research in CAD. This study fractionates lipoproteins according to size, relatively quantitates the proteome constituency of each fraction, and correlates quantitative elution profiles of individual proteins with known lipoprotein biomarkers to accurately determine lipoprotein proteomes.

Methods:
Asymmetric flow-field-flow fractionation combined with dynamic light scattering was used to fractionate serum samples and determine the average size of particles in each fraction. Following separation, each fraction was digested with trypsin and subjected to LC/MS/MS analysis in a Velos orbitrap mass spectrometer and isotopic dilution mass spectrometry on an ABI 5500 Qtrap. Database search results from each fraction were recombined to generate relative quantitation elution profiles for each identified protein. Correlation analysis was performed to reveal highly correlated elution profiles.

Results:
Quantitative elution profiles for apolipoprotein A-I (APOA1) and apolipoprotein B-100 reveal a size pattern consistent with known size ranges of high density lipoprotein (HDL) and low density lipoprotein (LDL). Proteins correlating highly with APOA1, the major structural protein of HDL, include apolipoprotein A-II, apolipoprotein A-IV, and apolipoprotein C-I, among many. A significant number of proteins previously reported as constituents of HDL do not have elution profiles correlating with apoA-1, including hemoglobin, haptoglobin and Complement C3, rendering their previous association with HDL questionable. For example, elution profiles of haptoglobin and hemoglobin peak at approximately 19 nm, significantly larger than observed HDL size ranges, yet consistent with the size of the known haptoglobin-hemoglobin complex. The protein components of HDL and LDL are reconstructed and presented according to correlation analysis.

P-663.00
SIMPLIFICATION STRATEGIES FOR THE CARDIAC PROTEOME
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Introduction and Objectives

The zebrafish (Danio rerio) is a powerful model for the investigation of cardiovascular development, injury and disease. The protein complement of the zebrafish heart is very complex with a few highly abundant proteins typically dominating the expressed proteome. In this study we have compared simplification strategies in order to characterise the zebrafish cardiac proteome more extensively.

Methods

Hearts were collected from adult zebrafish and soluble protein extracts were prepared. Proteins were fractionated using either ProteoMiner combinatorial peptide ligand library beads or an OFFGEL fractionator prior to separation by 1-D SDS-PAGE. Protein bands were subjected to in-gel digestion with trypsin. In addition, tissue extracts were processed using filtered aided sample preparation (FASP). The peptides were analysed by LC-MS/MS. The number of unique peptides and proteins using each method were compared to that obtained by 1-D SDS-PAGE alone. Different search engines and sequence databases were also evaluated.

Results and Discussion

The 1-D SDS-PAGE analysis revealed a large dynamic range in the distributions and intensities of protein bands. Using the ProteoMiner beads it was possible to abstract a substantial proportion of the high abundance proteins, allowing visualisation of additional protein bands by 1-D SDS-PAGE. OFFGEL analysis resulted in sequential fractionation of different protein populations with a concomitant increase in protein identifications although data from the FASP was inconsistent. These simplification strategies enhanced the number of proteins identified from the zebrafish cardiac muscle. In addition, greater sequence coverage was attained for many of the proteins leading to increased confidence in assignment.

Conclusions

This study has compared different experimental approaches for proteome simplification in zebrafish cardiac muscle. These strategies are able to improve the identification of low abundance soluble proteins by reducing the complexity of the protein complement of the tissue.

P-664.00
EXPLORING THE DYNAMICS OF THE ZEBRAFISH HEART PROTEOME

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Introduction and Objectives

The zebrafish (Danio rerio) is a powerful model for the investigation of cardiovascular development, injury and disease. In order to study the protein dynamics of the zebrafish heart we have developed a proteomics-based strategy to determine the rates of synthesis and degradation of individual proteins.

Methods

Adult zebrafish were fed with an experimental diet in which 30% of the L-leucine in the diet was replaced with crystalline [2H7] L-leucine. Over a six week period zebrafish were sacrificed and cardiac muscle samples collected. Soluble extracts of muscle homogenates were separated by 1-D SDS-PAGE. Protein bands were excised from the gel, subjected to in-gel digestion with trypsin and the resultant peptides were analysed by LC-MS/MS. The relative isotope abundance (RIA) of the precursor pool was determined and used to calculate the rates of synthesis of individual skeletal muscle proteins.

Results

Over 550 proteins were identified with at least two peptides at one or more timepoints. The labelling incorporation was calculated using RIA and MIDA based methods. The data were fitted to a single order of exponential revealing the absolute rate of synthesis of cardiac proteins. The rates differed by several orders of magnitude under steady state conditions. The rate of synthesis of the identified proteins ranged from 1.39x10-5 d-1 (cytochrome b5 reductase 1) to 2.112d-1 (myosin, heavy chain 14).

Conclusions

This study has determined the turnover of proteins on a proteome-wide scale in the heart muscle of zebrafish. This work has addressed a number of analytical and technical challenges delivers an understanding of proteome dynamics to a highly useful and widely studied model organism.

P-665.00

SEPSIS ASSOCIATED GLYCOPROTEINS IN PLASMA

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Sepsis is a potentially fatal disease caused by an overwhelming immune response to infection. Over half a million Americans are diagnosed each year, and mortality rates from severe sepsis exceed 20%. Despite extensive research efforts, treatments that dramatically impact mortality are lacking. The objective of this study was to identify potentially novel treatment targets in plasma.

Plasma was collected from survivors (n=10) and non-survivors (n=10) with severe sepsis in the emergency department shortly after hospital presentation, and analyzed using a glycoproteomics approach. The groups were matched for age (66±4 years), gender (40% female), race (40% African American), and severity of illness (based on the severity of illness assessment [SOFA] score). Solid phase extraction of glycopeptide using hydrazide chemistry was performed on 40 μL of plasma, followed by mass spectrometry analysis. Fold change was calculated and t-test was performed to compare the protein changes.

577 unique glycopeptides were identified with a 5% false discovery rate. A total of 10 proteins showed significantly different expression (fold change >=2 or =

Several sepsis related glycoproteins were identified in this study, including several novel proteins, demonstrating the feasibility of this glycoproteomics approach. Further studies are required to validate these findings, and to determine the roles of these targets in sepsis induced organ failure and mortality.

P-666.00

VINNCARDIO: HIGH-THROUGHPUT SCREENING FOR PLASMA BIOMARKERS IN CARDIOVASCULAR DISEASE

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Introduction and objectives

Cardiovascular diseases (CVD) are one of the leading death causes worldwide. Despite efforts to clinical practice in diagnosing the disease there is a great need for novel early markers to support clinical decisions and to enable an accurately recognition of patients at risk.

Methods

Nowadays, high-throughput antibody-based proteomics tools are applied for biomarker discovery in body fluids. In order to identify disease associated-proteins and offer new insights into development and progression of CVD, 3.000 available plasma samples from patients that had been collected pre- and posteriori to the cardiovascular event. The screening analysis was performed using antibody suspension bead arrays with reagents from the Human Protein Atlas, where 384 non-fractioned, biotinylated and heat-treated plasma samples were profiled on 384 protein-specific antibodies per batch.

Results and Discussion

Up to 40 batches with more than 10.000 HPA antibodies, were used during the untargeted discovery phase in 384 samples, with this single-binder assay. Several hundred interesting protein profiles were identified in relation to cardiovascular diseases, and followed up in targeted analysis of 3,000 samples. Currently in-depth statistical analysis is being performed including clinical data, as well as focused assays sandwich assays for validation of the biomarker candidates are being developed.

Conclusions

VinnCARDIO is a very large biomarker discovery project regarding numbers of both antibodies and samples. Moreover applying various proteomic techniques to confirm the candidates to an ultimately use of sandwich immunoassays for downstream validation of candidates. Considering a clinical implementation, these large screening approaches might allow identifying profiles related to CVD and offer possibilities to transfer technological advances into personalize and individualized clinical care.

P-667.00

PROTEOMIC ANALYSIS OF LEFT VENTRICULAR TISSUES IN DILATED CARDIOMYOPATHY

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Dilated cardiomyopathy (DCM) is an intractable disease and no radical treatment other than cardiac transplantation has ever been developed. Because a limited number of donors are available for the cardiac transplantation in Japan, left ventricular assist device (LVAD) is frequently implanted into patients with severe heart failure including DCM. Patients can be weaned from LVAD in recovered cases, but no explicit criteria for weaning has been available to date. To that end, novel biomarkers are required for evaluating the severity of heart failure and biochemical and physiological statuses of the cardiomyocytes. To identify these novel biomarkers, we performed proteome analysis of left ventricular tissues from heart transplant recipients or autopsy cases with DCM.

Frozen tissues were pulverized, denatured, and digested with trypsin in the presence of sodium deoxycholic acid. After desalting, the digests were analyzed by nanoLC-MS/MS (Triple TOF5600) and differential protein expression was evaluated with a 2DICAL software. Among over 1,500 quantitated proteins, 167 proteins showed significant alterations in DCM, compared with control non-heart disease group. Proteins associated with cell adhesion, remodeling, endoplasmic reticulum stress were increased, while those regulating cellular calcium levels or energy metabolism were remarkably decreased. We also evaluated gene expression levels of the same samples by transcriptome analysis. From these omics analysis data, we have listed many biomarker candidates. From them, we narrowed down the candidates based on their distinct functions in the calcium and energy metabolism, ER and oxidative stress, remodeling and hypertrophy, inflammation, and so forth, in addition to the heart specific expression and release. Tentatively, 12 candidates were selected and are being validated by measuring their tissue and plasma levels.

By establishing highly sensitive and specific measurement systems for these proteins and mRNAs, we may be able to estimate the severity of DCM and the probability of weaning from LVAD.

P-668.00

IDENTIFICATION OF A URINE MOLECULAR FINGERPRINT WHICH RESPONDS TO ALBUMINURIA CONDITION AND PROGRESSION IN HYPERTENSIVE PATIENTS CHRONICALLY RAAS SUPPRESSED

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Introduction and Objectives. The ability of renin-angiotensin-aldosterone system (RAAS) suppression to reduce albuminuria in cardio-renal disease is clear. However, chronic suppression of RAAS does not impede the development of damage and increase of cardiovascular risk in all cases. Microalbuminuria is one of the predictors of this evolution. With high prevalence, patients under chronic treatment show albuminuria or even develop de novo microalbuminuria, but there are not available markers able to predict patients' evolution.

The aim of the study is to discover a molecular fingerprint of prognostic targets in urine associated to albuminuria progression in hypertensive patients chronically treated, allowing the evaluation of patients' response to the action of RAAS inhibitors.

Methods. We have investigated changes in urine proteome and metabolome by DIGE and NMR, respectively. Urine was collected from 117 hypertensive patients under chronic suppression of RAAS (2 years follow-up), and 24 healthy subjects. Patients were classified as: normoalbuminuric who had remained stable (normoalbuminuric), normoalbuminuric who had progressed to microalbuminuria (de novo microalbuminuric) and microalbuminuric who had remained stable (microalbuminuric).

Results and Discussion. 11 proteins and three different sets of metabolites were found to significantly respond to albuminuria condition with different trends: responders to hypertension itself, progression of albuminuria, or microalbuminuria condition. Candidate markers variations were confirmed by SRM-LC-MS.

Conclusions. We have defined a specific protein and metabolite fingerprint in urine which responds to albuminuria condition and progression in hypertensive patients under chronic RAAS blockage. This molecular signature helps predicting patients' evolution and earlier clinical intervention.

P-669.00

MOLECULAR ALTERATIONS IN HUMAN URINE REVEAL ATHEROSCLEROSIS DEVELOPMENT AND CARDIOVASCULAR EVENT AT ONSET AND FOLLOW-UP.

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Introduction And Objectives:

The discovery and use of novel markers of cardiovascular disease will allow identification of individuals at risk for a cardiovascular event and those who will be targeted for preventive measures. We aimed to the identification of a molecular panel of proteins and metabolites in urine which responds to atherosclerosis development and cardiovascular risk.

Methods:

An animal model of atherosclerosis was carried out in rabbits. Urine samples from animals were comparatively analyzed by DIGE, 1D-NMR and 2D-NMR. Confirmation of significant variations was performed by SRM-(LC-MS/MS). Altered molecules were further investigated in human urine samples collected from individuals with acute coronary syndrome (ACS), both at onset and discharge.

Results And Discussion:

Four proteins were found significant altered in response to atherosclerosis silent development, one protein increased and three decreased. Two of them additionally showed reduced levels in response to an ACS, with normalized levels at discharge. These data are in agreement with a reduction of a cardioprotective effect mostly endothelium mediated. Twenty metabolites were found significantly varied in response to atherosclerosis development (eight increased and twelve decreased). In humans, a panel of six metabolites responded to an acute event at onset with the same trend. These data reveal a direct translation of animal response to atherosclerosis development, to human response to an acute event. Three metabolites responded to recovery. Metabolism changes of sugars, hydroxy acids, amino acids, cyclic alcohols, polyamines and imidazolidines are shown linked to atherosclerosis. Arginine and proline metabolism, glutathione metabolism and synthesis and degradation of ketone bodies are implicated in atherosclerosis and in the remodelling of vascular damage.

Conclusions:

Novel urinary panels of proteins and metabolites are here shown to be able to monitor atherosclerosis disease: silent progression, acute event and recovery. A specific molecular signature can be directly associated to cardiovascular risk.

P-670.00

THE DIRECT EFFECT OF CORTISOL ON CA2+ REGULATORY PROTEINS IN THE HEART

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Objective Context: Although the effects of glucocorticoid (cortisol) have been broadly examined on immunological and metabolic functions in various organs, the direct physiological or pathophysiological effect of cortisol on cardiac function is still unknown.

Aims: The aim of this study was to determine whether the cortisol has direct effect on cardiac hemodynamics and to examine the underlying mechanism at the cellular as well as organ levels.

Methods: To assess the direct effect of cortisol was to measure hemodynamics by using cardiac sonogragph and ex vivo Langendorff-perfusion heart. To investigate the underlying mechanism was to measure Na+ pump current using patch-clamp technique and Ca2+ imaging using confocal microscope.

Results and Discussion: Cortisol (10 ¥ìM) perfusion produced significant changes in heart. First coronary flow, diastole duration and left ventricular developing pressure (LVDP) were significantly decreased. Second myocyte contraction was also attenuated, which is resulted from the alteration of PKC activity. Third when applied ouabain (a specific inhibitor of Na/K-ATPase), cortisol suppressed the ouabain-mediated increase in cytosolic Ca2+ concentration in cardiomyocytes and LVDP of ex vivo perfused hearts. In addition, ouabain-mediated decrease in pumping activity of Na/K-ATPase was attenuated in presence of cortisol.

Conclusion: Cortisol influences cardiac contractility through the activation of PKC and Na/K-ATPase, which is related to the suppression of Ca2+ handling in rat heart.

P-671.00

MULTIPLE PTMS PLAY A ROLE IN THE REGULATION OF PLATELETS IN HEALTH AND DISEASE

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Introduction

Platelets act as safeguards of vascular integrity, and contribute to inflammation, tumor metastasis, liver regeneration and the genesis and progression of cardiovascular diseases. Due to the absence of a nucleus platelet (dys-)regulation is mainly attributable to posttranslational modifications. To better understand the role of platelets in thrombosis, we systematically analyzed phosphoproteome, disulfide and proteolytic cleavage patterns of human platelets, obtained from fresh blood donations, upon activation and inhibition in a time-resolved manner.

Methods

Platelets were freshly isolated from human blood and monitored for activation state and purity. Purified platelet aliquots either served as control or were treated with activating (ADP, thrombin) and inhibiting (iloprost) compounds as well as a combination of both (ADP+Iloprost) for 10, 30, and 60 s. For phosphoproteome analyses, samples were digested, labeled with iTRAQ on the peptide level, subjected to phosphopeptide enrichment and analyzed by nano-LC-MS/MS. For disulfide and N-terminal analyses, samples were subjected to dedicated preparation steps and enriched using our novel two-dimensional ChaFRADIC strategy.

Results

We monitored time-resolved changes of >4,000 phosphopeptides with phosphoRS probabilities >0.9%. This analysis yielded unprecedented insights into the dynamics of platelet activation and inhibition, indicating an important and central role of previously unanticipated proteins and pathways in the regulation of human platelets. Additional differential analyses of the phosphoproteome and N-terminal processing of platelets obtained from patients suffering from bleeding disorders confirmed some of these candidate proteins. In summary, our multi-pronged approach to tackle PTMs and PTM crosstalk paves the way to understand the initial and crucial steps that contribute to activation under physiological and pathophysiological conditions and thus develop improved tools for diagnosis and treatment of platelet disorders.

Conclusion

Time-resolved and multipronged PTM analysis of human platelets reveals new insights into regulatory nodes that contribute to physiological/pathophysiological conditions.

P-672.00

ALTERED PHOSPHORYLATION PROFILE OF SCOTT SYNDROME PLATELETS REVEALED BY QUANTITATIVE PHOSPHOPROTEOMICS

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Introduction: The Scott syndrome is a rare bleeding disorder, associated with a mutation in anoctamin-6. Blood cells from Scott patients are defective in Ca2+-induced phospholipid scrambling and Ca2+-induced Cl- conductance. When stimulated with strong agonists, the patient's platelets fail to expose phosphatidylserine (PS) and form membrane blebs, pointing to differences in membrane structure. We hypothesized that the altered responses are due to changes in protein phosphorylation.

Methods: Washed blood platelets from healthy control donors and a Scott patient, were activated with the Ca2+-mobilizing agonists, convulxin/thrombin or ionomycin. We quantified >2000 phospho-sites and >3000 proteins between 8 different conditions.

Results/discussion: In platelets from control subjects, stimulation with convulxin/thrombin or ionomycin resulted in a major fraction of cells showing exposed PS, inactivated adhesive receptors and a blebbing structure. These platelets were unable to aggregate, in contrast to thrombin-stimulated platelets. Thrombin stimulation changed 26% of the phosphorylation sites while convulxin/thrombin or ionomycin stimulation induced more drastic changes in phosphorylation sites (56% and 57%, respectively).

At baseline and after thrombin stimulation, the phosphorylation profiles of Scott platelets and control platelets were highly similar, with 99.3% overlap. Stimulation of Scott platelets with convulxin/thrombin or ionomycin gave negligible PS exposure, limited blebbing and increased aggregation. Markedly, Ca2+-mobilizing agonists reduced the overlap in phosphorylation pattern to 81%. Strongly activated Scott platelets showed more frequently increased (16.5-16.7%) than decreased (2.7-2.9%) phosphorylation. The top 50 of mostly altered phosphorylated proteins of activated Scott platelets were assigned to the following function or structure classes: actin-myosin cytoskeleton (20%), platelet adhesion (22%), signaling/adapter proteins (24%), receptor-linked cytoskeleton (8%), and microtubule cytoskeleton (8%).

Conclusion: These data reveal major alterations in the phosphorylation pattern of PS-exposing stimulated platelets. In Scott syndrome platelets, the inability to PS exposure and membrane blebbing is accompanied by changes in phosphorylation pattern, linked to cytoskeleton integrity and platelet adhesion.

P-673.00

THE REGENERATIVE ABILITIES OF RESIDENT CARDIAC STEM CELLS DEPENDS ON SECRETED PARACRINE FACTORS

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Introduction: Human adult c-kit+ resident cardiac stem cells (CSCs) recovers post-myocardial infarction left ventricle dysfunction in animal models and a Phase I clinical study shows encouraging results. We hypothesized that neonatal-derived c-kit+ CSCs have stronger regenerative abilities than adult-derived c-kit+ CSCs.

Methods: We explored whether there exists a regenerative difference between adult and neonatal-derived c-kit+ CSCs and using a candidate proteomic approach, we determined the essential secreted paracrine factors that contribute to their regenerative abilities in a rodent myocardial infarction (MI) model.

Results—Human specimens were obtained during routine cardiac surgical procedures. We developed a reproducible isolation method that generated c-kit+ CSCs with >90% homogeneity using immune-activated magnetic bead selection. CSCs from both groups showed a similar expression profile for c-kit+, Ki67, cardiac TnT, GATA4+ but did not express tryptase, collagen, CD45, CD34 and CD31. Neonatal-derived c-kit+ CSCs were significantly more proliferative when compared to adult-derived c-kit+ CSCs (2±0.4 folds, P

Conclusions—Neonatal-derived c-kit+ CSCs have a stronger regenerative ability when compared to adult-derived c-kit+ CSCs that may depend on selected secreted paracrine factors. Since the condition media contained the functional activity of CSCs, a further discovery based proteomic screen is warranted.

P-674.00

CHARACTERIZING QUALITATIVE AND QUANTITATIVE GLOBAL CHANGES IN THE AGING HEART USING PSMART, A NOVEL ACQUISITION METHOD

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The cardiovascular system has been shown to undergo significant changes as it ages. These changes range from genomic to structural. We have developed workflows for quantitative global profiling and targeted analysis of the cardiac proteome in aging mice using a novel hybrid data acquisition (hybridDA) method. Heart tissue was isolated and homogenized from both young (2 months old) and old (2 years old) mice.

Solubilized and digested protein samples were spiked with the PRTC peptide retention time trainer kit and analyzed using standard data-dependent acquisition (DDA) experimental methods to build a murine cardiac tissue spectral library. A novel hybridDA method was used to acquire data for each sample for automated qualitative/quantitative data analysis using one HR/AM MS and a series of smaller DIA mass windows. Initial characterization experiments using unbiased DDA facilitated the building of detailed spectral library. The spectral library information was used to create reference information to perform qual/quan determination in real-time. Our hybridDA resulted in 30% more peptide identifications per run than a standard DDA run. The hybridDA enabled quantitation of previously identified peptides as well as novel targets of aging.

By identifying and quantifying more targets, we were able to better characterize the dynamic proteomic changes in cardio-dysfunction in aging mice.

P-675.00

COMBINING MULTIPLE PROTEOMIC APPROACHES IN THE STUDY OF ATHEROSCLEROSIS: A NOVEL MECHANISM OF PLATELET-MONOCYTE INTERACTIONS

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Introduction: Atherosclerosis is the most frequent cause of cardiovascular diseases, the leading cause of death worldwide. Platelet activation and platelet-monocyte interaction facilitate monocyte activation and extravasation, which contributes to the early stages of plaque development. To better understand this process, we have established a novel mixed cell model consisting of human platelets, a monocytic cell line (THP-1), and primary human umbilical vein endothelial cells (HUVEC).

Methods: We used this model to investigate changes in protein expression and secretion, small GTPase activity, and glyco- phospho-, and redox subproteomes for up to 24 hours after stimulation with lysophosphatidic acid, LPA (16:0), or thrombin, the strongest platelet activators in atherosclerotic plaques and during haemostasis, respectively. Differential analysis involved SILAC labeling, subproteome fractionation, and LC-MS/MS.

Results: LPA activated platelets of a subset of donors, but did not significantly increase platelet-monocyte aggregate formation. Conversely, thrombin caused homogeneous platelet activation and aggregate formation. Platelet signaling studies linked these differences to different small GTPase activation profiles. Exposure of THP-1 cells to thrombin- and LPA-induced platelet releasate (PR) resulted in increased monocyte adhesion and migration, and higher reactive oxygen species (ROS) levels. Glycoproteomics revealed the up-regulation of integrin ?5 and ?1, a major adhesion complex. Redox proteomics identified proteins with altered cysteine redox state, including GAPDH. Phosphoproteomics indicated the involvement of RhoA and actin cytoskeleton signaling pathways. Increased monocyte adhesion was blocked by inhibiting ROS production and Cys oxidation in monocytes, but not by an antibody preventing platelet-monocyte binding via P-Selectin/PSGL-1.

Conclusion: We show that monocyte activation does not require platelet-monocyte binding via P-Selectin/PSGL-1, widely believed to initiate monocyte activation, and identify a novel pathway involving release of soluble factors upon platelet activation. This highlights the potential of our novel cell model for the study of cardiovascular disease mechanisms and the identification of novel prospective drug targets.

P-676.00

THE ROLE OF BLOOD INTENSITY IN EXPRESSION AND GLYCOSYLATION OF LDLR IN ENDOTHELIAL CELLS

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Changes in SS intensities are highly associated to the development and progression of atherosclerotic plaques. There are some regions on vascular trees where the vessels show bifurcations. In those places, the blood flow and SS intensities are decreased, increasing endothelium permeability, changing cytoskeleton organization and upregulating the expression of adhesive molecules. This work aimed identify and quantify proteins from endothelial cells (HUVEC) with differential expression between laminar and low SS, and connect these proteins to atherosclerosis development.

HUVECs were submitted to low SS with 5 dyn/cm2 (proatherogenic flow) and laminar SS with 15 dyn/cm2 (atheprotective flow) by 24 hours in a cone plate system. Proteins were extracted, digested and analyzed by nanoUPLC - Q Exactive. Raw file was processed with MaxQuant. The proteins changed were validated by western blot. In total, 2728 proteins were found in proteome analysis. We identified proteins already reported in literature using transcriptome techniques, as VCAM-1, beta-arrestin and E-cadherin. Interesting, was identified the LDLR as downregulated in low SS. Through western blot were observed that not only levels of expression, but also the glycosylation, changed with different intensity of SS. In low SS, there are unglycosylated LDLR whereas in laminar SS, all LDLR are glycosylated. Glycosylation of receptors is important for the good work of this, as well signaling downstream.

Aiming to analyze the activity of receptor, incorporation of label LDL followed by flow citometry were performed. Differences in incorporation of labeled LDL after submitted to different intensity of SS were observed. New experiments aiming to observe downstream signaling and LDLR- glycan analysis are being performed. All together our data suggest that intensity of SS have an important role atherosclerosis development and proteins could be useful as biomarker of vulnerability plaque.

P-677.00

SYSTEMATIC CHARACTERIZATION OF HUMAN PLATELETS IN CARDIOVASCULAR DISORDERS BY QUANTITATIVE PROTEOMICS

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Introduction and objectives

Dysfunctional platelets are associated with various disorders. Patients who suffer from cardiovascular diseases are commonly treated with antiplatelet drugs against abnormal blood clotting; nevertheless patients have been characterized in clinical studies who do not respond to antiplatelet therapy, resulting in an elevated risk for cardiovascular events as a consequence of proceeding arteriosclerosis.

Methods

In this study, we combine genome-wide association studies (GWAS) and proteomics to discover potential biomarkers for arteriosclerosis. For proteomic analyses global as well as targeted approaches are applied. The developed strategies moreover allow for a quantitative assay of low abundant key mediators in platelet activation, such as plasma membrane proteins. Membrane proteins are vital for important platelet functions and drug targets, but are underrepresented in standard proteomic approaches. Our designed targeted approach directed to membrane proteins exploits with its inherent sensitivity and selectivity to gain additional insight into the role of this important protein class in dysfunctional platelets.

For a global approach, we perform 8-plex iTRAQ experiments of patients and controls (relatives and spouses). As platelet proteins span a dynamic range of four orders of magnitude, analysis of low abundant proteins is challenging. To overcome this limitation, we have established targeted-MS/MS methods for two classes of proteins: (a) low-abundant proteins correlated with elevated risk for cardiovascular disease according to previous GWAS and (b) membrane receptor proteins.

Results and discussion

Here, we show an iTRAQ measurement and statistical data evaluation strategy for the reproducible and intercomparable analysis of 300 global measurements as well as a targeted MS/MS method with heavy-labeled peptides, allowing for absolute quantification of 39 low-abundant (

Conclusions

Biomarker discovery is challenging in clinical research. The powerful synergy of GWAS and proteomic strategies applied to patient and control cohort is providing the optimal environment for biomarker discovery.

P-678.00

ENDOMYOCARDIAL PROTEIN PROFILING OF DCM PATIENTS WITH DIFFERENT RESPONSE TO IMMUNOADSORPTION THERAPY

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Introduction and objective:
Removal of cardio-depressant antibodies using immunoadsorption therapy (IA) with immunoglobulin (IgG) substitution represents a novel therapeutic approach in the treatment of dilated cardiomyopathy (DCM). However, response to this therapeutic intervention shows wide inter-individual variability and a protein-based molecular classifier for the prediction of therapy outcome remains elusive. Thus, in this pilot study, we performed proteomic profiling of endomyocardial biopsies to screen for differences in the protein profile before therapy which might enable the identification and selective treatment of a subgroup of DCM patients.

Methods:
IA/IgG was conducted on five consecutive days using protein-A column on DCM patients, which were grouped as responder (R) (relative ΔLVEF ≥20% & absolute ΔLVEF ≥5%) and non-responder (NR), according to the improvement of myocardial function. Myocardial biopsy tissues of 16 patients were subjected to label free quantitative proteomic profiling. The spectra generated by mass spectrometry were analysed using Elucidator and Gene Data Analyst software whereas Ingenuity Pathway Analysis (IPA) was used for functional categorization of differentially abundant proteins.

Results and discussion:
Clinical characteristics of DCM patients like LVFE, LVIDd, inflammation and NYHA did not differ significantly at baseline which indicates that these parameters cannot be used as a predictor of IA/IgG outcome. Proteomic profiling covered 1452 proteins, 131 of which displayed significantly different abundance (FC|1.3|p≤0.05) in the heart of NR and R before therapy. Those proteins were assigned to glycolysis, gluconeogenesis, ketolysis, beta oxidation of fatty acids and mitochondrial dysfunction and predict an activation of peroxisome proliferator-activated receptor gamma (PPARG) in NR.

Conclusions:
DCM patients with different therapy outcome (NR, R) seem to differ at baseline in carbohydrate and lipid metabolism. Protein abundances indicate a difference in PPARG dependent pathways, and entangled proteins will be validated using western blot and MRM. The receptor and its ligands will be focus of further investigations.

P-679.00

PROFILING OF PLATELET PROTEIN IN CIRCULATION OF DIABETIC PATIENTS: IN VITRO EFFECT OF ISOLATED PLATELET-SPECIFIC PROTEIN MIXTURE ON ENDOTHELIAL CELLS

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Introduction and Objectives: Platelet proteome is vast and reported to vary in pathological conditions. In diabetic subjects, platelets circulate in activated condition; therefore, they may synthesize/secrete proteins which are otherwise absent in healthy condition. We hypothesize that once released into circulation these proteins may interact with vessel wall and may cause endothelial cells (ECs) dysfunction. Objective of this study was to compare profile of circulating platelet proteins from diabetic subjects (test) with that of healthy individuals (control). Further, mixture of proteins isolated from both test and control plasma was treated with EC in culture to identify their effect on cells.

Methods: The study was conducted with IEC approval. Test subjects were selected based on IDA definition and equal numbers of non diabetic controls were included in the study. Ten ml blood was collected from each subject. Major plasma proteins were eliminated by ethanol precipitation. Platelet proteins in plasma were then compared with whole platelet lysate. Proteomic analysis was done by 2D gel electrophoresis, Western Blot and MS-MS analysis. Isolated proteins were added into medium in which human umbilical vein endothelial cells (HUVEC) was cultured and expression of specific markers such as eNOS, tPA and vWF were estimated by real time polymerase chain reaction (RT-PCR)in both the group.

Results and Discussion: Spot identification after 2-D electrophoresis showed significant difference between platelet proteome of test and control. More than 400 proteins were identified in each group with high confidence score by MS-MS analysis. Around 130 unmatched proteins were detected in diabetic subjects as compared to healthy controls. Differential expressions of mRNA for vWF, tPA and eNOS were observed between cells treated with test and control proteins.

Conclusions: The differently expressed platelet proteins may cause endothelial dysfunction which in turn could be the reason of high sensitivity towards cardiovascular diseases in diabetic subjects.

P-680.00

A 2D-DIGE-BASED PROTEOMIC ANALYSIS REVEALS DIFFERENCES IN THE PLATELET RELEASATE DEPENDING ON THE PLATELET STIMULUS

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Introduction and objective: Platelets are small anucleate cells that play a fundamental role in haemostasis. Upon stimulation, platelets release a high number of proteins and other biomolecules, which is known as the releasate. There are clear indications that proteins secreted by platelets are involved in the pathogenesis of several diseases, such as atherosclerosis. A recent report showed that platelet secretion is kinetically heterogeneous in an agonist-responsive manner [Jonnalagadda et al., Blood 2012; 120:5209-5216]. We tried to confirm this since a proteomics point of view. To do so, we compared the platelet releasate following platelet activation with two major endogenous agonists: thrombin and collagen.

Methods: Platelets were activated in an aggregometer at 37oC with 0.75 U/mL of thrombin, or 30 µg/mL of collagen (minimum doses to achieve maximum aggregation). The releasate was concentrated and proteins precipitated. Differential proteomic analysis was based on 2D-DIGE. Image analysis was with SameSpots (nonlinear dynamics) and proteins were identified by LC-MS/MS. Validations were by western blotting.

Results and discussion: We detected 122 protein spots differentially regulated between both conditions (64 up-regulated in thrombin and 58 in collagen) with a fold change ≥ 2 and a $p < 0.05$. From those, we successfully identified 80. Besides fibrinogen, down-regulated in the releasate of thrombin-activated platelets, we identified another 42 differentially regulated protein features corresponding to 37 open-reading frames. We focused on vitamin K-dependent protein S (up-regulated in thrombin samples), and multimerin-1 (up-regulated in collagen samples) for validation studies. The former is an anticoagulant plasma protein, and multimerin-1 plays a role in thrombus formation.

Conclusions: In conclusion, we confirm the platelet releasate varies depending on the platelet stimulus. Overall, our results could have pathological implications given that some platelet-related diseases involve a primary role of some particular receptors.

P-681.00

ESTABLISHMENT OF PROTEIN PROFILE-BASED STAGE CLASSIFICATION FOR AORTIC ANEURYSM

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Atherosclerotic aortic aneurysm is one of serious cardiovascular diseases associated with sudden death. The purpose of this study is to establish staging criteria for aortic aneurysm in order to identify novel biomarkers for its presymptomatic phase.

Aortic media of vascular smooth muscles were dissected from thoracic aortic aneurysmal (TAA) tissue. Proteome analysis was performed by pulverization, denaturation, and tryptic digestion of tissues, followed by nano-LC/MS/MS and quantitative differential analysis with a 2DICAL software.

Cluster analysis of proteome analysis data revealed that aneurysmal tissues should be classified not by the morphological features but by the protein profiles into 3 stages (preclinical (P), intermediate (I) and advanced (A) stages). Histological disorder and disruption were observed strongly in stage A, and weakly in stage P, suggesting that the protein profile-based staging well matched pathological diagnosis of TAA. We added the non-disease control (stage (C)) the above-mentioned staging, and thus established the 4 stage classification (C-P-I-A) by using 3 sets of 5 benchmark protein-derived 10 tryptic peptides, whose expression levels were markedly changed between the stages. Thrombospondin1 and SOD1 were included in these benchmark proteins. Utilizing this staging method for classification of aneurysmal tissues, a 3-fold greater number of proteins showed significant changes compared with that of the morphologically-based tissue collection. Several proteins reported for the progression of TAA, such as periostin and MMP12, were dramatically increased in stage A, while more significant alterations of proteins involved in the inflammation, transcriptional and cell cycle regulation were observed between stages C and P. These proteins were detected only by the protein profile-based stage classification, verifying the utility of this staging method.

The protein profile-based staging of TAA established in this study provides a rational stage classification method of aneurysmal tissues, and will enable us to identify biomarkers for early diagnosis of aneurysm and atherosclerosis.

P-682.00

INTEGRATED PROTEOMIC AND METABOLOMIC ANALYSIS REVEALS THE ROLE OF DYSSYNCHRONY IN METABOLIC REMODELING IN TACHYPACING-INDUCED HEART FAILURE

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Introduction: Heart disease, the leading cause of death worldwide, is accompanied by complex alterations in myocardial energy metabolism.. Approximately 40% of heart failure patients have dyssynchronous electric activation and contraction, which reduces pump efficiency and is an independent indicator of mortality. The role of dyssynchrony in metabolic remodeling remains unknown.

Methods: Here we integrated LC-MS/MS-based label free quantitative proteomics, GS/MS-based untargeted metabolomics, and targeted analysis of acylcarnitines and high-energy phosphates in a canine model of tachypacing-induced heart failure. Proteins and metabolites were independently examined in cardiac tissues from animals in dyssynchronous (DHF) or synchronous (SHF) heart failure and compared to normal controls. Proteomics and metabolomics data were then merged to provide a comprehensive analysis of differentially regulated pathways and components.

Results and Discussion: Noteworthy alterations identified include a decrease in carnitine-parmitoyltransferase 1, the gatekeeper of β-oxidation flux, and two fatty acids (12:0 and 14:0) in DHF, but not SHF. TCA cycle enzymes were upregulated in both SHF and DHF, concomitant with a decrease in the TCA cycle intermediates. Two key glycolytic enzymes (phosphofructokinase1 and GAPDH) were upregulated in DHF, while some intermediates of glycolysis were decreased in both models. Nevertheless, phosphocreatine and ATP were significantly decreased in DHF, but not in SHF. Overall, a complex picture of metabolic remodeling emerges from these data, contrasting upregulation in many catabolic enzymes with a decrease in many metabolites mediating catabolic fluxes. Moreover, these results suggest that the energy deficit observed in DHF, but not SHF, may be related to a critical decrease in FA delivery to the β-oxidation pipeline, making it impossible to meet energy demands despite upregulation of the TCA cycle and glycolysis.

Conclusions: This study highlights the significant role of dyssynchrony in heart failure remodeling and underscores the importance of combined –omics approaches for achieving a systems view of complex diseases.

P-683.00

MRM ASSAY DEVELOPMENT WITH APPLICATION TO BIOBANK SAMPLES FROM PATIENTS WITH ACUTE MYOCARDIAL INFARCTION

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Introduction and objectives

Cardiovascular disease is the leading cause of death in the high-income parts of the world, even though preventive measures and acute treatments have improved substantially in later years. Despite better therapies of acute myocardial infarction (AMI) in certain patient subgroups the outcome remains especially poor. We focus our efforts to these groups where a large number of target proteins with putative links to cardiovascular and coexisting diseases are screened by using a quantitative MS-based assay. The Centralized Region Skåne Biobank provides the samples from the SWEDEHEART Patient cohort.

The aim of the study is to develop workflow that involves sample treatment, MRM analysis and biostatistical analysis, incorporating patient registry data.

Methods

Pooled and individual human plasma samples were handled and digested according to our previously optimized protocol. Plasma digests were spiked with heavy isotope-labeled peptide standards and analyzed by nanoLC-MRM-MS. The MRM assay was performed on a TSQ Vantage mass spectrometer equipped with an Easy n-LC II pump (Thermo Scientific). The assay optimization and data evaluation were done with the aid of Skyline v2.1 software (MacCoss Lab), further statistical analysis was done using Microsoft Excel, R and Matlab v7.11.

Results and discussion

MRM assay was developed for the quantification of 90 proteins by monitoring at least one unique tryptic peptide per protein. NanoLC-MRM-MS analysis was performed together with stable isotope dilution strategy for highly reproducible quantification of the target proteins. Evaluation of the digestion protocol, optimal standard amounts, measurement reproducibility and LOQ were done.

Conclusions

As part of a Swedish national cardiological research initiative, the development of a quantitative MRM assay is reported for the quantification of ninety putative cardiovascular disease markers. The assay has been utilized for screening high quality human plasma samples from the Centralized Region Skåne Biobank.

P-684.00

THE COMPARATIVE SECRETOME OF HUMAN HEPATIC HUH7 CELLS IN THE PRESENCE OF B-ESTRADIOL AND ITS AFFECT ON THE SECRETED PCSK9 INTERACTOME

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Introduction

A primary risk factor for cardiovascular disease (CVD), the leading cause of death worldwide, is hypercholesterolemia, a disorder characterized by high circulating levels of low-density lipoprotein cholesterol (LDLC). Additionally, there are gender-associated risks as premenopausal women have lower risk of CVD compared to age-matched men. The hormone β-estradiol (βE2) has been implicated in this protection, in part, since it enhances plasma clearance of LDL particles through upregulation of liver LDL receptors (LDLR).

Methods

Herein we employed spiked-in stable isotope labeling with amino acids in cell culture (SILAC) to compile the first, quantitative analyses of the differential secretome of βE2-stimulated HuH7 cells to identify other affected and potentially, cardioprotective proteins and pathways. In addition, we identified secreted proteins that differentially interact with proprotein convertase subtilisin kexin type 9 (PCSK9), a negative regulator of LDLR, to identify potential modifiers of this secreted cholesterol-regulating protein.

Results

We identified 1613 proteins from the HuH7 secretome with 254 that showed significant changes in expression following βE2-stimulation. These included alpha-enolase, ezrin and stathmin identified as βE2-sensitive in breast cancer cell lines, as well as lipoproteins ApoA2 and ApoC2 whose expression affects cholesterol levels and risk of atherosclerosis. Ingenuity Pathway Analysis revealed that the expression of a large number of differentially secreted proteins was governed by HNF1A and 4A transcription factors, including the aforementioned lipoproteins. Fifteen proteins were found to interact with secreted PCSK9. Upon βE2-stimulation 2 interactors were lost, 9 decreased and 2 increased.

Conclusions

In general, this study revealed the biological pathways affected by βE2 in a liver cell line, including those that may contribute its cardioprotective effects. More specifically, it revealed potential modifiers of the cholesterol-regulator PCSK9. Collectively, this information can be used to identify novel pathways for cholesterol-lowering, and in particular antagonists of PCSK9 – a target for lipid therapy.

P-685.00

QUANTITATIVE PROTEOME ANALYSIS OF SECTIONS OF FORMALIN FIXED-PARAFFIN EMBEDDED ARTERIAL SAMPLES FROM PATIENTS WITH TYPE 2 DIABETES: METFORMIN TREATMENT NORMALIZES ARTERIAL MATRIX COMPOSITION IN TYPE 2 DIABETES

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Introduction

Metformin is an anti-diabetic drug with beneficial cardiovascular effects, which in addition to glucose-lowering effects also mat influence cellular functions. The molecular pathology of the arteriopathy of type 2 diabetes mellitus is not well-understood, but includes alterations in the extracellular matrix. We hypothesized that metformin treatment influence the molecular composition of the arterial wall and aimed at analyzing arterial tissue by quantitative proteomics from patients with type 2 diabetes (T2DM), treated with or without metformin, and tissue from non-diabetic individuals as controls.

Methods

We analyzed non-atherosclerotic tissue from the internal mammary artery gathered at coronary by-pass operations from 30 patients with type 2 diabetes (15 treated with metformin, 15 without), as well as from 30 age- and gender-matched non-diabetic individuals. A novel extraction procedure was used for the extraction of hardly accessible extracellular matrix proteins from arterial sections (4 µm thickness, 2 mm outer diameter) of formalin fixed, paraffin embedded samples. The 60 samples were reduced and alkylated using iodoacetamide and labelled with iTRAQ 8-plex reagent. The labelled samples were mixed in equal amounts into a total of 10 8-plex samples that were fractionated by HILIC followed by nano-LC-MSMS analyses.

Results

We identified and quantitated 128 proteins that were present across all 60 samples, many of which were extracellular matrix molecules and cytoskeletal, filamentous and other smooth muscle cell components. We found that several extracellular matrix proteins were significantly changed in arterial samples from T2DM patients confirming that the molecular pathology of T2DM include alterations in the extracellular matrix. Moreover, the expression of type IV collagen, laminin components and a few other proteins were significantly lower in diabetic metformin users, compared to patients, not treated with metformin.

Conclusion

Metformin treatment leads to a normalization of the arterial matrix composition in T2DM

P-686.00

CARDIAC PROTEOMIC PROFILING IDENTIFIES NOVEL PLASMA MEMBRANE PROTEINS THAT REGULATE CARDIAC FUNCTION

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Introduction: Heart disease is a major source of global morbidity and death in the developed world and the development of novel therapeutic strategies is based on our understanding of the molecular basis of cardiac function. Analyzing cardiac membrane proteins is crucial to a better understanding of heart function since the majority of cardiac disorders can be attributed to genetic mutations that alter the expression, function or subcellular localisation of cardiac ion channels and associated membrane proteins. In this study we aimed to identify novel cardiac membrane proteins involved in critical cardiac functions.

Methods: We employed cationic silica-bead coating coupled with shotgun proteomics to enrich for and identify cell-surface associated proteins from mouse and human in vivo and in vitro ventricular cardiomyocytes as well as in vitro endothelial and smooth muscle cells.

Results: We identified >3,000 mouse and >2,500 human proteins by LC-MS MuDPIT on a Thermo LTQ Orbitrap. These proteins clustered uniquely to either a membrane enriched (P) or membrane depleted homogenate fraction (H). Orthologue mapping of proteins between mouse and human and QSpec statistical analysis calculated differential spectral counts between proteins in the P and H fraction. This provided a dataset of 555 cardiomyocytes proteins including known membrane proteins. Bioinformatic integration with transmembrane helix predictions, Phenotype Ontology (PO), and publically available microarray data, identified a rank order of previously understudied cardiac-enriched surface proteins. Top candidates were confirmed using confocal microscopy, immunogold electron microscopy, and sucrose density gradient biochemistry. In vitro (lentiviral-based shRNA) and in vivo (morpholino-based Danio rerio) knock-downs of select candidates demonstrated significantly altered Ca2+ dynamics and proved essential for normal cardiac function.

Conclusion: Using large scale proteomics we have provided the first comprehensive analysis of cardiac cell-surface associated proteins and provide a pipeline to validate surface proteins in cardiomyocytes that might be critically involved in Ca2+ dynamics.

P-687.00

EXPLORING PROTEOMICS TOOLBOX TOWARDS HUMAN CARDIAC STEM CELLS CHARACTERIZATION

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Introduction and objectives:

Heart diseases are the leading cause of death worldwide. The myocardium of acute myocardial infarction (AMI) patients presents severe deficit of myocytes. Stem cell-based therapies stand as a promising strategy for cardiac repair as the adult human heart homes a population of resident cardiac stem cells (hCSC) capable of regenerate contractile myocardium. Under a stress episode, hCSC are activated by secreted growth-factors (GF) that trigger an auto/paracrine loop which induces further expression of factors that keep cells activated.

Aiming at supporting the development of allogeneic cell-based therapies and providing new insights about molecules/pathways involved in cardiac repair, we investigated hCSCs receptome and secretome profiles.

Methods:

hCSC cultivated in environmentally controlled stirred tank bioreactors were characterized regarding cell viability, metabolism, phenotype, GF secretion and differentiation potential.

A high-throughput proteomics workflow was implemented, enabling identification of low abundance (receptors and GF) and highly hydrophobic proteins (membrane proteins- receptors). Enrichment of plasma membrane proteins was preformed prior to MCX-nanoLC-LTQ-Orbitrap for receptome analysis. For secretome characterization, conditioned medium of hCSC cultures was collected. Samples were run in a SDS-PAGE gel, each lane sliced in several fractions, individually digested and fractionated by nanoLC. The entire run was collected and spotted for further MS analysis (MALDI-TOF/TOF).

Results and Discussion:

hCSC cultured in bioreactors remained phenotypically and functionally similar to cells cultured in standard 2D culture systems. Receptome analyses lead to the identification of more than 2000 proteins/replicate, several hundred with numerous predicted transmembrane domains, from which around 100 were plasma membrane receptors. Cardiovascular system development and function was the top hit of functional analysis by IPA software. Secretome data analysis is on-going, with about 300 human proteins identified up to now.

Conclusions:

Proteomics approaches implemented allowed the identification of a wide-ranging list of receptors and secreted factors that are currently being further investigated.

P-688.00

QUANTITATIVE PROTEIN ANALYSIS OF ENDOTHELIAL PROGENITOR CELLS IN ATHEROSCLEROSIS

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Introduction And Objectives

Endothelial progenitor cells(EPCs) constitute a promising alternative in cardiovascular regenerative medicine due to their assigned role in angiogenesis and vascular repair. In response to injury, EPCs seem to promote vascular remodeling by replacement of damaged mature endothelial cells and/or by secreting certain angiogenic factors over the damaged tissue1. Nevertheless, such mechanisms need to be further characterized. Herein we have focused on the identification of proteins differentially expressed between EPCs of atherosclerotic patients and EPC from healthy individuals, in order to better characterize the response of EPC in the atherosclerotic pathology and provide potential candidates susceptible of manipulation to promote vascular regeneration.

Methods

EPCs were isolated from healthy donors or patients undergoing carotid endarterectomy and cultured following state of the art protocols, and cellular characterization of EPC markers was carried out by flow cytometry at days 4 and 7 after isolation. At day 7 EPCs were lysed, reduced/alkylated and sequentially digested with LysC and trypsin. The resulting peptides were then labeled using dimethyl labeling: EPC Patient (heavy, 13CD2O) and EPC Control (light, CH2O). Three technical replicates from samples of three different individuals were run on a Q-Exactive, using a 4h LC-gradient. Data analysis was performed with ProteomeDiscoverer 1.4. Differentially expressed proteins were defined as follows: Proteins identified in at least 2 replicates out of 3, p-value (t-test) < 0.05 and fold-change rates >2 for up-regulated or

Results And Discussion

Derived from this analysis 1196 proteins were identified, including 52 proteins which were up-regulated and 38 down-regulated in the EPCs of atherosclerotic patients versus the EPC control. Differentially expressed proteins appeared to be involved in several pathways regulating angiogenesis, cell mobility and cell-cell interactions. The function of these proteins will be discussed.

P-689.00

PROTEOMIC PROFILING OF THE HUMAN VENOUS EXTRACELLULAR MATRIX REVEALS A ROLE FOR MAST CELL PROTEASES IN THE PATHOGENESIS OF VARICOSE VEINS

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Venous hypertension in the hepatic, splanchnic and peripheral circulation is associated with significant morbidity and mortality affecting a large population of patients comprised of liver, renal and pulmonary disease. To understand the pathogenesis of human venous hypertension, normal and varicose veins were evaluated using a novel proteomic discovery approach targeting the extracellular matrix (ECM) proteins. ECM proteins were extracted from vascular tissues, deglycosylated, and identified using gel-LC-MS/MS.

Among the identified 84 ECM proteins, 13 proteins demonstrated significant differences in their quantity between the two types of venous tissue; 4 of which had not been previously detected in human venous tissue. Interestingly, chymase and tryptase beta-1, two mast cell proteases, were among the most differentially expressed proteins. To validate the proteomics data, the adjacent tissue from each sample was processed for histology (hematoxylin and eosin and Mason's trichrome) and immunohistochemistry (IHC). Additional Western blot analysis were also performed. Consistent with the proteomics data, IHC revealed that both proteases localized to the neointima of varicose veins. Western blotting results further confirmed the proteomics findings. The effect of these mast cell proteases on the vascular ECM have not been described so far. Incubation of normal veins with recombinant tryptase followed by LC-MS/MS analysis revealed extensive degradation of various ECM proteins including tenascin and fibronectin. In comparison, chymase was less potent, although degradation of periostin and tenascin was observed.

Our proteomics discovery approach suggests that mast cells play a pivotal role in the pathogenesis of venous hypertension. Besides their roles in inducing inflammation and apoptosis of smooth muscle cells, mast cell proteases participate in the degradation of structural components of the vascular ECM, a critical step in the development of varicosis.

P-690.00

PREDICTIVE BIOMARKERS FOR INCREASED RISK OF ARRHYTHMIC DEATH IN THE PREDICTION OF ARRHYTHMIC EVENTS IDENTIFIED BY LARGE-SCALE PLASMA PROTEOMICS PROFILING

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Introduction: Currently selection of patients to receive a primary prevention ICD is solely based on LV EF. Plasma biomarkers predicting sudden cardiac arrest (SCA) would be highly valuable but reliable biomarkers are absent. With a large-scale, novel and unbiased plasma proteomics strategy, we identified a cohort of promising biomarkers to predict cause-specific mortality from SCA (arrhythmic death or defibrillation for VT/VF).

Methods: We used a case-control approach in 20 patients with ischemic cardiomyopathy from the PAREPET trial. The EF, age and creatinine levels were matched in those experiencing SCA vs. survivors. Proteomic profiling was performed using a novel tandem affinity depletion (IgY14-SuperMix) method to quantitatively reduce high- and medium- abundance plasma proteins, followed by extensive and well-controlled ion-current-based biomarker discovery. Statistical analysis and functional annotations were performed.

Results: SCA developed 1016±735 days after sampling. Stringent cutoff criteria (0.3% peptide identification FDR; ≥2 unique peptides/protein) and a threshold change of 1.4 demonstrated 89 plasma proteins to be differentially expressed in SCA vs. survivors(p

Conclusions: Plasma proteomic profiling identifies a large number of novel candidate proteins that are differentially expressed many months prior to the development of SCA. This unbiased approach confirms several known biomarkers and identifies many others that can be tested retrospectively in larger populations as well as integrated into multiple biomarker risk models for the prediction of SCA.

Topic **18**

Neurological disorders

OP082 - BROAD-SCALE ANTIGEN ARRAY PROFILING PROVIDES NOVEL INSIGHTS INTO AUTOIMMUNE TARGETS IN MULTIPLE SCLEROSIS

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Untargeted strategies hold great promise towards broad exploration of the self-antigen repertoire and identification of novel antigenic targets in various autoimmune diseases such as multiple sclerosis (MS), which is the most common disabling neurological disease of young adults.

The increasing availability of human protein fragments within the Human Protein Atlas (http://www.proteinatlas.org/), combined with highly-multiplex and high-throughput array platforms provides a unique opportunity for untargeted discovery and high-throughput verification of autoantibody targets. We previously employed this strategy to screen for IgG reactivity against 11520 human protein fragments in an MS-related plasma cohort and reported 51 antigens, which were differentially recognized across different MS subtypes and controls [1].

Here, we present data from screening of a large and independent plasma cohort (n=2210) on the suspension bead array platform for a biological verification for these 51 antigens. The target set for this follow-up study was also complemented up to 384 antigens by including protein fragments representing autoimmune targets which are known or suggested within the MS literature (e.g. KIR4.1).

Interestingly, the statistically most significant differences between the reactivity profiles for the 1106 MS patients and 1104 non-diseased controls were revealed by 3 of our 51 follow-up targets: ANO2, PGAM5 and GPR62. Since the autoimmune reactivity towards these 3 antigens could be biologically verified in this large and independent plasma cohort, we propose these targets as novel autoimmune target candidates in MS.

Furthermore, the utility of our protein fragments for broad-scale autoimmunity screening could be demonstrated by confirming autoimmune reactivity against 3 literature-based targets (SRSF7, IFNB1 and KIR4.1), yet with less degree of statistical significance. As exemplified here in the context of MS, using protein fragments and complementary high-throughput protein array platforms facilitates an efficient route for discovery and verification of disease-associated autoimmunity signatures.

[1] Ayoglu B et al. 2013 Mol Cell Proteomics

OP083 - TARGETING SYNAPTIC PATHOLOGY IN ALZHEIMER'S DISEASE – AFFINITY MASS SPECTROMETRY AS A TOOL IN BIOMARKER DISCOVERY AND CLINICAL DIAGNOSIS

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Introduction and objectives

Synaptic loss occur early in neurodegenerative diseases and biomarkers of active synapses could be indicators of degree of synaptic damage. An interesting target is the SNARE complex which regulates synaptic neurotransmitter release. Animal models suggest that changed expression or modification of SNARE proteins (SNAP-25, syntaxin-1 and VAMP) alters synaptic function. Cerebrospinal fluid (CSF) is the optimal body fluid to analyze biochemical changes in the brain as it is in direct contact with the brain and isolated from the blood by the blood-brain barrier. However, hitherto relatively large quantities of CSF pooled from several patients have been necessary in order to detect synaptic proteins. Here we report a strategy to study synaptic pathology in CSF from individual patients by combining affinity purification and mass spectrometry.

Methods

SNAP-25 was immunopurified from biochemically fractionated brain tissue or CSF and trypsinized. Stable isotope labeled standards were added and the peptide mixtures analyzed by nanoLC-MS/MS on a QIT-FTICR mass spectrometer (LTQ-FT Ultra). A top-down approach was utilized to identify novel proteoforms of soluble SNAP-25. Quantification of SNAP-25 was performed by selected reaction monitoring (SRM) on a QqQ instrument or by high resolution selected ion monitoring (HR-SIM-MS) on a Quadrupole-Orbitrap Mass Spectrometer (Q Exactive).

Results and Discussion

Novel, soluble proteoforms of SNAP-25 were identified and characterized. These SNAP-25 proteoforms were found to be quantifiable in less than 1 mL CSF. Levels of CSF SNAP-25 were increased in early as well as later stages of AD in three independent case-control cohorts.

Conclusions

Affinity mass spectrometry has been utilized to identify novel target proteoforms of the presynaptic protein SNAP-25 and to measure the levels of these proteoforms in three independent clinical cohorts. This approach could tentatively be used for assessment of disease progression and to monitor drug effects in treatment trials.

OP084 - A NEW CEREBROSPINAL FLUID 16PLEX ASSAY FOR NEURODEGENERATION

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To expand the utility of cerebrospinal fluid (CSF) in the management of dementia we have developed a 16plex protein assay based on our prior proteomics discovery projects, review of the literature and consultation with key opinion leaders.

Uniquely, this is the first assay to benefit from our proprietary TMT-SRM Universal Reference concept. Herein, Selected Reaction Monitoring (SRM) mass spectrometry is used in combination with a bulk isotopically labelled Universal Reference Cerebrospinal Fluid. The use of SRM allows the precise selection of digested protein fragments for high sensitivity and specific measurement using a triple quadrupole mass spectrometer and the use of the heavy TMT-labelled universal reference ensures measurement consistency across multiple studies and over time.

In total, the assay quantifies 31 peptides from Amyloid-like protein 1, Amyloid beta A4 protein, Beta-2-microglobulin, Complement C3 alpha and beta, Chromogranin A, Complement factor H, Cystatin C, Serum amyloid P-component, Clusterin alpha and beta, Apolipoprotein E, Alpha-2-macroglobulin, Secretogranin-2, Gelsolin as well as Fibrinogen gamma. Each patient CSF sample is labelled with a light TMT tag and then spiked with the heavy TMT-labelled universal reference CSF. The lighter endogenous peptides co-elute with their equivalent heavier reference peptide and quantified by integrating the MS peak area for the light compared to heavy signals in a classical SRM workflow.

Following reproducibility testing using ten analytical repeats of 1:1 mixtures, the diagnostic utility and performance of the assay for Alzheimer's disease was subsequently assessed in a cohort of 62 CSF samples comprising 31 cases of clinically diagnosed Alzheimer's disease and 31 neurologically healthy age- and sex-matched controls.

A remarkable level of differentiation between the study groups was obtained with class separation of AD from controls giving similar performance to that obtained using any of the clinically recognised markers such as amyloid beta 1-40/1-42 ratio, total tau and pTau levels.

OP085 - ISOLATION OF MULTIPROTEIN COMPLEXES AND SYNAPSE PROTEOMES FROM HUMAN POST-MORTEM BRAIN.

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The molecular analysis of the human brain is severely hampered by the limitations on biopsy availability and post-mortem (PM) tissue degradation. Although measures of RNA integrity have been developed for PM studies, there are very few proteome quality markers and none described that assess synapse proteomes.

Here we report a suite of methods including a rapid quantitative biochemical screen that measures a Human Synapse Proteome Integrity Ratio (HUSPIR), streamlined isolation of synapse proteome fractions from small amounts of tissue and the first affinity isolation and proteomic analysis of synapse multiprotein complexes from neurosurgical biopsy and PM human brain.

This reveals human MAGUK Associated Signalling Complexes are involved with over fifty human brain diseases. These methods can be applied to a wide range of biochemical and proteomic studies of the normal and diseased human brain.

OP086 - A SINGLE DOSE OF THE GAMMA-SECRETASE INHIBITOR SEMAGACESTAT ALTERS THE CEREBROSPINAL FLUID PEPTIDOME IN HUMANS

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Introduction and Objectives
In Alzheimer's disease (AD), beta-amyloid peptides in the brain aggregate into plaques, a process linked to the neuronal degeneration, memory loss, and cognitive decline characteristic of the disease. One potential therapeutic strategy for treatment of AD is the use of gamma-secretase inhibitors; by decreasing the production of beta-amyloid peptides they may halt plaque formation. The development of disease-modifying drugs will benefit from the identification of biomarkers that reflect the drug activity in the brain, both to verify target engagement and to detect unwanted side effects. We hypothesize that the CSF peptidome may be a valuable source of biomarkers, particularly for studying events involving proteolytic processing. In the current study we test if a CSF peptidomic approach can be used to detect treatment effects of a gamma-secretase inhibitor, to identify substrates affected by the drug and quantify the effect.

Methods
15 human healthy volunteers were included in the study. Five participants received a single dose of 140 mg of the gamma-secretase inhibitor Semagacestat, another five 280 mg, and five received a placebo. CSF sampled at six time points after drug intake were analyzed using a peptidomic workflow based on TMT 6-plex labeling, isolation of CSF peptides by MWCO ultrafiltration, and LC-MS. Peptide relative abundances were compared longitudinally, as well as against the placebo group.

Results and Discussion
Several peptides in CSF were affected by gamma-secretase inhibition. Peptides from amyloid precursor protein and amyloid precursor-like protein 1, known ã-secretase substrates, were lowered by treatment. Other peptides were not derived from transmembrane proteins but were nevertheless affected.

Conclusions
These results confirm our hypothesis that the peptidomic approach used here is able to detect relevant changes among large numbers of peptides in response to drug treatment, and that this analytical technique may be valuable to include in future clinical trials.

P-691.00

INNATE AND ADAPTIVE IMMUNE SYSTEM ALTERATIONS IN PRE-MANIFEST STAGES OF TRANSGENIC PORCINE MODEL OF HUNTINGTON'S DISEASE

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Introduction and objectives

Huntington's disease (HD) is a rare autosomal dominant inherited neurodegenerative disease caused by a CAG repeat expansion in the Htt gene leading to the polyQ extension of huntingtin protein. The onset of the disease, which inversely correlates with polyQ extension, is usually diagnosed by clinical symptoms such as movement disturbances, decline in cognitive functions and eventual dementia. At present, there is no cure to slow or reverse the progression of HD and only palliative care can be provided. We established a model of HD using transgenic minipig expressing human mutant huntingtin to facilitate long-term studies including pre-clinical biomarker search and drug safety and efficacy studies. This work focused on the role of innate and adaptive immune system in HD.

Methods

Using transgenic minipig HD model bearing an N-terminal fragment of mutant human huntingtin, the sera of 18 months old animals prior to onset of clinical symptoms were utilised. The activity of complement system was scrutinised using Wieslab® Complement system Screen kit and the assessment of adaptive immune system was performed using Luminex xMAP multiplexing technology.

Results and Discussion

The main pathophysiological mechanisms triggered in the development of HD include abnormal protein aggregation, DNA and mitochondrial damage, excitotoxicity, oxidative stress, and neuroinflammation. Our results indicate that complement system was activated with possible prevalence to the alternate pathway. Despite complement activation, several TH1 and TH2 cytokine levels did not show marked increases at the pre-clinical stage. However, altered IL-8 level in HD minipigs suggested the role of chemotaxis, involving primarily neutrophils, the mediators of innate immune system.

Conclusion

The activation of complement system together with IL-8 at pre-symptomatic stages of HD may trigger many deleterious processes with possible consequences leading to neurodegeneration.

P-692.00

ALTERATIONS OF PROTEIN COMPOSITION ALONG THE ROSTRO-CAUDAL AXIS AFTER SPINAL CORD INJURY: PROTEOMIC ANALYSES

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Based on proteomic analyses we investigated the differences of released molecules from conditioned media (CM) and molecules present in tissue from the spinal cord central lesion and adjacent rostral and caudal segments. Temporal-spatial proteomic analyses have been performed 3, 7, 10 days after SCI in order to specify the molecular environment within greater extent of tissue damage.

To better understand the role of immune response in secondary damage processes, a balloon-compressive technique was used to produce spinal cord injury at thoracic Th8-9 spinal level in adult rat. Proteins from tissue and CM were analyzed by shot-gun analyses using nanoLC coupled to LTQ- orbitrap XL.

Results showed some specific proteins at each site of the lesion. Among proteins from rostral and lesion segments, some are related to chemokines e.g. CCL2, CCL3, CCL22, CXCL1, CXCL2, CXCL7 or to neurogenesis factors. In contrast, proteins from caudal segments are more related to necrosis factors.

Neurotrophic factors have been highly identified at 3 days, diminished at 7 days and disappeared at 10 days e.g (CTGF, NOV, FGF-1, BMP3, NGF, TGF beta (1-3), periostin, GAP-43, neurotrimin). However at 10 days, proteins related to synaptogenesis have been detected e.g. R–SNARE synaptobrevine, Q–SNAREs (SNAP25), GTPases (Rab proteins family), syntaxines, synaptotagmine. These data showed that at 10 days proteins involved in axonal reconnection and synaptic transmission reflecting that a neurorepair process has started. In contrast, at the caudal segment, the protein profile is always inflammatory and apoptotic whatever the days after SCI.

Taken together, these data shown that polarization in terms of inflammatory and neurotrophic responses occurs between rostral and caudal segments in SCI between 3 to 10 days post-lesion.

P-693.00

DIFFERENTIAL PROTEOMICS OF HUMAN CSF FROM TRAUMATIC SPINAL CORD INJURY PATIENTS REVEALS PERTURBED MOLECULAR PATHWAYS IN SECONDARY INJURY PHASE

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Introduction and Objectives: Traumatic spinal cord injury (SCI) is one of the leading causes of mortality and morbidity worldwide. Based on severity of the injury, which is classified by American Spinal Injury Association (ASIA) Impairment Scale (AIS), recovery outcomes vary widely among patients. In the secondary phase of injury, which may persist from 24 hours to days and months post injury, many well established pathways are functional. Our objective is to determine which of these pathways are perturbed in severe SCI (AIS A) as compared to less severe SCI (AIS C/D).

Methods: We compared cerebrospinal fluid (CSF) from AIS A and AIS C/D patients for differentially abundant proteins by 2 Dimensional Difference Gel Electrophoresis (2D-DIGE) and identified them by Matrix Assisted Laser Desorption Ionisation (MALDI-TOF-TOF). Subsequently, we constructed a protein-protein interaction network (SCI-PPIN) taking into account the identified proteins from AIS A CSF samples and their secondary interactors. The SCI-PPIN was then modularised to reveal biological pathways that are predominant in the secondary phase post SCI.

Results and Discussion: The differentially abundant proteins belonged to DNA repair, protein phosphorylation, tRNA transcription, iron transport, mRNA metabolism, immune response, lipid and ATP catabolism pathways. These pathways highlight mechanisms that the system may adopt to repair itself depending on the injury severity. Consequently, the different severity based recovery outcomes can be explained and external interventions may be attempted in future.

Conclusions: We have approached the problem of delineating the differentially regulated molecular pathways post traumatic SCI by analysing CSF, an easily attainable body fluid. Our results have revealed a set of active pathways in the secondary phase post SCI. These pathways have enables us to build a real time picture at the vicinity of the injured cord and have opened avenues for validation of the work in animal models and subsequent clinical application.

P-694.00

QUANTITATIVE PROTEOMICS OF HUMAN LRRK2 (R1441C) DROSOPHILA MODEL OF PARKINSON DISEASE

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Parkinson's disease (PD), occurring in late mid-life, is the second most prevalent neurodegenerative disorder after Alzheimer's disease. Drosophila melanogaster, the fruit fly, is already an accepted PD model and has been investigated vastly in the last couple of years. Stable isotope labeling of amino acids in cell culture (SILAC) is a widely used powerful tool for the quantification of proteomics based on mass spectrometry and recently SILAC was also extended for labeling of living animal including mouse, zebra fish and fruit flies.

In this study we showed a quantitative proteomics study of human LRRK2-R1441C overexpressing transgenic Drosophila melanogaster as a PD model at different disease stages. In total 3570 proteins were identified from fly head and 1753 proteins were quantified. Overall 87 (>10%) proteins were found to be significantly regulated at 30 days old flies. Up-regulated proteins are mostly associated with cytoskeleton and mitochondrion that may presage the development of advanced PD. Regulated ATP synthase proteins make it reasonable to hypothesize that alternation in ATP synthase may be responsible for the malfunction of mitochodria.

The cluster analysis also revealed the elevated expression of GstS1 which is known as a suppressor for neurodegeneration in dopaminergic neurons. In the fraction of down-regulated proteins, we reidentified protein punch, henna and purple which are substrates for Dopamine and catecholamine synthesis and these proteins are also dis-regulated due to alpha-synuclein toxicity in fly. Therefore the burning question would be, is there any common pathways for neurodegeneration due to αS and LRRK2 toxicity? As a summary, this study provides dynamic and temporal proteomic changes due to over-expressed human LRRK2 (R1441C) in Drosophila melanogaster as a PD model that may shed light on the fundamental etiology of PD.

P-695.00

INDUCTION MECHANISM OF NEURAL DIFFERENTIATION OF HUMAN BONE MARROW MESENCHYMAL STEM CELLS BY ELECTROMAGNETIC FIELD

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Introduction and Objectives

Electromagnetic field (EMF) is a well-known mechanical stimulation that induces neural differentiation. This is an affordable and effective way to treat neurodegenerative disease. Even though its function is distinctive, the underlying cellular mechanism of neural differentiation remains unclear

Methods

Human bone marrow-derived mesenchymal stem cells (BM-MSCs) which exposed to 50 Hz, 1 mT for 12 days were differentiated into neural cells. Besides differentially expressed proteins, especially ferritin light chain (FLC), were verified using 2-DE analysis. FLC is an important element in controlling iron ion homeostasis and is abundant in the specific region of central nervous system (CNS).

Results and Discussion

This study demonstrates that EMF triggers up-regulation of FLC in BM-MSCs. Up-regulated FLC has positive effects on the differentiation of BM-MSCs to neural cells compared with control group. EMF also induces activation of downstream candidates of FLC in novel neural differentiation mechanism. Intracellular iron level was down-regulated and ferritin heavy chain (FHC), iron regulatory protein-1 (IRP-1) and cofilin were up-regulated in EMF exposed group. Up-regulated cofilin triggers actin filament reorganization in neural morphogenesis.

Conclusions

These results suggest that EMF induces neural differentiation through an activation of ferritin regulated mechanism.

P-696.00

A PROTEOMICS PERSPECTIVE ON NEURODEGENERATION IN AMYOTROPHIC LATERAL SCLEROSIS

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Introduction and objectives

Fatal neurodegenerative disorders are becoming an increasing health problem in industrialized societies. They constitute a broad spectrum of different disease entities and share characteristics such as protein miss-folding. Despite intensive research efforts, major aspects of the pathology in neurodegeneration remain elusive. We used unbiased approaches to describe motoneurons at the proteome level and compared them to widely used motoneuronal cell model systems. Furthermore, we investigated pathogenic mechanisms of protein aggregation in amyotrophic lateral sclerosis (ALS) and other neurodegenerative diseases using quantitative mass spectrometry (MS) based proteomics.

Methods

Whole proteomes were measured of primary motoneurons and cell lines. To analyze the interactions of ALS associated proteins and aggregates we performed immunoprecipitation of tagged proteins. We used a single shot-strategy on a quadrupole Orbitrap MS (Q Exactive) mass spectrometer. MaxQuant and Perseus software were used to quantify cellular proteins with a label-free algorithm and to compare different motoneuronal model systems to primary motoneurons at the levels of annotations and individual proteins.

We characterized the proteome of motoneurons at a depths of more than 10,000 proteins. A comparison of primary motoneurons to motoneuronal model cell lines revealed distinct pattern of expressed proteins in neuron and ALS associated pathways. The proteome characterization of these cellular systems provides an unbiased benchmark for motoneuron research and a valuable resource for the community. Misfolded proteins accumulate in many neurodegenerative disorders, however, whether these are toxic entities or a byproduct of disease is still under debate. We quantified soluble interaction partners as well as co-aggregating proteins of disease associated proteins. Our results reveal proteins involved in common pathways linked to neurodegeneration as well as unexpectedly sequestered proteins, expanding our knowledge about generality and specificity of neurodegenerative disease mechanisms.

P-697.00

USE OF MULTIPLE REACTION MONITORING LC-MS FOR THE VERIFICATION OF PARKINSON'S DISEASE PROTEIN SIGNATURES

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Diagnosis of Parkinson's disease is based on the appearance of motor symptoms. A 2-DE analysis panel of proteins in the T-lymphocyte proteome was recently proposed as a disease signature. Here, an LC-MS based method was used to quantitatively evaluate this signature by MRM in T-lymphocytes and peripheral blood mononuclear cells.

A discriminant function was applied to MRM data from T-lymphocytes protein extracts, assigning seven controls out of nine as true negatives and nine patients out of nine as true positives. Good discriminant power was obtained by selecting a subset of peptides from the protein signature (GELS, MOES, SEPT6, TWF2, LSP1, VIME, TALDO), with an ROC AUC of 0.877.

The signature was not able to classify subjects by analyzing whole mononuclear cells. The results suggest portability of the method to large cohort validation using alternative technologies such as LC-MS.

P-698.00

RELATION BETWEEN CERVICAL CORD LESIONS AND DISK HERNIATION IN THE PATIENTS WITH MULTIPLE SCLEROSIS

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Introduction:
Multiple sclerosis (MS) is the most frequent chronic autoimmune demyelinating disease of the central nervous system (CNS). Purpose of this study is to determine the relationship between the site of cervical disk herniation and cervical spinal cord plaque in our outpatient's clinic for MS patients, Shiraz, Iran.

Methods:
All patients with definite diagnosis of multiple sclerosis at our outpatient clinic, Shiraz, Iran; from Sept. 2004 to Sept. 2011, which were 536, were involved in this prospective study. All patients underwent cervical MRI for primary investigation of the disease .The patients with cervical cord lesion, 471 patients, were selected and their MRIs were evaluated for detection of any site of cervical disk herniation. Any correlation between the site of lesion and disk herniation was recorded.

Results:
Over all 536 patients were involved in the study. 441 (82.3%) of the patients were females and others were males. Mean age of the patients was 28.2 years. Disk herniation was seen in 214 (40.9%) of the patients. 148 (28.3% of the all patients) had cervical plaque at the same site of cervical disk herniation. In 66 patients cervical plaque and disk herniation didn't have any correlation regarding the site of the lesion. The number of patients with plaque and herniation at same site was significantly higher than those with these lesions at different sites ($p < 0.05$).

Discussion:
With regards to these facts, it is possible to consider a relation between cervical disk herniation and cervical MS plaque.

P-699.00

EPILEPSY IN THE PATIENT WITH MYASTHENIA GRAVIS, A CASE REPORT AND REVIEW OF THE LITERATURES

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Introduction: Associations between myasthenia gravis (MG) and CNS functions have been diagnosed for many years. It was seen that there is an increased incidence of psychiatric disorders and epilepsy as well as electroencephalographic (EEG) abnormalities and abnormal evoked responses in patients with MG. there are few reports about the co incidence of MG and epilepsy in literatures. Here in we report a 47 year old man with MG and epilepsy.

Case presentation: The patient is a 47 year old man a known case of MG who came with several episodes of starring and then generalized tonic clonic convulsion. Epilepti- form discharge was seen in EEG.

Discussion: although there are some few reports of co incidence of MG and epilepsy, in all cases, patients with Epilepsy developed MG in the follow up of treatment, which may be on the base of medication, while in our case the patient is a known case of MG and after that developed epilepsy. In this setting anti epileptic medications are not responsible for presenting MG.

P-700.00

PLASMA LEVELS OF IL-17 AND IL-23 IN THE PATIENTS WITH MULTIPLE SCLEROSIS

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Introduction:

Multiple sclerosis (MS) is the most common demylinating disease of central nervous system. Recently a subset of T-helper lymphocytes named TH17 are known to be involved in the induction and progression of EAE (Experimental autoimmune encephalomyelitis) . these cells produce IL-17 under stimulation of IL-23 secreted by antigen presenting cells that both of them are pro inflammatory cytokines. The aim of this study was to measured plasma level of IL-17 and IL-23 and investigating association of their plasma concentrations with sex, age at disease onset, the disease severity, disease subtype and MRI enhancement of Iranian patients suffering MS.

Patients and Methods:

A total of 41 patients with MS were enrolled in the study.and were compared with 41 age and sex matched control subjects. IL-17 and IL-23 in plasma samples were determined by enzyme linked immuno sorbent assay method. (ELISA)

Results:

MS patients had approximately similar plasma levels of IL-17 and IL-23 compared to the controls. Male patients had significantly higher levels of IL-23 compared to their healthy controls. (P value : 0.047)

Discussion:

increased plasma levels of IL-23 in male patients comparing to controls may be suggestive of role of IL-23 in differentiation and function of Th 17 cells which enhances IL-17 production in the MS patients .

P-701.00

ASSOCIATION OF PEROXISOME PROLIFERATORS-ACTIVATED RECEPTOR-GAMMA (PPAR Γ) ON PROGNOSIS OF MULTIPLE SCLEROSIS

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Introduction:

Multiple sclerosis (MS) is a progressive autoimmune neurodegenerative disorder of the CNS that characterized with inflammation and demyelination of the CNS, which culminates in neurological deficits and paralysis. Numerous studies have revealed that agonists of peroxisome proliferators-activated receptor-gamma (PPAR γ) exert anti-inflammatory effects both in vitro and in vivo and may play a role in MS. We examined the association of PPAR γ Pro12Ala polymorphism with susceptibility to and severity of MS in Iranian people.

Patients and Methods: In a case-control analysis that included 254 multiple sclerosis cases and 217 matched controls, we checked peroxisome proliferators-activated receptor-gamma (PPAR γ) by PCR method and compare it between cases and controls.

Results: we found significant difference in distribution of this polymorphism between patients and controls (P value=0.029). but not significant association between sex (in female group P value=.816, in male group P value =.722), disease type (P value=0.690). And disability index (EDSS) (P value=0.943) progression index (EDSS/duration) (P value=0.878) disease onset age (P value=0.486).

Discussion: we conclude that the Pro12Ala polymorphism does appear to play a significant role in susceptibility to MS in this study.

P-702.00

THE MONTH OF BIRTH AND THE INCIDENCE OF MULTIPLE SCLEROSIS IN THE IRANIAN POPULATION

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Introduction: There are many studies which are suggested seasonal factors as a potential risk factor for existing MS even before birth. Recent studies on seasonal time of birth of multiple sclerosis patients showed a spring peak and an autumn nadir. In this study we examined month of birth (MOB) and season of birth (SOB) and risk of multiple sclerosis in later life in Iranian population.

Patients and methods: During a case control study, in Fars, Southern Iran, between 2005 to 2012 , more than 1500 patients with definite multiple sclerosis (according to Mc Donald's Criteria) in outpatient clinic were involved in the study. We randomly selected 2000 individuals from Fars province populations who were matched with case group in aspects of age and sex. The dates of birth, gender, were identified for each patient and control. The results were compared in the groups. We used a χ (2) analysis to compare the distribution of MOB in MS patients versus age-sex adjusted controls.

Results:
1020 females and 538 males, age between 15-65 years were involved in the study. Female to male ratio in this study was 1.9.Overall we detected that rate of birth among cases was significantly higher in March, April and October than controls. Moreover it seems that this rate is significantly lower in January in case group. We didn't detect any significant differences among cases and controls in time of birth in different seasons of a year.

Discussion: such as European countries we detected that rate of birth among cases was significantly higher in March, April and October than controls. Possible explanation is Decreased exposure to sun in the winter which leading to low vitamin D levels during pregnancy.

P-703.00

THE EFFECT OF FREE TESTOSTERONE ON COURSE, SEVERITY, DISEASE ACTIVITY AND DISABILITY IN MULTIPLE SCLEROSIS

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Introduction:

Sex related difference in the course and severity of Multiple Sclerosis (MS) could be mediated by several sex hormones. This study aimed to investigate The relation between free testosterone concentrations and course , severity , brain damage and disability in Iranian patients with MS.

Methods:

37 women with MS and 25 healthy subjects were included in the study as case and control groups.

Free testosterone level was assessed by ElISA method. Brain MRI with and without contrast was performed. Expanded Disability status scale (EDSS) and MS subtypes in the patients were collected via a questionnaire.

Results:

Serum testosterone was significantly lower in women with MS than controls (P value: 0.026). The free testosterone levels were not associated with EDSS, MS subtype and MRI findings.

Discussion:

The hormone related modulation of pathological changes dose not supports the hypothesis that sex hormones play a role in the inflammation , damage and repair mechanism in MS, Although serum level of these hormones in the patients was significantly lower than controls.

P-704.00

NEUROBRUCELLOSIS IN A PATIENT WITH MULTIPLE SCLEROSIS; A CASE REPORT

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Introduction: Neurobrucellosis is an uncommon complication of brucellosis. Acute meningitis and encephalitis are the most common clinical manifestations, however symptoms may be protean and diagnosis requires a high index of suspicion in patients from endemic areas.

Case Presentation: Herein we report a 30 year old lady a known case of MS who came with ataxia and drowsiness, in physical examination and para clinical evaluations Neurobrucellosis was detected.

Discussion: Diagnosis is often based on neurological symptoms, serology, and suggestive brain imaging because cerebrospinal fluid culture yields are low. Multiple sclerosis (MS) is a chronic autoimmune disorder affecting the central nervous system (CNS) through demyelination and neurodegeneration.

P-705.00

PLASMA PROTEIN MARKERS IN MAJOR DEPRESSIVE DISORDER TREATED WITH ANTIDEPRESSANTS

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Introduction and Objectives

Major depressive disorder (MDD) is the one of the largest cause of burden of disease. Recently, genetic variations and protein biomarkers to predict the efficacy of antidepressants have been reported, however, clinical studies of a lot of biomarkers are still insufficient and the clinical utility of previously described markers has not been clearly defined. Therefore, we performed the mass spectrometry (MS)-based plasma protein profiling of pre- and post-treatment plasma from MDD patients treated with either selective serotonin reuptake inhibitors (SSRIs) or non-SSRIs treatment, and healthy individuals.

Methods

We screened 135 candidate proteins acquired from database and literature search and carried out multiple reaction monitoring (MRM) to detect these markers using Q-TRAP 5500 (AB Sciex, Foster City, CA, USA). Among these proteins, we selected 10 proteins and measured each protein using isotope labeled peptide in pre- and post- treatment samples from 35 patients treated with SSRIs, 43 patients treated with non-SSRIs, and samples from 10 healthy individuals.

Results and Discussion

In patients treated with non-SSRIs, changes of plasma thyroxine-binding globulin (TBG $p = 0.025$), transthyretin (TTR, $p = 0.037$), and coagulation factor XI (FXI, $p = 0.008$) concentrations was observed after treatment. Among these proteins, TBG ($p = 0.013$) and FXI ($p = 0.004$) were only changed in patients who responded to SSRI treatment. Comparing MDD patients with healthy individuals, alterations of TTR ($p = 0.010$) and FXI ($p = 0.002$) were observed in patients.

Conclusions

Our data demonstrate that thyroid-related proteins and coagulation factors have the potential to contribute to understandings of pathogenesis and therapeutic response in MDD.

P-706.00

LOW-DOSE TOTAL BODY IRRADIATION IN NEONATAL MICE CAUSES LONG-TERM IMPAIRED COGNITIVE PERFORMANCE ASSOCIATED WITH CHANGES IN CREB / MIR-132-MEDIATED SYNAPTIC PLASTICITY

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High doses of ionising radiation (> 20 Gy) as used in radiotherapy of brain cancer patients are known to result in short- and long-term memory loss. Epidemiological evidence suggests that even considerably lower doses (< 1.0 Gy) may lead to persistent alterations in cognition if the exposure occurs at young age attributed to the multifaceted remodelling of the immature brain in this critical phase. However, the molecular mechanisms causing such alterations are scarcely studied.

We investigated the long-term effects of low doses (20 and 100 mGy) as used during medical imaging procedures and moderate doses (0.5 Gy and 1.0 Gy) of total body gamma radiation on neonatal NMRI mice. Significant alterations in the spontaneous behaviour were observed 2 and 4 months after the exposure to 0.5 and 1.0 Gy. Alterations in the brain proteome via Isotope-Coded Protein Label (ICPL) approach, transcriptome and miRNAome were analysed 7 months post-irradiation in the hippocampus, dentate gyrus and cortex. The molecular analysis demonstrated that signalling pathways related to actin-remodelling such as Rac1-cofilin pathway were significantly altered at the moderate doses in the cortex and hippocampus. Further, the level of synaptic proteins MAP-2 and PSD-95 increased in the dentate gyrus and hippocampus (1.0 Gy). The expression of synaptic plasticity genes as well as transcription factor CREB was persistently altered at 1.0 Gy in the hippocampus and cortex. These changes were coupled to epigenetic modulation via altered levels of miRNAs such as miR-132.

Further, we observed increased level of microglial cytokine Tnfα indicating radiation-induced neuroinflammation. These data may be linked to many neurocognitive disorders observed in adults exposed to low and moderate cranial doses of ionising radiation early in life.

P-707.00

CLINICAL WORKFLOW FOR ANALYSIS OF HUMAN CSF PROTEINS USING HIGH RESOLUTION MASS SPECTROMETRY PARALLEL REACTION MONITORING MULTIPLEX ASSAYS

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Introduction and Objectives:
Mass spectrometry (MS) is well suited for biomarker analysis diagnosis, prognosis, and theragnosis of many neurological diseases, including Alzheimer's disease (AD). The MS based assays are flexible allowing a high degree of customizability. The objective was to develop MS based assays to monitor cerebrospinal fluid (CSF) proteins using relatively little CSF. The assays should be robust, sensitive, and fast enough to allow analyzing larger study sets.

Methods:
Three different panels were developed. Panel 1 consist of six high-to-medium abundant proteins, cystatin C, beta-2-microglobulin, chromogranin A, secretogranin-2, neurosecretory protein VGF, and amyloid precursor protein (APP). Panel 2 contains another seven protein. Panel 3 is an APP panel consisting of 18 peptides along the protein. CSF samples were divided into AD and non-AD cases based on amyloid â, tau, and p-tau biomarkers. Trypsinated CSF was analyzed using microflow liquid chromatography coupled to an electrospray ionization hybrid quadrupole–orbitrap mass spectrometer. Hundred microliter CSF was sufficient for analysis using all three panels.

Results and Discussion:
Panel 1 was evaluated in two pilot studies. In the first, digestion replicates and technical replicates were analyzed for 3 AD and 3 non-AD over ten consequtive days. The CVs were
For complex samples high resolution instrumentation is advantageous because of the superior ability to handle signal interferences.

Conclusions:
The assay is reproducible and consumes little amount. Preliminary results should be interpreted with caution due to the limited sample material. Data from a larger study, using clinically well-characterized samples, is under way and will be presented.

P-708.00

VALIDATION OF PROGNOSTIC BIOMARKERS FOR CONVERSION TO MULTIPLE SCLEROSIS IN PATIENTS WITH CLINICALLY ISOLATED SYNDROME

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Introduction and objectives
Multiple sclerosis is a chronic inflammatory disease of the central nervous system probably caused by the interaction of genetic and environmental factors. In most patients, the disease initiates with an episode of neurological disturbance known as clinically isolated syndrome (CIS). However, not all patients with CIS develop multiple sclerosis. Therefore, identifying proteins that differentiate CIS patients who will convert to multiple sclerosis from those who won't, will allow the development of prognosis tools for disease progression.

Methods
Initially, a quantitative mass spectrometry approach using iTRAQ was performed to identify potential biomarker candidates in pooled cerebrospinal fluid from CIS patients (n=60). The validation of these candidates was conducted with selected reaction monitoring (SRM) in individual patients (n=127).

Results and Discussion
In our initial discovery analysis using pooled cerebrospinal fluid from CIS patients, several proteins showed different protein abundance levels when comparing patients who eventually converted to multiple sclerosis and patients who remained as CIS. These candidate proteins together with others described in the literature were included in a targeted validation study (SRM) in individual patients. Our results confirm that CIS patients who convert to multiple sclerosis have significantly decreased levels of Semaphorin 7A and beta-ala-dipeptidase, proving the association of these proteins with disease progression. More importantly, when used in combination some of the quantified proteins are able to predict conversion to multiple sclerosis in CIS patients becoming good biomarkers of disease progression.

Conclusions
A mass spectrometry discovery study followed by targeted validation of the resulting candidates has led to the identification of prognostic biomarkers for multiple sclerosis disease progression.

P-709.00
STUDY OF THE EXPRESSION OF NUCLEOSIDE AND NUCLEOTIDE RECEPTORS IN MOUSE SPINAL CORD THROUGH MULTIPLE REACTION MONITORING.

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Introduction and Objectives: The molecules of the purinergic system act as neurotransmitters and modulators. In neurodegenerative diseases and traumatic injuryin the nervous system, the massive release of nucleosides and nucleotides from damaged cells induces excytotoxic processes and necrotic and apoptotic cell death of neurons and oligodendrocytes and activation of microglia and astrocytes. These mediators act through the activation of specific transmembrane receptors that can be classified as metabotropic nucleoside receptors (P1 or AdoR) and nucleotide receptors, the ionotropic P2RXs and the metabotropic P2RYs. Depending on the receptor expression, purinergic molecules can have both pro and anti-apoptotic effects. Therefore, the main goal of the present work is to determine the expression of the different kind of receptors in the mice spinal cord as first step to the study of the time-course of changes in expression after injury.

Methods: Predicted MRM transitions of 15 purinergic receptor subtypes were calculated in silico using MRMPilot software (ABSciex). A mouse spinal cord protein extract was analyzed in a 4000QTrap mass spectrometer, in order to validate these theoretical transitions.

Results and Discussion: 87 transitions from the 15 receptors analyzed were validated in mouse samples of spinal cord. Using this approach, we could confirm the presence of the 4 nucleoside receptors (AdoRA1, A2A, A2B and A3), and among the nucleotide receptors, the 7 P2RXs (1 to 7) and 4 of 8 P2RYs (4, 6, 12 and 14). This study might be a step forward in our understanding of the molecular changes taking place in the purinergic system after spinal cord injury.

Conclusions: The presence of a wide variety of nucleoside and nucleotide receptors in the spinal cord opens the possibility to study changes in their expression at different times after SCI and design strategies to modulate these changes and reduce the deleterious effects of traumatic SCI.

P-710.00

IDENTIFICATION OF POTENTIAL BIOMARKERS OF ISCHEMIC STROKE BY INTEGRATING 2D-DIGE AND LABEL FREE-MS/MS

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Introduction and objectives

Stroke is the major cause of disability worldwide and one of the main causes of death in industrialized countries. Ischemic stroke (IS) accounting for more than 87% of the total number of strokes cases imposes an important burden on society in terms of health care. In the present study we hypothesized that specific stroke proteins may be released and detected in plasma of patients and be useful markers for diagnostic and prognostic.

Methods

For protein analysis, plasma samples were depleted of the 14 most-abundant proteins and analyzed by two-dimensional differential in-gel electrophoresis (2D-DIGE) and label free-MS/MS methodologies, as complementary techniques, seeking to identify lesser abundant proteins involved in the pathological process. Results were validated by WB and SRM and significantly altered proteins were analyzed with DAVID Bioinformatics Resources (NIH), in order to characterize the pathways in which these proteins are involved.

Results and discussion

More than 1200 proteins were identified. We observed that 9 proteins were found altered in IS by both techniques. Several of these proteins were detected in IS by an orthogonal technique (WB and SRM) therefore reinforcing the proteomic results performed.

The identification of biomarkers for IS is a very active area of research. To date various biomarkers of stroke have been studied; however none of them have reached the clinical setting due to the lack of sufficient sensitivity and specificity. In this study important subset of proteins involved in immune response, inflammation and transport were found altered, process that are activate early in the disease.

Conclusions

Novel proteins are here highlighted which could be potential diagnostic, prognostic and therapeutic targets in clinical practice, some of them were validated by more sensitive assays such as SRM.

P-711.00
SEARCH FOR PERIPHERAL BIOMARKERS IN PATIENTS AFFECTED BY ACUTELY PSYCHOTIC BIPOLAR DISORDER: A PROTEOMIC APPROACH

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Introduction and objectives
Bipolar disorder (BD) and severe clinical depression are mental diseases whose aetiology is multifactorial. Diagnosis relies on interview-based methodology and treatment options depend on the clinician's personal experience. The aim of the present study was to carry out a nonhypothesis-based proteomic investigation, in order to identify potential disease-related biomarkers in acute psychotic BD (PBD). Biological markers could improve the current classification of mood disorder subtypes.

Methods
We performed a comparative proteomic study of lymphocytes obtained from patients affected by PBD (n=15), major depressive episode (MDE) with no history of psychosis (n=11), and a group of demographically matched healthy controls (HC) (n=15). Blood lymphocytes were obtained by gradient separation, and two-dimensional electrophoresis coupled to nanoLC-ESI-MS/MS was carried out on protein extracts. Differential expressions of proteins were validated by western blot, and their involvement in canonical pathways was investigated with Ingenuity software analysis.

Results and Discussion
Twenty-five proteins were found differentially expressed in patients compared to controls. Twenty-one of these proteins failed to discriminate between PBD and MDE, suggesting common signatures for these disorders. Nevertheless two of the remaining proteins, namely LIM and SH3 domain protein1, and short-chain specific acyl-CoA dehydrogenase mitochondrial protein, resulted significantly upregulated in PBD samples suggesting additional mechanisms that could be associated with the psychotic features of BD. This result supports recent findings that propose mitochondrial dysfunction in patients with BD. Therefore, our interest will be to study mitochondria isolated from lymphocytes since our data support an involvement of mitochondria in the pathophysiology of BD.

Conclusions
Our results confirm that lymphocytes are a peripheral model useful to study psychiatric disorders, and to prove the difficulty in defining specific biomarkers considering the overlap of clinical profiles. However, we believe that our results might provide new insights into the molecular pathways involved in full-blown PBD symptoms.

P-712.00

COMPARATIVE PROTEOMIC ANALYSIS OF DIFFERENT FRAGILE X SYNDROME CELL LINES

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Introduction and objectives: Fragile X syndrome (FXS), the most common monogenic form of developmental intellectual disability, is caused by absence of the FMRP protein due to expansion over 200 repeats of the CGG tract at the 5' UTR of the FMR1 gene and subsequent DNA methylation. Rare individuals of normal intelligence with CGG expansion over 200 repeats without DNA methylation (unmethylated full mutation, UFM) have relatively normal transcription and translation, and represent the status of FXS cell lines prior to gene silencing. Comparing three types of cell lines (normal control WT, FXS and UFM fibroblasts) with a proteomic approach might elucidate possible differences in order to clarify the molecular events through which the rare UFM cells remain unmethylated and transcriptionally active.

Methods: Differential protein expression was investigated by LC-ESI-LTQ Orbitrap tandem mass spectrometry of cellular protein extracts after mono-dimensional SDS-PAGE separation and trypsin digestion. Bioinformatics interpretation of the three proteomic dataset shows that some metabolic pathways are deregulated in UFM cells when compared to FXS cells. Some of deregulated proteins were validated by Western blot and their mRNA levels were probed. The interaction of target mRNAs with FMRP was assessed by RNA immunoprecipitation.

Results and Discussion: The comparative proteomic characterization of WT, FXS and UFM fibroblasts allowed us to designate some cell type-specific proteome signatures (specifically mitochondrial SOD, SOD2) and, among the altered pathways identified, mitochondrial metabolism (oxidative stress) is of particular interest considering its role in neurodegenerative diseases (like FXTAS) and particularly in epigenetic regulation.

Conclusions: Our proteome profiling study suggest that mitochondrial metabolism is likely to have a role in DNA hypomethylation of UFM cell lines.

P-713.00

PROTEOMIC CHARACTERIZATION OF CLUSTER SPOTS DOWN REGULATED IN PARKINSON DISEASE.

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Introduction

In order to identify promising candidate biomarkers contributing to the onset and progression of Parkinson disease (PD), proteomic analysis of primary skin fibroblasts from two patients affected by an early-onset PD, with parkin compound heterozygous mutations, compared to neurologically unaffected, healthy controls cells has been conducted.

Methods

Proteins were separated by 2-DE. Spot differentially expressed were identified using a Micro- mass M@LDI-LR time-of-flight mass spectrometer (Waters MS Technologies, Manchester, U.K.) and/or a Bruker Autoflex (Bruker Daltonik, Bremen, Germany), both equipped with a nitrogen UV laser .

Results and discussion

Proteomic analyses revealed variations in the expression level of several proteins in the two patients cells as compared to controls cells. Our attention has been focused on a well-resolved cluster of six spots, down regulated in both the two patients' fibroblasts, identified as vimentin, constituent of the cytoskeleton intermediate filament. These vimentin isoforms show a particular separation pathway that can be ascribed to different post-translational modification (PTMs). The study show shows phosphorylation on serine or tyrosine of the "R.QVQSLTCEVDALK.G" protein peptide at m/z = 1570, supported by an internal dephosphorylation signal at m/z = 1490, and acetylation of "K.VRFLEQQNKILLAELEQLKGQGK.S" at m/z = 2808 of proteins, supported by an increase of missed cleavage.

Conclusion

It has been reported that vimentin is overexpressed in various cancers cells where its overexpression correlates well with accelerated tumor growth and that vimentin phosphorylation is enhanced during cell division at which time vimentin filaments are reorganized. The down expression of vimentin in PD cells can be ascribed to reduction of its phosphorylation and can be related to decreased of filament reorganization process. This protein could be valuable candidate as PD biomarkers. Further analysis will be performed to confirm our data and gain information's about their relation with PD disease.

P-714.00
DOWN REGULATION OF PLASMA APOLIPOPROTEIN A-IV IN SUICIDAL ATTEMPTERS: A MASS SPECTROMETRY BASED APPROACH

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Introduction and Objective:
Suicide is a major cause of mortality and a significant health problem globally. Discovery of a biological marker could help in diagnosing subjects at high risk of attempting suicide and understanding the molecular event associated with suicidal behaviour that might help in early intervention.

Methods:
In the present study differential expression of plasma proteins in suicidal attempters were analysed using two dimensional gel electrophoresis and protein identification was done using MALDI mass spectrometry.

Results and Discussion:
The differential expression analysis based on spot intensities in 2D gel showed Apolipoprotein-AIV, Retinol Binding Protein-4 and Complement C 4A protein in plasma were significantly down regulated in suicidal attempters compared to age and gender matched healthy controls. The clinical characterisation of suicidal attempters showed that all attempts were impulsive and in response to life stressors. Apo A-IV has multiple functional roles that involves cholesterol transport as well as activation of plasma enzyme lecithin:cholesterol acyltransferase (LCAT). LCAT is involved in esterification of free cholesterol and subsequent binding to HDL. Population based studies have shown low cholesterol levels to be associated with aggressiveness, impulsiveness and violent suicide attempts. Many studies have linked serotonin dysfunction to suicidal behaviour. The serotonin-cholesterol hypothesis suggests that the lower cholesterol level may result in lower lipid microviscosity and subsequent decrease in the exposure of serotonin to its receptors on the membrane surface. Hence the reduced level of cholesterol esterification in turn is associated with impulsivity, depression, and suicidal behaviour. The down regulated proteins, RBP-4 and Complement C 4A were shown in association with inflammation responses in psychiatric disorders.

Conclusions:
Therefore the observed down regulation of Apo A-IV in plasma of suicidal attempters might indicate a low levels of esterified cholesterol and subsequently decreased activity of serotonin receptors. This finding could be a missing link in cholesterol-serotonin hypothesis.

P-715.00

AMYLOID-INDUCED TAU PHOSPHORYLATION AT MOUSE NEURONAL SYNAPSES ANALYZED BY QUANTITATIVE PHOSPHOPROTEOMICS

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Introduction and objectives:

The missing link between amyloid pathology and tau pathology is a critical issue in the etiology of Alzheimer's disease (AD). In AD brains, both Aβ and tau oligomers accumulate at the neuronal synapse, which represents a plausible site for amyloid-tau interaction. Such interactions may affect the transmission of tauopathy across the synapse into interconnected brain regions. Our goal is to understand if Aβ could drive tau hyperphosphorylation at synapses.

Methods:

Using a mouse model (APP/PS1) of amyloid pathology, we investigated changes in tau phosphorylation in isolated synaptosomes. We applied phosphopeptide enrichment and label-free quantitative mass spectrometry to study phosphorylation changes across all synaptic proteins.

Results and discussion:

We observed a pattern of subtle phosphorylation increase on synaptically distributed tau molecules in amyloid model mice, clustered around the central and the C-terminal regions of tau protein. The most significant change is elevated double phosphorylation corresponding to residues Ser199/Ser202 on human tau protein.

Conclusion:

Increased tau phosphorylation in APP/PS1 mice were relatively modest compared to tau hyperphosphorylation in human AD conditions, but they may reflect initial impacts of Aβ on tau protein and the harbinger of full-blown AD tauopathy. Phosphorylation at Ser199/Ser202 may be an early key event. We will apply this type of analysis to human brain tissues for comparison.

P-716.00

CHARACTERIZING THE UNIQUE PROTEIN COMPLEXES FORMED BY PSD95 AND SAP102 IN THE POST-SYNAPTIC DENSITY OF THE BRAIN BY IP-LC-MS/MS

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Introduction and Objectives:
The membrane-associated guanylate kinases (MAGUKs), also known as the Discs Large homolog (Dlg) family of postsynaptic scaffold proteins, directly bind NMDARs and interact with numerous other proteins to orchestrate the formation of signalling complexes found in the postsynaptic terminals of brain synapses. Both the mouse and human genome encode four Dlg family members: Dlg1 (SAP97/hDlg), Dlg2 (PSD93/Chapsyn110), Dlg3 (SAP102) and Dlg4 (PSD95/SAP90). Mutations in the genes encoding the Dlg family of synaptic scaffolding proteins as well as their interacting partners and the NMDAR subunits themselves cause various diseases with a broad spectrum of psychiatric, cognitive and motor phenotypes. We have used various proteomics methods to characterise Sap102 synaptic signalling complexes in the PSD in order to uncover how the deletion of this Dlg family member results in mental retardation and the perturbation of signalling pathways critical to cognition in the brain.

Methods:
Transgenic mice expressing eGFP-PSD95 and mKO2-Sap102 were generated in our laboratory. PSD proteins were isolated from transgenic mice expressing GFP-PSD95 and mKO2-Sap102. These proteins were analysed by BNP western blotting, 2D BN-to SDS-PAGE western blotting, IP-western, IP-LC-MS/MS and label-free quantitation and profiling by mass spectrometry.

Results and Discussion:
IP-LC-MS/MS data indicate that Sap102 and PSD95 interact with entirely unique sets of proteins in the brain. BN-PAGE to SDS-PAGE results clearly demonstrate that PSD95 protein complexes are between ~2.0 MDa – 0.8 MDa, while Sap102 complexes are between 0.8 MDa - 0.3 MDa and NMDARs are most abundant in the ~2.0 MDa – 0.8 MDa complexes.

Conclusions:
For the first time, we have shown that PSD95 and Sap102 form unique protein complexes in the adult brain. These complexes are very distinct in their molecular weight distribution and their composition. Our results clearly indicate that these two key post-synaptic signalling molecules govern cognition through unique pathways.

P-717.00

KRABBE DISEASE: FIRST INVESTIGATION AT THE PROTEOMIC LEVEL

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Introduction and objectives: Krabbe disease (KD) is an autosomal recessive disorder caused by the deficiency of galactocerebrosidase (GALC), involved in the catabolism of myelin-enriched-sphingolipids. The erroneous catabolites accumulation in oligodendrocytes and Schwann cells has a cytotoxic effect inducing demyelination and neurodegeneration of the CNS and PNS. To date, the biochemical processes regarding this pathology are poorly studied. Therefore, we performed a proteomic analysis in whole brain and serum samples from twitcher (twi/twi), heterozygous (H), and wild type (WT) mice in order to deepen the KD study.

Methods: WT, H and twi/twi samples were investigated by a 2DE/MS approach. Then, identified differences were functionally processed performing MetaCore pathway analysis and some interesting data were proved by Western blot.

Results and Discussion: The identified differentially expressed proteins, detected among the tested conditions, resulted mainly involved in cytoskeleton rearrangements, glycolysis and gluconeogenesis, protein folding and proteolysis. Our results, in line with previous morphological investigations about oligodendroglia, report an up-regulation of tubulin in twi/twi mice explaining the increased number of microtubules. We also detected over-expression in twi/twi mice of some proteins required for vesicular transport between ER, Golgi apparatus and/or plasmalemma such as SNAB and SEC13, along with ER resident chaperon, HYOU1 and CRYAB, and factors involved in protein-ubiquitination. All these findings may, at least in part, be considered an attempt by the cell to respond to high stress conditions. Worthy of note, ATP5B and Alpha crystallin B are the central hubs of the statistically significant generated net, underlining the attempt to respond to cytotoxic condition.

Conclusion: To our knowledge, this is the first proteomic attempt to characterized KD. MetaCore analysis of the differentially expressed proteins suggests the biochemical contest in which the proteins of interest act, and how their aberrant expression, not yet investigated in KD, may alter cellular and/or tissue biology.

P-718.00

QUANTITATIVE MASS SPECTROMETRY BASED COMPARISON OF THE VENTRICULAR
AND LUMBAR CEREBROSPINAL FLUID (CSF) PROTEOME

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The diagnostics of neurodegenerative diseases are commonly years delayed compared to the putative onset of the diseases themself. In regard to the demographic change biomarkers that could be used for diagnostics of neurodegenerative diseases become even more important. As of the human brain itself is relatively inaccessible for taking samples, the cerebrospinal fluid (CSF) is the logical substitute. CSF is produced in the four brain ventricles and enters from the fourth ventricle the subarachnoid space in which it surrounds the brain. It can be routinely taken out at the lumbar vertebrae.

As seral proteins can diffuse into the CSF along the flow path of the CSF, their concentration is higher with increasing distance from the source of CSF. Nevertheless brain-derived proteins show a distinct behavior. By comparing the ventricular CSF to the lumbar CSF of patients with a normal pressure hydrocephalus our goal is to establish a library for brain specific proteins. Some of these proteins could also have the potential to become a biomarker for future diagnostics. Principally, our experimental strategy relies on mass spectrometry based relative and absolute quantification. In a first exploration phase we use a Q-Exactive mass spectrometer. By ITRAQ (isobaric tags for relative and absolute quantification) labeling we are able to compare lumbar to ventricular protein abundance within each run on a relative level.

The subsequent in depth characterization is then taken out by multiple reaction monitoring with a Q-Trap 6500. GO-Term analysis and carefully examining proteins known to be either blood or brain specific revealed that the difference between ventricular and lumbar proteins indeed relates to the difference in abundance of blood derived and brain derived proteins. So far we have ITRAQ measured 4 patients and detected 195 out of 2000 proteins which have the potential to act as biomarkers

P-719.00

PPROD1 DOWNREGULATION: A MAJOR DEPRESSION BIOMARKER THAT REDUCES HIPPOCAMPAL NEUROPROTECTION THROUGH DECREASING AKT-NFĸÊB PATHWAY ACTIVITY

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Introduction and objectives: Major depressive disorder (MDD) is diagnosed by subjective clinical evaluation, leading to underdiagnosis and misdiagnosis. State-dependent biomarkers for MDD would be efficacious in improving diagnostic accuracy, but are not yet available. Stress-induced hippocampal apoptosis has been previously reported in MDD and PEDF has been shown to be neuroprotective against stress, but PEDF¡¯s mechanism in MDD, if any, remains unknown.

Methods: Proteomic analysis was applied to identify differential plasma proteins from MDD subjects relative to healthy controls. These proteins were validated by Western blotting, ELISA and Multiplex Immunoassay in MDD, bipolar, schizophrenic, nervous system diseases and healthy control subjects. The most accurate biomarker, PEDF, was further investigated through an eight-week longitudinal antidepressant study, a chronic unpredictable mild stress (CUMS) rat model of depression, and rat hippocampal neuronal cultures.

Results and Discussion: Thirty-three proteins were significantly altered in MDD subjects relative to healthy controls. Through Multiplex Immunoassay, PEDF downregulation distinguished MDD subjects from bipolar, schizophrenic, and healthy control subjects with an 88% sensitivity and 81.2% specificity. After an eight-week longitudinal antidepressant study, PEDF expression in antidepressant-responsive subjects (n=24) significantly increased, but there was no significant change in non-responsive subjects (n=7). In a CUMS rat model, PEDF downregulation was detected in the hippocampus and prefrontal lobe. In vitro, PEDF counteracted corticosterone-induced neuronal apoptosis by significantly increasing p-IĸÊB¦Á and p65 expression through the Akt-NFĸÊB pathway; the ERK1/2 pathway was ruled out as a PEDF-induced signaling cascade.

Conclusions: Plasma PEDF downregulation is an accurate, state-dependent biomarker for MDD. PEDF downregulation likely inhibits hippocampal neuroprotection in MDD patients through the Akt-NFĸÊB pathway.

P-720.00

APPLICATION OF TRANSLATIONAL PROFILING METHOD FOR THE MOLECULAR CHARACTERIZATION OF LPS-STIMULATED BV2 MICROGLIA

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Microglial cells are the resident macrophage-like cells of the brain and act as the first line defense in the central nervous system. Activation of this primary immune effector can be observed in almost all CNS pathologies, including stroke, epilepsy, neurodegenerative diseases (like ALS, AD and PD), as well as autoimmune and infectious diseases. However, the role of microglial activation still remains ambiguous.

To gain a better understanding of the molecular mechanisms involved in the activation of microglial cells, we used a translational profiling approach. This elegant method, developed in recent years, first elucidate the biological properties of distinct neuronal populations (Doyle et al., 2008; Heiman et al., 2008). Basically, the translating ribosome affinity purification (TRAP) allows the opportunity to capture gene expression after genetic alteration, disease or pharmacological perturbations. For this purpose, we generated a BV2 cell-line that stably expresses an double epitope-tagged form of the large subunit ribosomal protein L10a: Flag-EGFP-RPL10a. Cells were treated with lipopolysaccharide (LPS) (1ug/ml) to induce an inflammatory response. Cells lysates were immunoprecipitated with anti-Flag resin. Bound proteins were eluted with EDTA-elution buffer and tryptic fragments of ribosomes-associated proteins were sequenced by mass spectrometry. Scaffold was used to validate MS/MS based peptide and proteins identifications.

The TRAP analysis resulted in detection of several proteins that belong to different functional categories: cytoskeletal proteins, metabolic enzymes, chaperones, kinases, proteases and proteins involved in signalling and in proteins degradation. Interestingly, we found that some of these proteins are highly enriched in LPS treated cells compared to untreated cells.

In the present study, this differential proteomic analysis enabled us to characterize gene expression pattern within LPS-activated BV2 microglia. Finally, these observations may contribute to deciphering the molecular mechanisms involved in microglial cells activation and inflammatory response.

P-721.00

QUANTITATIVE PROTEOMIC ANALYSIS REVEALS MITOCHONDRIAL PROTEIN CHANGES IN MPP+-INDUCED NEURONAL CELLS

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Parkinson¡¯s disease (PD) is a common neurodegenerative disorder pathologically characterized by the loss of dopaminergic neurons in the substantia nigra pars compacta. To further explore potential functional mechanisms of PD, we performed a comparative proteomic analysis using stable isotope labeling with amino acids in cell culture (SILAC) combined with nano-LC tandem mass spectrometry (nano-LC MS).

In total, 1,740 proteins were identified in MPP+-treated SH-SY5Y cells. Our comparative proteomic analysis indicated that a total of 39 proteins were differentially expressed in SH-SY5Y cells responding to MPP+ treatment. Of these, 14 altered proteins were clustered in the mitochondria, 5 proteins were already reported as related to PD, and the remaining proteins were newly identified in this study.

Together, our data further define that the mitochondria play an important role in regulating PD through multiple and complex mechanisms and provide new insights into the functional contribution of mitochondrial proteins in PD.

P-722.00

A PROTEOMICS INVESTIGATION TO UNDERSTAND THE ROLE OF SUBVENTRICULAR ZONE IN THE SURVIVAL OF GLIOBLASTOMA PATIENTS

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Introduction and objectives
Glioblastoma multiforme is the most aggressive form of glioma. Imaging techniques revealed the association of subventricular zone (SVZ) region with the survival period of GBM patients. Subventricular zone is rich in neural stem cells and mutations in such stem cells may lead to the development of more invasive tumors. Recent studies have revealed the association of SVZ region with the survival of the GBM patients. Objective of our study is to decipher the serum and tissue proteomic alterations in SVZ+ (in proximity to SVZ region) and SVZ- (away from the SVZ region) GBM patients and to investigate the molecular alterations in these subtypes of GBM tumors.

Methods
The serum and tissue proteins were extracted using TCA-Acetone and trizol methods, respectively. The serum and tissue proteome of SVZ- and SVZ+ GBM patients were analyzed using 2D-DIGE and iTRAQ methods. The normal brain tissue proteome was compared with SVZ- & SVZ+ GBM patient's tumor tissue proteome using iTRAQ method. In order to reduce the complexity of the serum and brain tissue proteome, the in-solution digested and iTRAQ labeled proteome samples were subjected to off-gel fractionation followed by LC-MS analysis.

Results and Discussion
Serum proteome analysis revealed the significant alteration of acute phase proteins like hemopexin, alpha 1-antichymotrypsin and various lipid carrying proteins like apolipoprotein A1 and apolipoprotein E. The tissue proteomic analysis revealed the significant alteration of brain fatty acid-binding protein, fibrin, collagen VI, γ-Synuclein, brain acid soluble protein in SVZ+, which are associated with the survival of the cancer patients and invasive nature of the tumors.

Conclusion
We have performed a comprehensive proteomic characterization of SVZ+/SVZ- subtypes of GBM and our results provide the enhanced understanding of increased invasiveness of SVZ+ GBM tumors over SVZ- GBM tumors.

P-723.00

IDENTIFICATION OF ALTERED METABOLIC PROTEINS IN DISTINCT BRAIN REGIONS OF SCHIZOPHRENIA

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Introduction

Schizophrenia is a devastating multifactorial neuropsychiatric disorder with unknown etiology, although diverse neuropathological evidence suggests disturbance in neurotransmission system, neuro-anatomical abnormalities and impaired synaptic connectivity. In this era, proteomic findings are in prime focus because they reflect complex gene and environment interactions and is increasingly appreciated in pathophysiological mechanism of a disease state. The present study focused on the identification and elucidation of differentially expressed proteins which can be used as possible biomarkers for schizophrenia and as an indicator of disease-derived protein dysfunction.

Methods

In this study, comparative proteomic analysis of 9 human schizophrenic postmortem brain tissue (cortex hippocampus and substantia nigra) and their respective controls was performed by using two dimensional electrophoresis. The differentially regulated spots were then identified by mass spectrometry and validated by western blotting.

Results

Our data revealed differential expression of 10 proteins involved in energy metabolism, in brain tissue (cortex, hippocampus, and substantia nigra) of schizophrenics as compare to the normal controls. Among the 6 proteins of substantia nigra , phosphoglycerate mutase 1, ATP synthase subunit d, mitochondrial and malate dehydrogenase cytoplasmic are downregulted, while glyceraldehyde-3-phosphate dehydrogenase, 4-trimethylaminobutyraldehyde dehydrogenase, and alcohol dehydrogenase are upregulated. In cortex, triosephosphate isomerase, electron transfer flavoprotein subunit beta, and L-lactate dehydrogenase B chain are found to be downregulated, while phosphoglucomutase 1 showed upregulation in hippocampus.

Conclusion

The identification of these differentially expressed proteins of three distinct regions of schizophrenic brain provides a new insight for a better understanding of pathophysiology of schizophrenia. As the identified proteins are found to be potent metabolic proteins, further characterization will contribute to explore the defective metabolic pathways which may be involved in onset or/and progression of this devastating brain disorder.

P-724.00

PHARMACO-PROTEOMIC ANALYSIS OF IPS-04001 IN ALZHEIMER'S DISEASE MOUSE MODEL

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We investigated a novel mechanism of IPS-04001 on Alzheimer's disease(AD) via a pharmaco-proteomic approach using ProteoChip-based antibody microarray technology.

IPS-04001 inhibited AƒÒ plaque in AD mouse model. Proteomic analysis via antibody microarray showed upregulation of pERK, CREB, and BDNF, and downregulation of NFkB in AD mouse brain tissue treated with IPS-04001, resulting in suppression of Abeta-induced neurotoxicity. IPS-04001 appeared to be involved in inhibiting dying neuron cells in AD brain.

Taken together, these data strongly suggest that the pharmaco-proteomic approach using antibody-arrayed ProteoChip results in determining the selective molecular mechanism of IPS-04001 as a potent anti-AD agent.

P-725.00

EXPLORATIVE CLINICAL PROTEOMICS IN NEURODEGENERATIVE DISORDERS

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Introduction

Neurodegenerative disorders seem to be initiated by several slight alterations in the complex chemical machinery that is the human nervous system. Finding a functional therapy is therefore no easy task until a more exact understanding of the causal-pathway is established. Hence, there is a need for greater insight into which parts of the mechanism that are involved. In order to achieve such understanding an exhaustive study of the biochemical components of the nervous system is imperative. By ascertaining new neurochemical biomarkers it would be possible to increase the accuracy of the diagnosis as well as further mapping of the route of degenerative advancement and thereby also possibly expedite cure development.

Method and Discussion

Cerebrospinal fluid (CSF) is to constitute the main bulk of test sample utilised in this study since its composition resembles that of the extracellular environment in the brain, and LC-MS will be the principal pathway of analysis. However, CSF contains a large amount of material that is not just of little interest but that also, if not removed, makes efficient detection of low-abundance molecules impossible. Hence the primary goal of the study is to develop or improve protocols for purification of endogenous peptides hitherto undetected.

Standard reverse-phase LC will be utilised to some extent; however, in this study we will seek to develop functional and efficient protocols for CSF fractionation by means of turbulent flow chromatography (TFC) as well as reverse-phase LC over an alkaline gradient. ELISA-assays and immunoblotting will be utilised to visualise fractionation, the content will be confirmed by MS and MS2 and a comparison between controls and AD-patient will allow for determination of potential biomarkers.

P-726.00

THE PROTEOMIC LANDSCAPE OF THE SUPRACHIASMATIC NUCLEUS CLOCK REVEALS LARGE-SCALE COORDINATION OF KEY BIOLOGICAL PROCESSES

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The suprachiasmatic nucleus (SCN) serves as the master circadian pacemaker in mammals, coordinating the physiological responses of a myriad of peripheral clocks throughout the body and linking their rhythms to the environmental light-dark cycle.

In this study, we interrogated the murine SCN proteome across the circadian cycle using stable isotope labeling by amino acids in cell culture (SILAC)-based quantitative mass spectrometry. Among 3409 identified proteins in the SCN, 441 displayed a time-of-day-dependent (temporally regulated) expression profile, 100 fit a circadian expression profile with a sinusoidal waveform and a ~24 h period, and a surprising number of proteins were ultradianly expressed. Only 15% of the proteins that exhibited circadian fluctuations were also rhythmic at the transcript level, and of these there was a significant time lag (> 6 h) between the peak in mRNA and the protein rhythms.

A substantial proportion of the temporally regulated proteome exhibited abrupt fluctuations at dawn and dusk, and was involved in mitochondrial oxidative phosphorylation. Our study underscores the significance of post-transcriptional regulation, the surprising prevalence of ultradian protein expression, and the functional implications on mitochondrial energy metabolism.

P-727.00

ANTIGEN AND PEPTIDE MICROARRAYS REVEAL AUTOANTIBODY TARGETS IN NARCOLEPSY

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The incidence of narcolepsy was dramatically increased in Sweden and Finland after the national vaccination campaign against the pandemic H1N1 influenza in 2009. Children and adolescents were affected and symptoms were characterized by a higher prevalence of cataplexy as compared to narcolepsy patients with disease onset unrelated to vaccination. The disease is regarded as a rare sleeping disorder with yet unknown cause, however, the specific loss of hypocretin producing neurons together with a strong HLA association have lead to the hypothesis that autoimmune components are involved.

We therefore set out to do a broad scaled screening for novel autoimmunity targets by investigating the specificities of antibodies present in blood through antigen and peptide microarrays. In an initial screening stage, a Finnish cohort with 58 serum samples was analyzed for reactivity against 11500 antigens using planar microarrays with human protein fragments produced within the Human Protein Atlas project. Additionally, autoantibodies in narcolepsy samples were analyzed on ultra high density peptide microarrays containing 12-mer peptides with a six amino acid overlap representing all human proteins, as well as on a dedicated array for higher resolution mapping containing peptides of the same length but with an overlap of 11 amino acids. Antigens and peptides found with a higher reactivity frequency in the narcolepsy patients were selected and reactivities towards these were further investigated in an independent Swedish cohort including 179 narcolepsy patients and controls. This was performed in a suspension bead array setup where also previously published suggestions of autoimmune targets were included for profiling.

Results of the massive screening and second stage validation reveals several interesting potential autoantibody targets, which after even further exploration have the potential to enable increased understanding and could finally shed some light on the disease mechanism behind vaccine induced childhood narcolepsy.

P-728.00

PHYSICAL EXERCISE MODULATES HIPPOCAMPAL PROTEIN EXPRESSION WITH NEUROPROTECTIVE EFFECTS IN SENESCENCE-ACCELERATED SAMP8 MOUSE MODEL.

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Introduction and objectives: Regular and moderate physical activity exerts a positive influence on cardiovascular and brain health protecting cognitive and psychological functions, especially in aging and decreases the risk of developing severe neurodegenerative diseases (1-3). The molecular pathways that sustain these processes are unknown but recent data revealing positive influence of exercise on brain aging confirmed that neuroproteoma changes seem to be involved in the molecular mechanisms in cognitive processes, to prevent brain damage (4). Proteomic approach could define these mechanisms probably correlated to the benefits of physical activity on brain decline. The goal of our investigation is to enhance knowledge regarding biochemical processes that sustain the hippocampus response to exercise in aging using a mouse strain SAMP8 that has a low incidence of other phenotypic changes when it develops deficits in learning and memory.

Methods: We performed a comparative proteomic analysis between trained (3 sessions/week on treadmill) and sedentary animals at the age of 8 weeks up to 24 weeks, analyzing SAMP8 hippocampal proteome using 2-DE combined with MALDI LIFT-TOF/TOF MS experiments.

Results and Discussion: In the trained group, we identified relevant differences in protein expression when comparing it to the sedentary group. PANTHER analysis of neuroproteome changes revealed that most of the differentially expressed proteins are involved in metabolic processes, energy generation, cellular processes, apoptosis, transport, cytoskeleton organization and cellular response.

Conclusion: We conclude that different regulation of protein expression could be implicated in important cognitive functions, providing useful information for achieving and maintaining mental health, preventing the risk of future dementia and delaying the development of age-related impairment.

P-729.00

EFFECT OF VITAMIN D ON MULTIPLE SCLEROSIS: NEUROCOGNITIVE IMPAIRMENT IMPLICATIONS

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Vitamin D deficiency has been associated with multiple sclerosis (MS). Due to the high prevalence of MS in Puerto Rico and worldwide, there is an urgent need to study the synergism between this severe demyelinating disease and Vitamin D deficiency (VDD). The presence of cognitive impairment in MS is well-documented and includes deficits in memory and speed of information processing, among others.

The purpose of this study is to reveal the cellular and molecular underlying mechanisms of MS, to determine the severity and pattern of neurocognitive deficits and the consequence of VDD. The EIWA-III was used to measure neurocognitive impairment. Peripheral blood mononuclear cells (PBMC) from MS patients with deficient/insufficient serum levels of Vitamin D were compared to PBMC from control subjects.

Two-dimensional gel electrophoresis (2D-GE) followed by mass spectrometry was used to establish homologies and differences in protein expression. Differentially expressed spots that met the criterion for a 2-fold or greater change that were significant at p

Significant differences in Verbal IQ, Performance, Total and indexes EIWA-III, suggest that the level of Neurocognitive Impairment is higher for MS patients when compared to non-MS controls.

The role of these proteins and their correlation with MS patients with VDD and neurocognitive impairment will help elucidate MS conditions associated with immune alterations.

P-730.00

A CHEMICAL PROTEOIMCS FOR SEARCHING DIAGNOSTIC MARKERS OF PARKINSON□FS DISEASE.

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Introduction and Objectives□F

Early diagnosis of Parkinson□fs disease (PD) before occurring motor symptoms is highly desirable, because most of dopamine-generating cells in the substantia nigra are dead at that time. PD biomarkers are useful as the target for such diagnosis; furthermore cerebrovascular PD biomarkers are preferable in the point of better accessibility of diagnostic product. However such PD biomarkers are not established well. Here we searched PD biomarkers which are expressed on the cerebral blood vessel, by using a chemical proteomics which is a powerful method to identify proteins accessible from the blood flow.

Methods□F

Acute mouse MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) model was chosen as an early PD model. Membrane-impermeable reactive biotin was perfused in vivo to biotinylate proteins accessible from blood flow (e.g. proteins expressed on the vasculature). Biotinylated cerebral proteins were captured on the streptavidin-coated magnetic beads; then tryptic digests were labeled with iTRAQ reagent. These samples were prepared from normal mice and PD model mice; then analyzed by LC-MS/MS.

Result and Discussion□F

We confirmed that biotinylation of the vasculature was accomplished in both normal mice and PD model mice using immunostaining. A total of 1138 proteins were identified as the result of identification of biotinylated proteins, 16 proteins showed high expression of more than 2.5 times in PD model mice and 12 of 16 proteins are classified as membrane proteins. These membrane proteins could be highly expressed in the blood vessels of the brain of PD model mice. We are now trying to validate the candidate proteins and to evaluate the usefulness of such proteins as cerebrovascular marker of PD.

Conclusions□F

These results indicate that we were able to identify candidates of PD cerebrovascular markers by using a chemical proteomics.

P-731.00
STUDY ON THE TREATMENT MECHANISM OF VITAMIN D IN MS/EAE

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Introduction and Objectives
Recently, the relationship between Vitamin D (VD) and multiple sclerosis (MS) has been a hot area of research. But there is a lack of a systematic study, and the mechanism of action of VD in MS is not yet clear. Our study is to investigate the protective mechanisms of VD and to identify additional factors that might limit VD efficacy.

Methods
EAE rats were separated into two groups based on their diet, supplemented with vitamin D (+D) or without vitamin D (-D). We performed 2-D DIGE-based proteomic analysis in spinal cords from EAE rats(-D/+D), followed by MALDI-TOF mass spectrometry. In the +D EAE rats, gelsolin (GSN), DBP and some other proteins showed significant difference in the spinal cords levels compared with ¨CD group. Subsequently, we chose GSN and sphingosine 1-phosphate (S1P: a lipid molecule which could interact with GSN) as the main target with deep reaserch.

Results
Our study demonstrates that S1P is upregulated in serum and spinal cords of EAE rats, but that VD reverses the upregulation to alleviate inflammation. We assessed the effects of VD on plasma gelsolin (pGSN), a regulator of S1P that is downregulated in the CSF of MS patients. Our results also show that pGSN is downregulated in the serum of EAE rats, whereas its cellular form, cytoplasmic gelsolin (cGSN) is upregulated in the spinal cord of EAE rats. Importantly, VD causes a down-regulation of both pGSN and cGSN, which may counteract the positive effects of S1P decrease.

Conclusions
These results support a therapeutic effect of VD that is derived from its ability to reduce S1P, but is limited by its simultaneous effect in reducing pGSN and cGSN. Based on these observations, we postulate that combined therapy with recombinant human pGSN and VD may produce more beneficial effect in treating MS.

P-732.00

QUANTITATIVE PROTEOMICS TO STUDY NEUROMUSCULAR DISORDERS

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Introduction: Cellular accumulation and aggregation of misfolded proteins is connected to several neuromuscular disorders. Marinesco-Sjögren Syndrome (MSS; OMIM 248800) is a heterogeneous multisystemic disorder, although rare and genetic, the patients suffer from skeletal muscle, eye and brain abnormalities. Genetic studies show that mutations in the SIL1 are responsible for this disease (~50% cases). SIL1 is a nucleotide exchange factor (NEF) for HSP70 chaperone BiP (GRP78). The latter is associated with the biosynthesis of secretory and membrane proteins in the lumen of endoplasmic reticulum (ER). However, it is not clear why the loss of SIL1 affects only certain tissues/organs.

Methods: We therefore performed a comparative analysis using iTRAQ and label free approaches to investigate the underlying proteomic changes taking place due to disrupted protein folding mechanism in the lumen of ER.

Results: From our results we could confidently identify and quantify 4872 proteins from human lymphoblasts (unaffected tissue from patients) and nearly 1630 proteins from mice (woozy-mouse model of MSS) muscle tissue, respectively. In the both datasets, we found regulation of several proteins which are involved in the activation of unfolded protein response (UPR) pathway, like BiP and MHC class proteins.

Conclusions: Our data correlates with the morphological findings observed in electron microscopic studies and the Western blot results. Moreover, a part of these proteins are also related to other neurodegenerative disorders and hence will be specifically monitored using targeted MS/MS approaches. Currently, analysis of woozy mice brain proteome is carried out with quantitative proteomics approaches. Abbreviations: SIL1: suppressor of Δire1 Δlhs1 double mutant number 1; HSP70: heat shock protein 70; BiP: immunoglobulin heavy chain binding protein; iTRAQ: isobaric tags for relative and absolute quantification.

P-733.00

PROTEOMIC ANALYSIS OF THE FRONTAL CORTEX AND THE HIPPOCAMPUS MITOCHONDRIA IN THE ANIMAL MODELS OF DEPRESSION

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Introduction and objectives

Major depression affects increasing number of people each year. Recently mitochondrial dysfunction and oxidative stress have been shown to be associated with the pathogenesis of depression. Using proteomic approach we investigated the differences in mitochondrial proteins expression in animal models of depression - prenatally stressed rats and acute LPS–treated prenatally stressed rats.

Methods

Pregnant Sprague-Dawley rats were subjected to stress sessions from 14th day of pregnancy until the delivery. At 3 months of age, control and prenatally stressed rats were tested in forced swimming test. After behavioral verification single LPS injections were given intraperitoneally in a dose of 250 μg/kg body weight. Next, the frontal cortex and the hippocampus were dissected and mitochondria were isolated. 2-D electrophoresis coupled with tandem mass spectrometry were used to investigate differentially expressed mitochondrial proteins.

Results and Discussion

We identified several differentially expressed mitochondrial proteins in the frontal cortex and the hippocampus of prenatally stressed and LPS-treated rats. Proteins related to oxidative phosphorylation were downregulated in the mitochondria from frontal cortex and the hippocampus of prenatally stressed rats. Moreover, pyruvate dehydrogenase – enzyme, which links the glycolysis pathway to the Krebs cycle, was strongly downregulated in the hippocampus of prenatally stressed and LPS–treated rats. LPS administration resulted in downregulation of proteins related to oxidative stress in the hippocampal mitochondria, especially 3-mercaptopyruvate sulfurtransferase (MST) that produces hydrogen disulfide in the brain.

Conclusions

Our results indicate that prenatally stressed and LPS–treated prenatally stressed rats exhibit altered mitochondrial proteins expression in the hippocampus and frontal cortex. The exact functional consequences of the revealed alterations require further investigation.

P-734.00

APPLYING MASS SPECTROMETRY-BASED QUALITATIVE PROTEOMICS TO HUMAN AMYGDALOID COMPLEX

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The amygdaloid complex is a key brain structure involved in the expression of behaviours and emotions such as learning, fear, and anxiety. Brain diseases including depression, epilepsy, autism, schizophrenia, and Alzheimer`s disease, have been associated with amygdala dysfunction. For several decades, neuroanatomical, neurophysiological, volumetric, and cognitive approaches have been the gold standard techniques employed to characterize the amygdala functionality. However, little attention has been focused specifically on the molecular composition of the human amygdala from the perspective of proteomics.

We have performed a global proteome analysis employing protein and peptide fractionation methods followed by nano-liquid chromatography tandem mass spectrometry (nanoLC-MS/MS), detecting expression of at least 1820 protein species in the basolateral region of the human amygdala, corresponding to 1814 proteins which represent a 9-fold increase in proteome coverage with respect to previous proteomic profiling of murine amygdala. Gene ontology analysis were used to determine biological process represented in human amygdala highlighting molecule transport, nucleotide binding, and oxidoreductase and GTPase activities. Bioinformatic analyses have revealed that nearly 4% of identified proteins have been previously associated to neurodegenerative syndromes, and 26% of amygdaloid proteins were also found to be present in cerebrospinal fluid (CSF). In particular, a subset of amygdaloid proteins was mainly involved in axon guidance, synaptic vesicle release, L1CAM interactome, and signaling pathways transduced by NGF and NCAM1.

Taken together, our data contributes to the repertoire of the human brain proteome, serving as a reference library to provide basic information for understanding the neurobiology of the human amygdala.

P-735.00

QUANTITATIVE MASS SPECTROMETRY IDENTIFIES AGE-DEPENDENT CONTROL OF PROTEIN TRANSLATION BY FRAGILE X MENTAL RETARDATION PROTEIN

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Introduction and objectives

Fragile X Syndrome (FXS) is an X-linked monogenic disorder with devastating neurodevelopmental abnormalities including mental retardation. The disease-causing gene encodes FMRP, a RNA binding protein that suppresses the activity-dependent translation of a large number of proteins in neuronal synapses. Although mRNAs that associate with FMRP has been extensively studied, little is known regarding the protein targets that are directly influence by FMRP. This study aims to profile proteome alterations in the neocortex of a mouse model of FXS (fmr1 knockout, KO), and to further measure alterations in de novo protein synthesis between KO and WT mice.

Methods

We utilized N15 labeled mouse brain (SILAM) as an internal standard, combining at 1:1 protein ratio with either WT or KO cortices for mass spectrometry analysis to quantify proteome changes. We further cultured cortical neurons from WT and KO mice, and applied 3-plex pulsed SILAC after mGluR5 activation to labeled newly synthesized proteins. As a complementary method, we purified polyribosomes from WT and KO cortices, and applied the ribosome run-off assay by biotinated puromycin to capture nascent polypeptides for mass spectrometry analysis.

Results and Discussion

We discovered that while numerous proteins are upregulated in the absence of fmr1 in young mice when active synaptogenesis is ongoing, this upregulation largely diminished in adult mice. Furthermore, we found that neurons derived from KO mice produce nascent proteins at a higher ratio than that of WT mice, many of which are direct FMRP targets. These observations were corroborated by Western blot analysis of acute cortical slices, as well as puromycin captured nascent polypeptides from cortices.

Conclusions

Our study found that FMRP control of protein synthesis is age dependent, and this dependency is largely due to the expression changes of FMRP that confers the translation control at the elongation stage.

P-736.00

HIPPOCAMPAL PROTEIN CONCENTRATION GRADIENTS – THE KEY TO AUTOIMMUNITY IN ALZHEIMER'S DISEASE?

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Alzheimer Disease (AD) is the most common form of dementia. Regarding the last 20 years of scientific research we conclude that the reason for AD might be more complex then known so far. Engelhardt and colleges (Engelhardt et al., 2000) demonstrated that the injection of human IgGs from AD patients into the basal forebrain of living rats results in a loss of cholinergic neurons. Giving special tribute to this work we postulate that AD is a disorder with a autoimmune character which starts in the early lifespan of human beings.

Furthermore Braak et al. (2000) observed that neuronal damage of the different regions of the human hippocampus (CA1, CA2, CA3, fascia dentata) occurs in a time dependent matter, a process which might be also forced by an autoimmune reaction. It is known that the hippocampal region CA1 is affected early in AD while damage of the CA2, CA3 region and the fascia dentata is observed at later stages. Taken all this together we decided to analyze the content of these hippocampal regions of interest and perform a differential proteomic study by a label free LC-MS/MS approach combined with a couple of functional analyses.

Laser-microdissection was used to separate the hippocampal regions of interest. Then proteome identification and differential quantification follows. We used 6 biological replicates and were able to identify specific hippocampal protein concentration gradients of prominent candidate proteins, which reflect the differences of the hippocampal regions of interests.

P-737.00

CROSS-LINKED SITES MS ASSIGNMENT IN A PHOTOCHEMICAL INDUCED-COVALENT AB DIMER

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Introduction and objectives

Amyloid-β (Aβ) dimers from the brains of Alzheimer´s disease (AD) patients play a key role in this pathology. Characterizing the structure of Aβ dimers is therefore crucial in the AD field. We are using well-defined synthetic Aβ dimer mimics to study their structure. Here we present a method for the characterization of cross-linked sites in Aβ dimers based on LC-MS/MS and parallel searches by using two different search engines.

Methods

Cross linked Aβ dimer samples were prepared using photo-induced cross-linking of unmodified proteins (PICUP) [1-2]. CL Aβ dimers were purified by either HPLC-PDA or SEC and digested using different enzymes (trypsin and Glu-C). The resulting peptides were analyzed by nano-LC-MS/MS. To identify CL peptides and determine cross-link positions, all data was processed using two search engines: MassMatrix [3] and StavroX [4]. To achieve final positive identification of CL sites, a cutoff value was established.

Results and Discussion

The analysis of CL digested peptides by LC-MS/MS revealed several peaks consistent with the same mass, meaning that distinct peptides with the same amino acid sequence but having different cross-linked sites were present in the sample. Data analysis was performed by using two different search algorithms and CL positions were assigned. Those CL sites involved residue pairs such as Y-Y and Y-H. One set of cross-links was consistent with the two Aβ chains having a face-to-face arrangement, whereas another set was not.

Conclusions

A method for CL sites determination was established and used for the characterization of synthetic CL Aβ dimers. This approach could be used to assign CL sites in native samples.

P-738.00

VITAMIN D BINDING PROTEIN ISOFORMS AND APOLIPOPROTEIN E IN CEREBROSPINAL FLUID AS EARLY PROGNOSTIC BIOMARKERS OF DISEASE AGGRESSIVENESS IN MULTIPLE SCLEROSIS

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Introduction and Objectives: The study of inter-individual differences in cerebrospinal fluid (CSF) proteome may contribute to the discovery of markers useful for the diagnosis and prognosis of multiple sclerosis (MS).
To identify such biomarkers, if any, a double-blind study was performed on the proteome of CSF coming from diagnostic lumbar puncture in 24 untreated women with possible relapsing-remitting MS.

Methods: A bi-dimensional electrophoresis (2-DE) study was performed according to standardized procedures. Patients were followed up for 5 years and subdivided into subsets according to pre-established clinical criteria.

Results and Discussion: On the basis of the percentage volume (%Vol) of 240 spots, hierarchical cluster analysis was carried out and a panel of spots was highlighted and identified by mass spectrometry analysis. Among them, Apolipoprotein E (ApoE) and two isoforms of vitamin D binding protein (DBP) stratified the patients in three clusters which correlated with the disease clinical course. Statistical analysis confirmed a strong ability of this method to predict the aggressive forms of MS (80%).
The two DBP isoforms were inversely related, suggesting a post-translational modification (PTM).

Conclusions: Our study suggests that ApoE and the newly described isoforms of DBP in CSF could represent early biomarkers of disease aggressiveness.

P-739.00

THE HUMAN TORSIN A IN DROSOPHILA ACTIVATES THE UPR AND INCREASES SUSCEPTIBILITY TO OXIDATIVE STRESS

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DYT1 dystonia is caused by a glutamic acid deletion (ΔE) mutation in the Torsin A in humans (HTorA). We found that the amount of proteins and transcripts of an ER resident chaperone Heat shock protein cognate 3 (HSC3) and a mitochondria chaperone HSP22 were significantly increased in the HTorAΔE–expressing brains compared to the HTorAWT-expressing brains.

The physiological consequences of altered HSC3 and HSP22 in HTorAΔE flies included increased susceptibilities to oxidative and ER stress compared to the HTorAWT flies. The alterations of transcripts of IRE1-dependent spliced Xbp1, several ER chaperones, a nucleotide exchange factor, ATG8b and components of the ER associated degradation (ERAD) and increased expression of the Xbp1-eGFP in HTorAΔE brains strongly indicated the activation of the unfolded protein response (UPR). In addition, perturbed expression of the UPR sensors and inducers in the HTorAΔE Drosophila brains induced significantly reduced life span of flies.

Furthermore, the quality and quantity of proteins present in the anti-HSC3 positive microsomes in the HTorAΔE brains were different from those of the HTorAWT brains. Taken together, HTorAΔE in Drosophila brains may activate the UPR and increase the expression of HSP22 to compensate for the toxic effects caused by HTorAΔE in the brains (KBSI E34300).

P-740.00

INVESTIGATING THE ROLE OF LG72 IN SCHIZOPHRENIA BY 2D-GEL ELECTROPHORESIS AND 15N METABOLIC LABELING

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Introduction and objectives
G72/G30 is a primate-specific gene locus which encodes the LG72 protein. Genetic studies have correlated the G72/G30 locus with major psychiatric disorders such as schizophrenia and bipolar disorder. However, the function of the LG72 protein remains controversial, with recent evidence pointing towards a mitochondrial localization/involvement. Our aim was to investigate the role of the LG72 protein using quantitative proteomics.

Methods
To investigate the role of the LG72 protein in vivo, we generated transgenic mice (G72Tg) which exhibited schizophrenia-like symptoms. To assess the effects of the LG72 protein at the proteome level, we compared the cerebella of G72Tg and wild type mice with two different quantitative proteomics approaches, in vivo 15N metabolic labeling and 2D-gel electrophoresis. Proteomics data were analyzed by in silico pathway approaches and validated by immunochemical assays.

Results and Discussion
We found partially overlapping protein expression differences by the two quantitative proteomics methods, indicating their complementarity. Protein expression alterations were involved in myelination, oxidative stress and mitochondrial pathways. In silico analysis revealed a regulatory function of apoptosis.

Conclusions
Our data support a mitochondrial role of the LG72 protein in brain circuits and reveal pathways relevant to pathomechanisms of psychiatric disorders.

P-741.00

DETECTION AND QUANTIFICATION OF TAU PROTEOFORMS IN THE CSF OF ALZHEIMER'S DISEASE PATIENTS USING HIGH RESOLUTION TARGETED MASS SPECTROMETRY

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Introduction And Objectives

Detection of biomarkers in the cerebrospinal fluid (CSF) has proven to be useful to follow and understand the metabolism of molecular actors of Alzheimer's disease (AD), as well as, for diagnosis purposes. Immunodetection, which is mostly used, has sometime a poor specify and a limited differentiation of proteoforms which are often very informative. This is particularly true for the tau protein which is subjected to many modifications including truncation, hyper-phosphorylation or aggregation. Importantly, the understanding of tau metabolism and implication in AD pathology is a major challenge as this molecule is now a major therapeutic target and diagnosis biomarker.

Methods

We developed a strategy termed Sequence Quantitative Analysis (SEQUANA) that combined the in-depth protein sequence analysis by sensitive multiplexing peptide capability of high resolution targeted mass spectrometry (PRM on Q-Exactive) with protein absolute quantification (based on recombinant proteins). It allowed after acidic precipitation combined to solid phase micro-extraction (µSPE), but without immunoprecipitation, the highly reproducible, sensitive, quantitative follow-up of 22 peptides covering tau N-terminus, central core and C-terminus; some of them being exon/isoform dependent. This representative set of peptide allowed monitoring stoichiometry profile linked to the presence of the different tau proteoforms in the CSF. Correlation with tau and p-tau ELISA results confirmed the relevance of the assay.

Results And Discussion

We uncovered the human physiological CSF tau proteoform expression profile characterized by a predominance of central core fragments and 1N/3R isoform expression. In pathological conditions (AD, FTLD, LBD..) the global profile was conserved, but specific peptide stoichiometry modifications, most likely linked to phosphorylation, were observed depending on the diagnosis.

Conclusions

Our new SEQUANA method can be seen as an intermediate between top-down and bottom-up approaches. Applied to tau in the CSF it revealed tau proteoform diversity which eventually allowed us to identify Alzheimer's disease specific pattern.

P-742.00

MS STRATEGY FOR IDENTIFICATION OF ALPHA-SYNUCLEIN PROTEOFORMS

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Introduction and objectives
Strong genetic and neuropathological evidence suggests that α-synuclein has a central role in the development of synucleinopathies, of which the most common is Parkinson's disease. The molecular factors that contribute to triggering α-synuclein aggregation and Lewy body formation remain unknown, however there are some indications that genetic mutations, posttranslational modifications promote α-synuclein aggregation.
Our aim is to establish a mass spectrometry-based assay for identification and quantification of α-synuclein and it´s different forms initiated by truncation, alternative splicing, mutation, etc.

Methods
Brain tissues from different mouse models were used in our experiments. Targeted and non-targeted MS analyses were performed on a TSQ Vantage and/or a QExactive mass spectrometer following nano-LC separation. Proteome Discoverer and Skyline software were used for database search and quantitative data evaluation, respectively.

Results and discussion
Various brain regions of human α-synuclein expressed mouse models were analyzed in this study. In order to get better sequence coverage different proteolytic enzymes were used in sample digestion. The digested samples were analyzed by nano-LC-MS/MS using different data acquisition modes, such as DDA with and without inclusion list for identification and targeted-SIM for quantification of α-synuclein forms. In addition nano-LC-MRM-MS were performed targeting signal peptides representing α-synuclein isoforms and natural variants. Database search were done against a modified mouse database containing all the possible human α-synuclein proteoforms with the aid of Proteome Discoverer.

Conclusions
Mass spectrometry-based qualitative and quantitative data supplement results obtained by other methods; and the analysis of animal models and human samples by the adapted MS technique helps better understand disease mechanism.

P-743.00

ORBITRAP PROTEOMICS ANALYSIS OF CSF TO IDENTIFY NOVEL MARKERS FOR PROGRESSION AFTER A FIRST ATTACK OF DEMYELINATION

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Background
The course of the disease after clinically isolated syndrome (CIS), the first presenting symptom of Multiple Sclerosis (MS), is highly variable, and therefore a strong need for biomarkers exists.

Methods
We included 47 CIS patients with CSF samples, clinical and MRI data collected within 2 months after symptom onset, and 64 controls. CSF samples were enzymatically digested and subsequently separated using a 180 minute chromatographic gradient on a 50 centimeter C18 column before measurement on an Orbitrap mass spectrometer. The mass spectra were analyzed using specialized software (Progenesis LC-MS, version 4.0), and the identified proteins were analyzed for statistically significant abundance between groups.

Results
A total of 3009 peptides were identified, relating to 555 proteins. Only 2 proteins were significantly more abundant in CSF of CIS patients than in controls: chitinase 3-like protein 1, and Ig kappa chain VII region. Eleven proteins were lower in CIS patients than controls (Voltage-dependent calcium channel subunit alpha-2/delta-1, Seizure 6-like protein 2, Calsyntenin-3 , Neurosecretory protein VGF, Superoxide dismutase [Cu-Zn], Ribonuclease pancreatic, Trans-Golgi network integral membrane protein 2, Extracellular matrix protein 1, Transmembrane protein 132A, Cerebellin-3, Xylosyltransferase 1). There were no significant differences in protein levels between patients who did and did not reach a diagnosis of clinically definite MS. We also found no differences related to the number of MRI lesions, type of CIS or fatigue.

Conclusions
This study confirms the earlier finding of chitinase 3-like protein 1 in the pathology of MS when compared to controls, but does not confirm its association with conversion to clinically definite MS. A striking finding is that most found proteins were actually lower in CIS patients than in controls, which is counterintuitive but might lead to new insights into the pathophysiology of MS.

P-744.00

CHANGES IN THE HUMAN HIPPOCAMPAL PROTEOME DURING ALZHEIMER'S DISEASE

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Introduction. Alzheimer's disease (AD) is the most common form of dementia for which currently no cure is available. To gain insight into the molecular mechanisms of AD we have determined the proteome of the CA1 and subiculum regions of human hippocampal brain tissue. This region represents one of the most vulnerable and early affected sites in AD brains. In this study we aim to identify early changes in the human proteome and underlying disease mechanisms.

Methods. A total of 56 patients representing the seven stages of AD (Braak 0 to VI) were selected based on the clinical and neuropathological diagnosis. Hippocampal subregions were isolated from human post-mortem brain tissue using laser microdissection and protein lysates were subjected to SDS-PAGE, followed by in-gel trypsin digestion. Extracted peptides were analysed by LC-MS/MS using Orbitrap mass spectrometry. MaxQuant software was used for protein identification and quantification. Validation was performed by immunoblotting and immunohistochemistry.

Results. We identified a total of 3283 proteins. A subset of 300 proteins showed either progressive up or down regulation from Braak 0 to Braak stage VI. As expected, proteins like GFAP and tau were increased with increased Braak stage. We also identified proteins that have not been associated with AD before. Cluster analysis revealed groups of proteins with distinct profiles like an "early up late down" pattern. Signalling pathways that are regulated during the early stages of AD pathology were identified.

Conclusions. Combining laser microdissection and quantitative proteomics enabled us to obtain an extensive representation of the human proteome of the hippocampal subregions at different stages of AD pathogenesis. The validity and reliability of our approach is illustrated by the identification of a number of proteins previously shown to change during the progression of AD. Newly identified proteins and pathways provide insight into the complex biology of AD pathology.

P-745.00

ITRAQ-BASED QUANTITATIVE PROTEOMICS STUDY ON THE NEUROPROTECTIVE EFFECTS OF BUYANG HUANWU DECOCTION IN CEREBRAL ISCHEMIC STROKE MICE MODEL.

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Introduction and objectives: Stroke is one of the top five leading causes of death in the worldwide, and most of the cases are caused by ischemic stroke. In view of unmet need for the safety and efficient treatment in ischemic stroke, there is great urgency to develop drug with more potency and lower toxicity. Buyang Huanwu Decoction (BHD), a famous traditional Chinese medicine (TCM) prescription, has long been used for improving neurological functional recovery in stroke by inducing neuroprotective effects against cerebral ischemia-reperfusion (CI/R) injury.

Methods: We characterized the neuroprotective effects of BHD on CI/R mice by iTRAQ proteomics approach using the nanoUPLC/MS/MS, and further confirmed the target proteins by Western blotting.

Results and Discussion:
Results showed that treatment with BHD (1 g/kg, p.o., twice daily) significantly ameliorated the damage to brain function caused by CI/R injury. BHD dramatically recovered the locomotor activity by enhanced the tracking distance and showed a normal mice behavior compared with that of the sham group. After iTRAQ analysis, 1310 and 1206 proteins were identified and quantified, respectively. Among them, 877 quantitative proteins were in the intersection of three biologic repeats, and 10.26% (90/877), 1.71% (15/877) and 2.62% (23/877) proteins significantly changed in CI/R, BHD treatment, and tissue plasminogen activator (TPA) treatment, respectively. Moreover, BHD plays as a GSK-3 inhibitor via activated Akt and its downstream MAPK pathway in ischemic stroke mice.

Conclusions:
In this study, the quantitative proteomics was performed to investigate the effects of TCM and unraveling the mechanically effects of BHD on the stroke mice, and provide the molecular evidences that BHD is beneficial for the ischemic stroke.

P-746.00

PROTEOMICS OF SYNAPTOSOMAL FRACTIONS TO MAP PREFRONTAL CORTEX PROTEINS DURING ANHEDONIA

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Introduction

The search for molecular markers of depression is important for broadening our knowledge on the biology of depression. The chronic mild stress model is a highly validated rat model of depression in which both behavioral and molecular studies can be performed. To study the biology of neurotransmitters, one can use synaptosomes, which are artificial structures obtained by homogenization and centrifugation of neuronal tissue. Synaptosomes maintain function and contain the presynaptic terminal, mitochondria, cytoskeleton, and parts of the postsynaptic terminal. Protein expression studies in synaptosomes are therefore suitable for the elucidation of the detailed molecular mechanisms behind the depression phenotype.

Objectives

The two main objectives were: 1) to map proteomic composition in three different synaptosome gradient fractions 2) to compare synaptosome proteomics data from anhedonic, control and resilient animals.

Methods

A discontinuous Percoll gradient was used to prepare synaptosomes from homogenized brain prefrontal cortex tissue from control rats and rats exposed to chronic mild stress. Tree gradient fractions (F2, F3, F4) were studied by iTRAQ based proteomics using nanoLC-MS/MS (LTQ Orbitrap).

Results

Our proteomics results confirmed previous functional studies describing that fraction F3 and F4 contain proteins important for synapse function. However, our data also show that for proteomics studies, e g expression and PTM studies, also F2 fraction is important since it contains high amounts of synaptic proteins. The differentially regulated proteins in brain cortex during depression were shown to belong mainly to mitochondrial functions (e g respiratory chain), cytoskeleton (tubulins, glial fibrillary acidic protein, spectrin etc), but also to oxidative stress (peroxiredoxins).

Conclusions

Synaptosome proteins from prefrontal cortex were shown to be regulated by the chronic mild stress induced depression. In addition to proteins directly involved in neurotransmission also cytoskeletal and mitochondrial proteins were shown to be related to the depression phenotype.

P-747.00

BIOMARKER IDENTIFICATION IN ALZHEIMER´S DISEASE BRAIN TISSUE AND RELATIONSHIP TO GENETIC RISK FACTORS

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Alzheimer's disease (AD) is the most prevalent cause of dementia in developed countries. The vast majority of affected individuals are sporadic cases with no inherited familial history. Hence, new genome-wide association studies evidence novel genomic risk factors. All of these aspects make AD a big challenge for research community in order to find early and specific diagnosis markers, therapeutic targets as well as effective treatment.

The search of new gene, protein, signaling pathways candidates becomes indispensible to achieve these objectives. In parallel to genomic investigation, the proteomics field offers a wide range of possibilities to identify new biomarkers and also bridge the proteome modifications to genomics risk factor. We have developed an optimized a protocol to perform two dimensional fluorescence difference gel electrophoresis (2D-DIGE) of human and mice brain tissues. Using this method, the proteome of control and AD brain tissues were compared as well as the mouse brain tissue of our transgenic AD model of neurofibrillary degeneration called Thy-Tau22. Markers observed to be modified in AD and in Thy-Tau22 were selected and further studied. The use of immunohistochemistry allowed us to establish the potential direct or indirect association of these biomarkers with the neuropathological hallmarks of AD. Our data point out the fact that 2D-DIGE allows to observe protein expression changes but further validation is needed since many of the variations were due to post-translational modifications and not only to a expression changes as exemplified by the genetic risk factor PTK2B.

These markers showed in this study shed light into the pathways underlying NFD in human throughout the common findings observed in this mouse model. Besides protein identification, supplementary characterization is needed to increase the suitability for the use of these biomarkers in the prognosis and their utilization as eventual pharmacological targets in AD.

P-748.00

AN ION MOBILITY MASS SPECTROMETRY APPROACH TO ELUCIDATE THE MOLECULAR MECHANISMS UNDERLYING AGGREGATION IN NEURODEGENERATIVE DISEASES

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Several neurodegenerative disorders, such as amyotrophic lateral sclerosis (ALS) and familial amyloidotic polyneuropathy type I (FAP-I) are characterized by the aggregation of a specific protein. However, the molecular mechanisms underlying aggregation are not clear yet. Some authors have proposed that aggregation could be triggered by protein misfolded intermediates or certain proteoforms (isoforms, glycoforms, etc.), as well as by monomerization when the protein biomarker is an oligomer.

In this study we propose to use a novel analytical technique, nano-electrospray ionization-ion mobility mass spectrometry (nanoESI-IM-MS), as a reliable tool to gain insight into the protein oligomers found under near native conditions, while also collecting information about the amino acid sequence, the metal content or the post-translational modifications.

We will present our results with superoxide dismutase 1 (SOD 1) and transthyretin (TTR). SOD 1 aggregates are characteristic of ALS. The native form of this metalloenzyme is a homodimer whose dissociation to form the monomer has been proposed as a trigger of abnormal oligomerization and later aggregation. TTR is a homotetrameric protein which is known to aggregate in FAP-I. FAP-I is associated with a TTR variant that presents a single amino acid substitution of valine for methionine at position 30 (Met 30). However, as in ALS, the mechanism that triggers the onset of protein aggregation and disease is unknown.

In both cases we demonstrate that using the optimum sample pretreatment methods and analysis conditions to minimize protein complex dissociation, nanoESI-IM-MS allows the characterization of SOD-1 and TTR at near native conditions from standards and blood samples of control individuals and patients. The information collected is crucial to find significant differences on the abundances of the monomeric and oligomeric SOD-1 and TTR proteoforms between control and patient samples.

P-749.00

VERIFICATION OF A PARKINSON'S DISEASE PROTEIN SIGNATURE IN T-LYMPHOCYTES BY MULTIPLE REACTION MONITORING

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Diagnosis of Parkinson's disease, the second most common neurodegenerative disease, is based on the appearance of motor symptoms. A panel of protein biomarkers in the T-lymphocyte proteome was previously proposed as a Parkinson's disease signature. Here, we designed an LC-MS based method to quantitatively evaluate this protein signature by multiple reaction monitoring (MRM) in T-lymphocytes and peripheral blood mononuclear cells from a new cohort of nine patients with Parkinson's disease and nine unaffected subjects.

Patients were classified using the discriminant function obtained from two-dimensional electrophoresis and protein amounts measured by MRM, thus assigning seven controls out of nine as true negatives and nine patients out of nine as true positives. A good discriminant power was obtained by selecting a subset of peptides from the protein signature, with an area under the receiver operating characteristic curve of 0.877. A similar result is achieved by evaluating all peptides of a selected panel of proteins (Gelsolin, Moesin, Septin-6, Twinfilin-2, Lymphocyte-specific protein 1, Vimentin, Transaldolase), with an area under curve of 0.840.

Conversely, the signature was not able to classify the enrolled subjects when evaluated in whole mononuclear cells. Overall, this report shows the portability of the proposed method to a large-scale clinical validation study.

The proteome quest
to understand
biology and disease

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Topic **19**

Technological horizons. MS imaging, targeted proteomics, others

OP087 - APPLICATION OF A NOVEL INTEGRATED MICROFUIDICS DEVICE FOR HIGH-THROUGHPUT LC-MS MRM DISEASE PROTEIN MARKER VERIFICATION

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Biomarker discovery and validation are the first steps in understanding disease and drug development. Validation is technology challenged since it requires high-throughput analysis of a large number of samples with high sensitivity, high resolution, large dynamic range and excellent selectivity. Targeted LC-MS based assays afford protein quantification with the reproducibility and throughput required in order to improve marker acceptance and multiple reaction monitoring (MRM) using tandem quadrupole mass spectrometers one of the explored enabling technologies. One of the major challenges using MRM for candidate marker verification in mammalian body fluids is the required sensitivity for the quantification of low-abundance proteins. Miniaturized LC systems offer improved mass-sensitivity but often lack the required throughput, robustness and reproducibility.

Here, the application of a novel microfluidics platform for the quantification of marker peptides and proteins is presented, considering speed, sensitivity and selectivity.

A 14 peptide species mixture, present as light and heavy labeled analogues and at varying amounts, giving an in-sample dynamic range of 1.25e3, was used for assessment. This mixture was spiked into an E.Coli tryptic digest in order to represent a high-throughput validation study and access quantitative precision and accuracy. An MRM method was programmed to monitor three transitions from each of the light and heavy peptide species and the sample separated using a nanoACQUITY system interfaced to an integrated microfluidic device and Xevo TQ-S tandem mass spectrometer. Data interrogation showed that the data exhibited excellent technical and quantitative reproducibility and that increasing the MS quadrupole resolution in order to observe effects of interfering background matrix did not adversely affect quantitative measurements. Excellent limits of detection were achieved for targeted peptide, which allowed good quantitation measurements down to 3.2 amol injected on column, whilst maintaining at least four order of quantification dynamic range.

OP088 - A NOVEL SWATH-MS PLATFORM FOR COMPREHENSIVE CHARACTERIZATION OF THE EPIGENETIC HISTONE MODIFICATIONS

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Histone post-translational modification (PTMs) sites regulate gene transcription, thus making reliable quantification a high priority in epigenetic studies. Quantification of histone PTMs is performed by shotgun proteomics favoring discovery of novel/low level PTMs, or targeted analyses of known PTM sites. Each workflow has strengths and weaknesses for quantification. Data independent acquisition (DIA) such as SWATH acquisition for comprehensive data generation combined with targeted data processing has recently been demonstrated to provide very high quality quantitative data. The advantages of this approach for targeted PTM quantification include no upfront assay development, quantitative data on all analytes and no dynamic exclusion of isobaric peptides

A key issue in analyzing histone peptide mixture is the presence of various isoforms of peptides, primarily from PTMs on the same peptide, but localized on different amino acids. Quantitation of these isoforms in a data-dependent mode using MS1 quantitation has been very challenging due to co-elution of isobaric forms. The peptide, GKGGKGLGKGGAKR from histone H4 is found to be acetylated at 4 sites: Lys2, Lys5, Lys9 or Lys13. In a 90 min gradient at 650nl/min flow rate, extracted ion chromatogram (XIC) of its precursor mass of m/z 768.9465 resulted in 2 peaks and a third that partially overlapped.

This made it difficult to distinguish and quantify each isoform based on MS1. However, with SWATH acquisition, all the MS/MS fragments are collected in one data set, so unique/combination of MS/MS fragments of each isoform were extracted post-acquisition and used for sequence assignment and quantification. Based on the SWATH analysis, the peak at 39.2 min was identified as acetylated Lys 2, while Lys5 and Lys9 coeluted at 41.7min and partially overlapped with Lys13 peak at 42.1min. Replicate SWATH analysis resulted on average a 10% CV. This work illustrates the advantage of using SWATH analysis for characterization of histone modifications.

OP089 - TOWARDS QUANTIFICATION BASED MS IMAGING: FILLING THE GAP BETWEEN MALDI MS IMAGING AND TISSUE MICROPROTEOMICS

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Introduction and objectives
MALDI MSI reseals high potential for clinical studies looking for biomarkers discovery or deciphering physiopathological mechanisms. Such an application of MALDI MSI requires the possible identification of the protein markers detected. Proteins identification in MALDI MSI remains a difficult task because of the specific strategies used.

Methods
To overcome this limitation we have developed new approaches involving on tissue micro-digestion followed by Liquid Microjunction micro-extraction (LMME) followed by Shot-Gun proteomics. This strategy has demonstrated to be powerful for high confidence identification of proteins of various abundances from small tissue area. LMME was then included in the MS imaging workflow in order to combine imaging and identification data.

Results and Discussion
We identified 1500 unique proteins in high confidence from each area. We then worked to reduce the size of the studied area down to one MSI image pixel dimension. However, we need again to increase the number of proteins both identified and imaged. To fulfill this gap between conventional proteomics and MALDI imaging we searched for providing image reconstruction based on identified proteins quantification. We have recently developed a strategy allowing for micro-dissection of the tissues by mounting the sample on a parafilm-covered glass slide allowing the facile excision before subjected to shot-gun. Using this strategy, 1140 proteins were identified from millimeter-sized regions on a rat brain tissue section. Applied to rat brain section allowed identification and quantification by label free. Their distribution was mapped by plotting the spectral count of the protein on each piece and correlating the pieces to their original locations on the tissue.

Conclusions
We now combine LDI with droplet capture in automatized mode and obtained from 50 µm laser spot the proteome and its quantification of thousand proteins per spot opening the door of the novel MS imaging: Quantification based MS imaging.

OP090 - TARGETED PROTEOMICS VIA AUTOMATED, HIGH PRECISION TRYPTIC DIGESTION AND SISCAPA-MS QUANTIFICATION OF HUMAN PLASMA PROTEINS USING THE AGILENT BRAVO PLATFORM

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[1]SISCAPA Assay Technologies

Introduction and objectives

Targeted proteomics is achieved via MRM mass spectrometric quantitation of proteotypic surrogate peptides in relation to heavy internal standards. Anti-peptide immunocapture enrichment (SISCAPA) has been demonstrated to provide improvements in assay sensitivity, specificity and analytical throughput versus non-enriched, depleted or anti-protein immunocapture enriched samples.

Our objective was to develop a simple and automated process for protein digestion, SISCAPA enrichment and quantitation of proteotypic peptides. The process had to be robust and easy to use, and the results highly reproducible.

Methods

A workflow was established and automated in 96-well format on the Agilent Bravo Automated Liquid Handling Platform. An "addition only" trypsin digestion protocol followed by multiplexed SISCAPA peptide enrichment on magnetic beads is driven through a simple software user interface. The combined protocols, taking as little as four hours total, were coupled with high throughput LC-MRM for multiplexed quantitation of plasma proteins over a wide range of abundance. The complete workflow was demonstrated for suitability for routine quantitation of target plasma proteins based upon sensitivity, multiplexing capability, scalability, reproducibility, and throughput.

Results and Discussion

This automated workflow provides all the benefits of SISCAPA in a simple-to-use format that requires minimal technician time. Robotic handling delivers single-digit CVs and unprecedented throughput with up to 500 protein samples prepared and analyzed in a working day. The method is generic across peptides and across dynamic range of abundance and provides simplicity of multiplexing.

Conclusions

We report here the development of a highly reproducible, automated "addition only" method for tryptic digestion of plasma, followed by bead-based SISCAPA enrichment of target peptides and quantitation via LC-QQQ. The workflow has been commercialized by SAT and proven in laboratories around the world to provide superior data for targeted protein quantitation.

OP091 - JUST ANOTHER FISHING EXERCISE? STABLE ISOTOPE LABELLING STRATEGIES TO INVESTIGATE THE DYNAMIC PROTEOME.

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The proteome of an organism is a dynamic entity, constantly changing in response to environment, stress and disease. Even under steady-state conditions, proteins are in constant flux, cycling between constituent amino acids and cognate proteins. To achieve a holistic view of the biology of a system it is essential that we can fully describe the relationship between transcript, protein, metabolite and phenotype. To that end, we have a devised a sophisticated mass spectrometric methodology based on monitoring the incorporation of stable isotope labelled amino acids into proteins.

Cells are cultured in media where a specific amino acid is replaced with a stable isotope labelled analogue (Cambridge Isotopes Laboratory) and the incorporation (or removal in a 'pulse-chase' experiment) is monitored by LC-MS/MS. This allows the first order rates of protein synthesis and / or protein degradation to be directly calculated on a proteome wide scale. In animal models, the stable isotope labelled amino acid is administered via the diet.

This approach has been applied to human cells in culture, chickens and most recently fish including the zebrafish, a model organism for human disease. We have determined the rates of protein synthesis for hundreds of zebrafish proteins, using a modified diet that is enriched to only 30% with stable isotope labelled leucine. This technology is now being applied to determine the effects of changing diet and induced stress.

Our data show that it is possible to determine protein synthesis rates using diet where only 30% of the amino acid is replaced with a labelled analogue. The approach we have developed is applicable to a range of biological systems and is a critical tool in the integration of systems-level data.

P-750.00

SIMULTANEOUS PROTEIN DIGESTION AND 18O LABELING BY A GRAPHENE OXIDE BASED IMMOBILIZED ENZYMATIC REACTOR FOR HIGH THROUGHPUT QUANTITATIVE PROTEOME ANALYSIS

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For 18O labeling based comparative proteome analysis, enzyme-catalyzed protein digestion and peptide labeling are two crucial sample preparation steps for protein identification and quantification. However, traditional in-solution protein digestion and enzyme-catalyzed O18 labeling not only might suffer from time-consuming, sample loss and incomplete labeling due to back exchange, but also it was difficult to integrate traditional in-solution digestion protocol with HPLC-MS for on line protein analysis, which might result in irreproducibility and low analysis throughput.

Therefore, to solve these problems, in our recent work, a graphene oxide based capillary immobilized enzymatic reactor (IMER) was developed, by which proteins could be simultaneously digested and 18O labeling. To evaluate the performance of such an IMER, bovine serum albumin (BSA) as a model protein was digested and labelled, compared to those obtained by traditional off-line protocol, not only the protein pretreatment time was greatly reduced from 36 h to 2.5 min, but also the labelling efficiency of peptides by 18O could be increased from 95% to 99%.

Furthermore, such an IMER was also coupled with 2D nano-HPLC-ESI/MS/MS system for quantitative analysis of identical aliquots of protein extractrs from highly and lowly metastatic typed HCA cell lines, and more than 1750 proteins were quantified with CVs of the ratios

P-752.00

IMMUNO-PROTEOMICS USING POLYCLONAL ANTIBODIES AND STABLE ISOTOPE LABELED AFFINITY-PURIFIED RECOMBINANT PROTEIN FRAGMENTS

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The combination of immuno-based methods with mass spectrometry detection has great potential in the field of quantitative proteomics. Here, we describe a new method (immuno-SILAC) for absolute quantification of proteins in complex samples based on polyclonal antibodies and stable isotope labeled recombinant protein fragments to allow affinity enrichment prior to mass spectrometry (MS) analysis and accurate quantification. We take advantage of the publicly available resource of antibodies from the Human Protein Atlas project covering more than 85% of all human protein-coding genes.

Epitope mapping revealed that a majority of the polyclonal antibodies recognize multiple linear epitopes and based on these results, a semi-automated method was developed for peptide enrichment using polyclonal antibodies immobilized on protein A-coated magnetic beads. A protocol based on simultaneous multiplex capture of more than 40 protein targets showed that approximately half of the antibodies enriched at least one functional peptide detected in the subsequent mass spectrometry analysis. The approach was further developed to also generate quantitative data by addition of heavy isotope-labeled recombinant protein fragment standards prior to trypsin digestion. Here, we show that small amounts of antibodies (50 ng per target) can be used in this manner for efficient multiplex analysis of quantitative levels of proteins in a human HeLa cell lysate. The results suggest that polyclonal antibodies generated by immunization of recombinant protein fragments could be used for enrichment of target peptides to allow for rapid mass spectrometry analysis, taking advantage of a substantial reduction in sample complexity.

Today, over 35,000 recombinant protein fragments with corresponding antibody exist within the Human Protein Atlas resource, eliminating the time needed for antibody generation normally associated with assay development towards novel targets for peptide enrichment using anti-peptide antibodies prior MS analysis.

P-753.00

COMPOUND-CENTERED PROTEOMIC APPROACHES TO DEFINE THE PROTEIN TARGETS OF THE ANTITUMORAL NATURAL COMPOUND ORIDONIN.

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Oridonin is a promising anti-cancer ent-kaurane diterpene; its ability to induce apoptosis and/or autophagy in tumor cells, either in vitro or in vivo, has been repeatedly demonstrated (1). This compound has been reported to modulate activity and/or level of many oncoproteins, but its specific intracellular targets are still not known. This lack of information limited oridonin use as a drug, and prevented the design of optimized molecules.

To ride out this problem, we have used two orthogonal compound-centric proteomics approaches to define oridonin targets. Firstly we performed chemical proteomics experiments (2), using a biotinylated oridonin. Secondarily, we used an approach called DARTS (Drug Affinity Responsive Target Stability) (3): this is based on the idea that a small molecule drug would stabilize its target protein's structure. Therefore, measuring a stability-responsive property (such as susceptibility to proteolytic agents) for all the proteins of an entire proteome, in the presence or in absence of the investigated drug, it is possible to identify the drug targets.

This research allowed suggesting the molecular chaperone Hsp70, the multifunctional protein Nucleolin and Thioredoxin reductase - a protein playing a pivotal role in cell redox homeostasis -, as the main targets of oridonin. Several in vitro and in cell tests have been performed in order to validate the interaction of oridonin with these proteins, and to evaluate the effect on protein activities.

Achieved results provide complete information on the mechanism of action of oridonin, opening the way to a direct use of this compound in some anticancer therapies, and permitting to start a rational design of new optimized therapeutic agents.

P-754.00

DEVELOPMENT OF ABSOLUTE QUANTIFICATION METHOD FOR DKK1 IN HUMAN SERUM USING LC-MS/MS

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Introduction and objectives

Serum concentration of Dickkopf-1 (DKK1) has been reported to be an important parameter for diagnosis of hepatocellular carcinoma (HCC), gastric cancer, lung cancer and so on. The concentration of DKK1 is usually measured by ELISA. In general, ELISA is a highly sensitive method, but also has disadvantages of cross-reactivity and non-specific bindings. In this study, we are developing a novel method for absolute quantification of DKK1 using LC-MS/MS.

Methods

Serum samples and calibration standards were prepared through processes of denaturation, chymotrypsin digestion and solid-phase extraction. LC-MS/MS analysis was conducted by Prominence UFLC system coupled with QTRAP5500 mass spectrometer. Surrogate peptides were selected by Skyline software.

Results and discussion

A novel quantitation method for DKK1 in human serum using LC-MS/MS technique was developed. The lowest limit of quantification was achieved to 100 ng/mL. It might be still not enough to measure the concentration of DKK1 in human serum, because the concentration of DKK1 in healthy human serum is mostly less than 10 ng/mL. Further efforts for developing more sensitive method will be presented and discussed.

P-755.00

HUMAN AORTIC VALVE STENOSIS ANALYZED BY MALDI-TOF IMAGING MASS SPECTROMETRY (MALDI-IMS) USING "LANGARTECH", A CUSTOM MADE MATRIX SPRAYER.

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Introduction

MALDI-MS imaging has recently emerged as a powerful technique for analyzing the spatial distribution of peptides and small proteins within biological tissues. Obtained signals can be correlated with underlying tissue architecture without any geometrical distortion, enabling the so-called Molecular Histology. This technique offers several advantages over other imaging methods like autoradiography, such as the high specificity of MS detection, minimal sample preparation, and applicability to a wide variety of analytes. Matrix spray stage is a critical step determining the final quality of the obtained images. Recently, many advances in the practice of imaging mass spectrometry have taken place, like automated matrix sprayers, making the technique more sensitive and robust.

Methods

In this work we have used an in-house made MALDI matrix sprayer: "Langartech"; made by IDEKO-IK4 research group in collaboration with CIC bioGUNE. The sprayer is made of several mass production parts, with economy and best performance in mind. The spray is located in a fixed position while operating (adjustable when needed) and the sample moves by pneumatic action in a computer controlled routine. The operation routine was empirically settled by sizing the droplet deposition. Using this sprayer, we have analyzed a sample of human aortic valve cryosection (10 µm) with a moderate degree of stenosis (calcification), using standard sinapinic acid (SA) preparation for peptidomics (mass window of 1,000 to 20,000 kDa).

Results

The images obtained are comparable with other higher priced sprayers in market, with an achieved lateral resolution of 75 µm, giving highly detailed images of the different structures and lesions present in tissue.

Conclusion

With the obtained preliminary results we are confident enough to further increase the lateral resolution to 50 µm in future analyses and to tackle the differential comparison among stenotic tissue samples.

P-756.00

REPRODUCIBLE PROTEIN SPOT COORDINATE DETECTION IN 2D-PAGE USING A PROTEIN MARKER GRID

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Introduction and objectives

The comparison of proteins separated on 2D-PAGE is hampered by gel-to-gel variability. Comparative fluorescence gel electrophoresis (CoFGE) is presented, which allows the generation of an artificial protein grid in parallel to the separation of an analytical sample on the same gel.

Methods

Fluorescent stains were used to distinguish sample and marker on the gel. For vertical (v) electrophoresis, special gel combs with V-shaped teeth were placed in a stacking gel above the pI strip. Proteins separated on the pI strip were electrophoresed at the same time as marker proteins placed in the V-wells. For horizontal (h)CoFGE, holes for the marker proteins were punched into the gel. Escherichia coli lysate was the test proteome. Sample grids for each gel were individually mapped to the ideal grid mix using Delta 2D.

Results and Discussion

Protein marker grids providing ~100 nodes as landmarks for the determination of protein spot coordinates were generated in parallel to E. coli images. For a set of 47 samples spots (2 vCoFGE experiments a 3 gels) the deviation of the coordinates was improved from 7% to < 1% applying warping using the marker grid. Use of hCoFGE eliminates some drawbacks of vCoFGE. When all gels of 8 experiments (4, 2, 2 gels, 30 landmark spots) were compared, the variation of the mean was < 1.3% (for 4 best gels < 0.7%). We introduced a third dye for pI-control. After warping, mean of x-coordinates was slightly improved (0.1%), but in general the influence due to pI in CoFGE was minor.

Conclusions

hCoFGE is an emerging technique in gel-based proteomics. As long as the same technology is used, gel matching is reproducibly possible. This is important for comparison of 2D-gels produced over several years and in different laboratories. Electrophoresis 2014, DOI: 10.1002/elps.201300507.

P-757.00
DETECTION OF BACTERIA AND COMPARATIVE PROTEOMIC ANALYSIS BY MASS SPECTROMETRY

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Mass spectrometric analysis is brilliant solution of bacterial identification and revealing its many biological systems, including the subcellular localization of carbohydrates, proteins and lipids, which can give us exact clue of identification and function.

We analyzed various bacterial cells and its products for the revelation of biological roles and processes. There are so many types of secondary metabolites and also related synthetic pathways and engaged enzymes. After construction of Genome Database, the industrial application of bacterial proteomics was widely used in this field for the bio production. Most goals of these approaches were optimization of bacterial production and commercial pathway reconstruction by proteomic analysis. For this purpose, construction of display proteome database after building bioinformatics programs are the most important tools of resolving these steps. Recently we begin studying rapid and direct identification of bacteria by mass spectrometry either.

As a same manner, but goal is different in use of mass spectrometry, we did mass analysis whole bacteria just after simple collection and compare those peaks by pattern matching algorithm. Results were quite good as up to 95 % accuracy in bunch of genus and we developing further technology with DESI and pyrolizer.

P-758.00

A PROTEIN BIOMARKERS PANEL TO DISCRIMINATE PROGRESSIVE FROM NON-PROGRESSIVE CHRONIC KIDNEY DISEASE USING A TARGETED PROTEOMICS METHOD

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Introduction and objectives
Chronic kidney disease (CKD) affects up to 13% of the general population and is associated with a high risk for progression towards end-stage renal disease. Currently used markers such as serum creatinine are unreliable both for early diagnosis and for prognostication, because they are affected by non-renal factors. Several candidate protein biomarkers emerged recently that outperform traditional markers because they potentially could detect kidney damage earlier and more accurately. A panel of 33 protein biomarkers was selected from the literature and included in a targeted proteomics method.

Methods
For each protein, proteotypic peptide(s) were selected that were unique and could be obtained by trypsin cleavage. An online SPE-UPLC-MS/MS method was developed to follow MRM transitions of the selected peptides together with their corresponding labelled heavy peptides, used as internal standards. The developed targeted proteomics method, preceding by an in-solution digestion protocol, was able to detect 44 peptides (corresponding to 26 different proteins) in urine of CKD patients. Finally, 140 CKD samples, 70 from patients with non-progressive disease and 70 from patients with progressive disease were analysed blindly, together with 20 control samples.

Results and discussion
A discriminant analysis shows that the panel of 44 peptides predicts the attribution of the 160 samples into their respective groups with a predictive power of 100%. A restricted panel of 30 peptides corresponding to 17 different proteins still gives an absolute prediction of all 3 groups, while a set of 7 peptides is able to distinguish healthy from CKD patients with a predictive power of 95%. An independent cohort of 50 CKD samples will be analysed to confirm the obtained mathematical model.

Conclusions
The proposed method represents an interesting alternative to measurement of traditional markers to detect kidney problems earlier and stratify CKD patients at risk for progression more accurately.

P-759.00

OPTIMIZATION OF THE MALDI IMAGING LASER REPETITION RATES USING AN ORTHOGONAL MALDI MASS SPECTROMETER

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MALDI mass spectrometry imaging (MSI) is gaining importance in clinical, Omics and pharmaceutical research areas due to the significant technological improvements achieved over the past decade. Here, proof-of-principle experiments have been carried out on standard spots consisting a 4 protein digest. Data were acquired using a MALDI SYNAPT G2-Si mass spectrometer equipped with a solid-state ND:YAG laser (repetition rate from 100Hz to 2.5 KHz). MS and MS/MS data were acquired and the data processed/visualized using High Definition Imaging (HDI) 1.2 software.

Initial experiments were performed with a fixed number of laser shots (200) per pixel; however, the laser repetition rate and time of acquisition were varied. Namely, for a repetition rate of 100 Hz, the pixel acquisition time was 2 s. At 500 Hz, a pixel acquisition time was 0.4 s was used. Lastly, at 1,000 Hz, pixel acquisition time was 0.2 s and at 2,500 Hz, the pixel acquisition time was 0.08 s. The experiment was designed at such that the same number of approximately 7,800 pixels was acquired. Moreover the peaks MS resolution for all experiments was around 18,000 in "Resolution" mode of the instrument. Using HDI software a combined single spectrum was reconstructed with all the pixels for each experiment to compare intensities. Finally comparing the peak intensities between each experiment, there were some disparities mainly due to sample preparation but in average the intensities were in the same order of magnitude.

A second experiment was performed with a fixed laser repetition rate of 2,500 Hz; however, here the later pixel resolution varied from 15µm, 30µm and 45µm. The acquisition rate went from 3.1 pixels/sec with the 45µm MALDI imaging experiment up to 4.5 pixels/sec with the 15µm MALDI imaging experiment.

P-760.00

LIPID VISUALISATION AND IDENTIFICATION THROUGH COLLISION CROSS SECTION AIDED CORRELATION OF MALDI IMAGING AND MS/MS FRAGMENTATION DATA SETS

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MALDI mass spectrometry imaging (MSI) is fast becoming an established technique within lipidomics research. Using MSI, a huge number of species can be visualised within a tissue section. Subsequent identification can though be extremely challenging. Lipids can be identified by extracting them from the same or consecutive tissue section and performing MS/MS. However, due to the large number of isobaric or near isobaric species, confidence can be lost when assigning identifications to peaks within the imaging data set by accurate mass alone.

Here, we demonstrate the use of ion mobility to differentiate ions and calculate collision cross sections (CCS) along with high accurate mass, adding confidence to peak assignment when relating fragmentation information from extracted lipids to imaging data.

Data were acquired using a MALDI SYNAPT G2-Si mass spectrometer equipped with a tri-wave ion mobility cell. CCS calibration was conducted prior to imaging a section of rat brain. Post imaging, lipids were extracted from the same tissue and deposited onto a stainless steel target plate. A second CCS calibration was acquired and the extracted lipids analysed by MS/MS. Identifications were assigned to imaging information using accurate mass and calculated CCS values.

Rat brain section was produced using a cryotome and deposited on a standard microscope slide. A nebulising spray device was used to apply CHCA evenly in several coats. Using polyalanine for CCS calibration, the CCS areas of ions present within the imaging data set were determined and red phosphorous was used as external lock mass. A mixture of 2:1 v/v CHCl3/MeOH could be used to extract lipids directly from the imaged tissue section in significant quantities for MS/MS identification. CCS was calculated for the extracted lipids and combined with high mass accuracy values, peaks identified by MS/MS were correlated to the peaks present in the imaging data set.

P-761.00

EXPLORING IMPACT OF DYNAMIC ACCUMULATION FOR IMPROVING MS/MS QUALITY OF QQTOF DATA

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Recent innovations in QqTOF instrumentation has resulted in a large increase in MS and MS/MS acquisition speed providing deeper coverage of complex proteomes. Some workflows, such as iTRAQ® reagent quantitation or PTM characterization, benefit more from higher spectral quality than traditional data-dependent workflows. Here, a QqTOF acquisition strategy that uses precursor intensity to adapt the MS/MS accumulation time (dynamic accumulation) was explored for its utility in improving these proteomic datasets.

Analysis of complex protein digests was performed using nanoflow LC/MS analysis on a TripleTOF® system. Data collection was done in data dependent mode with prototype acquisition software to explore a range of acquisition rates and precursor intensity combinations for optimal coverage and spectral quality. Protein identification data was processed using ProteinPilot™ Software and results assessment was performed using Excel tools. A number of areas of improvement were investigated, impact on MS/MS quantitation for iTRAQ reagents, effect on number of acquired spectra and therefore subsequent processing time, impact on protein identification rates, and impact on the generation of SWATH™ acquisition spectral ion libraries.

Ecoli lysate was labeled with 8plex iTRAQ® regents and mixed with equal loading in all channels. The sample was analyzed using three different acquisition strategies and the identification yields were characterized for both ID and quantitation. The dynamic accumulation approach provided a small increase in total protein/peptide identifications and significant improvements in the quantitation quality. The median reporter ion intensity was shifted higher by 34%, and the variance of protein ratio distributions was reduced (16% improvement in quality). The peptide variation about the protein was constant across the peptide intensity range, indicating improved quantitation of lower signal peptides.

P-762.00

PRECISION AND NORMALIZATION STRATEGIES FOR LABEL FREE MRM ANALYSIS OF PLASMA PEPTIDES OVER AN 18 MONTH TIME COURSE STUDY

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MRM analysis of enzymatic digested peptides is being used widely for targeted quantitation of plasma proteins. While it is desirable to use stable isotope labelled (SIL) peptides as internal standards, it is practically not always possible when a large number of proteins are to be quantitated. In the absence of SIL peptides, normalization is needed to allow relative protein quantity comparisons among samples. In this study, we investigated normalization and precision of MRM analysis for plasma samples.

62 peptides from 32 plasma proteins were quantitated by nanoLC-MRM repeatedly for a standard plasma sample during an 18 month period. We followed stringent SOPs for sample storage, sample preparation, data acquisition and data analysis protocols. Normalization methods such as normalizing to total peak intensity, normalizing to total peak intensity but excluding proteins of large variations, normalizing to peak intensity of a spiked protein or normalizing to peak intensity of a spiked peptide were assessed. The magnitude of the variations due to sample storage, sample preparation, and mass spectrometry instrument stability were evaluated.

When MRM data were acquired within a short period of time (within a few weeks), variation due to sample preparation was the greatest contributor to CV. However, over a longer period of time, variations due to mass spectrometry tuning and sensitivity changes became dominating, resulting much larger CV. Our data showed that normalizing to a spiked protein or a spiked peptide did not improve the MRM data precision. Instead, normalizing to total peak intensity was found to be the optimum way to compensate run to run variations in label free MRM analysis.

P-763.00

RECONSTRUCTION OF HUMAN PROTEOME: REVIVAL OF TARGETED PROTEOMICS FROM SHOTGUN MASS SPECTROMETRY

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A huge number of proteins represent interest in proteomic and medical researches, but few of them are available for qualitative and quantitative analysis. Low and ultralow concentration of much of proteins is the major obstacle on a way of rapid development of quantitative proteomics. Simplification of biological samples by multidimensional fractionation dangers that there is a possibility of loss the target proteins. Affinity purification may cause non-specific binding.

The main approach in proteomic part we support is targeted proteomic employing selected reactions monitoring (SRM) with ordinary sensitivity limit up to 10-14 M (for qualitative analysis) and high resolution data-dependent MS/MS. But even with high selectivity a portion of proteins remained undetected and, consequently, unavailable for quantitative assay.

We developed sample preparation approach to enrich ultralow abundant proteins and bioinformatics approach to validate the signal obtained after SRM analysis. Human plasma (280 mg initially) was used as biological diversity in this work. The plasma sample was depleted onto Hu-14 (MARS) system and digested. Resulting peptides were separated by their pI using off-gel fractionation. Further samples preparation included complex of chemical N- and O-deglycosilation (TFMS – triflourmethansulfonic acid) and concentration of the resulted peptide fractions using irreversible covalent binding, thus, increasing the sensitivity in 2-3 orders of magnitude. The final samples were analyzed on QExactive in ddMS/MS mode within several detection m/z ranges. Identified proteins were quantified and the rest of proteins were registered and reconfirmed using SRM and triggered-SRM on QqQ mass spectrometer. The pooled data from ddMS/MS and SRM experiments increased the possibility of quantitative imaging of plasma proteome and prediction of proteins concentration dynamic representation when they occasionally undetected.

P-764.00

DEVELOPMENT OF A WORKFLOW FOR THE DETECTION OF DRUG-DERIVED ADDUCTS ON PROTEINS

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Introduction and objectives:

Busulfan is a bi-functional alkylating agent used as a component of conditioning regimens before hematopoietic stem cell transplantation in children. Busulfan therapy needs to be carefully monitored due to the inter-individual variability in pharmacokinetics and toxicity. Being an electrophile, busulfan can interact with proteins. However, no data is available on protein adducts formed by busulfan. The aim of our study was to develop a workflow to detect busulfan adducts on albumin and hemoglobin.

Methods:

The workflow, relying on MS and bioinformatics, consisted in: 1) in vitro incubation of albumin or hemoglobin with the drug of interest, 2) trypsin digestion and LC-MS/MS analysis in an orbitrap hybrid instrument with high mass accuracy measurements, 3) standard protein search and generation of spectral libraries, 4) use of the open modification search software QuickMod to define mass shifts and possible modification sites from the generated spectral libraries, 5) new protein search including the potential drug-induced modifications provided by QuickMod , 6) manual validation of MS2 spectra with high identification score matching.

Results and Discussion:

As a proof-of-concept, the workflow was validated for identifying the well-known adduct derived from NAPQI (mass shift: 149.05 Da), a toxic metabolite of acetaminophen. For busulfan, a mass shift of 150.03 Da was detected on several acidic residues of albumin and hemoglobin. This mass shift was absent in the negative control and when proteins were incubated with NAPQI or sulfolane, a non-alkylating busulfan metabolite. The chemical composition of busulfan adducts was calculated from the mass shift, allowing the formulation of the hypothetical structure ($C_5H_{11}O_3S$). Samples from busulfan-treated patients need now to be analyzed to evaluate the clinical relevance of the in vitro identified adducts.

Conclusions:

We developed a workflow to identify busulfan adducts on proteins, which could be applied to similar agents in the future.

P-765.00

EVALUATION AND PROTEOMICS APPLICATIONS OF NEUTRON-ENCODED TMT10-PLEXING

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Isobaric mass tag-based quantitative proteomics strategies such as iTRAQ and TMT utilize reporter ions in the low mass range of tandem MS spectra for relative quantification. The recent extension of TMT multiplexing to 10 conditions has been enabled by utilizing neutron encoded tags with reporter ion m/z differences of 6 mDa. Initially this method was set up for Orbitrap Elite.(1,2) Here we further evaluate the performance and optimized the performance on other instruments such as the Q Exactive.

Methods:
Tryptic digest from K562 cells was labeled using TMT-10plex. This mix was used for the evaluation of Q Exactive (Thermo Scientific) settings.(3)

Results:
With the standard setting initially optimized for TMT-6plex we observed that the proximate TMT10 reporter ion pairs become prone to coalescence on Q Exactive instruments. The fusion of the different reporter ion signals into one has a detrimental effect on protein quantification. We tested different target and resolutions setting. As expected for this phenomenon increasing the resolution did not resolve the two peaks. However the coalescence artifact was completely removed by lowering the maximum ion target for MS2 spectra from 1e6 to 2e5 without any losses in identification depth.

Conclusions:
TMT10-plex can also be used on Q Exactive instruments. We observed ion coalescence which can be overcome by adjusting the acquisition method without compromising on the analytical depth. TMT10-plex is beneficial in wide range of applications where higher multi-plexing rates are desired such as chemoproteomic dose response experiments.

P-766.00

MALDI-TOF/TOF-IMS ON FORMALIN FIXED PARAFFIN EMBEDDED (FFPE) HUMAN BRAINSTEM TISSUE; EXAMINATION OF PEPTIDE EXPRESSION IN THE ROSTRAL (OPEN) MEDULLA

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The brainstem contains both specific and interacting groups of neurons (nuclei) that have unique and critical roles in the central regulation of respiration, cardiovascular activity, metabolic/energy homeostasis, and the control of the transition between sleep and wakeful states. This study aimed to optimise MALDI-TOF/TOF-IMS for use on archived (1-6 years) FFPE human brainstem tissue, and applying it at the level of the rostral (open) medulla, to determine differences in peptide expression amongst the major nuclei.

We hypothesised that large scale analysis of peptide expression would identify markers of neurons, nuclei and neuronal tracts. Experimentally citrate buffer rather than Tris for antigen retrieval better maintained tissue structure for these proteins. The age of the archived tissue negatively correlated with the peptide output. Tryptic digestion of intact tissue and automated matrix/typsin spraying were employed in this method. Comparisons between the nuclei identified common peptides for neuronal and non-neuronal structures and unique peptides allowing for the identification of specific nuclei. Refining these techniques will allow the study of new neuronal peptide markers in the brainstem and improve the ability to identify specific brain regions using MALDI-TOF/TOF-IMS.

This study is the first to show that MALDI-TOF/TOF-IMS is able to be performed on FFPE archived human brain tissue, and with correct optimisation, can differentiate amongst important physiologically different regions of the human brain.

P-767.00

PEPTIDE TISSUE IMAGING MASS SPECTROMETRY ON TISSUE MICRO ARRAYS FOR CANCER DIAGNOSTICS

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Introduction and objectives
Imaging Mass Spectrometry (IMS) is typically used to determine the distribution of proteins in fresh frozen tissue. Tryptic Peptide Imaging has some advantages over imaging of intact proteins. These include peptide level analysis provides the possibility for identification by matching accurate m/z and in situ MS/MS to high quality LC-MS/MS data obtained through digestion of relevant laser dissected tissue. Finally, formalin-fixed paraffin embedded (FFPE) tissue can be analysed after antigen retrieval.

Methods
Here we present the latest developments within our group, including up-to-date methods for analysis of formalin-fixed tissue (e.g. tryptic peptide MALDI-IMS), a method for linking LC-MS/MS data to MALDI-IMS data using internal calibrants as well as the generation of the first data for a MALDI-IMS patient and disease specific tryptic peptide database and the use of tissue micro arrays.

Results and Discussion
Metastasis is a crucial step of malignant progression and remains the primary cause of death from solid cancers. In cancers of the female genital tract (vulval, cervical and endometrial cancers) lymph node metastasis is a crucial factor in the choice of treatment and prognosis of patients. As it is impossible to accurately predict lymphatic metastasis in individual patients, a large number of women who would be cured by local treatment alone, undergo radical surgery including lymph node dissections. Peptide Imaging Mass Spectrometry could be able to distinguish if patients have metastasis by analysing the primary tumour using FFPE tissue from large patient cohorts.

Conclusions
The potential for tryptic peptide imaging on FFPE tissue micro arrays as a diagnostic tool for the metastasis of gynaecological cancers will be discussed on preliminary data using FFPE endometrial tumour cancer tissue from patients with and without metastasis.

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Topic 19: Technological horizons. MS imaging, targeted proteomics, others

P-768.00

ELEVATING PROTEIN IDENTIFICATIONS PER MINUTE BY USING SPELC-MS/MS IN COMBINATION WITH ULTRA-SHORT GRADIENTS

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Introduction and objectives

To tackle real biological questions ideally bottom-up proteomics should be able to analyse proteomes much faster. Longer gradients as well as prefractionation techniques combined with the latest fast mass spectrometers have improved proteome coverage tremendously. There is also a need for a rapid analysis strategy for protein or protein complex samples such as those originating from purifications, affinity experiments and fractionation. Robust procedures that can deliver large sample throughput have been somewhat neglected and are urgently needed.

Methods

Here, we are demonstrating a novel LC system capable of ultra-short (9 min) analysis times and combine it with different offline prefractionation methods, namely affinity purification, electrophoresis and chromatography, to achieve fast protein identification. The setup consists of an autosampler harbouring 96 stage-tips which functions as precolumn, and a short RP analytical column (5 min gradient) interfaced with a QExactive mass spectrometer.

Results and Discussion

36 SCX fractions of AspN digested Hela cell lysate were analysed with this setup leading to >3600 protein groups in only 3h gradient time. By calculating the identification per minute of gradient time, the 2D approaches we developed were even superior to 1D UHPLC runs. Applying medium complex samples from pull-down experiments hundreds of interactors, many of them novel, could be determined in less than 1h accumulated gradient time.

Conclusions

The new fast LC system is a powerful tool for very efficient, robust and reasonably comprehensive proteome analysis, making the setup suited for fast sensibly deep proteome screenings.

P-769.00

IMPROVED ANALYSIS OF PHOSPHOLIPIDS USING GRAPHENE OXIDE AS A MATRIX FOR MALDI IMAGING MASS SPECTROMETRY

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Matrix assisted laser desorption/ionization (MALDI) imaging mass spectrometry (IMS) is an increasingly important technique for investigating the distribution of molecules across the tissue section. Appropriate matrix for generating ions from analytes of interest is extremely important for a successful IMS analysis.

Therefore, in the past decade, great efforts have been made to discover suitable matrices for analysis of different molecules. In this study, graphene oxide (GO) was introduced for the first time as a MALDI matrix to enhance the ionization of various phospholipids in positive ion mode. Compared to the commonly used matrix (DHB), the use of GO significantly improved signal-to-noise ratios of phospholipids (6, 22, 97 and 15 fold for PC, PE, PG and PS, respectively) and afforded a homogeneous crystallization resulting in better shot-to-shot, spot-to-spot reproducibility.

More importantly, GO was proven to improve the detection of some phospholipids species which are suppressed by the presence of phosphatidylcholine. Finally, GO was successfully applied to identify the species of phospholipids from rat brain. Therefore, it is believed that GO could be a promising matrix for MALDI imaging of phospholipids on tissue.

P-770.00

SINGLE-TUBE SAMPLE PREPARATION WORKFLOWS USING PARA-MAGNETIC BEADS FOR ULTRA-SENSITIVE PROTEOMICS

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Introduction and objectives
Proteomic sample preparation typically is a multi-step process that suffers from protein losses during sample handling and transfer. This becomes detrimental especially when sample amounts are limited. The aim of this study was to develop a methodology for true single-tube sample preparation that minimizes protein losses and that allows in-depth proteome coverage even of rare samples. As a result, here we present a completely novel and versatile workflow leveraging para-magnetic bead technology.

Methods
Whole-cell lysates from yeast, HeLa and Drosophila were prepared by boiling in SDS (1%) containing buffer. To remove SDS, proteins were immobilized on paramagnetic beads and then rinsed. After tryptic digestion, peptides were re-immobilized on the paramagnetic beads. Optionally, peptides were fractionated on-bead or by high-pH reversed phase chromatography before analysis by Orbitrap mass spectrometry.

Results and Discussion
To overcome limitations of current methods in proteomic sample preparation we have developed a single-tube workflow based on para-magnetic beads that includes cell lysis in detergent-containing buffer (SDS, up to 10%), detergent removal, digestion, labeling (TMT, dimethyl), and peptide fractionation. We successfully demonstrate that proteins and peptides can be immobilized on para-magnetic beads in an unbiased fashion. Moreover, immobilized peptides can be fractionated off of the beads before MS, thus enabling a true-single-tube proteomics workflow. We apply this to analyses where a limited amount of sample is available, identifying 3000 unique proteins starting with just 1000 HeLa cells or a single fruit fly embryo. Aside from reducing sample losses by minimizing sample handling and transfer, this workflow requires just 15 minutes each for protein and peptide clean-up, and is compatible with robotics and microfluidic automation platforms.

Conclusions
We have developed a true single-tube proteomics workflow using para-magnetic beads allowing in-depth proteome analysis at unparalleled sensitivity. This approach should find broad applicability in any area of proteomics.

P-771.00

A NOVEL MULTIPLE REACTION MONITORING METHOD OF QUANTITATIVE PROTEOMICS BY USING COMBINATION OF MULTIPLEX LABELING AND QCONCAT.

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Targeted multiple reaction monitoring (MRM)-based approaches with stable isotope-labeled standard (SIS) is the ¡°golden standard¡± method in the quantification of small molecular. In the past years, Western blotting and ELISA technique has been a principal method for the absolute quantification of protein. Since MRM based protein quantification approach is not limited by antibody development.

In decade, there exists a growing demand in the quantitative proteomics through MRM approach. However the high cost of the synthesis of stable isotope-labeled standards (SIS) limited the application of protein quantitcation. A Quantification concatamers (QconCAT) method comes into being developed as a high throughout and economic absolute quantification approach of protein. Although, the QconCAT protein has a high throughout, it sometimes gives variable result which introduces a poor accuracy. Our method will use QconCAT protein and synthetic peptides in parallel and labeled with mTRAQ plex and determine protein by MRM assay.

The incorporation of QconCAT and synthetic peptides allows the absolute quantification of each peptide in the QconCAT protein to be calibrated. mTRAQ labeling increase the throughput and cut off the cost. Using QconCAT, synthetic peptides and mTRAQ in combination can quantify 160 peptides in one analyse and cut the cost off 80%. The resulting method also should be applicable to other clinical diagnose.

P-772.00

TUNABLE ISOELECTRIC FOCUSING VIA MOVING REACTION BOUNDARY FOR HIGH RESOLUTION TWO-DIMENSIONAL GEL ELECTROPHORESIS AND PROTEOMICS

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Introduction: The current top-down immobilized pH gradient isoelectric focusing (IPG-IEF) and two dimensional gel electrophoresis (2DE) are still suffering from unsatisfactory resolution, untunability, repeatability (due to protein precipitation) and poor sensitivity for the characterization of intact proteins (e.g., isoforms and posttranslational modifications).

Methods: To address these issues, a novel non-IPG-IEF system was firstly proposed based on the concept of moving reaction boundary (MRB). By choosing proper pairs of catholyte and anolyte, one could achieve an ideal cathodic or anodic migrating pH gradient as well as no migrating pH gradient in non-IPG-IEF, effectively improving resolution and sensitivity of IEF and eliminating protein precipitation and uncertainty of protein quantitation existing in IPG-IEF. Then the non-IPG-IEF was combined with polyacrylamide gel electrophoresis (PAGE) to develop a novel 2DE system. The developed 2DE was evaluated by testing model proteins and real colon cancer samples.

Results: The experiments revealed that (i) a tunable pH gradient could be designed via MRB; (ii) up to 1.65 fold improvement of resolution was achieved via the non-IPG system, and the different subunits of model proteins that could not be separated in a typical 2DE were well separated via the developed 2DE system; (iii) the sensitivity of the newly-developed system was increased up to 2.7 folds; and (iv) about 30% more protein spots could be observed via combination strategy of non-IPG-based 2DE as compared with IPG-based 2DE.

Conclusion: Therefore, the developed non-IPG IEF and 2DE systems might contribute to complex proteome research, especially different protein isoforms.

P-773.00

INTEGRATING HEAT STABILIZION WITH MALDI IMAGING ENABLES MOLECULAR DISTRIBUTION IMAGES OF HIGH QUALITY TISSUE

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Introduction and objectives

MALDI imaging (MSI) is gaining increasing interest in pharmaceutical research, as it is a valuable tool when mapping molecules in situ on tissue sections. Due to residual enzymatic activity in tissue samples, degradation and changes occur, altering the molecular composition and leading to misinterpretations and erroneous conclusions. Rapid heat stabilization using the Stabilizor system (Denator) prevents such changes and reveals a molecular composition closer to in vivo. In the presented work a number of heat stabilization - and sectioning parameters have been evaluated for their effect on section morphology in order to establish a standard protocol for working with heat stabilized tissue in the IMS workflow.

Methods

Brain from mice were used as a challenging model tissue to asses the impact of heat stabilization, freezing, sectioning parameters and the use of tape transfer system on the morphology of cryosectionen sections and suitability for subsequent MSI analysis.

Results and Discussion

Key parameters for good quality sections are: fresh tissue, embedding in 2.5% CMC; rapid freezing, transfer of sections to the slide with an artist brush and unidirectional thaw mounting. The tape transfer system CryoJane (Leica) can be used to further improve section quality if needed.

Conclusions

Overall it was shown that sections of good quality can be produced from heat stabilized tissue and distribution profiles for neuropeptides and small molecules could be obtained.

This demonstrates that the Stabilizor system can be introduced into the MSI workflow without loss of section quality while preserving distribution profiles of sensitive molecules.

P-774.00

COMPREHENSIVE RELATIVE QUANTIFICATION OF THE CYTOCHROMES P450 USING SWATH(TM) ACQUISITION

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Metabolism of drugs by the Cytochrome P450 superfamily is pivotal in determining their disposition, safety and efficacy. Since drugs may induce expression of several isoforms of Cytochrome P450, they may enhance their own turnover, increasing the risk of toxic metabolite formation or adverse interactions with co-ingested compounds. Thus P450 profiling is a fundamental aspect of drug safety evaluation. The Cytochromes P450 share extensive sequence homology, so that antibodies are incapable of discriminating every isoform, plus mRNA levels do not correlate well with protein. SWATH(TM) is a data-independent MS method for label-free quantification which enables closely-related proteins to be quantified retrospectively through post-acquisition extraction of specific peptide ions, and is thus perfectly suited to P450 profiling.

Methods
Mice were exposed to inducers of the Cytochromes P450, and pooled microsomal fractions were prepared from the livers. Following protein extraction and digestion, a database of microsomal proteins was generated by 2D-LC-MS/MS using information-dependent acquisition on a TripleTOF 5600 (AB SCIEX, Framingham, USA). Individual samples were then processed and LC-MS data were acquired using the SWATH(TM) approach. PCA analysis was performed using MarkerView(TM) software (AB SCIEX) to identify differentially expressed proteins.

Results and Discussion
PCA analysis separated induced and non-induced mice based on their overall protein expression pattern, and that of the P450s. Relative quantification of uniquely discriminatory P450 peptides enabled the induction profile of each compound to be ascertained in unprecedented detail. For instance, it was possible to identify and quantify peptides unique to Cyp2C50 and Cyp2C54 despite the fact that the proteins share 92% sequence identity.

Conclusions
SWATH(TM) technology will facilitate the identification of drug candidates with undesirable properties early in the drug development pathway. Since the approach enables even highly homologous proteins to be discriminated, it may also refine our understanding of enzyme function leading to improved drug design.

P-775.00

ULTRA-LOW FLOW ESI-MS FOR THE DETECTION OF POST TRANSLATIONAL MODIFICATION (PTM) SITES AND THE INCREASED SEQUENCE COVERAGE OF PROTEINS IN A SINGLE ANALYSIS

Stephen Lock[1]
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Introduction

ESI-MS in the low nanoliter per minute range has long been thought to provide increased ionization efficiency and reduced ion suppression, resulting in increased sensitivity and a better representation of all analytes in a particular sample. CESI-MS is a revolutionary technology which combines capillary electrophoresis (CE) with electrospray ionization (ESI) by a dynamic process in a single device: the sprayer interface. CESI-MS provides robust ionization at the low nL/min range. The purpose of this presentation is to show how CESI-MS can be used to sequence proteins and identify modifications in a single analysis and offer a complementary separation technique to nano LC.

Methods

In this study the CESI 8000 system was coupled to QqTOF instruments and orbitrap systems for the analysis of proteins. Protein digests were prepared using standard tryptic digest protocols and samples injected electrokinetically and separated over a silica capillary using capillary zone electrophoresis.

Results

Initial results have shown that this technique is capable of generating increased sequence coverage of proteins with also the ability to detect PTMs, including deamination, oxidation of methionine as well as glycosylation sites in a single run. This was due to lower losses of larger and smaller peptides.

Conclusion

CESI-MS has been shown to effectively provide robust ultra-low ESI-MS flow. This reduces ion suppression and increases ionization efficiency such that from a single analysis PTM sites can be detected, a full peptide map and identification of glycosylation variants can be obtained.

P-776.00
CAN MRM-BASED SECRETOME PROFILING BE USED AS DRUG EVALUATION ASSAY?

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Introduction and Aim
Drug effects are usually evaluated by measuring effects on target molecules, typically focusing on enzyme inhibition. However, such strategies cannot uncover other effects on the target cells. Proteome profiling is a powerful screening technology which allows us to detect responses to cells to challenges posed by drug applications. We have observed that alterations of the secretome may reflect altered functional cell states. Targeted analysis by multiple reaction monitoring (MRM) is a fast and reliable technique to quantify a considerable number of analytes with high accuracy and sensitivity.

Methods
Primary human cells including white blood cells, umbilical vein endothelial cells and fibroblasts were stimulated in vitro for inflammatory activation and then treated with various antiphlogistic drugs. Secreted proteins characteristic for inflammatory cell states were identified using a Thermo QEXACTIVE orbitrap and evaluated with a label-free quantification method. Furthermore, we established MRM assays for cytokines, chemokines and growth factors which were found specifically induced in response to inflammatory activation. MRM assays were accomplished using an Agilent nano-flow Chip-HPLC coupled to the Agilent 6490 triple quadrupole mass spectrometer

Results and Discussion
MRM analysis was used to test the reproducibility and robustness of protein secretion with respect to cell culture conditions, functional cell states, individual cell donors, biological as well as technical replicas. A set of molecules was thus identified for each cell type with expression features significantly dependent on the functional cell state and the drug applied. Each drug tested showed unique features with respect to the efficiency of regulating the secretion of inflammation-related molecules.

Conclusion
Secretome analysis based on MRM is demonstrated to be a powerful analysis method supporting the screening for desired drug effects in meaningful cell culture model systems.

P-777.00

EXTENDING PROTEOME COVERAGE THROUGH AN ON-LINE 2D SCX-RP ULTRA-HIGH PRESSURE SYSTEM

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Introduction and objectives

Ultra-high pressure liquid chromatography combined with state of the art mass spectrometers has pushed the limit of deep proteome sequencing. One path to success is combining long gradients and long columns packed with sub 2 um particles providing high peak capacities. However, the relationship between gradient length and the number of proteins identified is not linear and as gradient time increases, performance gain diminishes. Multidimensional approaches, using orthogonal separation, provide the ideal way of increasing the overall peak capacity but typically come at the expense of sample loss and increased analysis time. Here, we explore an automated online 2D UHPLC/MS workflow and demonstrate with special attention to time-to-performance considerations the overall favorable performance when compared to alternative workflows.

Methods

An EASY-nLC 1000 system (Thermo Fisher Scientific, Odense, Denmark) was setup to accommodate an automated 2D-LC/MS in the UHP regime. The system consists of an SCX column, an RP trap column and a 50 cm analytical EASY-Spray column. The performance was evaluated using human cell tryptic digests with mass analysis performed on a Q-Exactive.

Results and Discussion

The 2D SCX-RP UHPLC-MS/MS workflow allowed us to identify almost 37000 unique peptides and 6000 proteins in a total analysis time of ~7 hours, while 57000 peptides and 7500 proteins were identified in ~21 hours. On the identical system a 1D combined triplicate RP UHPLC-MS/MS analysis gave about 24000 peptides and 4400 unique proteins in 9 hours.

Conclusion

We report, that with this fast online SCX-RP UHPLC-MS/MS workflow proteome coverage can be substantially extended without significantly compromising analysis time and sample usage.

P-778.00
TARGETED PROTEOMICS WITH A HIGH-PERFORMANCE TRIPLE QUADRUPOLE MASS SPECTROMETER AND NEXT GENERATION ESI SOURCE

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Introduction
Qualitative proteomics for clinical biomarker discovery as well as targeted quantitative proteomics for biomarker verification requires versatile instrumentation capable of robust, high-throughput quantitation from complex biological samples such as plasma or global proteome digests where the dynamic range of protein and peptide concentrations can exceed six-orders of magnitude. To achieve these technological challenges a triple quadrupole with extended dynamic range (EDR) and a new versatile low-flow (300nl- 5µl/min), next generation electrospray ionization (ESI) source has been developed and applied to BSA quantitation in E.coli matrices. Notable improvements in throughput, dynamic range and sensitivity were observed.

Methods
Experiments are performed on a triple quadrupole mass spectrometer equipped with EDR, the next generation ESI source and a UHPLC (EVOQ-ER, advanced Captive Spray Ionization source (CSI 2), NanoAdvance UHPLC, Bruker Daltonics). RPLC are performed with a 0.3 mm X 100 mm, 300A, C18 column operated at 5 µl/min with a gradient from 5-45% ACN in 12 min. Two sample sets were used: 1) A dilution series containing tryptic digest mixtures of BSA spiked in 10 ng E.coli digest matrices. 2) A dilution series containing bovine angiotensin 3.

Results/Conclusions
As a control, LCMS analysis of BSA tryptic peptides ((++582.40) LVNELTEFAK, (++ 464.5) YLYEIAR) on the QQQ were initially performed without matrix or EDR. First results demonstrate four to five orders of linear dynamic range for BSA peptides with %CV values below 10 and LLOQ values in the low attomole range. It is expected that the sensitivity measurements of BSA digest peptides will not be hindered by matrix interferences. Considering these initial results, we expect the combination of EDR and the new CSI 2 source to improve sensitivity, throughput, reproducibility and linear dynamic range.

P-779.00

QUANTIFYING CYTOCHROME P450 ENZYMES INVOLVED IN XENOBIOTIC METABOLISM – SCREENING OF DRUG-INDUCED PROTEIN EXPRESSION BY TXP-IMMUNOAFFINITY MASS SPECTROMETRY

Frederik Weiss[1], Bart van den Berg[1], Hannes Planatscher[1], Helen Hammer[1], Thomas O Joos[1], Oliver Poetz[1]
[1]NMI

One central issue in pharmacokinetics and -dynamics is the induction of drug metabolizing enzymes by drugs and drug candidates. The expression of cytochrome P450 enzymes can increase by the factor 100 after drug administering. As a consequence the drug availability is drastically reduced. The concentration of these proteins can be assessed by mass spectrometry-based immunoassays with highest specificity.

Here we present a method employing TXP-antibodies specific to short C-terminal peptide epitopes capable of enriching peptide groups. We generated antibodies targeting common epitopes present in signature peptides derived from members of the cytochrome P450 system. These antibodies were applied in an immunoaffinity step prior to an MRM-like readout. Thus we were able to quantify CYP1A1, CYP1A2, CYP2A6, CYP2A13, CYP2B6, CYP2C8, CYP2C9, CYP2C18, CYP2C19, CYP2D6, CYP2E1, CYP2F1, CYP2S1, CYP3A4, CYP3A5, CYP3A7, CRP, and MDR1 as toxicologically relevant proteins directly from proteolytical digests of hepatocytes, microsomes and tissue.

The established assays were used to analyze the CYP450 induction of 450 FDA-approved drugs. Cryo-preserved hepatocytes were seeded in 96 well plates and treated with the drugs at 10 µM concentration. Based on the CYP450 induction profiles drugs were clustered into classes and conclusions about nuclear receptor to drug interaction could be drawn. The data-set might allow predictive conclusions about novel drug candidates and their behavior in terms of CYP induction.

P-780.00

PNGASE F-CATALYZED 18O-LABELING FOR QUANTITATIVE GLYCOMICS AND GLYCOPROTEOMICS SIMULTANEOUSLY

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Introduction and objectives

Changes in glycosylation abundance and corresponding glycan structures correlate evidently with many disorders and diseases. Quantitative profiling of glycosylation variations in either glycan level for glycome or glycopeptide level for glycoproteome has been arousing considerable attentions. None of quantitative methods so far can be used for quantifying glycome, glycoproteome and proteome simultaneously. To bridge this methodological gap, we developed a new method for simultaneous quantitation of glycans, glycopeptides and non-glycopeptides.

Methods

The aliquots of protein solution were deglycosylated by PNGase F prepared in $H_2^{16}O$ /$H_2^{18}O$. During the deglycosylation, the N-glycans and N-glycosylation sites were labeled with one ^{16}O /^{18}O atom. Then the deglycosylated proteins were digested by trypsin in $H_2^{16}O$/ $H_2^{18}O$ prepared buffer and labeled with two ^{18}O atoms.

Results and Discussion

In the present work, PNGase F-catalyzed complete N-glycan 18O-labeling was realized for the first time, which showed good linearity and high reproducibility within at least 2 orders of magnitude in dynamic range. By combination of this labeling reaction with glycosite 18O-labeling and peptide 18O2-labeling, a novel enzymatic 18O4-labeling strategy was developed for comprehensive N-glycosylation quantification, achieving simultaneous quantification of glycan, glycopeptide and glycoprotein in single workflow. Furthermore, glycosylation changes in human hepatocellular carcinoma (HCC) associated immunoglobulin G (IgG) was analyzed by utilizing 18O4-labeling as an example, and quantitative information concerning glycan structure and glycosite of Ig G was obtained, confirmed the feasibility and efficiency of this strategy for quantitative glycomic and glycoproteomic investigation.

Conclusion

The new approach we reported, in which PNGase F is used for the first time to catalyze glycan labeled with 18O, realized comprehensive N-glycosylation quantification in a single experiment for both glycomics and glycoproteomics simultaneously.

P-781.00

AN AUTOMATED DUAL-ONLINE MULTIFUNCTIONAL ULTRAHIGH PRESSURE LIQUID CHROMATOGRAPHY SYSTEM FOR HIGH THROUGHPUT AND EXTENSIVE PROTEOMICS ANALYSIS.

Hangyeore Lee[1], Sang-Won Lee[1]
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Introduction

The bottom-up proteomic approach utilizing liquid chromatography-tandem mass spectrometry (LC-MS/MS) has become the most powerful platform for proteome analysis. To analyze high complex peptide mixtures, methods of high separation efficiency and sensitivity are required. In large-scale proteome research, especially for clinical studies, experimental throughput is also becoming an important factor in method development.

Methods

For high throughput proteomics analyses, a fully automated dual-online multifunctional ultrahigh pressure liquid chromatography (DO-MULTI-UPLC) system has been developed. The system employees one binary pump and one isocratic pump, two capillary reverse phase (RP) columns (75 ¥ìm inner diameter x 100 cm length), two online SPE columns (150 ¥ìm x 3 cm), and a strong cation exchange (SCX) column (150 ¥ìm x 15 cm). Single-dimensional RPLC and online two-dimensional (SCX/RPLC) were performed selectively by the automated operation of six switching valves. Two RP columns were used independently and alternatively to remove the dead time for column equilibration between experiments.

Results

The advantageous features of the current system were demonstrated by its application to proteome samples of varying complexities. The DO-MULTI-UPLC system provides an increase in experimental throughput by about two folds, while keeping the retention time reproducibility in less than one percent of gradient time. The 2DLC-MS/MS experiments using tryptic gastric tissue peptides resulted in a significant increase (ca. 56%) in peptide identification compare to 1DLC-MS/MS experiment.

Conclusions

In this study a dual-online multifunctional ultrahigh pressure liquid chromatography system was developed. The system was successfully applied to both the 1DLC and 2DLC-MS/MS for the complex proteome sample, demonstrating its benefits in large-scale quantitative proteome research.

P-782.00

SRM VS HR-MS QUANTITATIVE PROTEOMICS; MATRIX EFFECT AND LOQ IN SERUM

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Introduction and objectives

Proteomic targeted quantitative approaches using SRM-based methods is an emerging tool to confirm discovery results or to target specific proteins. However, heavy bioinformatics treatments are required for selection of specific peptide transitions. Recently, HR-MS based approaches were introduced with advantage of producing full MS/MS high mass accuracy spectra. However analysis of samples having high concentration dynamic range, like serum or plasma, remains challenging due to the important matrix effects observed. This study aims at comparing both quantitative approaches to target exogenous proteins in human serum.

Methods

4 Standard proteins and serum samples were digested and injected on an UPLC. Using the same gradient, UPLC was coupled either to a Xevo-TQ-S triple-quadrupole (Waters) or to a Q Exactive (Thermo).

Results and Discussion

The LOD of exogenous peptides in serum was determined on triple-quadrupole mass spectrometer and was in the 5-500 fmol range (loaded on column). We next spiked 1 to 300 fmoles of this protein digest mix in a constant amount (20 µg) of serum digest in order to select the best sample preparation method. Two proteins purification methods were compared. The matrix effect was generally lower using the 2D clean-up kit (GE). Using this kit, the limit of quantification was 1-5 fmoles for most of the peptides. The importance of matrix effects was evaluated; this was achieved by spiking 300 fmoles of standard proteins digest in various total quantities of serum (1 µg to 100 µg). Signal to noise ratio obtained dropped dramatically between 1 and 40 µg of matrix. Beyond that threshold, signal to noise ratios of the targeted peptides were almost stable.

Conclusion

The matrix effect is the major issue in targeted proteomics in serum. Results of the comparison between SRM and HR-MS using the Q-Exactive will be presented.

P-783.00

AUTOMATED ISOFORM DETECTION BY CUSTOMIZED PEPTIDE IDENTIFICATION METHODS AND NOVEL HIGH THROUGHPUT LCMS INSTRUMENTATION

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Introduction and objectives
Mass spectrometry can now identify the vast majority of the expressed protein species of for example the human cell and while post translational modifications has been studied extensively for the past decade, we believe isoform specific experimental data is often hidden or ignored in bottom-up proteomics.

Methods
Isoelectric focusing (IEF) gels are used to separate protein isoforms from organelle fractionated (by ultracentrifugation) samples. Thin bands are excised, digested separately by a selection of proteases and analysed by LCMS by short gradients on a Solid Phase Extraction (SPE)-LC / QExactive setup. Custom made software combine identification and quantification data. Validation procedures are proposed by the software and targeted LCMS analysis is performed.

Results and Discussion
Automated examination of peptide quantification data from excised IEF bands provides a method for detecting isoform specific peptides from proteins present at multiple variants without a-priori knowledge besides the canonical protein sequence. When sequence variation alters the isoelectric point of proteins, resulting peptides can be grouped as either shared or isoform specific and multimodal distributions can be extracted from LCMS peptide quantitation data. While other protein modifications (e.g. PTMs) modify the isoelectric point, they are easily distinguished from isoforms and the peptide data often explain or suggest modifications not covered by the database search as shared peptides infer the proteoform presence.

Conclusions
Isoform specific peptides are presented as detected by this method and validated by both alternative digestion and know literature. Biologically relevant isoforms are furthermore investigated by utilizing the SPE-LC setup in a targeted approach.

P-784.00

PROBING PROTEIN STRUCTURAL TRANSITIONS IN COMPLEX BIOLOGICAL BACKGROUNDS ON A LARGE SCALE

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Protein structural transitions in response to environmental or genetic factors, such as those associated to allosteric regulation, misfolding, or post-translational modifications, can strongly impact protein function, with drastic consequences on the phgysiology of cells. However, monitoring protein structures on a large scale and directly in their biological environment has so far not been feasible.

We developed a novel approach that couples proteolytic probes and a targeted proteomic workflow to quantitatively analyze protein structural rearrangements directly in their biological context and on a large scale. It is based on the use of proteases with broad specificity applied under controlled conditions such that the initial cleavage sites are dictated by the structural features of the protein substrate. The approach allows probing of both pronounced and subtle conformational transitions of proteins, with an average resolution of about 10 amino acids. We validated it by applying to model proteins with well-characterized structural transitions. Further, we applied it to the systematic identification of in vivo protein structural transitions in the proteome of cells subjected to a metabolic perturbation. We probed the structural features of more than 1,000 proteins simultaneously and detected novel stimulation-induced structural transitions for about 300 proteins.

Our analysis suggested a highly modular nature of metabolism, with certain metabolic branches preferentially controlled transcriptionally and others by enzyme structural changes, including cases of allosteric regulation. We also identified novel structural transitions of prion-like, aggregation-prone proteins and showed the functional relevance of one of these proteins to the considered environmental change. This structural proteomics method enables a variety of novel applications in biology and biomedicine and provides an additional layer of 'omic data for systems biology analyses.

P-785.00

COMPARISON OF GROUP SPECIFIC IMMUNOAFFINITY MS BASED QUANTIFICATION OF PLASMA BIOMARKERS FROM DRIED BLOOD SPOTS, DRIED PLASMA SPOTS AND BLOOD PLASMA

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Monitoring multiple plasma biomarkers in preterm infants is not possible using established clinical assays due to the limited blood volume of the patients (ca. 125 mL). However, routine analysis of plasma proteins could be a decisive factor in early diagnosis of inflammation or sepsis, monitoring organ development and nutritional status as well as in assessing the success of therapeutic interventions.

This could in turn both improve preterm infants' health, development and survival rates, as well as helping to drive down treatment costs. The use of dried blood spots (DBS) is well established in the screening of inborn errors of metabolism in infants. Sample acquisition is relatively simple and non-invasive. Here we compare the efficacy of the quantification of selected protein biomarkers inn DBS, dried plasma spots (DPS) and plasma   samples.
Blood and plasma samples spiked with stable isotope standard proteins (SILP) were spotted onto DBS carriers. Extraction of proteins from DBS/DPS was performed based on published protocols (Chambers et al., 2012, J.Mol.Cell.Prot, 12,781-791). Tryptic digestion of extracts and original plasma was followed by group specific immunoaffinity enrichment (TXP-IP) of signature peptides. Captured peptide groups were subsequently analysed by nano-UHPLC-high resolution ESI-MS.

Immunoaffinity enrichment could not fully prevent interference from RBC components on analyte protein quantification. Although DBS and DPS are easy to handle in the clinic and storage and logistics of DBS/DPS is much easier and cheaper compared to blood or plasma samples, RBC bursting must be avoided during DBS preparation. Preparation of cell free plasma spots can be one option to enhance recovery and reproducibility of analyte protein quantification compared to DBS. In addition, further optimization of protein elution from DBS prior to TXP-IP-enrichment could help to improve protein biomarker quantification from DBS.

P-786.00

COMPARING ION THERMAL FOCUSING ELECTROSPRAY AND NANOSPRAY LC-MS/MS FOR CHARACTERIZING HUMAN EMBRYONIC STEM CELLS AND NEURAL PROGENITOR CELLS

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Large scale LC-MS/MS analyses on various mass spectrometry platforms have been carried out to understand the proteome of human embryonic stem cells before and after differentiation. Increased sensitivity and faster scan rates are essential for rapid and deep proteome scans of stem cells. This is also important for quantitative measurements of differentially expressed proteins among stem cells at different stages of differentiation. Varieties of neuronal and glial cells derive from progenitor cell populations (NPs).

Hence extensive proteome analysis of ESC and NPs and their comparison reveal global differences and associated functional roles. In the present study we have used high flow chromatography and ion thermal focusing electrospray ionization (AJS-ESI) and nano-HPLC-Chip technology for the proteome analysis of ESCs and NPs. Protein samples were overnight trypsinized and subjected to basic reversed-phase (RP) fractionation. Concatenated 30 fractions were analyzed on UHPLC/AJS-ESI and nano-HPLC-Chip/iFunnel-QTOF mass spectrometer using 30min and 60min gradient respectively. The data was analyzed using Spectrum Mill and MPP software.

We have identified ~12,700 unique human proteins from ESCs and NPs using UHPLC/AJS-ESI and nano-HPLC-Chip MS analysis including 1,100 phosphoproteins at 1% FDR. Nearly 1,200 proteins (10%) showed increased levels of expression in NPs with an overlap of ~450 common proteins between two types of LC-MS/MS analysis. Nearly 2,000 proteins (16%) showed increased levels of expression in ESCs with an overlap of 1,200 proteins common between two LC-MS/MS methods. This large proteome dataset constituted >260 protein pathways involving differentially expressed protein between ESC and NPs. We have identified novel differentially expressed proteins expressed in ESCs and NPs along with known markers of these two cell types. Several novel proteins were found elevated in NPs including NEURON NAVIGATOR 3 (NAV3), S100A6 and SERPINE1 involved in the calcineurin/NFAT pathway in addition to elevated levels of SOX2, NESTIN and LAMIN B1 compared to ESCs.

P-787.00

MASS DEFECT-BASED PSEUDO-ISOBARIC DIMETHYL LABELING METHODS FOR PROTEOME QUANTIFICATION

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Dimethyl labeling is widely used for proteome quantification, but it suffers from low accuracy and dynamic range because the S/N is lower at the MS level than that at MS/MS level.

In this report, mass defect-based pseudo-isobaric dimethyl labeling (pIDL) method based on the subtle mass defect differences between 12C/13C and 1H/2H were introduced. Tryptic/Lys-C digests were labeled with CD2O/13CD2O and reduced with NaCNBD3/NaCNBH3 as heavy and light isotopologues, respectively. The fragment ion pairs with mass differences of 5.84 mDa could be resolved by high-resolution MS/MS and used for quantification.

When the MS/MS resolution was set as 30000, the fragment ions with m/z All these results indicate that the pIDL method is better than commonly used dimethyl method in accuracy, wide dynamic range and throughput. We believed that these results are attributed largely to high labeling efficiency, precursor-specific fragment ions and enhanced purity of fragment ions by high-resolution MS/MS. We believe the pIDL method might become a promising technique to achieve the large scale accurate proteome quantification.

P-788.00

ABSOLUTE QUANTIFICATION OF 26 CYTOCHROME P450 ENZYMES AND UDP- GLUCURONOSYLTRANSFERASES IN HUMAN LIVER MICROSOMES USING QCONCAT/MRM APPROACH

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Introduction and objectives£ºQuantification of drug metabolizing enzyme expression has an important role in drug metabolism and safety. Conventional immunequantitation methods have poor dynamic range, low throughput, and a limited number of specific antibodies. This study aimed to establish a rapid and high throughput method to quantify 26 drug metabolizing enzymes (20 CYP450s, 6 UGTs) based on QconCAT/MRM approach. Methods£ºProteotypic peptides for 26 target proteins were selected from the human PeptideAtlas based on their suitability score. The QconCAT DNA construct was synthesized de novo.

The recombinant QconCATs in labeled and unlabeled form were purified. MRM was using to quantifying CYP and UGT Isoforms. Results and Discussion: 56 tryptic peptides were used to quantify 26 proteins. The calibration curves of each peptide showed linearity (coefficient of regression, $r>0.99$) over the concentration range of 2-500 fmol. The inaccuracy values (expressed as relative error) were 1.33% to 8.50%, whereas imprecision values (expressed as RSD) were within 3.09% for quality control (QC) samples ranged from 8-80 fmol. In two separate panels of HLM examined (n=50 pool and n=10 separate), 26 drug metabolizing enzymes were determined reproducibly, giving values of 2.83-161.66 and 3.08-194.82 pmol/mg microsomal protein. Protein concentration between two panels of sample have good correlation ($r2=0.84$). CYP2C9, UGT2B7, UGT1A4, UGT1A6 and UGT2B4 were determined as high abundant top five drug metabolizing enzyme, expressing 194.82, 184.73, 87.22, 71.40 and 58.47 pmol/mg microsomal protein. CYP27A1, CYP4F3, CYP4F12 and CYP51A1 concentration were first given in the present work. And the established method correlated well with the frequently-used immunequantitation method, such as for CYP3A4 ($r2=0.76$).

Conclusions: Taken together, our MS-based method provides a specific, sensitive and reliable means of P450 protein quantification and should facilitate P450 characterization during drug development, especially when specific substrates and/or antibodies are unavailable.

P-789.00

EXPANSION OF ION LIBRARY FOR MINING SWATH DATA THROUGH FRACTIONATION PROTEOMICS

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Introduction and Objectives

The strategy of sequential window acquisition of all theoretical fragment-ion spectra (SWATH) is emerging in label-free proteomics. Mining the MS data acquired from SWATH is critically depended on the quality of ion library. Herein, we proposed an approach to build up an expanded ion library using the data dependent acquisition (DDA) data generated from fractionated samples.

Methods

We took a bacterial, T. tengcongensis (TTE) sample and fractionated the lysate proteins by SDS-PAGE, followed by tryptic digestion and protein identification upon DDA with 5600 TripleTOF (ABSCIEX). The peptides derived from the same lysate proteins without fractionation was employed for proteomics analysis upon DIA using SWATH. The RTs of the peptides from DDA data were corrected against those from SWATH using linear regression, and the expanded ion library were constructed by combining the corrected peptides from the fractioned samples.

Results and Discussions

It was found that three technique elements were important to achieve a satisfactory library: to well correct the retention times (RTs) gained from fractionated proteomics data, to appropriately integrate the fractionated proteomics into an ion library, and to fairly judge the impact of the expanded ion libraries to data mining in SWATH. As compared with the library built from the unfractionated proteomics data, a same SWATH data could be extracted with approximate 20% more peptides against the expanded ion library. Moreover, the peptides extracted from the expanded ion library were qualified for quantitative analysis of proteomics. With two sets of bacterial samples which were cultured at different temperatures, the relatively quantitative data based upon iTRAQ was well correlated with that gained from SWATH. Fractionation approach therefore was proven not only suitable for enlarging proteins identified through LC-MS/MS, but also feasible for expanding ion library in SWATH.

P-790.00

USE OF MONOCLONAL ANTIBODY TO AID DIAGNOSIS AND PROGNOSTIC EVALUATION OF HEPATOCELLULAR CARCINOMA

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Introduction and objectives
Hepatocellular carcinoma (HCC) is one of the major causes of illness and mortality in China. Monoclonal antibody (mAb) is a useful tool in auxiliary diagnosis, treatment and prognosis evaluation. This study generated monoclonal antibody against two potential biomarkers of HCC for aiding diagnosis and prognosis judgment purpose.

Methods
Potential biomarker proteins vitronectin and clusterin were recombinant expressed. mAbs were generated by cell fusion and hybridoma screening, mAb cell lines that can be paired were selected by Octet red96 system. HCC patients¡¯ sera and pathological tissue were collected and tested by Enzyme-Linked Immuno Sorbent Assay (ELISA) and immunohistochemistry staining, respectively using our mAbs.

Results and discussion
HCC Patients¡¯ sera can be distinguished from normal sera by anti vitronetin mAb in ELISA assay, and HCC Patients¡¯ pathological tissue can be recognized by anti clusterin mAb with immunohistochemistry staining. More clinical samples should be adopted next step and more efforts should be made on sandwich ELISA detection using paired mAb.

Conclusions
mAb against vitronectin and clusterin can specifically identify HCC clinical pathological samples, which is helpful for diagnosis and prognosis of HCC.

P-791.00

DEVELOPMENT OF A "UNIVERSAL" MASS SPECTROMETRY METHOD BRINGS THE END OF TIME-CONSUMING DATA-DEPENDENT METHOD OPTIMIZATION FOR ACHIEVING MAXIMAL IDENTIFICATIONS FROM ANY SAMPLE TYPE

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Introduction

Achieving the maximum identifications from different peptide samples requires optimization of MS methods. Optimizations are time/sample intensive to determine the best balance of scan rate and number of ions per spectrum. This is particularly true when accurate sample concentration, complexity, and dynamic range are unknown, often the case following fractionation/enrichment. Unfortunately, optimizations are often not performed due to sample/time restraints. Here we present a universal method which adjusts parameters "on-the-fly" according to spectral complexity/intensity, eliminating the requirement for optimization.

Methods

Here, we analyzed various samples including HeLa digests and immunoprecipitations. Analysis was performed on an Orbitrap Fusion MS. The resulting LC-MS/MS data were searched using Proteome Discoverer, matches were filtered to 1% FDR. Each sample was analyzed with varying ion targets and maximum injection times to determine optimal parameters.

Novel instrument control software, now implemented on Orbitrap Fusion MS, was used to develop a Universal Method which makes "on-the-fly" decisions about length of injection time per precursor based on the ion flux, complexity of full scan and available cycle time without user input.

Results

Maximum identifications are obtained by reaching a balance between scan rate and quality of spectra. With 1 microgram HeLa digest, Orbitrap Fusion achieves maximal identifications (~25,000 unique peptide identifications (UPI)) using 35 ms maximum injection time and 1e4 ion target. At 1 ng, however, maximal identifications are achieved (~700 UPI) using 500 ms maximum injection time and 1e4 ion target. Depending on the sample load, complexity, and dynamic range, optimal values change dramatically. A single Universal Method achieved maximal identifications in all sample types.

Conclusions

Our results show that it is possible to achieve maximal peptide identifications from samples with unknown concentrations without method optimization and lengthy reanalysis, thereby, increasing the throughput of the instrument while simultaneously improving the quality of the data acquired.

P-792.00

SIMS AND MALDI IMAGING METHODOLOGIES REVEAL LIPID MARKERS OF CHONDROGENIC DIFFERENTIATION

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Introduction and objectives: Lipid distribution and modulation during cartilage formation or chondrogenesis could be used to improve the mesenchymal stem cells (MSC)-based cartilage therapy by the discovery of new chondrogenic markers. In this work, we have used two different imaging techniques, TOF-SIMS and MALDI-IMS, for the characterization of lipids in three-dimensional MSC cultures or micromasses at different time points (day 2 and 14) of chondrogenesis.

Methods: Micromasses were cut in sections and dried in a vacuum desiccator prior the metal and matrix deposition. TOF-SIMS experiments were performed in positive mode on a Physical Electronics TRIFTII secondary ion mass spectrometer with and Au1+ primary ion beam. The raster size and the acquisition time were set at 68.8 µm and 63.8 s per tile, respectively. MALDI-MSI was performed using a MALDI-Q-TOF instrument (Synapt HDMS, Waters, UK). Data were acquired in the mass/charge (m/z) range of 100-1000 and at a raster size of 100 µm (positive and negative V-reflectron mode). After data analyses, m/z of interest were selected and identified by tandem mass spectrometry (MS/MS).

Results and Discussion: MALDI-IMS and TOF-SIMS revealed a specific peak profile between the two time points. Specific masses of two day-samples were attributed to phosphatidylcholines (PC) (m/z 739.5, m/z 798.5 and m/z 826.6) and several sphingomyelines (SM) (m/z 725.5, m/z 741.5, m/z 767.5 and m/z 826.6) after MS/MS fragmentation. Some two day-specific peaks obtained with SIMS, such as 577.5, 599.5 and 666.5, complement the profile obtained with MALDI. A high content of phosphatidylinoisitols (PI) (m/z 885.6 and m/z 943.5) and phosphatidyletanolamines (PE) such as m/z 750.5 and 794.5 were found specifically at day 14.

Conclusions: Using SIMS and MALDI methodologies we were able to identify putative chondrogenic markers. Phosphatidyletanolamine and phosphatidylinositol pathways are increased in chondrocyte-differentiated MSC, whereas phosphocholine-related lipids could be markers of the undifferentiated stage.

P-793.00

A RIBONUCLEIC ACID BASED STRATEGY FOR RNA-BINDING PROTEOME ANALYSIS

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Protein-mRNA interactions are extensively found in many key biological processes, including mRNA transcription, splicing, nuclear export, translation and degradation. RNA-binding proteins (RBPs) not only serve as structural elements for mRNA packaging and transportation, but also have essential roles in mediating the function of mRNA in its entire life cycle.

Discovering new RBPs may largely facilitate the understanding of unknown functional roles of mRNA. Hereby, we report a systematic strategy for the comprehensive enrichment and identification of RNA-binding proteins from mammal tissue by using endogenous RNA baits and mass-spectrometry. Among the ~3000 identified proteins from mouse liver using this method, 439 were annotated RBPs which covers 52.6% of known Mus musculus RBPs. Furthermore, 645 proteins were found to have close association with these identified RBPs via 1109 protein-protein interactions demonstrating the capability of this method for efficient coverage of RBPs and associated proteins.

Further analysis using two protein-RNA interaction prediction algorithms leads to consistent identification of more than 600 highly confident potential RBPs with diversified functions providing a useful resource for the study of protein-RNA interaction network in mammals.

P-794.00
DIRECT TISSUE ANALYSIS BY MALDI-MASS SPECTROMETRY IMAGING (MSI) REVEALS A MOLECULAR PATTERN OF ARTERIAL LAYERS IN ATHEROSCLEROSIS DEVELOPMENT

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Introduction:
MALDI-MSI allows in situ visualization of a tissue's molecular map. The aim of this study was to set-up a protocol for MALDI-MSI analysis of healthy and atherosclerotic arteries, as a novel direct approach into the ongoing molecular changes that occur during atherosclerosis development. Protocol should guarantee spatial integrity and high resolution in order to visualize the molecules present at the intima and media arterial layers, where major biomolecular changes are known to occur.

Methods:
MALDI-MSI protocols for proteins, lipids and metabolites were set-up using human atherosclerotic arteries (carotids) and control arteries (mammary). For comparison of molecular maps of pathologic and healthy arteries an early rabbit model of atherosclerosis was used. Aortic tissue was dissected, histologically characterized and analyzed by MALDI-MSI in an UltrafleXtreme MALDI-ToF/ToF and a Solarix FT-ICR with high spatial resolution (30 µm).

Results:
Fourteen proteins ions were found that were statistically confined to the media or intima layer. Six of them were significantly altered between animal groups, 4 m/z in intima layer (m/z 3010, m/z 4762, m/z 4777, m/z 6241) and 2 m/z in the media (m/z 5621, m/z 6182). Metabolites and lipids analysis resulted in 18 m/z values responding to different lipid classes, 8 of them being significantly altered with atherosclerosis. Ten m/z values were localized in the whole intima (saturated fatty acids, lysolipids, glycerophospholipids and sphingolipids), which are related to endothelial dysfunction and inflammation, and 8 m/z values specifically defined the plaque region (glycerophospholipds, sphingolipids and triacylglycerols).

Conclusions:
We have developed a novel protocol to in situ visualize the molecular map of healthy and atherosclerotic arteries. A total of 32 molecules (m/z), 15 of them significantly altered, were found to be specifically localized in the different arterial layers or in the plaque area, helping to elucidate internal mechanisms and arising as novel potential molecular targets.

P-795.00
IMPROVED IONIZATION EFFICIENCY USING ACETONITRILE AS A DOPANT TO NANO-ELECTROSPRAY FOR THE ANALYSIS OF CLINICAL SAMPLES

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Introduction and objective
Analysis of clinical samples is one of the major goals of proteomics research, but it remains challenging for various reasons. One of these is the often very limited amount of material that is accessible. High sample complexity and dynamic range of the clinical proteome therefore require high performance mass spectrometry instrumentation with high sensitivity. An important and critical aspect is the peptide ionization step that is neither very efficient nor very well understood.

Methods
To systematically investigate the benefit of acetonitrile we acquire a dilution series from several µg to several ng of a peptide mixture from a human cancer cell line. It is added to the nano-electrospray via the gas inlet system of a sub-atmosphere electrospray source (CaptiveSpray) coupled to a time-of-flight mass spectrometer (impact HD, Bruker). For data analysis the MaxQuant software environment was adapted and optimized for the analysis of time-of-flight data, including novel mass recalibration, and identification of peptides and proteins was performed using Andromeda.

Results and Discussion
Here we investigate the use of acetonitrile as dopant to nano-electrospray to improve the sensitivity of the analysis for clinical samples. We observe that acetonitrile-assisted ionization increases the signal intensity and boosts the number of unique peptide identifications for low ng sample amounts by up to 80%. This leads to a fourfold higher amount of protein identifications and improved quantification in bottom-up proteomics.

Conclusion
Adding acetonitrile as a dopant in the electrospray ionization process can significantly improve ionization efficiency, which is particularly promising for low level clinical samples.

P-796.00
QUANTITATIVE EVALUATION OF GSTS IN MOUSE SERUM BY MRM

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Glutathione S-transferases (GSTs) belongs to a protein superfamily and involves in many reactions of detoxification and anti-oxidation. It is generally recognized that the GST abundance is a sensor to diseases, while the serum GSTs changes in response to disease have been identified by enzymatic and immunochemical assay. Although several GSTs are found in serum, there is lack of an efficient method that enables global and quantitative measurement of GSTs.

Herein, we have developed a quantitative method to evaluate the abundance of GSTs in serum. To make a sample prepared easily, mouse serum was selected as a GSTs source. Four different enrichments, such as SDS-PAGE and affinity resin, were used to treat the serum followed by identification of GSTs with Triple-TOF MS. Based upon the GST profiling data, the GST targets with appropriate peptides and transitions were selected and delivered to Triple-quadrupole MS for quantitative assay at MRM mode. The enrichment results revealed that magnetic GSH affinity was the best material in this project, while the GST profiling demonstrated that 10 GSTs, at least, present in mouse serum, such as A3, A4, M1, M2, P1/2, Z1, M3, M5, M7, and MGST1. The reproducibility test was further conducted in 6 individual mice serum, and all the GSTs were consistently detected in the samples with high quality MRM signals. Furthermore, the sample volume was carefully evaluated to minimize the sample usage for identification of all serum GSTs. A volume of 200ƒÝl serum was basically required to acquire all the quantitative information of mouse serum GSTs.

Finally, synthesized GST peptides were taken for generation of calibration curves corresponding to the varied GSTs. Thus, for the first time we have established a MRM method to quantitative measurement of serum GSTs, which is potentially useful for evaluation of human serum GSTs in later application.

P-797.00

INTERFACING SURFACE ACOUSTIC WAVE NEBULIZATION WITH LC-MS

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Introduction: Although electrospray ionisation is widely used for mass spectrometric analysis, alternative nebulization methods may offer advantages of softer ionisation to study labile molecules, or in negative ionization mode. Surface acoustic wave nebulisation (SAWN) has been shown to be a low energy method for bringing molecules into the gas phase and has been interfaced with mass spectrometry. We have investigated the use of a flow interface using SAWN to nebulize the sample, and further characterized the effects of a range of parameters in this system.

Methods: The nebulising apparatus consisted of a signal generator and a RF amplifier driving a gold interdigitated transducer (IDT) fabricated on a $LiNbO_3$ wafer. The frequency and amplitude of the driving system were adjusted to optimise signal intensity. The SAWN system was placed in front of a Bruker HCT Ultra mass spectrometer with a modified source to optimise performance. Sample was flowed onto the SAWN chip via a silica capillary accurately positioned using an xyz stage, using an HPLC system to generate steady flow rates and sample separations.

Results and Discussion: SAWN nebulisation of standard peptides could be detected at nmole amounts in infusion mode using flow rates from 30 uL min-1 down to nanoflow. Signal to noise ratios were maintained across a wide range of flow rates, and high-quality MSMS spectra could be generated. SAWN was coupled to LC separation used for MSMS analysis of and a tryptic digest of human serum albumin (HSA). Chromatographic resolution was maintained and HSA peptides were detected with good sensitivity HSA identified with a good protein score.

Conclusion: We demonstrate that SAWN can be used as a nebulisation method for peptide and protein analysis using liquid flow to deliver the sample, and can be fully integrated with standard LC-MSMS techniques for peptide and protein identification.

P-798.00

DEVELOPMENT OF SELECTED REACTION MONITORING (SRM) ASSAYS FOR QUANTIFICATION OF ANGIOTENSIN-II SIGNATURE PROTEINS IN HUMAN URINE

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Angiotensin-II (AngII), the main effector of the renin-angiotensin system, mediates kidney disease progression. However, there are no specific markers of renal AngII activity. We previously defined 83 AngII-regulated proteins in vitro, which reflected renal AngII activity in vivo. We now examine whether these AngII-regulated proteins measured in urine represent markers of renal AngII activity in patients with kidney diseases.

SRM assays were developed for 37 peptides corresponding to 18 previously identified AngII-regulated proteins. Methods were developed by spiking 37 crude unlabeled peptides into normal urine. We also spiked in bovine serum albumin (BSA) and chicken ovalbumin (OVA) proteins, and 7 heavy-labeled peptides unique to these two proteins. We processed urine samples with four different methods and compared peptide concentrations. These four methods included combinations of 1)protein precipitation using 3kDa-filter (Amicon) or acetonitrile, and 2)protein digestion with lys-C/trypsin or trypsin alone. After digestion, 20μg of total protein/sample was subjected to C18 microextraction and analyzed on triple-quadrupole mass spectrometer (TSQ-Quantiva). We repeated the four methods in 2-biological and 2-technical replicates. Biological replicate CVs were ≤11% for both BSA and OVA peptides for each method. Technical replicate CVs between the four methods were 82% for BSA and 52% for OVA peptides. Similarly, biological replicate CVs between the methods were 90% for BSA and 69% for OVA peptides. Protein precipitation with acetonitrile followed by lys-C/trypsin digestion enabled detection of most AngII-regulated peptides (16 of 37).

In conclusion, we developed and optimized a protocol for SRM measurement of protein biomarkers in urine. Although all four methods performed well, there was substantial technical variability between the methods. Protein precipitation with acetonitrile and digestion with lys-C/trypsin appeared most effective at detecting AngII-regulated peptides. Presented sample preparation protocol coupled to the multiplex SRM assay will facilitate verification of AngII-regulated proteins in urine samples from patients with kidney diseases.

P-799.00
DEVELOPMENT OF A HIGH-THROUGHPUT BINDER SELECTION AND SCREENING PLATFORM

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Reliable methods for the selection and screening of a large number of affinity reagents in parallel are a prerequisite to provide specific binders for proteomic analyses. In order to increase the throughput of these selections and screenings, our laboratory focuses on the development and optimization of a streamlined pipeline, consisting of parallel Ribosome Display selections and various semi-automated high-throughput screenings.

Aiming at an improved robustness of the overall process, while decreasing its time and cost requirements, we can perform simultaneous selections against 94 targets and screen several thousand binders in parallel for their biophysical characteristics. In binder generation, the characterization and validation of individual candidates is the major bottleneck of selections and screening pipelines. Recent fine-tuning of experimental setups permitted the miniaturization of assays and the reduction of required reagents and thus of the final cost of the screening pipeline. The biggest savings, however, can be achieved through improvements in sample throughput and the time required for the various steps in our pipeline. Therefore, we focused on improvements in the sample throughput while reducing the associated costs. Moreover, we investigated various assays allowing predictions about the quality and properties of candidates at early stages of the screening process, resulting in an increased percentage of binders passing the strict requirements of the various validation steps and in the need of testing fewer candidates.

With the help of technology developments, a robust pipeline could be established that already generated valuable DARPins (Designed Ankyrin Repeat Proteins) for applications like pull-downs, immunohistochemistry or intracellular biosensors, amongst others. These binders did not just cover a variety of different target families but also met the high quality criteria important for most proteomic projects: monomeric binders that specifically recognize different, non-overlapping epitopes at their targets with high affinities, also allowing applications requiring sandwich assays.

P-800.00

TARGETED ANALYSIS IN PRM MODE TOWARDS CLINICAL APPLICATION: OPTIMIZATION OF EXPERIMENTAL SETUP AND DATA PROCESSING FOR ACCURATE AMYLOID DIAGNOSIS

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Amyloidosis is a disease whose prevalence is rare and which suffers from a lack of accurate diagnosis tools. This disease results from a wrong folding of specific amyloidogenic proteins and their identification is essential for proper medical care. Today most patients' cases are identified thanks to immunohistochemistry analysis after surgery or biopsy on the defective tissues. However they can be inconclusive on certain cases, leading to a lack of information about the underlying ethiology. We showed that ultrasonic treatment could help for the completion of enzymatic proteolysis (60s instead of 15h incubation) of either fixed or raw biopsy samples and to get closer to the clinical routine application for amyloidosis subtyping.

In discovery phase, abundance species were evaluated according to the Top 3 Protein Quantification and data were manually classified. Protein quantification has also been assessed with label-free MaxQuant software and classified with their statistical Perseus Tool.
In order to implement our approach in the French clinical departments, a targeted proteomics method has been evaluated. Parallel reaction monitoring (PRM) based on high resolution and accurate mass (HR/AM) measurement in MS/MS mode was performed with a hybrid quadrupole-Orbitrap mass spectrometer.
Up to 25 amyloidogenic proteins with a maximum of 8 peptides per proteins have been simultaneously targeted. When less than 8 peptides were experimentally identified in the discovery phase, the list was completed using the Peptide Atlas repository.

We propose a specific protocol that allows to blindly discriminate healthy and pathological biopsies first and to classify the pathological samples according to the nature of the amyloidogenic protein. The number of peptides and transitions should be then minimized and optimized for a rapid and accurate amyloid subtyping with a clinical benchtop mass spectrometry (Triple-Quadrupole). Statistical processing will be also validated to ascertain the diagnostic.

P-801.00

LOCALIZING AND CHARACTERIZING DISULFIDE BONDS IN MS-BASED PROTEOMICS USING ONLINE LC–ELECTROCHEMISTRY–MS

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Identification of large numbers of proteins is, without doubt, powerful and useful, yet not sufficient for meeting the aim to fully describe a proteome. In fact, the analysis of protein isoforms, or proteoforms, experiences a growing interest in proteomics. Post-translational modifications (PTMs) can be considered as an "additional layer" of information similar to the epigenetic code that may switch a gene on-and-off. Disulfide bonds are an important PTM because these bonds, or "S–S bridges", stabilize the three-dimensional structure of proteins and as a result are crucial elements for their biological function. Peptide backbone characterization by MS/MS is often hampered by the presence of a disulfide bond.

As a consequence, offline reduction of disulfides with a reagent, such as dithiothreitol, has become a pivotal step in obtaining full sequence coverage of a protein in a bottom-up experiment. However, this type of approach leads to a loss of information regarding the presence and connectivity of disulfide bonds. A recently reported alternative for the characterization of disulfide bonds involves reduction via online electrochemistry, as has been exemplified for lactoglobulin, lysozyme, oxytocin and hepcidin (Zhang et al. Anal Chem 2012; Nicolardi et al. JASMS 2013). In the current study, we further report on the application of electrochemistry to overcome disulfide bridge complexity in the middle-down analysis of proteins. To this end, an electrochemical cell (microprep-cell, Antec) was coupled directly to an electrospray ionization (ESI) source and a Fourier transform ion cyclotron (FTICR) mass spectrometer equipped with a 15 tesla magnet.

By performing online electrochemistry-assisted reduction of disulfide bonds, peptides that initially contained S–S bridges could be identified from their accurate masses. LC–MS/MS experiments including online electrochemical reduction were performed for further validation of the identified disulfide-linked peptides.

P-802.00

EXPLORING THE EFFECT OF DMSO AS A MOBILE PHASE ADDITIVE FOR IMPROVING PROTEIN IDENTIFICATION AND SWATH ACQUISITION BY NANOLC/MS/MS

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NanoLC/MS/MS is the current method of choice for high sensitivity identification of proteins, e.g. for the discovery of biomarkers in plasma or other biological fluids. It has recently been demonstrated that solvents like DMSO can increase electrospray ionization efficiency and improve sensitivity. In this paper we will discuss optimization of the concentration of DMSO, the effect on chromatography, and the improvements on proteomics applications.

A nanoLC-MS set-up using chip based columns was used, with DMSO either added post-column, or added directly to the mobile phase. For adding DMSO post-column a nanoLC chip column was developed with an integrated post-column Tee. The effect of adding DMSO was studied by looking at peptide sensitivity and the overall number of peptides/proteins identified in a complex mixture, using a QqTOF type mass spectrometer.

Using post-column addition of DMSO, the optimal % DMSO after addition was found to be 5%, consistent with previous observations in the literature. An increase in peak area for 13 Beta-galactosidase tryptic peptides was observed that varied from 1.2 to 2.6 x, with a median of 1.9 x. For a Yeast digest, a significant increase in peptides and proteins identified was seen (30 and 20% respectively). Next, adding 5% DMSO directly to the mobile phase was explored. A slight effect on retention times, and minimal impact on peak widths was observed The effect on sensitivity as a function of peptide properties and the effect on protein identification is currently under investigation.

The addition of DMSO promises to improve the number of proteins identified in complex samples using nanoLC/MS/MS, benefiting biomarker discovery research. Next steps will be to characterize the impact of this workflow on SWATH acquisition results.

P-803.00

A NOVEL AND ROBUST WORKFLOW FOR PROTEIN QUANTITATION AND LARGE-SCALE BIOMARKER STUDY IN HUMAN PLASMA

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Introduction and objectives

Biomarker study requires large clinical sample analysis. Successful analysis of hundreds and thousands of samples by Multiple or Selected Reaction Monitoring (M/SRM) needs speed and tight quality control. The core sample preparation steps consisting of protein denaturation, reduction, alkylation, digestion, and desalination are both time and labor consuming. Moreover, these steps induce the greatest technical variation. Here, we demonstrate a novel workflow with robotic sample preparation and on-line desalination for quantitation of 8 proteins in 320 clinical plasma samples.

Methods

In this workflow, sample preparation including protein denaturation, reduction, alkylation, and digestion was accomplished by a Biomed NXP automation workstation. A HPLC-Triple quadruple mass spectrometer system was used for sample analysis. 8 proteins (24 peptides) of potential biomarkers were quantified in human plasma samples. Beta-galactosidase (Beta-Gal) protein from E Coli was spiked in the samples, and served as a quality control protein standard. Synthesized stable isotope-labeled peptides were used as internal standards.

Results and Discussion

320 human plasma samples were processed and analyzed by this workflow. Sample processing was optimized to ensure maximum digestion, 4 runs on the 96 well plate format were required for total sample processing (less than 4 days). Each sample was run in triplicate with a standard curve at the beginning and 3 quality control samples were run across the cohort samples. The average of CVs of each peptide based on quality control samples was under 7%, and CV for spiking in control, Beta-Gal (consisting of two peptides), was

Conclusions

The combination of robotic sample preparation and LC-MS/MS analysis overcome several technical bottlenecks in large-scale biomarker verification and validation.

P-804.00

EVALUATION OF NEW KIDNEY INJURY BIOMARKERS USING ABSOLUTE QUANTITATIVE PROTEOMICS (PSAQ AND SRM)

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For more than 50 years, diagnosis and monitoring of kidney diseases relies on the dosage of functional biomarkers, mainly serum creatinine and blood urea nitrogen. Also widely used, these biological parameters provide little information about etiology and are poorly sensitive to the loss of kidney reserve. During the last decade, extensive efforts have been directed to the identification and evaluation of novel biomarkers to detect kidney injury at early stages with improved specificity. Hence, NGAL, L-FABP, KIM-1 and IL-18 have emerged as promising biomarkers for the diagnosis of a variety of kidney diseases. However, none of these new biomarkers has gained approval of health agencies for clinical use.

The goal of this study was to identify and evaluate new biomarkers that will improve the detection and monitoring of acute kidney injury (AKI). The selected panel combined 3 new biomarker candidates, selected by our laboratory using a data mining approach, as well as NGAL and L-FABP. To develop a quantitative and reliable assay, isotopically labeled protein standards (PSAQ standards) were synthetized for each biomarker candidate. Such quantification standards can be added to clinical samples at early stages of the analytical process and provide accurate results. Then, sample biochemical prefractionation was optimized for sensitive quantification of the 5 biomarker candidates in urine samples.

To specifically quantify the 5 targeted biomarkers, a LC-SRM (Liquid-Chromatography and Selected Reaction Monitoring) pipeline was developed based on narrowbore LC column and scheduled SRM acquisition on a QTRAP6500 mass spectrometer. Once the assay was developed and assessed using titration curves, urine samples from 10 patients suffering AKI were analyzed and compared to urine samples obtained from 15 healthy donors. We confirmed the clinical relevance of NGAL and L-FABP as AKI biomarkers. We also qualified one of our candidates for AKI diagnosis.

P-805.00

IMMOBILIZED MONOLITHIC ENZYMATIC REACTORS FOR ONLINE DIGESTION OF PROTEINS SECRETED BY DEVELOPING HUMAN EMBRYOS

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Introduction and objectives

The process of IVF (In-Vitro Fertilization) involves implantation of fertilized oocytes following long medical preparation upon optical investigation of developing oocytes. Analysis of proteins secreted can help providing a more accurate prediction of the implantation success and embryo development. Classical proteomics approaches involve enzymatic hydrolysis of a protein (either separated by polyacrylamide gels or in solution) followed by peptide identification using LC-MS/(MS) analysis. Enzymatic digestions can take up to several hours to complete. In case of clinical analysis, it is of utmost importance to provide fast and reproducible analysis with a minimum of manual sample handling.

Methods

A specific multivalve switching device was designed and used to setup a complex parallel LC system. The Immobilized Monolithic Enzyme Reactor (IMER) was integrated into parallel nano LC system to perform online digestion followed by reverse phase HPLC separation. An Amazon Speed ETD mass spectrometer was used to record MS spectra. The digestion conditions of IMER were extensively investigated using bovine serum albumin and dynamic protein mix to obtain optimized conditions. Upon system optimization biological samples were applied to the system.

Results and Discussion

Herein we report the method development for online protein digestion on an enzymatic reactor (IMER) to accelerate protein digestion, reduce manual sample handling, and provide reproducibility to the digestion process in clinical laboratory. The composition of digestion solution, including pH, buffer salt and organic solution percentage, were extensively investigated to obtain best digestion. The online digestion conditions, including flow rate, flow-through volume, column temperature, and washing volume, were also optimized.

Conclusions

By sequentially developing and improving the methods applied, we were able to establish an automated sample digestion method, reduce the time for protein digestion from overnight to one hour (including all sample preparation steps), and analyze and detect proteins secreted from developing embryos.

P-806.00

EVALUATION OF DATA-INDEPENDENT ACQUISITION APPROACHES FOR SPIKED PEPTIDES IN HELA DIGEST ON Q-OT-QIT MASS SPECTROMETER

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Introduction and Objectives

Data-independent acquisition (DIA) is becoming increasingly important for quantitative proteomics, providing better reproducibility, acquiring all product ions, and breaking through the limit of throughput. On latest Q-OT-qIT mass spectrometer, tribrid architecture provides potential novelty for data acquisition. Herein three DIA strategies on Q-OT-qIT MS were tested using 10 spiked peptides in HeLa digest.

Methods

Ten non-human peptides were spiked into 100 ng/ìL HeLa digest and analyzed by DIA, WiSIM-DIA and Full MS-DIA. DIA collected high-resolution MS/MS spectra by sequential 20 amu isolation windows. WiSIM-DIA combined three 200 amu window high-resolution SIM scans with sequential 12 amu window ion trap MS/MS scans. Full MS-DIA inserted four high-resolution MS full scans into sequential 3 amu window ion trap MS/MS scans.

Results and Discussion

LOQs of the 10 peptides reached amol level with good linearity (R2>0.99) at about 3 orders of magnitude, suggesting good performance of these DIA methods. Classic DIA utilizes high-resolution MS/MS to generate transitions, similar to SRM on triple quadrupole and thus has better selectivity for high-complicated sample. WiSIM-DIA uses high-resolution SIM for quantification and ion trap MS/MS for sequence confirmation, enabling more sensitive detection for high-complicated sample. Full MS-DIA decreases isolation window to 3 Da, closing to DDA isolation window, and thus can be directly used for database searching, realizing the integration of DDA and DIA modes. SEQUEST searching results show that a total of 2835 proteins were identified from 100 ng HeLa digest (1% FDR) in 90 minutes LC gradient (single experiment).

Conclusions

Various DIA approaches based on Q-OT-qIT MS were evaluated, and their powerful quantification abilities were demonstrated.

P-807.00

BARCDB: A NEW RESEARCH TOOL FOR THE ANALYSIS OF BIOSPECIMENS

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We report the development of a new database of analytical technology services and products designed for the use of hsamples in biomedical research. BARC, the Biobanking Analysis Resource Catalogue (www.barcdb.org), is a freely available web resource, listing expertise and molecular resource capabilities available at research centres and biotech companies. Initially focused on service providers in the Nordic region, BARC is currently being extended to the rest of Europe.

The database is designed for researchers who require information about how to make best use of valuable biospecimens from biobanks and other sample collections, focusing on choice of analysis techniques and the demands they make on the type of samples, pre-analytical preparation or amount needed. BARC has been developed and managed as part of the Swedish biobanking infrastructure (BBMRI.se), but it now welcomes submissions from service providers throughout Europe. BARC can contribute to matching resource providers with potential users, stimulating transnational collaborations, and promoting more optimal use of European resources in general, both with respect to standard and more experimental technologies as well as for valuable biobank samples.

This article describes how service and reagent providers of relevant technologies are made available via BARC, and how this resource may contribute to strengthening biomedical research in academia and in biotech and pharma industry.

P-808.00

HIRIEF COUPLED WITH MS/MRM FOR ANTIBODY FREE PROTEIN QUANTIFICATION IN HUMAN PLASMA

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Introduction: Proteins quantification in biological fluids has been performed primarily by methods relying on antibodies, such as ELISA. Despite those methods offer high sensitivity, the lack of antibodies to target proteins and cross reactivity set limits to detect and quantify targets in biomarker validation studies. Recently Multiple Reaction Monitoring (MRM) targeted mass spectrometry (MS)-based approach for analysis of peptides and proteins has become popular in quantitative proteomics studies. In few cases MRM methods have demonstrated the limit of quantification into the attomole range. However, complex matrices such as human plasma require sample pre-treatments/fractionation processes. Peptide isoelectric focusing (IEF) based fractionation has demonstrated to be powerful method in terms of decreasing of proteome complexity prior MS-analysis.

Aim of this project is develop an antibody free method based on high resolution isoelectric focusing (HiRIEF) coupled with MS-MRM to quantify breast cancer associated biomarkers in human plasma. Method: A mix of standard peptides previously selected has been spiked-in in cell lysate and in plasma. Peptides were separated by HiRIEF. A peptide isoelectric point calculation algorithm tool is used to predict the pI of the targeted peptides and used to guide selection of the fraction for analysis. Peptides were extracted from the strip dried and re-suspended with a known amount of heavy labeled peptide.

Results: A method to quantify biomarker in plasma based on HiRIEF-MRM study has been developed and tested on 30 peptides belonging to proteins relevant in pathways involved in breast cancer. Extraction conditions were optimized to obtain high recovery of peptides from IPG strips (about 80%) and MS acquisition parameters have been optimized in order to measure the most intense transitions. HiRIEF fractionation combined to MS-MRM is a promising approach to develop and improve the sensitivity of antibody free MS-based quantitative assays for the detection of low abundant biomarkers in plasma.

P-809.00

IELEMENT: NEW ULTRA-HIGH RESOLUTION MS (UHR-MS) UHRMS SIGNAL HANDLING APPROACH FOR MORE ACCURATE METABOLITE IDENTIFICATION

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The field of metabolomics has become increasingly important in the functional genomics. Until now, the large scale identification of metabolites is still challenging. The key challenging step for the metabolite identification is on the determination of accurate elemental composition. An accurate elemental composition determination can largely reduce the number of possible chemical structures need for further validation. The specificity for the determination of metabolite elemental composition is based on the mass and isotopic ratio accuracy. To date, the current development of ultra-high resolution MS (UHRMS) can facilitate the separation and accurate molecular weight information of the metabolites. Unfortunately, most of UHRMS cannot obtain accurate isotopic pattern because of the ion-decay effects during the mass measurement.

In consideration of the effects of noise level, signal stability and ion-decay effects, a new signal processing software was developed. There are three major functions: First, the adaptive background subtraction function can improve the noise removal in different m/z range. Second, the use of two-dimensional spectra smoothing can improve signal stability of individual mass spectrum. Third, the isotopic pattern error cause by ion-decay effect can be corrected using polyfit regression.

In this study, a sample with ten fatty acids was analyzed by LC-OT-MS (Orbitrap Elite) in 480,000 resolution. With conventional approach, the average relative isotopic abundance (RIA) error determined by Orbitrap directly was ~5%. With noise removal, spectra smoothing and RIA correction by iElement, the RIA error can be reduced to 1%. With the sub-ppm mass accuracy and ~1% RIA error, the possible compound candidates for each metabolite signal were reduced from 21 to 1. This reduction can significant reduce to effort for unknown metabolite identification and highlight the application of UHRMS in metabolomics application

This new approach can reach sub-ppm mass accuracy and

P-810.00

MANIPULATION OF THE ISOTOPIC COMPOSITION OF PROTEINS AS A POWERFUL TOOL IN HIGH RESOLUTION HIGH SENSITIVITY MASS SPECTROMETRY ANALYSES

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Advances in high resolution, high sensitivity mass spectrometers provide new analytical methods with unprecedented power in protein and metabolite characterization. Most of the protein/peptide identification issues rely on accurate measurement of the monoisotopic mass of the cognate ions to limit the parameter space in sequence database search.

Owing to the relative abundance of the naturally occurring stable isotopes of carbon, hydrogen, nitrogen, oxygen and sulfur, the probability of presence of the monoisotopic ion decreases rapidly when the number of atoms in the biomolecules increases, making it virtually impossible to access experimentally to the monoisotopic mass of large proteins without dedicated ultra-high resolution instruments. One way to overcome this difficulty may be to lower the isotopic complexity of the biological samples. To test this hypothesis, we grew Candida albicans cells on a defined synthetic medium (Yeast Nitrogen Base) using 1% [U]12C-glucose as the sole source of carbon, or on normal glucose (98.93% 12C) as a control. Cell free extracts from both conditions were processed for both classical bottom-up proteomic approaches and preliminary top-down analysis of intact proteins. The bottom-up procedure involved a tryptic digestion of total protein samples, separation and analysis of the resulting peptides by LC-MSMS, using a LTQ-Orbitrap Velos ETD and an Orbitrap Fusion ETD mass spectrometer.

We investigated the information content of the different runs, and pointed out a number of potential difficulties to optimize the acquisition methods, with special emphasis on the determination of the charge state of the ions and the selection of appropriate precursors for MSMS analyses. Provided appropriate processing of the experimental data, this unusual type of metabolic labelling proves to be extremely promising to analyze the dynamics of proteomes with an exquisite sensitivity, and a great potential in top-down experiments.

P-811.00

ABSOLUTE AND MULTIPLEX QUANTIFICATION OF THERAPEUTIC MONOCLONAL ANTIBODIES IN SERUM SAMPLES BY LC-SRM USING PSAQ STANDARDS.

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Since the first approval of a therapeutic monoclonal antibody drug in the late nineties, over 400 therapeutic monoclonal antibodies (mAbs) were undergoing in preclinical development or clinical evaluation. Due to high specificity and efficacy, therapeutic mAbs have become a major class of therapeutic compounds for the treatment of cancers, infectious diseases, allergies, inflammation, and auto-immune diseases. To date, immunoassays, such as ELISA, remain the most sensitive, specific and selective technologies used for quantifying mAbs in biological fluids. However, it requires specific developments for each mAb, suffers from matrix-effect and is species-dependant, what is time-consuming, costly and not really suited for early phases of developments.

Thus, the development of innovative and fit-for-purpose MS-based quantification methods represents a challenge for pharmacokinetics studies. Hence, we have developed a method combining liquid chromatography tandem mass spectrometry (LC-MS/MS) and PSAQ strategy (Protein Standard Absolute Quantification) strategy. The approach, we developed, is faster to set up than ELISA, is an excellent and powerful tool for early estimation of PK in a multiplexed evaluation of mAbs and can be easily transferable to various animal species commonly used in preclinical studies. Moreover, the use of selective-reaction monitoring mode brings optimal specificity in compound selection and sensitivity comparable to ELISA. Full-length stable isotope-labeled mAbs were synthetized using an optimized mammalian expression system. A generic pre-fractionation protocol was developed and calibration curves were performed to assess accuracy and reliability of the method using the labeled mAbs as internal standards. Finally, MS data were compared to data obtained with classic ELISA on serum samples from various species.

We showed that our MS-based quantification approach, based on PSAQ mAbs, provides accurate and reliable quantification data, even when mAbs are co-injected. It is a powerful tool for early selection of best mAbs clinical candidates, thus de-risking pharmaceutical development.

P-812.00

CHARACTERIZATION OF AN IMPROVED ULTRA-HIGH RESOLUTION QUADRUPOLE TIME OF FLIGHT (UHR-TOF) INSTRUMENT FOR PROTEOMICS APPLICATIONS

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Introduction and objectives

In shotgun proteomics it is desirable to identify and quantify a large number of individual peptides from complex samples. Complexity and concentration range, however, pose a great challenge to the MS instrumentation in terms of sensitivity, resolution and dynamic range. Several hardware modifications of a bench-top UHR-TOF instrument were carried out and evaluated addressing these particular performance aspects.

Methods

To test the impact of these modifications on proteomics performance, different complex tryptic digests were mixed with stable isotope labeled peptides or digests of standard proteins at known concentrations, spanning a range of several orders of magnitude. Samples were analyzed with nano-UHPLC MS/MS on the modified UHR-TOF. For peptide identification and quantitative analysis the MaxQuant software package was used.

Results and Discussion

For higher sensitivity at fast acquisition speed, a novel collision cell design was used. Increased resolution could be achieved with a modified reflectron. In addition, a faster detector led to further improvements in resolving power.

Using an optimized detector digitizer combination, a threefold higher dynamic range was observed. However in complex samples, the dynamic range is also limited by the capability of the instrument to resolve nearly isobaric compounds. The performance improvements were analyzed in a label-free quantification experiment, evaluating the number of quantifiable peptides over the entire dynamic range. As a defined model system, a mixture of 48 standard proteins spanning a concentration range of five orders of magnitude (UPS-2, Sigma) was spiked into samples of 500ng E. coli, S.Cerevisiae (higher complexity) or human plasma (wider dynamic range) digests at a concentration of 1:2. During separation with two hour gradients, the UPS-2 peptides could be quantified scans at levels from 500fmol down to the low attomole range.

Conclusions

Improvments to several hardware components allow quantification of complex proteomics samples with very high dynamic range

P-813.00

HIGH QUANTIFICATION EFFICIENCY IN PLASMA TARGETED PROTEOMICS WITH A FULL-CAPABILITY DISCOVERY Q-TOF PLATFORM

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Introduction and objectives Targeted proteomics experiments have traditionally been performed on triple quadrupole instruments, using a multiple-reaction monitoring approach. This requires a priori knowledge of the target precursor/product ion transitions and time-consuming method preparation. However, until a recent past, it was the only approach capable of delivering the dynamic range and sensitivity required for quantitation in complex biological samples. High resolution systems are now able to address these limitations, while providing post-analysis data mining capacities. We report here the characterization of a Q-TOF system for the targeted quantitation of peptides in a plasma tryptic digest.

Methods 7 plasma tryptic digests samples were spiked with 43 Stable Isotopically labelled Standards (SIS) peptides, spanning a 10000 fold concentration range. Samples were measured in quadruplicate with a 60 min nano-UHPLC separation coupled to an impact benchtop UHR-Q-TOF system, operated in the high resolution extracted ion chromatogram (HR-XIC) or Data Independent Analysis (DIA) modes. All results were processed in Skyline and R software.

Results and Discussion Preliminary results reveal high quantification efficiency for the target peptides using both a pure MS-based data acquisition method (HR-XIC) and a DIA acquisition method, where quantification was based on specific fragment ions . The system setup enabled reproducible quantification with a 4.5% average coefficient of variation for detected peak areas. The maximum detectable linear range of the assay was 10^4 for both approaches. A broad quantification range could be covered for natural plasma peptides from 180,000 ng/ml to 230 ng/ml for targeted HR-XIC and from 210,000 ng/ml to 360 ng/ml for DIA.

P-814.00

A COMPARISON OF LABEL FREE DDA-HRXIC AND DIA ACQUISITION ON A BENCHTOP UHR-Q-TOF

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Introduction

The lack of publically available, software and the need of a fast scanning and high-resolution mass spectrometer, have hampered the development of the DIA (Data Independent Analysis) method [1] until the last two years. Today, a DIA method is available on most last-generation ESI-MS/MS systems. We present here a comparative study performed on a Bruker Impact HD to evaluate the benefit of DIA acquisition over DDA-HrXIC (Data-Dependent Analysis with MS/MS for ID coupled to the integration of highly specific MS traces), for label free quantification in complex protein mixture. A yeast extract was used for this benchmark study.

Method

The DIA method has been optimized in order to have a cycle time of 3 seconds resulting in 45 selection windows of 20 m/z to cover the mass range from 400 m/z to 1300 m/z.

For the DDA method, the time cycle was fixed to 3.5 seconds, the associated MS/MS acquisition speed is consequently modulated between 3Hz and 25Hz. Data sets acquired with both approaches were loaded on Skyline software to obtain the quantitative information.

Results and discussion

Quantification of a series of key proteins with a large span of concentrations showed that in the conditions of the analysis:

1. DIA provides clearly a better sensitivity, with an increased signal to noise ratio
2. The repeatability study presented a lower CV% in the HrXIC

Conclusion

The Bruker Impact HD mass spectrometer proved to be reliable and sensitive for label free quantification in complex mixtures for both DIA and DDA approaches. Complementary results are obtained with the two methods, each approach having its advantages and drawbacks. In the conditions of our analysis, DIA achieved a better sensitivity providing an increased dynamic range of the proteome but HrXIC results presented lower CV%.

P-815.00
A SISCAPA IMMUNO-MASS SPECTROMETRIC ASSAY FOR QUANTIFICATION OF SOLUBLE TRANSFERRIN RECEPTOR (STFR) IN HUMAN SERUM

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Introduction
Elevated levels of Transferrin receptors (TfRs) are often associated with dysfunction of erythropoiesis due to iron-deficiencies or diseases such as hemolytic anemia and myelodysplastic syndromes. .We present an alternative approach to ELISA assays for automated quantification of soluble transferrin receptor (sTfR) in human serum based on analyte enrichment in microtiter plates followed by MALDI-MS quantification.

Methods
A sTfR proteotypic peptide was selectedand a stable isotope standard (SIS) version of this peptide was synthesized. To quantify sTfR in human serum, an automated SISCAPA workflow was used to digest the sample, enrich the endogenous and SIS peptides using a high-affinity rabbit monoclonal antibody against the target peptide, wash and elute the peptides from the antibody. The eluted peptides were then spotted onto a MALDI target for automated measurement. The data were analyzed by a software tool calculating the ratio of endogenous to SIS peptides from which the sTfR content of the sample was quantified.

Results
A 12-point standard addition curve where a mixture of labeled (heavy) and unlabeled (light) peptide was spiked into pooled serum samples was used to determine the endogenous peptide concentration : the heavy peptide concentration was kept constant at 500 fmol while the synthetic light peptide was titrated from 1000 fmol to 1.0 fmol with 0 fmol spiked in the 12th sample (true endogenous level).. A second 12-point 'reverse' curve was built where the SIS peptide was titrated from 1000 fmol to 0.5 fmol while the synthetic light peptide was spiked at a constant 500 fmol level. This curve enabled to determine the assay sensitivity. The average endogenous level of sTfR-[295-309] to be 185 fmol in 10 µl serum with total workflow CV of 6 % (at endogenous level). Using the reverse curve, the lower limit of detection was determined to be 4 fmol.

P-816.00

NEW WORKFLOWS FOR IDENTIFICATION AND PROFILING OF DISULFIDE BONDS IN BIOPHARMACEUTICALS

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Introductionand objectives

The spatial structures of biologics are crucial for their safety and efficacy and fundamentally determined by disulfide bonds (DSBs). We evaluated alternative approaches to the classical comparison of reduced and non-reduced digests using ESI LC-MS³ and MALDI LC-MS³ and non-reduced protein digests.. These workflows were applied to various model proteins (á-lactalbumin, RNAse, adalimumab)

Methods

Digests of were performed with a mixture of LysC and trypsin to obtain a good yield. Free cysteins were blocked with N-ethylmaleimide to avoid disulfide scrambling . The samples were separated using nano-LC followed by MS analysis. An ion-trap equipped with a CaptiveSpray ionization source was used in the ETD workflow, and a MALDI-TOF/TOF instrument was used in the ISD workflow. Software was developed to identify characteristic fragmentation patterns of disulfide-linked peptides in MS2 spectra and generate target lists for the MS3 step.

Results and discussions

To improve ETD efficiency, sheath gas was acetonitrile-enriched : the average charge state distribution (CSD) of the analyzed DSB-peptides was shifted by 30% towards the higher values, and their average intensity was doubled.

In this approach, we utilized a new targeted MS3 experiment to profile DSB-peptides by applying ETD in MS2 followed by targeted CID in MS3. we acquired retention time dependent full scan MS3 spectra in the IT, enabling simultaneous detection of many peptide fragments. .With this targeted workflow, 7 of 8 expected adalimumab DSB-peptides were identified.

MALDI-ISD was used to partially reduce LC separated DSB-peptides. For the identification of DSB-peptides, MS spectra were screened for triplets of the DSB-peptide and their two characteristic ISD fragments. All peaks were subjected to LC-MALDI-MS/MS analysis. For adalimumab, all DSB-peptides with one disulfide bond were identified with this method.

Conclusions

Protein disulfide bond analysis was performed using either an ETD-MRM workflow with an ion-trap or a new MALDI-ISD workflow.

P-817.00

THE PAN-HUMAN LIBRARY: A REPOSITORY OF ASSAYS TO QUANTIFY 10 000 PROTEINS BY SWATH-MS

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Mass spectrometry is the method of choice for the deep and reliable exploration of the (human) proteome. Targeted mass spectrometry reliably detects and quantifies pre-determined sets of proteins in a complex biological matrix and is frequently used in studies that rely on the quantitatively accurate and reproducible measurement of proteins across multiple samples. It requires the one-time, a priori generation of a specific measurement assay for each targeted protein. SWATH-MS is a mass spectrometric method that combines data-independent acquisition (DIA) and targeted data analysis and vastly extends the number of proteins that can be targeted in a sample compared to selected reaction monitoring (SRM), the prototypical targeting technique. To enable exhaustive and comparable analysis of SWATH-MS data, portable large-scale assay libraries are required.

Here we present the "Pan-Human Library", a compendium of highly specific assays covering more than 10 000 human proteins and enabling their targeted analysis in SWATH-MS datasets acquired from research or clinical specimens.
We combined identified spectra from over 300 measurements of distinctly fractionated cell lines, tissue and affinity enriched protein samples to generate a non-redundant, high confidence assay library for 140 000 proteotypic peptides, identifying over 10 000 human proteins.
We show that using this assay library in combination with an adapted data analysis strategy, more than 2 800 non redundant, canonical proteins with at least two proteotypic peptides can be identified at a 5% protein false discovery rate (FDR) in a single SWATH-MS injection acquired from digested, unfractionated lysate of a human cell line.

This resource supports the confident detection and quantification of 65.2% of all human proteins with experimental evidence and of 78.9% of the human proteome so far detected by the HUPO Human Proteome Project and is therefore expected to find wide application in basic and clinical research.

P-818.00
ROBUSTNESS STUDY OF PEPTIDES IN PLASMA USING NOVEL TECHNOLOGIES IN AN LC-MS INSTRUMENT

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Introduction and Objectives:
LC-triple quadrupole mass spectrometry is often the technique of choice for the analysis of peptides in plasma due to the sensitivity and the selectivity of the MRM analysis. However, the plasma matrix often leads to degradation of performance and loss of sensitivity with repeated injections, necessitating the need to clean the system frequently. In response, an LC-MS system has been developed with an active exhaust in the source and an orifice interface with dual ion funnels in the analyzer. The robustness of this new LC-MS system is evaluated with the analysis of peptides in plasma over a thousand runs.

Methods:
Bovine Angiotensin 3 digest was spiked into serum and separated on a Bruker Advance UHPLC system. The peptide was analyzed with the Bruker EVOQ Elite triple quadrupole mass spectrometer operating in MRM mode with a H-ESI source. Signal intensity was measured during the course of a thousand repeated injections.

Results and Discussions:
The new ESI interface design focuses the LC-eluent spray on the inlet to the mass spectrometer and away from the other surfaces of the ion source. The effectiveness of this design was evaluated with dye infusions to visualize the location of the ESI spray. Images of the system after infusion show a narrowly focused area around the inlet cone and no build-up in other areas of the ion source.
A peptide in plasma sample was repeatedly injected over several thousand runs. Signal intensities were measured throughout the course of the study with no measureable decrease in signal. Visual inspection of the ion source housing and cone showed only minor buildup of contaminants that did not lead to any deterioration in performance.

Conclusion:
An LC-QQQ with active exhaust, orifice interface and dual ion funnels technology provided robust analysis of peptides in plasma.

P-819.00

INCREASE OF SENSITIVITY WITH THE NANO-ESI CAPTIVESPRAY SOURCE ASSOCIATED TO NANOBOOSTER (BRUKER)

Marine Plumel[1], Diego Bertaccini[1], Agnes Hovasse[1], Alain Van Dorsselaer[1], Christine Schaeffer-Reiss[1]
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Introduction:
Current quantitative proteomics methods must still make significant progress and have to gain in sensitivity, precision and robustness in order to enable the quantification of all proteins of a biologic sample. Nanospray is a key tool in high sensitivity mass spectrometry required for these proteomics studies.
In this context, we have evaluated a new nanospray source solution, the CaptiveSpray, equipped with a nanoBooster (enrichment of nebulizing gas with organic solvent vapors, acetonitrile in our study) for the detection and quantification of low abundance proteins in complex mixture.
A comparison with the classical nanoESI sprayer, formerly used on the Bruker Q-TOF, was also conducted to determine the gain of sensitivity and linearity.

Methods:
A yeast extract was used for this benchmark study. 1µg of yeast digest was analyzed by LC-MS/MS using a nanoACQUITY Ultra-Performance-LC system (equipped with a Acquity column (250mm*75µm, 1.7µm, C18) coupled to an Impact HD benchtop Ultra-High Resolution Q-TOF (Bruker).

Results and Discussion:
The acetonitrile enrichment coupled to the CaptiveSpray source increased the identification rate of the yeast digest by around 15%. MS/MS Spectral quality was also increased for multiple states of charge (4+, 5+ and 6+). The use of the CaptiveSpray equipped with nanobooster resulted in doubling the number of identified yeast proteins compared to the use of the standard nanoESI Sprayer, and permitted to increase the linearity of detection (dynamic range) as well. The limit of detection has been lowered by a factor of five reaching the femtomol range.

Conclusion:
Using Acetonitrile as a doping agent for the nebulizing gas with the CaptiveSpray nanoBooster has permitted to increase both the sensitivity and dynamic range of the proteome analysis with no degradation of the robustness and reproducibility which are essential for quantitative analyses

P-820.00

ENRICHMENT OF CARBONYLATED PEPTIDES USING BIOTIN-LC (LONG CHAIN)-HYDRAZIDE

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Introduction and objectives

Irreversible cell damage through protein carbonylation is the result of reaction with reactive oxygen species (ROS) and has been coupled to many diseases. The precise molecular consequences of protein carbonylation, however, are still not clear. The localization of the carbonylated amino acid is an important piece of information in this puzzle. Mass spectrometry is here a superior tool; however, protein/peptide carbonylation is of low stoichiometry and the MS signal is decreased due to low ionization efficiency and signal suppression from non-modified peptides. In addition, other more common modifications are isobaric to carbonylation and it is often challenging to detect the weaker signal from carbonylated peptides necessitating enrichment step. We here present an optimized method for the enrichment of carbonylated peptides.

Methods

Bovine serum albumin (BSA) and isolated, oxidatively stressed mitochondria were used as model samples. For BSA, protein carbonylation was induced using metal-catalyzed oxidation. Biotin-LC-hydrazide was used to tag the carbonylated sites. The tagged peptides were enriched from protein digests using magnetic monomeric avidin beads and analyzed using LC-MS/MS. The peptides were identified by the mass tags and by the production of two specific biotin-LC fragment ions in the MS/MS spectrum.

Results and Discussion

Appling this method increased the number of identified carbonylated peptides 10-fold compared to the non-enriched samples. The peptides were identified with high confidence due to the production of two biotin-LC fragment ions and increased ionization efficiency of the tagged carbonylated peptides. Moreover, the tagged species constituted >40% of the enriched sample, significantly decreasing the suppression from non-tagged peptides.

Conclusions

The performance of this enrichment method is a significant improvement over current alternatives to analyze protein carbonylation in terms of enrichment efficiency and MS identification. This method will help to gain new insights into role of protein carbonylation in pathophysiology of oxidative stress-related diseases.

P-821.00

IMPROVED SPATIAL RESOLUTION IN THE ANALYSIS OF FFPE TISSUE AFTER TRYPTIC DIGESTION

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Introduction and objectives

In recent years, MALDI imaging has proven to be a powerful method for clinical research. Although imaging of protein distributions has had substantial impact in this area, three challenges does still have to be addressed: the detection of high molecular weight (>30kDa), the identification of m/z signals and the accessibility of formalin-fixed, paraffin embedded (FFPE) tissues. Trypsin digestion is seen as a promising approach for improving the above points and we present here an update on our efforts to develop a robust protocol for on-tissue digestion.

Methods

Mouse and rat brains tissue sections were mounted on conductive glass slides. Digestion was preceded by paraffin removal and antigen-retrieval treatment. Enyzme and matrix application was performed by a piezoelectric sprayer. Incubation at elevated temperatures (37 – 50 °C) was conducted in a custom-made humid chamber. MS data was acquired on a MALDI-TOF instrument operating in reflector mode. Results were visualized and evaluated using commercially available software.

Results and discussion

On-tissue digestion adds more complexity to sample preparation for MALDI Imaging, which makes development of a robust protocol challenging. Spatial resolution, number of peptides generated and identification rate are considered important for a successful experiment, but optimizing these can be diametrically opposed. We found that high humidity and extended digestion time increased the effectiveness of the digestion, but also on-tissue delocalization of peptides. Short (

Conclusions

We present an optimized protocol for on-tissue digestion, considering several parameters to achieve efficient digestion at good spatial resolution.

P-822.00

LC-MS/SRM ASSAY FOR THE QUANTIFICATION OF PROTEINS INVOLVED IN CELLULAR LIFE AND DEATH PROCESSES – ELUCIDATING THE UNDERLYING MECHANISMS OF MITOCHONDRIAL DYSFUNCTION

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Introduction and objectives:

Mitochondria are the powerhouses of the cell, and are involved in several key metabolic and signaling mechanisms. In cases of cellular stress the mitochondria can initiate a series of defense responses in order to maintain its homeostasis, which is obviously vital for the cellular survival. The responses are ranging from removal of reactive oxygen species and misfolded proteins over mitochondrial fission and fusion to apoptotic removal of the entire cell in cases where the mitochondrial function cannot be maintained or restored. Quantitating protein markers for mitochondrial homeostasis is therefore critical for studying the underlying mechanisms of mitochondrial dysfunction and associated diseases. Therefore we initiated the development of a quantitative mass spectrometric (LC-MS/SRM) assay for monitoring 25 proteins important in cellular survival and death mechanisms. The systematically selected pathways were chaperones, antioxidant enzymes, proteins involved in autophagy, mitochondrial fusion and fission, apoptosis, and the respiratory chain.

Methods:

Human dermal fibroblasts from three healthy controls and three Medium-chain acyl-CoA dehydrogenase (MCAD) deficient patients were studied. Whole cell lysate as well as enriched mitochondria were separated by SDS-PAGE, and proteins were in-gel digested using Trypsin, purified on C18-columns and analysed by LC-MS/SRM. Peptides were monitored using a LC-MS/MS TSQ Vantage triple quadrupole system. Two to four peptides were selected for each protein, and stable isotope heavy labelled peptide analogues were used for each of the peptides and four transitions were monitored.

Conclusions:

The developed assay gives insight to the underlying mechanisms of mitochondrial dysfunction. LS-MS/SRM enables simultaneous quantitation of multiple proteins in a single analysis, which is an advantage to western blotting. Enriching mitochondria is a necessary sample preparation step in order to monitor low-abundant proteins.

P-823.00

COMPARISON OF THREE MS2 BASED QUANTITATIVE METHODS FOR TARGETED PEPTIDE QUANTITATION

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Introduction and Objectives

To evaluate and compare targeted peptide quantitative performance of MS2 based quantitation medthod, including parallel reaction monitoring(PRM),data independent acquisition (DIA) workflow on Q Exactive hybrid quadrupole-Orbitrap TM and traditional selective reaction monitoring (SRM) on TSQ Quantiva TM triple quadrupole mass spectrometer.

Methods

For PRM, all the targeted precursor ions m/z were imported into the inclusion Mass list, MS/MS spectrum of each precursor ions with 2amu isolation window were acquired one by one. For DIA, 16 sequential MS/MS with 25 amu isolation windows were acquired to cover all precursor ions of 400 ¨C 800 m/z. Product ion was extracted from MS/MS spectrum with 10ppm mass tolerance, which provided quantitative information. All MS/MS fragment information was recorded for sequence confirmation of any peptide of interest by spectral library match.

As to SRM, multiple transition information for each targeted peptide was recorded. One of them was used for quantitation, the others were used for confirmation. The quantitative performances of these three approach were evaluated using 14 heavy isotope-labeled peptide spiked in 10ng E.coli tryptic digest matrix.

Results and Discussion

All data processing was performed in Pinpoint1.4 software. Calibration was generated for each peptide. 3.5 to 4 orders of linear dynamic range was observed in these three approaches with good precision. 1 attomole limits of detection (LODs) was observed for SRM method, with 5 attomole LOD for PRM and 20amol for DIA. The pattern of composite MS/MS collected from multiple transitions or real MS/MS scans was used for peptide verification by spectral library match.

Conclusions

Limit of detection (LOD) were achieved in the amole range and 3.5 to 4 orders of quantification linearity were achieved for all these three method.Compared with traditional SRM methods, novel quantitative method on high resolution mass spectrometer such as tHCD and DIA are promising quantitative methods.

P-824.00

IMPROVEMENT OF SELECTIVITY AND SENSITIVITY BY OPTIMIZED SWATH METHODS

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One of the challenges in quantitative proteomics is the high complexity on protein level in biological samples. Method optimization tailored to the sample under investigation will improve the quality of the results. Therefore, we optimized the data independent acquisition technique SWATH. It is a technique that outperforms Selected Reaction Monitoring (SRM) and allows the quantification of all analytes present in complex samples.

The aim of this work is to compare the performance of different SWATH method settings. Samples of different complexity were used to determine selectivity, limits of detection (LODs) and limits of quantification (LOQs). Therefore, methods were designed using precursor ion isolation windows between 10 and 35 m/z and accumulation times between 30 and 105 ms. As simple testing mixture, we used a commercial proteins standard (UPS1, Sigma) at different concentration levels (0.2 fmol – 20.8 fmol) and as complex testing mixture we used the UPS1 at different concentrations spiked in 1 μg yeast lysate. The samples were digested with LysC and trypsin. ProteinPilot and PeakView software were used for the computational analysis. The best sensitivity and adequate selectivity in the simple mixture was achieved with 35 m/z wide isolation windows and an accumulation time of 105 ms. For the complex mixture, the use of 35 m/z wide isolation windows resulted in higher noise and product ion interferences. Therefore, 25 m/z wide isolation windows provide best compromise between selectivity and sensitivity.

Optimization of isolation window width and accumulation time tailored to the sample of interest can indeed enhance sensitivity and selectivity of the measurement compared to just applying the state of the art method; 25 m/z wide isolation windows and 100 ms accumulation time.

P-825.00

ANALYSIS OF POST-TRANSLATIONAL MODIFICATIONS IN HUMAN TRANSTHYRETIN ASSOCIATED WITH FAMILIAL AMYLOIDOTIC POLYNEUROPATHY BY TARGETED LC/MS AND INTACT PROTEIN MS

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Transthyretin (TTR) is an amyloidogenic tetrameric protein (55kDa) present in human plasma, transporting T4 hormone and retinol, through the retinol binding protein (RBP). TTR is associated with several amyloidosis, namely familial amyloidotic polyneuropathy (FAP), familial amyloidotic cardiomyopathy (FAC) and senile systemic amyloidosis (SSA). Variability of TTR is not only due to point mutations in the encoding gene but also to post-translational modifications (PTMs) at Cys10, being the most common PTMs the S-sulfocysteinylation, S-glycinylcysteinylation, S-cystinylation and S-gluthationcysteinylation. It is thought that PTMs at Cys10 may play an important biological role in the onset and pathological process of amyloidoses related to TTR.

The objective of our study is to analyze the most significant Cys10 PTMs present in TTR human serum samples of patients with TTR-related amyloidoses and to study their relationship with the clinical profile of the patients. To this aim, we previously developed a targeted LC/MS and intact protein MS methodology, both performed on a UHR-QTOF instrument (Impact, Bruker), enabling the relative and absolute quantification of the selected PTM forms of TTR in serum, as well as the wt:V30M TTR ratio. We report here the application of this methodology to two different sets of clinical samples. Analysis of samples from FAP patients at different stages of the disease reveals changes in the distribution of the Cys10 PTMs along the progression.

Through the analysis of a time series from FAP patients having undergone liver transplantation (LT) and from domino liver transplantation (DLT) recipients from V30M carriers, we have characterized the progression of the wt:V30M ratios, as well as the evolution of the Cys10 PTMs, from transplantation and up to 9 years after. The results from this pilot study will be extended to a larger cohort of samples to reassess their potential clinical relevance.

P-826.00

MOTIF-TARGETING PHOSPHOPEPTIDE ENRICHMENT FOR QUANTITATIVE PHOSPHOPROTEOME ANALYSIS

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Phosphopeptide enrichment approaches such as IMAC and titania chromatography have been widely used to unveil cellular phosphoproteomes. In combination with multidimensional LC-MS/MS, it is relatively easy to identify thousands of phosphorylated peptides in single experiments. However, identification of lower abundant phosphorylated peptides such as tyrosine phosphorylated peptides by these approaches is still difficult in the presence of abundant phosphopeptides. So far, sequence/motif specific antibodies such as phospho-(Ser/Thr) kinase substrate antibodies and phospho-(Tyr) antibodies have been employed to enrich phosphopeptides including target sequences/phosphorylated amino acids. But the enrichment selectivity is not sufficient enough to exclude contaminant peptides. In addition, the required amount of samples is generally over mg range. In this study, we developed a novel motif-targeting approach to enrich target phosphopeptides without using antibodies.

HeLa cells treated with EGF and selumetinib were prepared. After tryptic digestion, stable isotope dimethyl labeling was carried out for selumetinib-treated samples (heavy label) and untreated control (light label). After mixing the samples, phosphopeptide enrichment was done and the phosphopeptides were dephosphorylated by phosphatase treatment. A kinase having target substrate motifs was spiked and phosphorylation reaction to the peptides with target motifs was carried out. After 2nd HAMMOC, the eluted phosphopeptides were dephosphorylated by the phosphatase and were subjected to nanoLC□|MS/MS analysis.

For the MEK inhibitor selumetinib-treated HeLa cells, 926 and 891 phosphorylated peptides were successfully quantified by the motif-targeting approach with MAPK and EGFR, respectively. As a result, more than 100 phosphopeptides with PXpS/TP or pY were up- or down-regulated including VADPDHDHTGFLpTEpYVATR(ERK2) and LEPVYpSPPGSPPPGDPR(S6Kf¿4), whereas these two phosphopeptides were not quantified by the conventional phosphoproteomics approach because of smaller signal-to-noise ratio. Since this approach can easily target the phosphopeptides of interest by changing spiked kinases, it would be helpful for target quantitative analysis of phosphopeptides in proteome-scale.

P-827.00

**IN VIVO DEEP PROTEOME MICRO/NANO-HARVESTING
EXPLORING INACCESSIBLE HUMAN TERRITORIES**

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Introduction

Biomarker discovery and screening using novel proteomic technologies is an area that is attracting increased attention in the biomedical community. However, the large dynamic range of serum/plasma proteins remains a critical issue for the development of MS based proteomics. Additionally, pre-analytical bias due to sample handling and stability is another raising concern of the reliability of proteomics profiling approaches in clinical applications. Here we aimed to demonstrate the advantage of using specific micro/nano devices in combination with the MS strategies, providing a technological breakthrough for relevant biomarker harvesting.

Method

We investigated the interest of magnetic nanoparticles (NPs) as harvesting surfaces to reduce the dynamic range of protein concentrations, allowing for the exploration of the deep proteome in complex body fluids. We further develop novel micro/nanotechnology based proteomic platforms for biomarkers discovery in circulating fluids and at the immediate contact with the pathological tissues.

Results

We performed Nano-LC-MS/MS analysis to validate the enrichment efficacy of our NP-harvesting method compared to untreated samples. Given the magnetic properties of the NPs, we evaluated the potential use of their biomarkers-harvesting capability in vivo. The NPs were injected into the blood circulation and re-collected using an innovative magnetic probe. This probe, magnetically patterned at the micron-scale, acts as an extremely efficient magnetic trap, able to attract magnetic nanoparticles with diameter below 10 nm injected in the blood circulation. Direct MS analysis of the proteins captured by the nanoparticles and trapped on the probe was performed, demonstrating the feasibility and efficacy of our in vivo bio-harvesting.

Conclusion

We developed and validated the efficiency of a micro vascular device designed for direct intra-vascular capture of proteins. This approach will permit to avoid pre-analytical biases of sample preparation and will favor the harvesting of potential biomarkers released by the pathological tissues.

P-828.00

HIGHLY-MULTIPLEXED PROTEIN QUANTIFICATION FROM DRIED BLOOD SPOTS

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Introduction and Objectives: Dried blood spot (DBS) sampling, combined with multiple reaction monitoring (MRM), has proven advantages for quantifying small molecules in drug development and biomarker screening. To extend these benefits to protein targets, we recently developed the first multiplexed DBS-MRM method for the quantification of endogenous proteins. (Mol. Cell. Proteomics, 2013, 12, 781.) We are now developing targeted MRM assays to quantify 120 proteins in DBS samples for clinical biomarker screening applications.

Methods: Proteins in DBS samples were simultaneously extracted and denatured prior to tryptic digestion. Stable isotope-labeled standard (SIS) peptides were then spiked in the sample to enable precise, relative quantification of targeted proteins by LC-MRM-MS (Agilent 6490 QQQ). MRM transitions were screened for chemical interference by monitoring the relative intensities for multiple fragments of the same peptide. The most abundant interference-free transition was used for quantification and calibration curves are employed for peptide targets in the final method.

Results and Discussion: Multiplexed quantitative MRM assays have been developed for 37 proteins in human DBS samples. These proteins span more than 4 orders of magnitude in concentration from albumin (at 76 mg/mL) to apolipoprotein C-I (at 5.9 µg/mL). High precision was achieved as full process technical replicates resulted in CVs of less than 15% for all peptides. Excellent stability was observed as 80% of the targets in DBS samples were within 20% of their original concentration after storage, even at elevated temperature (37°C). Expanding on these results, we have recently detected 120 proteins in DBS by MRM of 226 interference-free peptides and their SIS peptide analogues. Method optimization and full analytical characterization for all quantified peptides will be presented.

Conclusions: DBS sampling has been integrated into highly-multiplexed MRM-based protein quantification and holds considerable promise for clinical proteomics.

P-829.00

COMPARISON OF DRUG DISTRIBUTIONS IN IN-VIVO ANIMAL AND IN-VITRO EXPERIMENTAL MODELS BY MALDI-MS IMAGING

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Introduction

The spatial distribution of an anti-cancer drug in a tumour has a major effect on how effective the drug can be at tackling the tumour. This study provides data about the lateral distribution of sunitinib an oral multiple-receptor targeted, tyrosine kinase inhibitor and anti-angiogenesis drug. We have investigated whether the drug distribution in in-vivo treated tumours grown in mice could be matched using the same tumour cell line in untreated mice using an in-vitro application of sunitinib.

Materials and Methods

5 All tumour sections were examined by MALDI-MSI on a Thermo Scientific MALDI LTQ Orbitrap XL mass spectrometer, subsequently stained by H&E, scanned and the extracted ion maps of sunitinib were overlaid onto the histology images for analysis. Six tumours at 4 different time points after commencement of in-vivo treatment of sunitinib were examined to follow the effect to binding patterns as treatment progressed, whereas 2-2 tumours were investigated by two different in-vitro experimental models. The first experimental method involved deposition a solution of the drug on to tissue sections, while the other involved placing intact tumours in to a solution of the drug, refreezing and then cutting the tumour after diffusion had taken place.

Results

In the investigation of the tumours at different time points, it was found that early after the drug was given the centre of the tumour had no drug present, however by 9 days the entire tumour had drug present. It was also found that the levels of drug present varied in-vivo depending on tumour. The first experimental model gave similar results to what is expected. The second experimental model did not give as clear results as the first. In both experimental models the tumour to tumour variation in levels of drug was also present.

Conclusion

However the deposition a solution of the drug on to tissue sections appeared to best represent the in-vivo situation of a tumour. The tumour diffusion, tumour model appears to not accurately represent the in-vivo situation of an untreated tumour. There also appears to be a significant difference in the levels of the drug in tumours by both in-vivo and in-vitro methods caused by biological factors in the tumours.

P-830.00

DISTRIBUTION OF VEMURAFENIB IN MALIGNANT MELANOMA TISSUE BY MALDI MASS SPECTROMETRY IMAGING

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Introduction and objectives

Malignant melanoma (MM) is one of the leading causes of cancer death. The 5-year survival rate of MM patients with distant metastasis is less than 20%. Vemurafenib is a small compound with promising personalized therapeutic properties, which are specific to BRAF V600 mutation in MM patients. Vemurafenib has achieved improved rates of progression-free and overall survival in patients with the BRAF V600E mutation. MALDI mass spectrometry imaging is a powerful technology to analyze the distribution of endo/exogenous molecular in tissues. We applied MALDI mass spectrometry imaging, to provide direct evidence for the mode of action of vemurafenib in MM.

Materials and Methods

MM tissues were prepared in 10 ?m thin sections prior to be exposed to vemurafenib. Following extensive washing of the tissue sections by PBS and DW, samples were coated with a MALDI matrix, CHCA. These tissue sections were analyzed by a MALDI LTQ Orbitrap XL mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). The fragment ions of vemurafenib were visualized with ImageQuest software (Thermo Fisher Scientific, San Jose, CA).

Results

The intra-tumor localization of vemurafenib was examined. In addition, its signal intensity on the tissues was compared between with and without V600E mutation. The localization of vemurafenib showed heterogeneous distribution on MM tissue sections. The signal intensity of vemurafenib from the MM cells with BRAF V600E was higher than that of control tissue.

Conclusion

We demonstrated evidence of co-localization of the target protein and the drug. Taken together, our results indicated that MALDI mass spectrometry imaging has a predictive potential for efficient treatment for MM patients.

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Topic 20

Proteomics of neglected tropical diseases

OP104 - COMPARATIVE PROTEOMICS OF REPLETE FEMALE TICKS HIGHLIGHTS MOLECULAR MECHANISMS ASSOCIATED WITH BM86 AND SUBOLESIN VACCINE PROTECTION

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Cattle ticks, Rhipicephalus (Boophilus) microplus, are a serious threat to animal health and production in many regions of the world. Tick vaccines are a cost-effective alternative for tick control. Although the antibody response has been well characterized in hosts immunized with the tick protective antigens BM86 and Subolesin (SUB), little information is available on the molecular mechanisms affected in ticks fed on vaccinated hosts that can be associated with vaccine protection. Herein, we compared the proteome of R. microplus female ticks fed on animals vaccinated with BM86, SUB and controls.

The results of the proteomics analysis showed that although both vaccines reduce tick feeding and reproduction, they act through different protective mechanisms. The main protective effect of the BM86 vaccine is the effect on BM86 function and levels, which probably alters tick gut structure and function and consequently blood digestion and reproduction. For the SUB vaccine, the results showed that vaccination reduces protein levels and affects its function as a transcriptional regulator of genes involved in several biological processes important for tick feeding and reproduction.

These results have important implications for tick vaccine research, supporting the study of the molecular mechanisms associated with vaccine protection to combine antigens that act through different protective mechanisms to increase vaccine efficacy.

OP105 - ROLE OF STAGE SPECIFIC LEISHMANIA A600 PROTEINS IN CELLULAR DIFFERENTIATION AND METABOLISM

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Leishmaniasis, caused by infection with Leishmania, is a major public health concern affecting more than 20 million people globally. Leishmania has a digenetic lifecycle consisting of an extracellular flagellated promastigote, adapted to live in the mid-gut of the sand fly host and an aflagellated intracellular amastigote that resides within the macrophage of the mammalian host Differentiation of Leishmania from the promastigote- to the amastigote stage involves significant morphological and biochemical changes. As the vast majority of Leishmania genes are constitutively expressed in both life stages, protein expression levels may play a critical role in differentiation.

The Leishmania specific A600-gene family encodes a family of 4 small membrane bound proteins that show differential expression as they are up-regulated in the amastigote stage; and are essential for parasite replication in host macrophage cells. To determine A600 subcellular localization fusion constructs, A600.1-GFP and A600.4-RFP, were expressed in L. mexicana promastigotes.

Epifluorescence-, confocal, and electron microscopy demonstrated that both proteins localize to the inner mitochondrial membrane. To test whether A600 proteins play a role in cell respiration, ATP levels and the redox potential were quantitated in promastigote and amastigote cells of a wild type L. mexicana and the A600 deletion mutant. Promastigotes of wild type and mutant cells had similar ATP levels and similar redox potential. However, ATP levels in mutant cell amastigotes was significantly lower and redox potential impaired compared to wild type amastigote cells. Furthermore, the redox potential in wild type amastigote but not mutant cells was significantly reduced using LicochalconeA, a fumarate reductase specific inhibitor.

Thus, suggesting a role of A600 proteins in the anaerobic respiratory chain of amastigotes. To elucidate further the role of A600, protein-protein interaction studies are underway to identify whether A600 proteins are integral to a multi-protein complex within the Leishmania mitochondria.

OP106 -   DISCOVERING UNIQUE PEPTIDES WITHOUT A SEQUENCE DATABASE

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Parasitic tissue infection (cysticercosis) by pork tapeworm (Taenia solium) affects millions of people, with WHO estimating 11 million to 29 million cases in Latin America alone, listing it as one of 17 neglected tropical diseases. In 2012, the genome of T. solium was sequenced and a preliminary protein database was built. However, other Taenia species such as Taenia hydatigena also infect swine without causing disease in humans. T. hydatigena remains unsequenced. Since there is currently no diagnostic test to distinguish these two species in meat samples, it is crucial to find unique peptides as biomarkers to develop such tests.

Because the lack of protein sequence information makes peptide identification very difficult, we developed a novel pipeline to discover unique peptides from two MS/MS data sets without requiring a sequence database. This pipeline consists of four distinct steps, starting with the removal of any host or common contaminant derived spectrum. Then, non-peptide derived spectra are filtered out by applying an automatic spectral quality assessment. Subsequently, the MS/MS data sets of T. solium and T. hydatigena are compared on the spectral level to find unique spectra in each data set. The final step of the pipeline tries to identify these unique spectra through searching the six reading frame translated T. solium genome database, and through de novo sequencing. Starting from approximately 450.000 acquired MS/MS spectra in each data set, 42.000 and 26.00 unique spectra are found for T. solium and T. hydatigena, respectively. Furthermore, it assigned reliable sequences to 1253 T. solium spectra, yielding 479 unique peptides for T. solium.

These were sorted based on abundance as measured by spectral counting to rank the most promising candidates.

The pipeline is built to be generic and applicable in any situation where two MS/MS data sets are compared from poorly annotated or unsequenced species.

OP107 - QUANTITATIVE PROTEOMIC ANALYSIS OF HUH 7 CELLS INFECTED WITH DENGUE VIRUS BY LABEL-FREE LC-MS

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Introduction: Dengue is an important and growing globally public health problem, with an estimates of 390 millions of new infections annually. Despite numerous efforts to produce specific antiviral compounds or vaccines, it has not been developed yet. The liver cells constitutes one of the main targets of dengue infection, to gain a better understanding of the cross talk between virus and host cell proteins, here we used proteomics approach to analyze the early host response.

Methods: The differential proteomes were resolved 24 hours post-infection using label-free LC-MS/MS. Proteins that showed a significant alteration in amount during infection were examined using gene enrichment, pathway and network analysis tools.

Results and discussion: Quantitative analysis revealed 155 differentially expressed proteins, with 66 up-regulated proteins and 89 down-regulated proteins including some proteins previously implicated in dengue infection. The proteomic data reveal an important decrease in expression of enzymes in the glycolytic pathway, citrate cycle and pyruvate metabolism. These findings indicate that dengue infection of human hepatocyte cells (Huh-7) significantly perturbs host energy metabolism, the implication of these alteration in the carbohydrate metabolism of the infected cells need to be analyzed. This study thus provides large-scale protein-related information that should be useful for understanding the pathogenesis of dengue infection.

P-831.00

A SYSTEMS BIOLOGY APPROACH TO THE CHARACTERIZATION OF STRESS RESPONSE IN DERMACENTOR RETICULATUS TICK UNFED LARVAE.

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Background: Dermacentor reticulatus (Fabricius, 1794) is distributed in Europe and Asia where it infests and transmits disease-causing pathogens to humans, pets and other domestic and wild animals. However, despite its role as a vector of emerging or re-emerging diseases, very little information is available on the genome, transcriptome and proteome of D. reticulatus. Tick larvae are the first developmental stage to infest hosts, acquire infection and transmit pathogens that are transovarially transmitted and are exposed to extremely stressing conditions. In this study, we used a systems biology approach to get an insight into the mechanisms active in D. reticulatus unfed larvae, with special emphasis on stress response.

Principal Findings: The results support the use of paired end RNA sequencing and proteomics informed by transcriptomics (PIT) for the analysis of transcriptomics and proteomics data, particularly for organisms such as D. reticulatus with little sequence information available. The results showed that metabolic and cellular processes involved in protein synthesis were the most active in D. reticulatus unfed larvae, suggesting that ticks are very active during this life stage. The stress response was activated in D. reticulatus unfed larvae and microorganisms such a Rickettsia sp. similar to R. raoultii was identified in these ticks.

Significance: The activation of stress responses in D. reticulatus unfed larvae likely counteracts the negative effect of temperature and other stress conditions such as Rickettsia infection and favors tick adaptation to environmental conditions to increase tick survival. These results show mechanisms that have evolved in D. reticulatus ticks to survive under stress conditions and suggest that these mechanisms are conserved across hard tick species. Targeting some of these proteins by vaccination may increase tick susceptibility to natural stress conditions, which in turn reduce tick survival and reproduction, thus reducing tick populations and vector capacity for tick-borne pathogens.

P-832.00

PROTEOMICS DISCOVERY OF PLASMA TEST-OF-CURE MARKERS FOR TRYPANOSOMA BRUCEI GAMBIENSE HUMAN AFRICAN TRYPANOSOMIASIS

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Human African trypanosomiasis (HAT) is a parasitic disease affecting rural communities in sub-Saharan Africa. To detect relapses after treatment, especially when symptoms re-appear, patients need to undergo cerebrospinal fluid (CSF) examinations for white blood cell (WBC) counting and parasite finding.

To try to avoid lumbar punctures, we investigated plasma samples from two T. b. gambiense HAT patients using quantitative proteomics based on 6-plex TMT isobaric labelling. Samples taken before the treatment (BT) with Melarsoprol, at the end of the treatment (EoT) and at the time of the relapse (R) were analysed and compared to highlight new plasma test-of-cure (TOC) markers.

Out of the 248 plasma proteins that were identified and quantified, 11 resulted to be increased at the relapse with an R/EoT ratio > 1.5. Lumican showed the highest ratio (R/EoT>2.5) and was thus selected for further verification. Western blot performed on plasma samples taken during the follow-up from a higher number of patients (n=10) confirmed the significantly increased lumican concentrations at the time of relapse (R/EoT = 2.18, p-value < 0.05). In order to validate these results and to evaluate the potential of lumican as a plasma TOC marker for HAT, we are currently developing home-made ELISA and SRM assays to measure its concentration on a cohort comprising more than 250 HAT T. b. gambiense patients followed after treatment.

The validation of lumican as a plasma TOC marker would represent a major advance in HAT patients' management as it would avoid lumbar puncture, which represents an important barrier for patients' attendance to the follow-up and thus a risk for patients' health.

P-833.00

INCREASED ACUTE IMMUNE RESPONSE DURING THE MENINGO-ENCEPHALITIC STAGE OF TRYPANOSOMA BRUCEI RHODESIENSE SLEEPING SICKNESS COMPARED TO T. B. GAMBIENSE

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The characterization of the host central nervous system (CNS) response to T. b. gambiense or T. b. rhodesiense parasites, the causative agents of human African trypanosomiasis (HAT), is a hardly explored area of research. The two parasites are responsible for the chronic or the acute forms of HAT, respectively, both characterized by the progression from a haemolymphatic first stage (S1) to a meningo-encephalitic second stage (S2) due to the invasion of parasites into the CNS.

In the present study we applied a quantitative proteomics approach, based on 6-plex TMT isobaric labelling, to compare and characterize cerebrospinal fluid (CSF) samples taken from S2 patients affected by either T. b. gambiense or T. b. rhodesiense HAT. The gene ontology and pathway analyses performed on the 222 quantified proteins, revealed a predominant activation of the innate immune and the acute phase responses in the rhodesiense form of the disease. These results were further confirmed through the verification by ELISA of the over-expression of two proteins involved in these mechanisms, C-reactive protein (CRP) and orosomucoid 1 (ORM1), on a population comprising n=126 S2 HAT patients suffering either from the chronic or the acute form of HAT. Both proteins resulted to be highly significantly increased in the CSF of T. b. rhodesiense patients (Mann-Whitney U test, $p < 0.0001$).

The results presented in this work contribute to a better comprehension of the pathophysiological mechanisms of HAT late stage caused by T. b. gambiense or T. b. rhodesiense. Moreover, new molecules, CRP and ORM1, able to differentiate between the two forms of S2 HAT are proposed, paving the way for further studies to assess their clinical utility.

P-834.00

STRUCTURAL CHARACTERIZATION OF ECHINOCOCCUS GRANULOSUS IMMUNODOMINANT PROTEINS AND DEVELOPMENT OF A NOVEL IMMUNOASSAY FOR HUMAN CYSTIC ECHINOCOCCOSIS

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Introduction and objectives

Human cystic echinococcosis (CE) is a long-lasting infection caused by the cestode Echinococcus granulosus. Serological immunoassays should enable reliable diagnosis of CE, but currently available immunodiagnostic tests lack sensitivity and specificity, and their use needs standardizing.

Aim of this study was the structural and immunogenic characterization of the most abundant and immunogenic HCF proteins, in order to assess the antibody response to purified and synthetic antigens for the development of a sensitive and specific diagnostic kit.

Methods

Western immunoblotting, size exclusion chromatography, crosslinking experiments and mass spectrometry analysis were performed on sheep HCF, synthetic and native partly purified antigens. ELISA assays on almost 700 sera (368 patients and 253 healty donors) were carried out for validation.

Results and discussion

Western immunoblotting assays on HCF, performed against positive human sera, showed many protein bands, whose identities were investigated by mass spectrometry, revealing the presence of Ag5 and/or AgB, the most immunodominant proteins from Echinococcus granulosus.

In order to characterize AgB immunogenic properties, typically occurring in western blotting with a ladder like pattern, caused by the oligomerization of single subunits, the 4 synthetic subunits of AgB were produced and tested in crosslinking experiments. An Ag5 enriched fraction was obtained by HCF size exclusion chromatography, to which patient sera showed an unambiguous reactivity when compared to the heterogeneous results seen with crude HCF; moreover, a significant reduction in nonspecific signals was detected with control sera.

The suitability of the enriched Ag5 preparation to the ELISA format was then evaluated on all patient and control sera. A ROC curve confirmed the high specificity and sensitivity of the test.

Conclusions

The methods and results reported open interesting perspectives for the development of sensitive diagnostic tools to enable the timely and unambiguous detection of cystic echinococcosis antibodies in patient sera.

P-835.00
SCHISTOSOMIASIS INDUCES SIGNIFICANT CHANGES IN THE HOST BILE PROTEOME PROFILE.

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Introduction and objectives

Schistosomiasis is a disease caused by blood trematodes affecting man and animals that represents an important human health and veterinary problem in many tropical and subtropical areas of the world. Main damages caused by this infection are a consequence of the host inflammatory reaction against the parasite eggs trapped inside the host tissues, being the liver one of the most affected organs. Despite the hepatic pathology of schistosomiasis is very well known, there is no specific studies dealing with the schistosome infection effects on the biliary function. The purpose of this work was to initiate such a study by analyzing the changes induced by Schistosoma bovis infection in the protein composition of host bile.

Methods

The pooled bile vesicles from either uninfected healthy mice (C) or from 4 month S. bovis infected mice (Sb) were homogenized using an Ultra-Turrax T10 disperser and sonicated. The resulting homogenates were fractionated by centrifugation at 17,000 xg and 100,000 xg, and the pellets and supernatants were electrophoresed in SDS polyacrylamide gels. The proteins were in gel digested and analysed by LC-MS/MS for identification. The identified proteins were subjected to functional in silico analysis and comparison between control and S. bovis-infected mice.

Results and Discussion

As a whole, almost 2000 proteins were identified in the supernatant and pellet samples showing a range of proteins shared between both groups of mice as well as proteins only present in either the Sb or C group. Functional predictions of the identified proteins revealed clear differences in the bile proteome between non-infected and infected mice.

Conclusions

S. bovis infection in mice substantially modifies the protein composition of the bile, which could affect its functionality.

P-836.00

SCHISTOSOMIASIS: WORM-INDUCED CHANGES IN THE PROTEOME OF THE LUNG ENDOTHELIAL CELL SURFACE

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Introduction and objectives

Schistosomes are blood trematodes perfectly adapted to their intravascular habitat. They have evolved mechanisms to manipulate the immune and haemostatic responses of the host and to regulate the endothelial cell function favouring their own survival. The objective of this work was to analyse the changes induced by the Schistosoma bovis schistosomula larva in the proteome of the endothelial surface of lung vasculature expressed by infected hamsters over 20 days, which is the period of pulmonary migration of the parasite.

Methods

The lungs of non-infected and S. bovis-infected hamsters for 10 and 20 days were subjected to vascular perfusion with a biotin ester reactive. The biotinylated proteins from endothelial surface were purified by streptavidin affinity chromatography and analysed by liquid chromatography and tandem mass spectrometry analysis (LC-MS/MS).

Results

Overall, 459 non-redundant proteins with two or more unique peptides were identified: 148 in non-infected hamsters (L0); 120 in hamsters infected for 10 days (LS10) and 364 in hamsters infected for 20 days (LS20). Comparative analysis of the pulmonary proteomes of the infected hamsters (LS10 and LS20) and of the non-infected animals revealed that the greatest differences lay between the non-infected animals and the group LS20. Among the protein found in this group, we identified a particular set of proteins with important functions in the parasite-host relationships and lung physiology (namely, proteins involved in the immune response and anti-parasite defence mechanisms; proteins with angiogenic function, and proteins involved in inflammatory processes and leukocyte recruitment).

Conclusion

At 20 days post-infection, when most of the migrating larvae have left the pulmonary vasculature, the pulmonary endothelium displays an inflammatory, pro-angiogenic phenotype, with the formation of new endothelial vessels from pre-existing post-capillary venules and wound repair.

P-837.00

SHOTGUN ANALYSIS OF PLATELETS FROM DENGUE PATIENS: CONTRIBUTIONS TO THE PATHOGENESIS ELUCIDATION

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Dengue is the most frequent hemorrhagic viral disease and re-emergent infection in the world, infecting millions people and causing thousands of deaths every year. Infection can be asymptomatic or may lead to sickness whose intensity may vary, featuring from undifferentiated fever up to severe cases with bleeding and shock. Although thrombocytopenia is characteristically observed in mild and severe forms of dengue, the role of platelet activation in dengue pathogenesis has not been fully elucidated. Here we report a study of platelets isolated from healthy donors and DV-infected patients, using label-free mass spectrometry-based quantification in a proteomic approach.

For this purpose, isolated platelets were lysed, trypsinized and fractioned by OFFGEL system, followed by nESI-LTQ-Orbitrap XL. Protein identification/quantification was performed using the PatternLab software. As preliminary results, we identified more than 3000 proteins in two conditions (control and dengue) and several proteins were found as differentially expressed with q-value of 0.05. As examples, we can mention upregulated proteins in dengue samples as disulfide isomerase (PDI), required for platelet adhesion/aggregation; anti-oxidant superoxide dismutase (SOD1) and glucose-6-phosphate isomerase (GPI) involved in glycolytic pathway activation. Thrombospondin-1 (THBS1), a glycoprotein which mediates platelet adhesion to endothelium upon injury or inflammation and has anti-angiogenic function when secreted by activated platelets, was downregulated in dengue samples. Another downregulated protein is platelet glycoprotein V (GP5), a component of the von Willebrand factor (vWF) receptor that is cleaved from the platelet surface during activation. Secretion of THBS1 and GP5 by activated platelets explains their lower levels in platelets from patients. Also, we identified exclusively in dengue samples, proteins PCAM1, that mediates platelet-endothelial adhesion; and BID, a pro-apoptotic protein.

All these findings corroborate with our previous data of activation, mitochondrial dysfunction and apoptosis in platelets from patients with dengue and may further improve our knowledge on the pathogenesis of dengue disease.

Interactomics and beyond. Protein networks and pathways

OP096 - LARGE SCALE IDENTIFICATION OF PHOSPHO-DEPENDENT PROTEIN-PROTEIN INTERACTIONS

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Post-translational modifications (PTMs), such as phosphorylation (P), regulate protein activity, stability and protein-protein interaction (PPI) profiles critical for cellular functioning and thus dictate how dynamic information is processed in the cell. To better understand the flow of information through cellular networks we develop direct experimental approaches to analyze conditional, i.e. P-dependent PPI patterns. We use a modified Y2H system (employing human protein kinases) that is capable of detecting PTM-dependent interactions on a proteome wide scale. Complementary to existing strategies such as peptide arrays or AP-MS techniques for the identification of PTM-dependent protein interactions, our approach is examining the binary interactions of full-length human proteins at cellular concentrations in a cellular environment.

We have identified ~300 novel P-dependent PPIs that show high specificity with respect to human kinases and interacting proteins. Though linear sequence motifs do determine interaction specificity to some extent, network analysis of this data set suggests that most PPIs are mediated by unknown linear motifs or are governed by alternative recognition modes. P-dependent interactions are further analyzed in mammalian cell culture systems using e.g. co-immunoprecipitation, protein complementation and functional readouts. The more detailed characterization of selected P-dependent interactions will help to exemplarily demonstrate how PTM-dependent PPIs are dynamically and spatially constrained to separate simultaneously triggered growth signals which are often altered in oncogenic conditions.

OP097 - HIGHLY ACCURATE PROTEIN COMPLEX RETRIEVAL BY AFFINITY ENRICHMENT MS RATHER THAN AFFINITY PURIFICATION MS

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Introduction and objectives
Protein-protein interactions are fundamental to understanding biological processes. One of the most promising methods for their investigation is affinity purification coupled to mass spectrometry (AP-MS). Previously, in AP-MS experiments complexes were purified as much as possible, frequently followed by gel-based fractionation and identification of individual gel bands by MS. However today mass spectrometers are highly sensitive, and powerful quantification strategies can distinguish true interactors from unspecific binders, offering intriguing possibilities to enhance protein complex retrieval.

Methods
We developed a high-performance affinity enrichment - mass spectrometry (AE-MS) method for detecting protein-protein interactions in budding yeast. To that end, we performed single-step affinity enrichment of endogenously expressed GFP-tagged proteins and their interactors, followed by single-run, intensity-based label-free quantitative LC-MS/MS analysis on an Orbitrap classic mass spectrometer. Data analysis was performed using the MaxQuant and Perseus software.

Results and Discussion
In our AE-MS method no attempt at purifying complexes to homogeneity is made. Instead, we developed analysis methods that take advantage of the specific enrichment of complexes in the context of a large number of unspecific binders. Each of our pulldowns contains around 2000 of those background binders, which are reinterpreted from troubling contaminants to useful elements in a novel data analysis strategy. They are exploited for accurate data normalization, which in turn constitutes the basis for accurate quantification. Furthermore, interactors are not identified solely by comparison to an untagged control strain, but by comparison to a number of other tagged strains, and further validated by their intensity profiles across all samples. We demonstrate the power of our AE-MS method using several well-known and challenging yeast complexes of various abundances.

Conclusions
AE-MS is a new concept in interaction proteomics, which is not only highly efficient and robust, but also cost effective, broadly applicable and implementable in any laboratory.

OP098 - AP-MS AND BIOID ANALYSIS OF THE HBX VIRAL-HOST INTERACTOME

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Introduction
Worldwide, 2 billion individuals have been infected with hepatitis B virus (HBV). Despite advances in HBV prevention chronic hepatitis B remains an unsolved problem and a prime target for novel antiviral therapies. The HBV X protein (HBx) is a non-structural, regulatory viral protein with pleiotropic activities which is thought to play a central role in HBV persistence and HBV-associated hepatocellular carcinoma. Here we investigated the HBx interactome with two complementary Interactomic strategies in order to elucidate network structure and potential targets for pharmaceutical intervention.

Methods
Systematic reciprocal affinity purification mass spectrometry (AP-MS) and a proximity-dependent biotinylation of living cells coupled to affinity purification and mass spectrometry (BioID) strategy was used to investigate the HBx-host interaction network.

Results and Discussion
Combination of AP-MS and BioID strategies revealed a HBx-interacting network consisting of defined subnetworks. The identified subnetworks confirm and extend current knowledge on the pleiotropic activities of HBx pointing towards an involvement of HBx in multiple distinct cellular processes. Identified interactors show HBx involvement and potentially modulation of proteasome-dependent degradation, regulation of cellular proliferation, mRNA processing and nuclear translocation. Using two complementary approaches for network reconstruction not only enabled the informed filtering of commonly identified interaction partners, but also the retrieval of new, presumably lower affinity, interaction partners mainly due to the BioID proximity-tagging strategy.

Conclusions
Together, the HBx-Host interactome revealed new leads for pharmaceutical intervention and show the benefit of using a combined AP-MS and BioID strategy for probing biological network structure.

OP099 - UNTANGLING THE COLORECTAL CANCER EGFR/ERBB NETWORK USING A TRIPLE SILAC AP/MS APPROACH.

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Introduction and objectives

Protein-protein interactions (PPIs) are essential for regulating signaling networks by assembling functional complexes. In this work, we focused on how the EGFR/ERBB network is deregulated in colorectal cancer (CRC) in humans. The aim of this work is to provide a quantitative PPIs model of EGFR/ERBB network, in an oncogenic versus non-oncogenic cell line.

Methods

Our cellular model is based on the cell line HCT116 (G13DKRas +/WT) and its isogenic line HKE3 (-/WT). Triple-SILAC AP-MS approach was used. The bait proteins were flag-tagged; the complexes were immunoprecipitated, digested on-beads and analyzed on a Q-Exactive in biological triplicates. The resulting mass spectra were identified and quantified with the MaxQuant software suite. After further processing, with HiScAn software, final interaction fold changes and significance (Benjamini corrected), were used to construct a PPI network (P1.5).

Results and discussion

Comprehensive analysis of all candidate PPIs provides a framework for the understanding of biology as an integrated system. To date, 85 baits were analyzed (2103 nodes, 8244 edges) in order to maximize the coverage of a benchmark in silico generated EGFR core network. Although the topologies of HKE3/ HCT116 networks display similarities, there is significant variation in types of proteins (~15%) and unique interactions detected (~40%). This suggests that the effect of a single mutation deeply penetrates and radiates through the EGFR network.

Conclusions

Our central hypothesis is that PPIs assemble dynamic molecular machines, able to coordinate cellular responses when challenged by systemic perturbations. Our data suggest that the EGFR/ERBB network is significantly re-wired in oncogenic versus non-oncogenic cells. Understanding which nodes are not only re-wired in the presence of a single oncogenic mutation, but also predicted to re-direct information flow through the network, will provide new network-targeted therapies in order to overcome resistance observed in currently used drugs.

P-838.00

IMMUNO-COMPETITIVE CAPTURE MASS SPECTROMETRY FOR THE CHARACTERIZATION OF THE NS5A PROTEIN INTERACTOME IN A CELLULAR MODEL OF HEPATITIS C VIRUS REPLICATION

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Affinity purification coupled to mass spectrometry (AP-MS) using selective antibodies against a target protein has been commonly applied to study protein complexes. However, one major limitation is a lack of specificity as a substantial part of the proposed binders is due to nonspecific interactions. Here, we describe an innovative Immuno-Competitive Capture Mass Spectrometry (ICC-MS) method to allow systematic investigation of protein-protein interactions. We established a complete workflow and assessed its feasibility to specifically map the interactome of the HCV NS5A protein in a human hepatocyte-derived cellular model of HCV genome replication.

ICC-MS involves a competition step between free and bound antibody in the same cellular extract and quantitation using label-free MS. Instead of comparing only one immunoprecipitation with a control, the methodology generates a 12-concentration antibody competition profile. Combined with a robust statistical analysis of the quantified MS signals, the cellular endogenous interactome of a protein of interest can be extracted out of the background of hundreds of proteins.

We uncover seven new NS5A-interacting protein candidates along with two well known NS5A interacting proteins, VAPA and PI4KA, validating our approach. Among the identified candidates, LATS1 and LATS2 kinases were of particular interest. Follow-up biological validation experiments revealed that LATS1 and LATS2 are novel host kinases responsible for NS5A phosphorylation at a highly conserved position required for optimal HCV genome replication.
Whereas further investigations are needed to fully define the respective roles of each kinase, LATS kinases could be potential new host targets for developing HCV therapeutics.

These results are the first illustration of the value of ICC-MS for the analysis of endogenous protein complexes to identify biologically relevant protein-protein interactions with high specificity.
We expect that a broad application of this method together with additional available approaches will help to improve the accuracy and specificity of current protein-protein interaction databases.

P-839.00

ESSENTIAL AND NONREDUNDANT ROLES FOR DIAPHANOUS FORMINS IN CORTICAL MICROTUBULE CAPTURE AND DIRECTED CELL MIGRATION.

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Introduction and objectives:

Formins constitute a large family of proteins that regulate the dynamics and organization of both the actin and microtubule cytoskeletons. Previously we showed that the formin mDia1 helps tether microtubules at the cell cortex, acting downstream of the ErbB2 receptor tyrosine kinase during direct cell migration. Here we further study the contributions of mDia1 and its two most closely related formins, mDia2 and mDia3, to cortical microtubule capture and ErbB2-dependent breast carcinoma cell migration. Methods : We analysed contribution of each formin to erbB2-dependent chemotaxis in Dunn chambers assays. We analysed role of mDia formins in microtubule capture and asked what domain in mDia are required to microtubule capture. Finally, we investigated FH2-interacting proteins involved in microtubule capture using pulldown and mass spectrometry.

Results and Discussion

We find that depletion of each of the three formins mDia1, 2 and 3 strongly disrupts chemotaxis without significantly affecting actin-based structures. Further, all three formins are required for formation of cortical microtubules in a nonredundant manner, and formin proteins defective in actin polymerization remain active for microtubule capture. Using affinity purification and mass spectrometry analysis, we identify differential binding partners of the formin-homology domain 2 (FH2) of mDia1, mDia2, and mDia3, which may explain their nonredundant roles in microtubule capture. The FH2 domain of mDia1 specifically interacts with Rab6-interacting protein 2 (Rab6IP2). Further, mDia1 is required for cortical localization of Rab6IP2, and concomitant depletion of Rab6IP2 and IQGAP1 severely disrupts cortical capture of microtubules, demonstrating the coinvolvement of mDia1, IQGAP1, and Rab6IP2 in microtubule tethering at the leading edge.

Conclusions

Our results provide new insights into the molecular and cellular basis of mDia1-mediated capture of cortical microtubule and open up new avenues of research concerning the discrete functions of Diaphanous formins

P-840.00

STANDARD PROTEASOME AND IMMUNOPROTEASOME ASSOCIATE TO PREFERRED REGULATORS AS REVEALED BY LABEL-FREE QUANTITATIVE PROTEIN CORRELATION PROFILING

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Introduction and objectives:
One challenge of systemic biology is to characterize the complex nature of supramolecular protein machineries. Proteasome particles display high heterogeneity because they are formed by the dynamic association of several sub-complexes, a 20S core particle (20S CP), either single or associated to one or two regulatory particles (RPs) of identical or different protein composition. The 20S CP, which presents an á7â7â7á7 barrel-like structure, can itself be found in the eukaryotic cell as four different sub-types, the major forms being the standard proteasome and the immunoproteasome which differ in their catalytic beta subunits composition.
Identifying preferential associations within proteasome sub-complexes would therefore help to better characterize the functional relevance of proteasome diversity.

Methods:
Recently, protein correlation profiling associated to mass spectrometry, based on the mass separation of protein complexes and their analysis by quantitative MS (1), was used for the determination of protein complexes and their dynamics (2). We developed an integrated proteomic workflow based on proteasome immunopurification, label-free quantification using high resolution MS on LTQ Orbitrap Velos (3), protein correlation profiling, and statistical methods, to resolve the various complexes the different 20S CPs might be involved in.

Results and Discussion:
From a wide set of nine different human cell lines, we could highlight previously unreported preferential associations within proteasome sub-complexes. Our data indeed show that the interactions between the proteasome 20S subtypes, regulators (19S, PA28áâ, PA28gamma, PA200, PI31), and associated proteins (DUBs, Ecm29, shuttling factors,…) do not occur randomly. Some of these preferential interactions were validated using complementary approaches.

Conclusions:
This integrated proteomic workflow provides a valuable tool to better understand the heterogeneity of proteasomes and more generally of complex molecular systems.

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Topic 21: Interactomics and beyond. Protein networks and pathways

P-841.00

PROTEIN INTERACTOMICS BASED ON DIRECT MOLECULAR FISHING ON PARAMAGNETIC PARTICLES: PRACTICAL REALIZATION AND FURTHER SPR VALIDATION

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There is increasing evidence that proteins function in the cell as integrated stable or temporally formed protein complexes, interactomes. Previously, using model samples we demonstrated the applicability of direct molecular fishing on paramagnetic particles (PMP) for protein interactomics (Ershov et al. Proteomics, 2012, 12, 3295).

In present study we have used a combination of affinity based molecular fishing and subsequent mass spectrometry for investigation of human liver proteins involved in interactions with immobilized microsomal cytochrome b5 (CYB5A), also transthyretin (TTR) and BSA as alternative affinity ligands (baits). The LC−MS/MS identification of prey proteins fished on these baits revealed three sets of proteins: 98, 120, and 220, respectively.

Comparison analysis of these sets revealed only one protein common for all the baits. In the case of paired analysis, the number of common proteins varied from 5 to 8 (thus representing less than 10% of total number of identified proteins). The binding capacity of some identified proteins has been validated by a SPR-based biosensor. All the investigated proteins effectively interacted with the immobilized CYB5A (Kd values of ranged from 0.07 μM to 1.1 μM). Results of this study suggest that direct molecular fishing is applicable for analysis of protein-protein interactions (PPI) in normal and pathological conditions, in which altered PPIs are especially important.

P-842.00

MINIATURIZED CHEMOPROTEOMICS WORKFLOW ENABLES CHARACTERIZATION OF PATIENT SAMPLES

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Chemical proteomics is a powerful approach to identify proteins binding to small molecules and determine their respective affinities. In a generic approach, like Kinobeads, kinases from lysates are enriched by promiscuous inhibitors and dose-dependent competition with a free compound is used to determine IC50s. Typical protocols require 5mg to 100mg total protein per data point corresponding to 108 to 109 cells. This restricts experiments to cell culture systems. We aimed for evolving chemoproteomics workflows into miniaturized assays at 100 to 250 µg total protein per data point to enable work with primary cells and patient material. Pull-downs are performed in 384 well filter plates. Peptide fractionation is done either by high pH reversed phase chromatography or stage tip based SAX. Samples are analyzed by LC-MS/MS on a QExactive mass spectrometer.

We optimized the entire chemoproteomics workflow from cell lysis to MS measurement to enable dose-dependent inhibitor binding studies from 106-107 cells per data point. Using Kinobeads as test system we generated 7-point dose-response curves of 250 kinases from 2 mg total protein from a cell line mix in a single MS experiment thus yielding a performance comparable to what we typically achieve using 20-fold more cell extract. Monitoring small molecule affinities to kinases from primary patient material would allow testing of kinase engagement in a simple in vitro assay and could potentially uncover resistance mutations. As a proof-of-concept, we applied the miniaturized protocols to peripheral blood mononuclear cells (PBMCs) isolated from 50 ml blood of three separate human donors and determined dose-response curves for dasatinib for more than 150 protein kinases per donor.

In this study we show that by combining biochemical workflows optimized for small sample amounts with latest MS technologies, chemoproteomics assays approach applicability for primary patient samples and could become valuable tools for individualized therapeutic approaches.

P-843.00

CHARACTERISATION OF B-CATENIN CONTAINING PROTEIN COMPLEXES BY INTRACELLULAR CO-IMMUNOPRECIPITATION AND HIGH OUTPUT WESTERN BLOTTING.

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The canonical Wnt-/β-catenin pathway plays an important role in regulating central physiological processes such as cell proliferation, differentiation and apoptosis. Aberrant mutational activation of central players in this signaling pathway has shown to be closely linked to the development of cancer.

To functionally analyze the signaling state of the Wnt-pathway we chose an approach that focuses on the detection of protein complexes comprising the central regulator β-catenin.

Besides using the conventional co-immunoprecipitation approach we established a novel technique for "intracellular co-immunoprecipitation". This approach uses a recently developed class of intracellular binding molecules, called chromobodies. Chromobodies are composed of the antigen binding domain (VHH) of camelid antibodies connected to a fluorescent moiety (e.g. GFP). They are capable of recognizing and binding their target inside living cells. We generated high affinity binders targeting β-catenin and used them to visualize and monitor the sub-cellular localization and distribution of endogenous β-catenin. Furthermore, the intracellular functionality of chromobodies provides the basis for the intracellular co-immunoprecipitation approach by capturing endogenous protein complexes in living cells.

We show the precipitation of protein complexes consisting of chromobody, β-catenin and various interaction partners (e.g. α-catenin and GSK3β) from HEK293T cells after stimulation of Wnt-signaling and detect these complexes by immunoblot. To get a higher resolution picture of the isolated β-catenin complexes, a novel Western-blotting approach was used that allows probing of the precipitate with hundreds of antibodies. By using this high output Western-blotting (DIGI-West) novel interaction partners of β-catenin could be identified, that are part of protein complexes formed inside the living cell under physiological conditions.

In summary we demonstrate that the combination of intracellular immunopreciptation from living cells using target specific chromobodies with a novel readout system for immunoblots provide a versatile approach to study endogenous complexes and to identify dynamic protein-protein interactions in cancer relevant signaling pathways.

P-844.00

PROFILING OF CELLULAR SIGNAL TRANSDUCTION PATHWAYS USING A BEAD-BASED WESTERN BLOT

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The analysis of cellular signaling cascades is a valuable approach for understanding the processes that underlie cellular functions. Profiling of central signal transduction pathways requires the detection of protein expression and activation. In recent years, mass spectrometry based analysis systems showed their power by allowing unbiased discovery approaches, whereas novel image-guided analysis systems have given detailed information on the localization of proteins. Nevertheless the Western blot is still the most widely employed approach. In hypothesis driven research, this tool gives reliable results with good sensitivity. With the large efforts that have been put into the generation of binding molecules for array-based proteomics, large numbers of antibodies are available and screening approaches that take advantage of these valuable resources are of interest.

Here, we present a novel approach that uses the principles of the Western blot (protein separation by SDS-PAGE; protein immobilization on a solid support; detection by specific antibodies) and combines it with a multiplexed bead array as a readout system. The system allows the generation of hundreds of bead-based Western blot equivalents from a few micrograms of protein and thereby information on the expression and modification of hundreds of proteins is obtained. This new approach combines reproducibility and good linearity with large dynamic range.

Taking advantage of our approach, we performed comprehensive signal transduction analyses of clinical ovarian tumor specimen. Whereas for ovarian cancer first-line platinum-based therapy reveals high response rates (75 %), as many as 70 % of patients develop platinum resistance and relapse. Mechanisms of cellular resistance are still unknown as well are predictive markers. Using our system we compared tumor specimen from relapsed to cured patients directly on the protein level. Hundreds of antibodies were employed to analyze the activation state of different signaling cascades. Data on changes in signal transduction during resistance development is shown.

P-845.00

TARGETING CANCER RELEVANT SUBCELLULAR SPECIES OF Β-CATENIN IN LIVING CELLS WITH NEW INTRACELLULAR FUNCTIONAL ANTIBODIES

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β-Catenin is the key component of the canonical Wnt-/β-Catenin pathway and plays a crucial role in a multitude of developmental and homeostatic processes. Discrete cellular pools of β-Catenin are involved in various cellular mechanisms, such as cell adhesion, mitotic progression and transcriptional coactivation. The different tasks of β-Catenin are orchestrated by its participation on multi-protein complexes and cellular levels are tightly controlled by dynamic posttranslational modifications. Imbalances lead to stabilization of non-phosphorylated/activated β-Catenin in the cytoplasm which translocate to the nucleus and triggers the transcription of genes responsible for aberrant cell proliferation and development of cancer.

To gain a better understanding of β-Catenin's dynamic role in living cells we have generated new recombinant binding molecules (VHH-domains) derived from heavy chain antibodies of camelids. We show that these binders target distinct domains of β-Catenin with nanomolar affinities. By determining the epitope we selected two VHH-domains preferably recognizing the non-phosphorylated/activated species of β-Catenin. Here we demonstrate the application of these new β-Catenin binders in various applications including SPR measurements, sandwich immunoassays co-immunoprecipitations. Moreover we performed MS/MS analysis to identify protein-protein interactions of endogenous complexes by targeting the stabilized/activated species of human β-Catenin. For visualization of endogenous β-Catenin in living cells we combine the epitope-specific VHH domain with fluorescent proteins generating so called "chromobodies". Upon cellular expression those β-Catenin-chromobodies visualize the sub-cellular localization and nuclear translocation, of activated β-Catenin corresponding to compound treatment in real time.

The new single domain antibodies allow comprehensive studies on β-Catenin combining proteomic and live imaging approaches. Applied in tumor models we propose that β-Catenin-chromobodies can be used for MS-based interaction studiesand high content analysis in the field of the Wnt/β-Catenin pathway.

P-846.00

A COMPREHENSIVE PROTEOMICS SCREEN FOR SUBSTRATES OF THE SCFB-TRCP2 UBIQUITIN LIGASE

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Introduction and Objective: Poly-ubiquitination is elicited by the activating enzymes (E1), conjugating enzymes (E2) and ligases (E3), which dictates substrate specificity. Among the ~700 E3 ligases, the SCF family consists of the invariable subunits: Skp1, Cullin, Ring finger proteins and the variable F-box proteins that capture specific substrates to be ubiquitinated by E2. β-Trcp is a F-box protein that contains WD repeats which bind to the DSpGXX(X)Sp phosphodegrons of the substrates. We developed a comprehensive interaction proteomics assay for validating known and identifying new substrates of β-Trcp.

Methods: Based on the 47 published phosphodegrons, a consensus motif is built for mining the human genome. Wild type and mutant FLAG-HA tagged β-Trcp are used for co-immunoprecipitation with HEK293 cellular lysate followed by LC-MS/MS. The mutant β-Trcp harbors an Arg 447 to Ala mutation, abolishing the binding of substrates. To evaluate the interacting partners and to remove the non-specific binders, we use two quantitative proteomics pipelines: the spectra-count based CRAPome and the MS1 intensity-based Perseus. Both bioinformatics and proteomics datasets are then combined.

Results and Discussion: Our screen validated 22 published substrates such as β-catenin 1, Per1 and Rest in a single screen, demonstrating the robustness and reliability of our method. Besides these known interactors, we also identify 421 other proteins that interact specifically with wild type β-Trcp. Among these, 67 are DNA-binding and 90 are RNA-binding proteins. Finally, we present a list of putative novel substrates with their putative degron motifs as well as phosphorylation and ubiquitination sites from PhosphositePlus.

Conclusion: Our combined bioinformatics and proteomics approach identify 421 proteins specifically interacting with the wild type β-Trcp E3 ligase, many of which harbor sequences similar to the DSpGXX(X)Sp motif. Phosphorylation of these motifs and ubiquitination of these proteins have also been confirmed by data-mining of the latest Phosphosite Plus database.

P-847.00

PROTEOME-WIDE ANALYSIS FOR IDENTIFICATION OF FUNCTIONAL SNPS

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Genome-wide association studies (GWAS) have identified more than 50 chromosomal loci that are associated with large artery atherosclerotic stroke (LAS) or coronary artery disease (CAD). LAS and CAD share several risk factors and many aspects of their underlying pathophysiology. Most disease-associated variants are located in non-coding regions of the genome, indicating that these single nucleotide polymorphisms (SNPs) might exert their effects by impacting on transcriptional regulation. Our objective is to characterize the genome-wide molecular interaction landscape for all SNPs that are highly associated with CAD and LAS.

To systematically identify causal variants from non-coding regions and to delineate their molecular mechanisms we employ Proteome-Wide Analysis of Disease-Associated SNPs (PWAS). By combining a DNA-protein pull-down approach with single-shot LC/MS analysis we are able to detect differential binding of transcription factors in an allele-specific manner. Allele specific interactors are subsequently subjected to transcriptional reporter assays. Using this approach we will establish transcription factor-mediated regulation of target genes and gain insight into key molecular regulators and pathways of atherosclerotic phenotypes.

We demonstrate feasibility of a DNA-centric, genome-wide screen for functional SNPs in cardiovascular disease, an approach that can be applied to any complex phenotype.

P-848.00
FUNCTIONAL INTERACTOMICS IN ALZHEIMER'S DISEASE

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Introduction: Alzheimer's disease is a progressive neurodegenerative disorder and the most common form of dementia. It is the sixth leading cause of death in old age people. Despite recent advances, the medical treatment for the disease is hardly effective. Thus there is a need to understand the molecular mechanism behind the disease in order to improve the treatment aspects of the disease. Proteins often use complex networks of interactions to produce a sophisticated signalling network. Deciphering the structure and dynamics of complex network of protein-protein interactions is important for understanding many aspects of living systems in depth.

Aim: The present study aims to investigate the functional interaction of brain proteins and the altered expression of interacting components in a complex. Methods: The protein complexes were isolated from the autopsied human brain tissue cortical region of Alzheimer's patients and Control subjects. After quantification expression profiling was carried out initially on Blue Native PAGE in the first dimension followed by Two Dimensional SDSPAGE. After software analysis of differentially expressed proteins Mass Spectrometric analysis and validation by western blotting was performed. The protein-protein interactions in a single complex were further confirmed by STRING and MINT databases.

Results and Discussion: Thirteen protein complexes were isolated and separated on BNPAGE gel. Each protein complex was further resolved into five to eight components on SDSPAGE. A number of protein components in the second dimension were found to be differentially expressed between the AD and control brain. Mass spectrometric analysis in conjunction with protein-protein interaction database reveals significant interaction of proteins in a single complex which might be involved in the pathophysiology of the disease.

Conclusion: Current study expressed remarkable results that propose unique possibilities to investigate the interaction between protein in complexes in more detail with their modifications in AD for drug development and biomarker discovery.

P-849.00
DICER2-ARGONAUTE2-R2D2 NETWORK DYNAMICS DURING ANTIVIRAL RESPONSE IN D. MELANOGASTER CELLS.

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Introduction and objectives
Fighting viral infections is hampered by the scarcity of viral targets and their variability resulting in development of resistance. In drosophila, viral infections induce various and specific immune responses, including RNA degradation by iRNA mecanism. Viral RNA are recognized and processed into 21-nucleotide-long siRNA complexes by Dicer-2. siRNAs are then loaded onto AGO-2, a central component of the RNA Induced Silencing Complex (RISC), and one strand is discarded, while the other is used to guide RISC towards complementary RNA molecules. These mechanisms are allowed by R2D2, which helps directing Dicer-2 to viral RNA, and loading siRNA onto RISC. The present work aims to identify molecular partners of Dicer-2, Argonaute-2 and R2D2 by using a functional proteomic approach.

Methods
Gene of three major component of the iRNA pathway (Dicer-2, Argonaute2 and R2D2) have been transfected in S2 drosophila cell, the protein expressed and labeled with biotin. Cells were submitted to a RNA virus infection (DCV, FHV, VSV), and proteins bound to the baits have been purified at various time-point and identified by LC-MS/MS on a FT-ICR mass spectrometer. A label-free quantitification method was used to characterize the antiviral complex. The effect of the inhibition of the expression of the identified proteins have been studied by a functional assay.

Results and discussion
This experiment allowed identifying 134 proteins candidates. The effect of the inhibition of the expression of each of these 134 proteins on the viral expression has been studied. For half of them, the inhibition significantly modified the viral expression.

Conclusion
The network of proteins interacting with Dicer2, AGO-2 and R2D2 after infection with each virus have been studied to propose specific function in selective mRNA translation and uncover a promising target for the development of broad antiviral intervention.

P-850.00

REVEALING DJ-1 FUNCTION UNDER OXIDATIVE STRESS THROUGH ITS INTERACTORS: IMPLICATIONS FOR PARKINSON'S DISEASE

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The functions of DJ-1, a Parkinson's disease (PD) associated protein, are not fully understood, however evidences suggest an involvement in protection against oxidative stress, the proposed cause of death of dopaminergic neurons in PD and the trigger condition for DJ-1 function. Therefore, by providing a comprehensive characterization of DJ-1 dynamic interactome under oxidative stress conditions, this work intends to elucidate the mechanisms through which DJ-1 exerts its neuroprotective role in response to oxidative challenges.

Using affinity purification combined with SWATH (AP-SWATH), a dynamic interactomic screening of endogenous DJ-1 was conducted to identify and quantify DJ-1 interactions under resting and oxidative stress conditions. Experimental conditions were defined based on the kinetics of ERK and PI3-K/Akt pathways activation on SH-SY5Y cells under oxidative stimuli, which are dependent of DJ-1. The data generated was analyzed in order to infer the main mechanisms of DJ-1 action, monitoring the interactions changes and highlight groups of proteins with similar behavior, and identify DJ-1 interactors that are responsible for the discrimination between the different stress conditions.

The data obtained from this project largely contribute to the elucidation of the DJ-1 neuroprotective mechanisms. From the characterization of the dynamic interactome of DJ-1 under oxidative stress conditions, several novel DJ-1 binding pasterns were identified pointing to new mechanisms for DJ-1-mediated neuroprotection. Finally, many of the proteins identified are well established in distinct cellular functions implicated in PD, thus, these results will also contribute to a better understanding of the disease.

P-851.00

A CROSS-OMICS GLOBAL PHOSPHOPROTEOMICS AND METABOLOMICS ANALYSIS REVEALS A CONNECTION BETWEEN KINASE INHIBITION AND RNA PROCESSING IN BCR-ABL POSITIVE MYELOMA CELLS

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An integrated triple SILAC global phosphoproteomics and a targeted unlabeled and 13C labeled polar metabolomics approach were used to investigate the signaling of H929 multiple myeloma (MM) cells which possess a BCR-ABL fusion. From 11,880 phosphorylation sites identified, we confirm that H929 cells are primarily signaling through the BCR-ABL-ERK pathway and show that imatinib treatment not only down-regulates phosphosites in this pathway, but also up-regulates phosphosites on RNA splicing factors which causes an inhibition of transcription.

Metabolomics analyses reveal that BCR-ABL-ERK signaling in H929 cells drives the pentose phosphate pathway (PPP) and RNA biosynthesis, where imatinib treatment results in marked PPP impairment and an accumulation of RNA nucleotides that cannot be incorporated into transcribed mRNA. RNA immunoprecipitation studies illustrate that transcription is inhibited in the nucleus upon imatinib treatment, validating the cross-omics results. We also show differences in the metabolism between BCR-ABL transformed multiple myeloma cells and BCR-ABL transformed CML cells.

Overall, these data suggest a novel role for kinase inhibitors whereby not only do they down-regulate phosphorylation of their targets but also induce phosphorylation of RNA machinery that inhibit RNA processes.

P-852.00

ANDSYSTEM: AUTOMATED LITERATURE MINING AND INTERACTOME NETWORKS RECONSTRUCTION IN THE AREA OF BIOLOGY

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Introduction and objectives: To date, over 20 million abstracts highly relevant to biology and medicine are stored in the PubMed database and the number keeps increasing. Existing text mining systems can not completely extract all the information contained in scientific publications. At the same time, it can be assumed that combined use of different text-mining tools will reduce the information loss. Another significant disadvantage of existing systems is too generalized description of interactions between biological objects at which they lose their meaning. At the same time, along with the problem of information lack there is another side of the problem arising in the reconstruction of any particular molecular network using automated methods as well: the "information explosion". It is well known that in the case when the reconstruction process of the network includes the addition of all genes for which there is information that they interact with the started set of genes, very often the "galaxy" can appear on the second or third iteration.

The goal of this work was a development of ANDSystem providing a more complete extraction of knowledge and automated check of the specificity of networks features to the studied phenotypic trait or biological process limiting uncontrolled growth of the network during its reconstruction.

Results: ANDSystem was developed for the purpose of scanning literature for extracting relationships between diseases, pathways, proteins, genes, microRNAs and metabolites and automated networks reconstruction. ANDSystem incorporates utilities for automated knowledge extraction from Pubmed and analysis of factographic databases. ANDSystem is provided with tools supporting automated reconstruction of associative networks taking into account the specific relation of objects in the network to the studied phenotypic trait or biological process limiting uncontrolled network expansion.

P-853.00

MITOCHONDRIAL PROTEIN-PROTEIN INTERACTIONS REVEAL DIVERSE COMPLEXES AND DISEASE-RELEVANT FUNCTIONAL RELATIONSHIPS

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Due to the integrative role played by mitochondria in diverse cellular processes, mitochondrial dysfunction is emerging as a causative factor in a wide range of human diseases, including neurodegenerative disorders (NDs). Yet despite decades of research on MP complexes in isolation, how the nuclear-encoded mitochondrial proteins (MPs) that are widely conserved across eukaryotes interact remains poorly understood, and there are still many outstanding questions regarding the role of mitochondrial dysfunction in the development of human disease.

Our past and current work addresses this gap in two ways: First, a compilation of all existing mitochondrial physical (protein-protein) interaction data for over 1,200 experimentally defined yeast MPs identified hundreds of putative heteromeric MP complexes having extensive associations within mitochondria and with extra-mitochondrial protein-products. As more than one-third of the MP complexes are conserved in humans and contain subunits linked to clinical pathologies, we identified new candidate disease-genes with putative roles in malignancies or neurodevelopment abnormalities. Second, by conducting focused experiments on a selected subset of 130 ND-linked MPs (from a target index of ~600 putative disease-linked MPs), new molecular insights are expected to be gained into the complex clinical presentations of NDs, which has the potential to identify novel therapeutic targets for treating this debilitating chronic disease. As part of the latter effort, we have affinity purified roughly 80 ND-linked MPs in the mammalian non-neuronal (human embryonic kidney) and differentiated neuronal (embryonal carcinoma stem cell) cell lines. Interactors of the purified proteins were identified with an Orbitrap Elite mass spectrometer.

Our assay captured both previously known interacting proteins, as well as several new associations that have not been reported previously. By taking this systematic, integrated look at inter- and extra-mitochondrial protein function, our analysis can identify novel, disease-relevant mitochondrial gene functions and reveal new insights into the complex etiologies of ND disease.

P-854.00

VIROTRAP: ABDUCTING PROTEIN COMPLEXES FROM MAMMALIAN CELLS

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VIB-UGent Medical Protein Research

Affinity purification of protein complexes followed by mass spectrometry is a well-established strategy for the characterization of these protein assemblies. A critical part in this workflow is the homogenization of the cells followed by purification, typically through extensive washing of the protein complex, often resulting in an interaction network biased towards strong(er) interactions.

In Virotrap, we exploit the spontaneous particle formation initiated by expression and multimerization of the HIV-1 GAG protein. Specific protein complexes are sorted and trapped inside these particles by fusion of a bait protein to GAG. Using a novel purification protocol we demonstrate that Virotrap allows the detection of known binary interactions, as well as the identification of new interactions by mass spectrometry.

Virotrap thus provides a unique addition to the arsenal of protein interaction technologies by omitting the need for cell homogenization.

P-855.00

TOWARDS MORE COMPREHENSIVE MODELING OF FUNCTIONAL REGULATION AND PATHWAY ENRICHMENT

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A common goal in many biomedical studies is to try to pinpoint either the mechanisms that change or the level of change for such mechanisms between two conditions. In other words, we often seek the answer to what or how much any potential change is between two medically or biologically relevant conditions. These conditions might be healthy versus sick, treatment vs control, or samples that have received different treatments.

From the turn of the century and onwards numerous methods have been described to address this type of problem. With the rise of high-throughput omics data, questions of this character are more relevant than ever. Within proteomics context however, robust and efficient analysis of regulation on functional level (such as pathways) has additional challenges due to experimental variability, inferential propagation of uncertainties as well as incompleteness of datasets, in comparison to genomics or transcriptomics. On the other hand, proteins are primary actors in biochemical reactions and their activity is the foundation of the mechanisms in question.

We have previously described a method, named FEvER, which is developed in order to address some of the issues with respect to functional analysis based on shotgun mass-spectrometry proteomics data. Here we present a newer version of FEvER, which adds further functionality and aims to address one of the fundamental issues with mass-spectrometry data. Among the changes in FEvER, the highlight is the added ability to process data at peptide-level, instead of protein-level, alleviating problems with protein inference and quantification. Furthermore, peptide-level analysis has the potential to use meaningful PTM information that have been accumulating in the literature over the past decade. We try and demonstrate the proof-of-principle implementation of such an enrichment model, and thus display the potential for peptide-centric pathway analysis.

P-856.00

TOWARDS COMPREHENSIVE IDENTIFICATION OF PROTEIN-SPECIFIC INTERACTOMES VIA COMBINED USE OF AFFINITY MATRICES

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Identifying interacting proteins i.e., components of (functional) protein complexes, is central to mechanistic studies of proteins and their functional/biological processes. A common approach for these studies is immunoaffinity using specific bead matrices (eg: agarose/sepharose versus magnetic beads) followed by mass spectrometry/western blotting. However, it remains unclear if the choice of the bead matrix might affect ability to comprehensively identify components of protein complexes.

Here we address this question, by immunoaffinity and 2-dimensional electrophoresis (2-DE)/mass spectrometric (MS) analysis of the Hsp90 protein chaperone complex using Protein A-sepharose beads versus Protein A-magnetic beads. Equal amount of anti-Hps90 antibodies were cross-linked to above affinity matrices using NHS chemistry and incubated with equal amount of total protein extracts from HeLa cells. Hps90 interacting proteins were eluted from each treatment and visualized by 2-DE or identified by MS analysis.

2-DE analyses revealed a unique pattern of Hsp90-interacting proteins for each of the affinity matrices employed. MS (Velos-Orbitrap) analysis was done to identify Hsp90 interacting proteins. Approximately similar number of proteins was identified from sepharose and magnetic beads. However, the use of sepharose beads led to a higher proportion of cytoplasmic Hsp90-interactors, while that of magnetic beads yielded a higher proportion of nuclear/membrane Hsp90-interactors. Most of the Hsp90-specific interacting proteins fall within four main biochemical/functional classes - co-chaperones, protein kinases or their regulators, transcription factors, and steroid hormone receptors – and constituted a distinct profile for each of the affinity matrices in line with the 2DE and MS analyses. Western blotting is being used to validate identified Hsp90 interacting proteins.

Taken together, our results demonstrate the general benefit of using multiple affinity matrices for comprehensive identification of protein functional complexes via immunoaffinity and mass spectrometry/western blotting.

P-857.00

PATHOPHYSIOLOGICAL MECHANISMS IN COLLAGEN VI MYOPATHIES: CONTRIBUTION OF PROTEOMICS

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Introduction and Objectives. Mutations in each of the three collagen VI genes, COL6A1, COL6A2 and COL6A3 cause two types of muscle disorders: Ullrich congenital muscular dystrophy (UCMD), characterized by severe phenotype, and Bethlem myopathy (BM) with mild to moderate phenotype. Both UCMD and BM patients show dystrophic features with degeneration/regeneration and replacement of muscle with fat and fibrous connective tissue. At molecular level, UCMD patients, not BM, show autophagic impairment, PTP opening inhibited by cyclosporin A treatment leaving the question of pathophysiological mechanisms of these diseases still open. The muscle proteomic changes have been investigated with the aim to elucidate the biochemical mechanism adopted by the muscle to adapt to COL VI deficiency in BM leading to muscle wasting and atrophy in UCMD.

Methods. Qualitative and quantitative differences in the proteome were obtained by 2D-DIGE. The differentially expressed proteins were identified by MALDI-ToF/ToF or LC-ESI-MS/MS mass spectrometry. Altered cellular pathways resulting from proteomic data were studied by immunoblotting.

Results and Discussion. Results indicate alteration in UPR, hexosamine pathway, aminoacid and fatty acid metabolism demonstrating the association of ER stress, metabolic dysregulation, autophagic impairment and alteration in mechanotransduction signalling from ECM to muscle cell.
Overall, these results indicate that the signaling, provided by mutated Col VI, affects glycosylation processes both in BM and UCMD, in BM the protein quality control system remains active and is sustained by a metabolic adaptation supporting energy requirements for autophagic process maintenance, counteracting ER misfolded protein overload, in UCMD this multi layered system is disrupted and worsened by the metabolic rewiring which leads to lipotoxicity.

Conclusions. The identified dysregulated pathways could be targeted for possible intervention aimed to improve the phenotype in UCMD patients.

P-858.00
INVESTIGATION OF MOLECULAR MECHANISMS IMPAIRED IN WILSON

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Introduction: Wilson disease (WD) is an autosomal recessive disorder that is caused by toxic accumulation of Cu in the liver. The ATP7B gene, which is mutated in WD, encodes a multi-transmembrane domain ATPase that traffics from the trans-Golgi network to canalicular area of hepatocytes, where facilitates excretion of excess copper into the bile. Several ATP7B mutations (comprising the most frequent H1069Q and R778L variants) result in the still active protein products that, however, undergo retention in the endoplasmic reticulum (ER), fail to reach Cu excretion sites and, thus, trigger toxic build up of Cu in the liver of WD patients. In order to unravel the molecular mechanisms affected by presence of ATP7B(H1069Q) mutant, both proteomic and transcriptomic approaches has been employed.

Methods: Molecular pathways altered by expression of ATP7B(H1069Q) mutant were investigated by comparing interactomes of wild type ATP7B and the mutant. HepG2 expressing GFP-wt-ATP7B and GFP-ATP7B(H1069Q) were lysated and protein complexes immunoprecipited. The isolated proteins were identified by mass spectrometry methodologies and protein databases search. Analogously, comparative transcriptomic analysis was carried out by microarrays analysis on mRNA extracted from cell expressing wt ATP7B and mutant.

Results: Proteomics analysis revealed specific binding partners of wt-ATP7B and ATP7B(H1069Q). Most of the wild type protein interactors belong to intracellular vesicular trafficking while ATP7B(H1069Q) partners exhibited significant enrichment in proteins localized in ER and proteins involved in degradation proteasome dependent. Transcriptomic data indicate an enrichment in gene networks involved in signaling pathways mediated by p38 MAPK and JNK in cells expressing the mutant.

Conclusions: Proteomic and transcriptomic analyses revealed that ATP7B(H1069Q) is retained in the ER by interaction with specific proteins, some of these able to address the mutant protein to degradation. In particular this phenomena is further sustained by p38 and JNK pathway activation. By inhibiting these two pathways, the rescue of ATP7B function is observed.

P-859.00

OFD1 PARTICIPATE TO PRE-INITIATION COMPLEX AT THE CENTROSOME

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Introduction: Ciliopathies represent an emerging group of human genetic disorders associated to functional and/or structural abnormalities of cilia, specialized, evolutionary conserved organelles that protrude from the cell surface of almost all mammalian cells. Defects in cilia formation and/or function result in a wide spectrum of clinical phenotypes including renal cystic disease and disorders in which the renal involvement is associated to eye, skeletal and central nervous abnormalities such as the Oral facial digital type I syndrome (OFDI). This latter syndrome is associated to mutations in the OFD1 transcripten coding for a centrosome/basal body-associated protein [1] which exerts a critical role in cilia formation [2].

Methods: OFD1 interactome was investigated by a functional proteomic approach based on immunoprecipitation of 3xFLAG tagged protein involving complexes and nanoLC-MS/MS and protein database search protein identification.

Results: More than 100 proteins were identified as putative OFD-1 interacting proteins and clusterised in several functional categories by using information reported in recent literature and bioinformatics tools. A large number of OFD-1 protein partners belong to protein synthesis process, including several subunit of eukaryotic translation initiation factor 3 and ribosomal protein. The presence of these proteins belonging to so called translation pre-initiation complex (PIC) suggests for the first time a role in translation for OFD1. Immunofluoscence experiments show that OFD1 colocalizes at the centrosome with several elements of PIC.

Conclusions: Present data demonstrate that OFD1 localizes with PIC at the centrosome where controls, in the kidney, the translation of specific targets involved in cilia formation.

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Computational proteomics. Data analysis and biostatistics

OP100 - SEQUENCE VARIANT ANALYSIS WITH INCREASED SPECIFICITY AND MEANINGFUL CONFIDENCES

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Introduction

Sequence variant analysis (SVA) is becoming increasingly important. In proteome-scale research, SVA provides new information on human variation and disease, while at the other limit of complexity, SVA plays a critical quality control role in the production of biologics. With hybrid search approaches, putative variant detection has become easier, but assigning meaningful confidences to identify the small fraction of real variants remains a great challenge. Here we present significant progress on this challenge.

Methods

Development versions of ProteinPilot™ Software V5.0 served as the platform for implementing a new processing architecture to restructure database search results to be more amenable to SVA using the Paragon™ algorithm for database search. Machine learning approaches were evaluated using RapidMiner 5.

Results and Discussion

Parsimonious aggregation at three levels – the LCMS feature, the peptide group, and the 'protein feature group' level, was implemented to structure search results to provide a clear picture of relevant ambiguity. Multiple predictive metrics were calculated using this new framing and assembled using machine learning techniques. To measure the performance of variant classifiers, a carefully annotated set of 317 true variants and 320 false variants was generated by random mutations in the searched database. Using search engine confidence as the lone classifier yielded a receiver operator characteristic (ROC) area under the curve (AUC) of 0.873. Using eight additional predictive metrics enabled by the restructured results, a support vector machine classifier gave a clear gain with an AUC of 0.948 +/- 0.029, while an artificial neural network classifier yielded further improvement with an AUC of 0.967 +/- 0.018.

Conclusions

These results indicate this direction has great potential to reduce the amount manual inspection needed in SVA. Subsequent work will optimize separate substitution probability matrices for proteome-scale and recombinant expression applications and extend validation to include proteomics samples with known variants.

OP101 - RE-ANALYSIS OF PUBLIC DATA: DOES THE FUTURE OF PROTEOMICS LIE IN THE PAST?

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Acquired data from proteomics experiments are rarely analyzed beyond the scope of the experiment. However, due to the speed and sensitivity of instruments, discoveries remain hidden within these data. Indeed, the high fraction of unidentified spectra (often up to 80% of the spectra) hints at unexpected analytes in the sample that remain unassigned because analyses start from a limiting perspective, restricting possible sequences (e.g., through taxonomy) and modifications a priori.

Since proteomics data are collected in public repositories, these data remain available for further exploration. To maximize the (orthogonal) re-use of these public data sets, we therefore here present two complementary bioinformatics approaches for re-analysis.

The first approach decentralizes the reprocessing of public data by providing a very user-friendly, highly powerful interface to re-analyse public proteomics data through the freely available PeptideShaker software.

The second approach centralizes the reprocessing of the public data. For this, we built ReSpin, a fully automated, distributed infrastructure for reprocessing public proteomics data. ReSpin automatically extracts relevant information from public data sets stored in the PRIDE database using the pride-asap pipeline, delivering customized search settings. ReSpin also allows users to change these settings, providing customized data interpretation

By centralizing the reprocessing, and by carefully optimizing search settings per data set, a coherent, quality-controlled set of identifications is created that can be directly used to improve the annotation of UniProt, for instance by annotating splice isoforms and protein variants in the human proteome.

The growing amount of public proteomics data, coupled to PeptideShaker and ReSpin, allows a disruptive change in how proteomics data is interpreted, transforming proteomics data repositories from data graveyards into treasure troves of undiscovered analytes. There is a bright future for proteomics, and part of that future will be discovered in its past

OP102 - PINT: A NEW PLATFORM FOR THE COMPREHENSIVE INTEGRATION OF COMPLEX LARGE-SCALE PROTEOMICS DATA AND RESULTS

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Characterization of complex biological systems based on high-throughput protein identification and quantification through mass spectrometry commonly involves, among others, differential expression analysis between replicate samples originating from different experimental conditions. Bioinformatics tools are crucial to store and analyze data, especially in the context of collaborative and long-term research projects. In spite of the existence of several pieces of software performing such tasks, additional data analysis and investigations are required subsequent to application of these tools to get the final results publication ready.

For instance, it is common to obtain available biological protein annotations supporting a given set of findings or to compare results from significantly different experimental setups in order to build interesting and biologically relevant conclusions.

Here we present a user-friendly web-based platform (PINT: Proteomics INTegrator), deployable on any web server, as a new comprehensive system to store, visualize, and analyze data for proteomics results obtained under different experimental conditions. PINT provides an extremely flexible and powerful query interface that allows data filtering based on numerous proteomics features such as confidence values, abundance levels or ratios, dataset overlaps, etc.

Furthermore, proteomics results can be combined with queries over the vast majority of the UniprotKB annotations, which are transparently incorporated into the system. For example, these queries can allow rapid identification of proteins with a confidence score above a given threshold that are known to be associated to diseases or they may highlight proteins with a least one phosphorylated site that are shared between a set of experimental conditions. In addition, PINT allows the developers to incorporate data visualization and analysis tools, serving its role as a centralized hub of proteomics data analysis tools. PINT will thus facilitate interpretation of proteomics results and expedite biological conclusions and, by the same means, deal with the "big data" paradigm in proteomics.

OP103 - SCALING UP SPECTRAL CLUSTERING IN THE PRIDE DATABASE

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Introduction and objectives: The PRIDE (PRoteomics IDEntifications) database (http://www.ebi.ac.uk/pride) at the European Bioinformatics Institute is one of the main public repositories for MS-based proteomics data. In resources like PRIDE, it is often challenging to identify reliable identifications. We therefore developed the 'PRIDE-Cluster' algorithm (Griss et al., 2013, Nat Methods 10(2):95-96) which was used to cluster all identified spectra in PRIDE. Based on these results we were able to separate reliable from unreliable identifications.

Because of the big increase of data stored in PRIDE, the original algorithm could no longer be run in a feasible time frame. Therefore, we started to develop an updated version of the 'PRIDE-Cluster' algorithm that would be more efficient and able to continuously cluster all data submitted to PRIDE without impairing clustering quality.

Methods: The new 'PRIDE-Cluster-H' algorithm was designed to run in the Hadoop open-source software framework. The same three test datasets were used to assess the quality of the clustering algorithm as previously shown in the original 'PRIDE-Cluster' publication.

Results and Discussion: The new 'PRIDE-Cluster-H' algorithm performed at least equally well in the test datasets compared to the original algorithm. Based on the clustering results, target and decoy identifications (i.e. correct and incorrect identifications) could be reliably identified. In addition, the new algorithm was able to cluster all identified spectra in PRIDE in a fraction of the time used by the original version. Most importantly, we show how incorrect annotations found in PRIDE can be corrected using this approach. The assessment of peptide identification data will be propagated into the PRIDE-Q resource.

Conclusions: Spectral clustering is an approach that can be used to efficiently present the data available in public repositories and assess the quality of the identifications. A framework like Hadoop is needed to handle the huge data volumes present in PRIDE.

P-860.00

GLYCOPEPTIDEID: WEB TOOL FOR N-GLYCOPEPTIDE IDENTIFICATION WITH CID MS2 SPECTRA

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Introduction and objectives

Accurate glycopeptide identification sets additional demands for bioinformatics, but is essential in the full understanding of glycobiology. The GlycopeptideID is an open access web service aimed to ease the analysis of intact N-glycopeptide MS2 spectra. The service is automated, scalable and has an easy to use web interface.

Methods

The tool input is deconvoluted glycopeptide MS2 spectra. The prerequisite to identify a glycopeptide is that the spectra contain both peptide and glycan fragments. Peptides are identified from the peptide fragments and glycan structures or compositions from the glycan and glycopeptide fragments. The tool outcome is a combination of best matching peptides and glycans for each MS2 precursor. The peptides are searched against protein database (SwissProt) and the glycans against either glycan database (GlycomeDB) or calculated as de novo glycans. Other peptide modifications are also possible. The matching glycopeptides are ordered by probability based score values. For peptide identifications target-decoy false discovery rate estimation can be applied.

The service was tested with model N-glycoproteins with known glycosylation sites and glycan structures. The proteins were trypsin digested and MS2 spectra were acquired with Synapt G2-S (Waters) mass spectrometer in CID-mode with alternating collision energy. The following studies were a case control study of human pancreatitis and pancreas cancer as well as profiling of human urinary exosome surface N-glycoproteins. The analysis workflow included tryptic digestion followed by enrichments done by various lectins or size exclusion chromatography.

Results and Discussion

In both of the studies the results have provided already known glycoproteins and structures as well as novel entities, suggesting that the software is able to generate reliable and high quality results from high resolution CID spectra.

Conclusions

The authors believe that the GlycopeptideID web service can be a valuable tool for the glycomics community. The service is accessible at www.appliednumerics.com.

P-861.00

A CURRENT PERSPECTIVE ON USING R AND BIOCONDUCTOR FOR PROTEOMICS DATA ANALYSIS

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Introduction and objectives
With the continuous increase in data throughput and experimental designs complexity, the processing, analysis and interpretation of proteomics data becomes a major bottleneck that can be tackled by the appropriate use of statistical and computational tools. The R language and in particular the Bioconductor project have a major impact on other fields in high-throughput biology and benefited, in the recent years, from substantial contributions from the computational proteomics developers.

Methods
We summarise some of the latest R and Bioconductor developments in the field of proteomics, including the support of open community-driven formats for raw data and identification results, packages for peptide-spectrum matching, methods quantitative proteomics, mass spectrometry and quantitation data processing, visualisation and interpretation.

Results and Discussion
We provide figures of the number of new package submissions and downloads over the last Bioconductor releases to illustrate the recent interest of the proteomics community in the Bioconductor project. While the command line interface (CLI) represents a considerable novelty for many life scientists, numerous documentation and tutorials are available and an increasing number of tools also provide graphical user interfaces in addition to the CLI. We also discuss current needs and anticipated developments in the light of recent progress.

Conclusions
The R/Bioconductor environment addresses some important issues in computational proteomics and offers a unique set of interdisciplinary expertise, capabilities and flexibility in the existing proteomics software ecosystem. Noteworthy is also the introduction of tools and technique of R development and usage that permit open and reproducible computational research and data analysis, an area of increasing importance in the current data intensive area.

P-862.00

A STATE-OF-THE-ART MACHINE LEARNING PIPELINE FOR THE ANALYSIS OF ORGANELLE PROTEOMICS DATA

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Introduction and objectives

Organelle proteomics, or spatial proteomics, is the systematic study of protein sub-cellular localisation. Here, we focus on high-throughput quantitative mass spectrometry-based techniques such as LOPIT and PCP and demonstrate a robust and sound analysis pipeline using state-of-the-art and novel machine learning algorithms implemented in the pRoloc [1] R/Bioconductor package.

Methods

We illustrate the pipeline using relevant real-world data sets available from the pRolocdata package [1], documenting importing data available in spreadsheet formats into the R environment, missing data imputation, data quality control, facilitated organelle marker assignment, protein clustering, identification of new, non-labelled organelles using semi-supervised machine learning [2], protein classification and data visualisation.

Results and Discussion

While the pipeline automates some fundamental requirements such as parameter optimisation via cross-validation, imputation of missing values, organelle markers definition and allows the user to assess such crucial parameters, we also highlight the importance of informed user decisions and validation. Despite the requirement for elaborate and cross-disciplinary tool sets, the biologists must remain in control of the fate of their data and in a position to make informed decisions about the data analysis and validity of the results to produce biologically relevant and meaningful interpretation.

Conclusions

Complex high dimensional data analysis is a challenging task. While statistics and computer science provide the wider research community with several algorithms and best practice, their application is often difficult and may at times, when underlying assumptions are not met, lead to misleading claims. We show how such state-of-the-art methods can be applied on well-defined and annotated data in a coherent, traceable and reproducible pipeline.

P-863.00

ANALYSIS OF LABELED AND NON-LABELED DIA AND DDA PROTEOMIC DATA USING 'PROGENESIS QI FOR PROTEOMICS'

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LC-MS is routinely applied for the qualitative and quantitative analysis of complex proteomes to characterize biological processes and understand disease states. However, experiments can readily generate large and complex data sets with the analysis and interpretation of the results readily evolving into the rate determining steps. In turn, this has led to a demand for improved data analysis systems, including efficient and accurate data compression routines, intuitive software interfaces with menu-guided workflows, flexible experimental designs without sample number restrictions, consistent peak detection for improved accuracy and precision, complete data matrices without missing values for reliable statistics, and the ability to analyze fractionated samples. These features are illustrated for novel informatics for the quantification and identification of example isotopically-labeled and label-free proteomics datasets.

A number of experimental designs and cases were investigated, including ion mobility assisted DIA HDMSE experiments for the label-free quantitative analysis of samples with known differential protein expression values to assess quantification accuracy, SILAC and dimethyl labeled cases to assess peak pair detection efficiency and quantitative reproducibility across multiple technical replicates, and application examples to demonstrate identified biological relevance. Data from DDA based label-free studies were analyzed in detail to investigate peak matching efficiency across a complete experiment, indicating that co-detection affords near 100% feature intensity value measurement within individual runs.

The identification of significantly regulated peptides and proteins and the classification of samples are demonstrated through the use of embedded multivariate analysis tools, alongside analysis of variance scoring, which include unsupervised principal component analysis and hierarchical clustering techniques. The software also enables easy export of differentially regulated features to pathway analysis tools and flexible reporting options to produce publication-ready reports.

P-864.00

A SITE FOR DIRECT PROTEIN-PROTEIN INTERACTION FROM STRUCTURAL
MODELLING AND DOCKING FOR INTEGRIN AVB6 AND □UPAR

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Integrin avb6 is an epithelially-restricted heterodimeric transmembrane glycoprotein, known to interact with the urokinase plasminogen activating receptor (uPAR), playing a critical role in cancer progression. While the X-ray crystallographic structures of segments of other integrin heterodimers are known, there is no structural information for the complete avb6 integrin to assess its direct interaction with uPAR.

We have performed structural analysis of avb6.uPAR interactions using model data with docking simulations to pinpoint their interface, in accord with earlier reports of the b-propeller region of integrin a-chain interacting with uPAR. Interaction of avb6.uPAR was demonstrated by our previous study using immunoprecipitation coupled with proteomic analysis by mass spectrometry. Recently this interaction was validated with proximity ligation assays and peptide arrays. The data suggested that two potential peptide regions from domain II and one peptide region from domain III of uPAR, interact with avb6 integrin.

Only the peptide region from domain III is consistent with the three-dimensional interaction site proposed in this study. The molecular basis of integrin avb6.uPAR binding using structural data is discussed for its implications as a potential therapeutic target in cancer management.

P-865.00

UNLOCKING THE PUZZLING BIOLOGY OF THE BLACK PÉRIGORD TRUFFLE TUBER MELANOSPORUM

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The black Périgord truffle (Tuber melanosporum Vittad.) is a highlyprized food today, with its unique scent (i.e., perfume) and texture. Despite these attributes, it remains relatively poorly studied, lacking "omics" information to characterize its biology and biochemistry, especially changes associated with freshness and the proteins/metabolites responsible for its organoleptic properties.

In this study, we have functionally annotated the truffle proteome from the 2010 T. melanosporum genome comprising 12 771 putative nonredundant proteins. Using sequential BLAST search strategies, we identified homologues for 2587 proteins with 2486 (96.0%) fungal homologues (available from http://biolinfo.org/protannotator/blacktruffle.php). A combined 1D PAGE and high-accuracy LC−MS/MS proteomic study was employed to validate the results of the functional annotation and identified 836 (6.5%) proteins, of which 47.5% (i.e., 397) were present in our bioinformatics studies.

Our study, functionally annotating 6487 black Périgord truffle proteins and confirming 836 by proteomic experiments, is by far the most comprehensive study to date contributing significantly to the scientific community. This study has resulted in the functional characterization of novel proteins to increase our biological understanding of this organism and to uncover potential biomarkers of authenticity, freshness, and perfume maturation.

P-866.00

NON-REDUNDANT PEPTIDES DATABASE FOR CHR18 ENCODED PROTEINS QUANTIFICATION

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Despite the fact that SRM is the most productive and sensitive method of protein detection [Malmstr, L., & A, R. A. (2012).], there are many false-positive protein identifications caused from peptide interference [Sherman, J et al. (2009)]. While optimizing protein quantification, we combined several approaches to find non-redundant peptides, providing the most high-confident measurements.

Here we present method for SRM data validation. We analyzed published information about chromosome 18 encoded proteins abundance enriched with our original SRM data. In the next step we selected peptides used in SRM experiments with good reproducibility and stable results. For non-redundant peptides search we used UniProt and SRM Collider. Using For additional validation of protein quantification in the liver tissue and HepG2 cell line we used transcriptomic data.

Our database contain unique proteotypic peptides for all the protein of chromosome 18. About 35% of collected peptides (for 96 proteins) provide high-confident copy number measurements with low interference. We created the criteria for the validation of SRM measurement, and mined high-quality SRM results with the good correlation with the transcriptomic data ($R2>0,3$).

P-867.00

CONSOLIDATING CHR 18 DATA USING KNOWLEDGEBASE OF PROTEIN AND TRANSCRIPT ANNOTATIONS

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The Human Proteome Project (HPP) was started four years ago and the international consortia have indicated a number of informational resources to harbor the HPP data. Selected informational resources are currently used to elaborate the HPP baseline metrics, which was introduced to estimate in future contribution of HPP to the knowledge domain.

We developed the Web-based tool Gene-centric Content Management System (GenoCMS) for comparing public resources to the proprietary results by using the representation of proteins as a color-coded catalog. Within GenoCMS, the features of protein-coding genes are uploaded from the public sources and then appended by the additional features derived from the original experimental workflows. We describe the heat-map/traffic light representation of our proteomic and transcriptomic experiments on chromosome 18 as the background of the data taken from NextProt, MS/MS repositories, the Human Protein Atlas and the RNAseqAtlas.

The system presented at www.kb18.ru comprises a collaborative knowledge base for annotating the gene sets and disseminating these annotations through the Web.

P-868.00

CONSTRUCT: THREE-DIMENSIONAL VISUALIZATION OF SEQUENCE CONSERVATION

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Proteins are an ever growing source of information to gain insights on the biology of life. Integrating the information of the protein structure with the outcome of a multiple sequence alignment provides even further insights on how proteins evolve. This integrated method can also tell us how protein evolution relates to protein function and changing function and immunogenicity.

In order to combine the information of 3D structure and its sequence conservation, we built ConStruct, a web-based tool that maps protein sequence conservation on 3D structures. The ConStruct tool calculates the sequence conservation for each residue from the multiple sequence alignment of a protein and subsequently maps this conservation on the related protein 3D structure. The structure of the analyzed protein can originate either from the Protein DataBank (PDB) or can be an in-house created PDB-file (via homology modelling, etc…). The built-in export function produces a modified PDB-file – the B-values are replaced by the percentage of conservation of each residue of the given protein - which can then be used in further analysis.

This tool can provide us with critical information on which regions of a protein show high variability and can be used in immunological questions such as vaccine development and identifying antigenic diversity hotspots. ConStruct can also provide information on which parts of the protein are essential for its function.

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Topic 22: Computational proteomics. Data analysis and biostatistics

P-869.00

PEPSHELL: VISUALIZING CONFORMATIONAL PROTEOMICS.

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Proteins are dynamic molecules; they undergo crucial conformational changes induced by post-modifications or binding of e.g. cofactors that modulate their biological function. Cofactor induced subdomain communication and the corresponding modulation of the scaffold increases the complexity of a biological molecule. Recently, this principle has been rediscovered in the field of small molecule drug discovery of ATP mimetic protein kinase inhibitors. Kinase inhibitors can act either as antagonists or even agonists. Depending on the mechanism in which they trigger subdomain communication, these ATP mimetics can be responsible for resistance formation and ineffectiveness.

Here, we want to analyze and visualize the conformational plasticity of proteins to increase biological knowledge. The classic methods to obtain protein structure information are inadequate to solve this puzzle. The result of a protein crystallographic experiment is a static picture of the protein. An NMR experiment does provide dynamic information but is only applicable for proteins up to 50 kDa excluding many proteins. We therefore use conformational proteomics to solve this question. In conformational proteomics, limited proteolysis is combined with structural knowledge. During limited proteolysis, the proteins are in their native conformation and the protease is removed from the sample after only a short incubation period. Hence, only cleavage sites located at the surface of the protein can undergo cleavage. So, conformational proteomics allows the identification of surface exposed residues and flexible regions within a protein.

Here we present PepShell, a tool that allows interactive data analysis of conformational proteomics experiments by visualizing the generated peptides both at the sequence level and structural level. It especially allows the comparison of experiments under different conditions; for example different time of proteolysis or binding of different substrates/inhibitors. PepShell not only supports classic proteolysis data but also data from HDX experiments. An export function produces publication-quality illustrations of the data.

P-870.00

RESPONSE OF PEPTIDE INTENSITY TO CONCENTRATION IN ESI¨CMS-BASED PROTEOME

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High-throughput quantification with label-free methods has received considerable attention in electrospray ionization (ESI)¨Cmass spectrometry (MS), but the manner by which MS signals respond to peptide concentration remains unclear in proteomics.

We developed a new mathematical formula to describe the intrinsic log¨Clog relationship between the MS intensity response and peptide concentration in an analytical ESI process. Experimental results showed that the calibration curve is fairly fit to the log¨Clog formula with a linear dynamic range of approximate four to five orders of magnitude. However, we found that the ionization of analytical peptides can be severely suppressed by coexisting matrix peptides, such that the calibration curve can be poorly leveled off on both ends.

Our study suggests that the interferences from coexisting matrix peptides should be reduced in the ESI process to use the log¨Clog calibration curve successfully for the high-throughput quantification.

P-871.00

A SPATIALLY-AWARE PEAK PICKING METHOD FOR MALDI-IMAGING DATA FROM TOF AND FTICR MASS ANALYZERS

Jan Hendrik Kobarg[1,2], Lena Hauberg-Lotte[1,3], Michaela Aichler[4], Michael Becker[5], Janina Oetjen[3], Judith Berger[6], Stefan Heldmann[6], Axel Walch[4] Dennis Trede[2,7] Theodore Alexandrov[1,2,3,7]

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Introduction

MALDI-imaging is a spatially-resolved mass spectrometric technique which can obtain the spatial distribution of hundreds of molecules in a thin tissue section. Manual analysis is time-consuming since it requires visual examination of all m/z-images. We introduce a novel method that automatically detects structured m/z-values without specifying a region of interest and without manual visual examination.

Methods

The new approach automatically selects spatially structured m/z-images by ranking all m/z-images by their level of spatial structure. The ranking is based on our original measure of spatial chaos. We illustrate the idea of the measure of spatial chaos by applying it to MALDI-imaging data sets detected with different types of mass analyzers. We apply it to a 2D TOF data set of a rat brain section, to a 3D TOF data set of a mouse heart after myocardial Infarction, and to a 2D FTICR data set of a rat brain section.

Results and Discussion

The application of our novel spatially-aware peak picking method to MALDI-TOF data shows that the algorithm can be used to automatically discover m/z-values corresponding to structured images in two and in three spatial dimensions. For FTICR data one typically faces the problem of having too many peak candidates. We show how the number of peaks can be reasonably reduced with the structure detection approach by restricting to those peaks showing spatial structure in the corresponding m/z-image.

Conclusion

Selecting structured m/z-images after visual examination is the well-accepted approach of manual analysis and it is a part of everyday work. Our parameter-free and unsupervised method supports the imaging mass spectrometrist at this task. The method also complements spectrum-wise peak picking increasing its sensitivity, as it does not depend on peak intensity, but only on the measure of spatial chaos of the corresponding m/z-image.

P-872.00
APPLICATION OF NON-SELECTIVE PHOTOREACTIVE CROSS-LINKING IN MASS SPECTROMETRY-BASED STRUCTURAL PROTEOMICS

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Structural Proteomics is combination of protein chemistry and mass spectrometry techniques to study the protein structure, assembly, and protein-protein interaction. The chemical cross-linking reaction was applied in order to ¡§fix¡¨ protein complex structures. According to the covalent linkages identified by mass spectrometry, the spatial coordination of linked amino acids in protein molecules can be revealed. Since protein molecules in solution show more actual biological condition, the major purpose of acquiring spatial information of linked amino acids is for refining the protein structures determined by x-ray crystallography.

The non-selective photoreactive cross-linking reagents which provide higher flexibility in formation covalent linkages were utilized. They provide spatial information without consideration of the distance limitation for linked amino acids with certain functional groups. Two bi-functional photoreactive cross-linking reagents including sulfo-LC-SDA (succinimidyl-diazirine) and sulfo-SANPAH (Sulfosuccinimidyl 6-(4'-azido-2'-nitrophenylamino) hexanoate) were utilized in this study. They contain two functional groups, the NHS ester group reacts with primary amines to form covalent amide bonds, and the photoreactive groups which are activated by UV irradiation (330-370nm) and form linkages with amino acid side chain. We used hemoglobin as a model protein to test our method. After separation by SDS-PAGE, monomer, dimer, tetramer bands of hemoglobin were cut for in-gel digestion followed by LC-MS/MS analysis. The non-selective cross-linkages were analyzed by MassMatrix. MassMatrix is currently the only available program providing the non-selective cross-linkage identification for LC-MS/MS data. The model protein, hemoglobin, is a heterodimer protein including two alpha and two beta chains; therefore, the cross-linkages could be formed either within or between chains. Most identified cross-linkages were intra-chain and we suggested that was because the photo-reactive reaction is too fast to form inter-chain linkages of hemoglobin.

The current results showed that the MassMatrix can successfully identify the non-selective cross-linkages and the application of this method is under development.

P-873.00

AUTOMATED AND COMPREHENSIVE SITE-SPECIFIC CHARACTERIZATION OF GLYCOPROTEINS USING PROTEOLYTIC ENZYMES WITH HIGH AND LOW SPECIFICITIES

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Successful application of site-specific protein glycosylation analysis with respect to bio-activity is hampered by inconsistent analysis, difficult to repeat results, and low throughput. Difficulties include peptides too large for accurate mass determination, hard to confirm structures due to multiple sites, and resistance to proteolysis. Our method provides a workaround to these problems by using a toolkit of proteases including nonspecific proteases, broad specificity proteases, and site specific proteases like trypsin to generate a range of glycopeptides depending on the specific need.

Pronase is used for completely nonspecific digestions is required while elastase can be use used when broad specificity is needed but some level of sequence prediction is desirable. Trypsin is still used when highly repeatable glycopeptides are needed, especially in cases where quantitation is a goal. Using our own software platform, Glycopeptide Finder 3, we are able to analyze all of the data in a directly comparable manner. Mass matching glycopeptides are scored by tandem mass spectra and rescored by comparison to common motifs in high scoring glycopeptides. Assignments are filtered using a false discovery rate cutoff generated through use of a mass addition decoy library.

The N-glycosylation sites of many common human and mammalian glycoproteins are currently being systematically mapped. Bovine fetuin is a useful example of why multiple proteases should be used to elucidate site-specific structures. Using tryptic digestion, N-glycan sites 99, 156, and 176 are detected with limited heterogeneity. Using elastase as a proteolytic enzyme, peptides are much shorter and greater heterogeneity can be found for some sites. With nonspecific digestion, peptides are observed from length 2 to 10 at all glycosylation sites including o-glycosylation sites, but signal is split between multiple peptides making confirmation of individual glycopeptides difficult.

P-874.00

AN HMSILAC-BASED ANALYSIS PIPELINE WHICH ENABLES ACCURATE ASSIGNMENT
AND QUANTIFICATION OF METHYLATED SITES BETWEEN DISTINCT FUNCTIONAL
STATES

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Protein methylation is a post-translational modification by which a variable number of methyl groups are transferred predominantly to lysine and arginine residues. There has been increased interest in global identification of this modification due to observations of its reversible nature and emerging roles in a diverse set of pathways beyond chromatin. In our reported study (Bremang et al., Mol. Biosyst. 2013), we described 501 methylation events in the human proteome, obtained through classical separation techniques and a broad assessment of immuno-affinity enrichment. We employed heavy methyl stable isotope labelling by amino-acids in cell culture (hmSILAC) as a means to identify and quantify high confidence in vivo methylated sites, globally. This approach however generated strong abundance biases through the requirement for both heavy and light peptide sequences. We have sought to: 1) Develop and apply methods that address this source of bias; 2) Re-evaluate statistical scoring thresholds for identification and site localisation, in the hmSILAC context and 3) Quantify changes in methylation levels that occur upon siRNA-mediated depletion of different arginine methyltransferases.

Our analysis pipeline, developed in Perl, uses the result tables from MaxQuant/Andromeda database searches. Methylated peptides are assigned based on the detection of a mass shift at the precursor level and the MS/MS-level identification of the heavy or light peptide. Normalised extracted ion intensities are used to quantify relative methylation levels.

We have addressed abundance biases in the hmSILAC approach and provide an extended reference set of high confidence, in vivo methylation events. Our method has been applied successfully to the quantification of methylation levels upon PRMT5 depletion, where we have since validated candidate sites of symmetric di-methylation.

hmSILAC offers a reliable means to identify high confidence in vivo methylation events in global studies and can be used for accurate relative quantification of methylation levels, between distinct functional states.

P-875.00

A SPATIALLY-AWARE PEAK PICKING METHOD FOR MALDI-IMAGING DATA FROM TOF AND FTICR MASS ANALYZERS

Jan Hendrik Kobarg[1,2], Lena Hauberg-Lotte[1,3], Michaela Aichler[4], Michael Becker[5], Janina Oetjen[3], Judith Berger[6], Stefan Heldmann[6], Axel Walch[4] Dennis Trede[2,7] Theodore Alexandrov[1,2,3,7]

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Conclusion

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P-876.00

INTEGRATED AND COMPARATIVE ANALYSIS OF 3-OMICS DATA TO IDENTIFY A PANEL OF DIFFERENTIALLY EXPRESSED MEMBRANE PROTEINS IN BENIGN AND METASTATIC PROSTATE CANCER CELLS LINES.

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Introduction and objectives: Proteins associated with plasma membrane play critical roles in cellular communications, cell cycle regulation, differentiation, cell migration, and drug resistance. In this study we used integrative and comparative analysis of 3-omics (genomics, transcriptomics, and proteomics) datasets from benign and metastatic cell lines to elucidate the repertoire of cell surface proteins of prostate cancer cells relevant to circulating tumor cells (CTCs) capture, targeted delivery of cytotoxic agents, tumor imaging, and identification of new pathways and targets for therapies.

Methods: Plasma membranes proteomes of benign (PNT1A, PNT2) and metastatic (PC3, 22RV3, LNCap) prostate cell lines were analyzed using one-dimensional gel liquid chromatography coupled to nano spray liquid chromatography and high resolution tandem mass spectrometry. Microarray analysis was performed using Affymetrix HG-U133_Plus_2 microarrays on the same cell line cultures used for proteome data generation. Genomics analysis was performed using Illumina HiSeqTM 2000 to capture primary sequencing reads for paired-end RNA Sequencing. All data were processed using technology specific workflows to generate data matrices for combined statistical analysis in Genedata Expressionist Software.

Results and Discussion: Multivariate statistical analysis of the combined 3-omics datasets from benign and metastatic cell lines revealed differential relationships of transcripts and proteins expressed between the metastatic and benign cell lines. Statistical analysis also showed differentially expressed proteins and transcripts between the metastatic cell lines. Analysis of genomic data generated from deep sequencing of RNA Transcripts provide confirmation of gene regulation and additional insight.

Conclusions: 3-omics based common and discriminatory signatures should elucidate cancer biology, particularly metastasis, and guide future development of novel drug targets. In addition, biomarkers panels originating from these studies will improve early detection, prognosis, and prediction of treatment response.

P-877.00

IDENTIPY, AN OPEN-SOURCE MS/MS DATA SEARCH PLATFORM FOR SHOTGUN PROTEOMICS

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Database search is a key component of the shotgun proteomics approach based on LC-MS/MS analysis. The overall sensitivity of this approach depends strongly on the sensitivity of peptide identification performed by a search engine. The efficiency of peptide identification depends on a number of procedures, including spectrum filtering, PSM (peptide-spectrum match) filtering, PSM scoring, and search settings. However, the vast majority of developed search engines provide limited capabilities for customization of the search process, which may introduce ambiguity in the results. Here we present a fully customizable, open-source search engine "IdentiPy" written in Python programming language. IdentiPy allows seamless plugging of third-party scoring functions and/or introduction of extra search steps, automatic tuning of search parameters, and easy extension with add-on features.

We compared IdentiPy with X!Tandem, Mascot and OMSSA search engines using experimental data sets of varying complexity, including both shotgun and middle-down proteomics experiments. Because of the pluggable scoring functions implemented in IdentiPy it can be used for search engine prototyping. We further demonstrated this ability by using Python-written implementations of scoring algorithms employed by X!Tandem, Morpheus and other search engines within the IdentiPy framework. One of the useful abilities of IdentiPy is the efficient use of experimental information complementary to MS/MS during the identification process.

We demonstrated herein that the use of complementary experimental information inside the scoring function improves significantly the sensitivity of the MS/MS search. Finally, we show that the automatic parameter tuning implemented in IdentiPy increases peptide identification specificity.

P-878.00

REANALYSIS OF PUBLIC DATA TO FIND NON-STANDARD PHOSPHORYLATIONS IN HUMAN SAMPLES

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Introduction: Phosphorylations can chemically happen on 9 amino acid residues producing phosphoesters (serine, threonine, tyrosine), acyl phoshates (aspartate, glutamate), phosphoramidates (histidine, lysine, arginine) and phosphocysteine. Standard phosphoesters are intensively studied while the other 6 "non-standard" phosphorylated residues have only been reported sporadically in eukaryotes. Searching against many variable modifications (>5) at the same time presents two main problems: i., scaling up the search to include all possible modifications, and ii., the posterior validation of the results. The aim of the current study is twofold: to scale up modification searches using the distributed search engine Hydra and to apply this approach to find potential non-standard phosphorylations by reanalyzing public datasets.

Methods: Two samples (~250 000 spectra each) of the phospho-enriched, publicly available PXD000089 ProteomeXchange dataset have been used for the reanalysis. First, Hydra generated a candidate human peptide database including all variable phosphorylations on 8 amino acids: S, T, Y, H, R, K, D, E and methionine oxidation. A 1% peptide FDR cutoff was used to export the identified spectra (1.5-3% of all spectra). They were then searched using native X!Tandem and Mascot/Percolator. The PSMs identified shared by the three search engines were manually annotated and researched individually using MS-GF+ for confirmation purposes.

Results and Discussion: Using the 3 search engines yielded several hundred PSMs each containing non-standard phosphorylations out of which only a couple tens of individual PSMs and modifications were overlapping. The majority of overlapping hits do not contain enough site determining ions to enable modification localization. Nevertheless there are several cases, including a phosphohistidine, phosphoglutamates and phosphoaspartates where non-standard phosphorylation is the most probable interpretation.

Conclusions: We have developed a search strategy to identify a low number of potential non-standard phosphorylation candidates in human samples. Our ongoing efforts concentrate on further validation of the generated results.

MZJAVA: AN OPEN SOURCE MASS SPECTROMETRY LIBRARY

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In order to provide bioinformatics support for new and experimental mass spectrometry (MS) experimental techniques, or to make better use of the information obtained from contemporary techniques, it is often necessary to write custom data analysis software. To make MS software development easier and faster we have developed MzJava, an open-source Java library that provides well engineered building blocks that are common to most MS data processing software.

MzJava was designed to be extensible, flexible and efficient so that it can be used to write research software and to analyze large data sets. MzJava provides algorithms and data structures for representing and processing mass spectra and their associated biological molecules, such as metabolites, glycans and peptides. The library contains algorithms to perform peak processing (e.g. centroiding, filtering, transforming), mass calculation, protein digestion, fragmentation of peptides and glycans and scoring of spectrum-spectrum and peptide-spectrum matches. For data import and export MzJava implements readers and writers for the commonly used data formats.

MzJava has been used to develop algorithms for positioning post translational modifications, clustering data independent MS/MS spectra[1], spectral library searches [2,3] and infrastructure code for EasyProt [4] a mass spectrometry software platform. MzJava is distributed under the AGPL v3.0 license and can be downloaded from http://mzjava.expasy.org.

P-880.00

THE SEAMASS PLATFORM: A NEW LC-MS SOFTWARE PIPELINE BASED ON IMAGE ANALYSIS AND BAYESIAN MODELLING

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Introduction and Objectives: We present the seaMass platform, a new approach to the analysis of LC-MS data for quantitative proteomics. With seaMass, LC-MS datasets are processed as massive images. The image analysis pipeline consists of denoising and image creation ('image restoration'), alignment ('image registration'), peptide feature detection ('sparse mixture modelling'), differential analysis ('functional mixed-modelling') and Google Earth-style visualisation of the results fused on the raw data. The workflow is highly sensitive, as detailed information is preserved from the beginning of the workflow to the end.

Methods: The core concept is to transform irregularly-binned raw LC-MS data into a sparse image representation constructed from overlapping Gaussian-like building blocks at multiple scales. The resulting image accurately reflects the raw data whilst rejecting ion-counting noise and requiring significantly less disk/memory space. It also enables incremental image construction, allowing us to create an efficient visualisation tool able to load, zoom and pan LC-MS data in real-time. We account for retention time inhomogeneities through a new group-wise image registration/normalisation technique, and subsequently apply either a linear mixed-effects model directly to the aligned images, or consensus peptide-detection followed by novel Bayesian mixed-modelling for differential protein-level analysis.

Results and Discussion: seaMass was objectively validated against Progenesis LC-MS (published in Liao et al, Proceedings of ISBI2014) using spike-in label-free datasets. Advantages are: (i) seaMass does not suffer from false-positives due to feature detection errors. (ii) seaMass identifies large numbers of differential expression below the detection limit of Progenesis. (iii) Differential expression that overlaps with interfering compounds is characterised.

Conclusions: We have demonstrated a novel workflow for differential analysis of LC-MS data with significantly improved sensitivity/robustness compared to the state-of-the-art.

P-881.00

OPTIMAL SELECTION OF SAMPLES FOR MULTIPLEX SEROLOGICAL ASSAY VALIDATION

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Serological assays indirectly prove diseases by the detection of antibodies towards viral, bacterial, or patient´s own protein repertoire. Array-based assay systems are used to discover novel antigens and to set-up multiplex assay panels for several auto-immune diseases. However, the validation of such multiplex approaches requires proof that low, medium and high levels of antibodies can be reliably and reproducibly measured. Reference samples cannot be easily obtained in the realm of serological assays, because the target analyte - the antibodies - cannot be synthesized. The only correct way to validate such an assay is to identify suitable patient samples.

For multiplex serological assays, a whole assay panel needs to be validated. Every analyte needs to be validated on its own, so at least three suitable samples would have to be identified for each analyte in the panel. Considering multiple replicates, intra-, inter-assay variation and stability check as multiplicators, the data points required can quickly run out of a manageable size. If it is possible to choose a few samples such that the combination of its profiles would cover low, medium and high concentration for most analytes, validation work would drop significantly.

Integer programming can be used to select samples for validation in order to achieve a resource-optimal result, using data from a previous screening. Resource-optimality is given if no other combination of samples would lead to validation of more analytes from the panel using the same resources.

P-882.00

A COMPLETE WORKFLOW FOR ACHIEVING HIGH ACCURACY MULTIPLEXED N-TERMINOMICS DATA USING TMT10PLEX.

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Proteolytic cleavage is a critical PTM that can significantly affect the behavior and function of a protein and its interaction partners. However, identifying, quantifying, and confirming the substrates and specificities of the known proteases, many of which are only vaguely categorized, has proved to be a challenging task1. A breakthrough methodology in this line of analysis by LC/MS takes advantage of a negative enrichment strategy coupled with amine-reactive labeling of newly formed (neo) N-termini to yield relative abundance information2. This study presents a complete workflow for this analysis using TMT10plex tags with accurate Synchronous Precursor Selection (SPS) MS3 quantitation and a new data analysis pipeline, Proteome Discoverer 2.0, employing the Byonic3 search engine.

The Byonic searches of MS spectra from two replicate analyses yielded identifications for nearly 3000 protein groups and 18000 unique peptides before enrichment and 2500 and 5500 respectively following enrichment, a two-fold increase when compared to Mascot search results. In the enriched sample, over 800 TMT10plex N-terminally labeled peptides were detected with a quantitation rate exceeding 90%, These represent high confidence sites of protease activity. The high-multiplexing capabilities of TMT10plex enabled the use of additional tags as technical replicates within the same LCMS experiment enhancing quantitative reproducibility and confidence.

The magnitude of observed fold changes increased using the SPS MS3 method (compared to data collected using MS2 based quantitation) due to elimination of interference, co-isolated with the parent ion. Using Proteome Discoverer 2.0 with Byonic, enabled facile identification of biologically relevant substrates showing significant changes in abundance. In conclusion, combining negative N-terminal peptide enrichment and higher multiplexing TMT with high resolution SPS MS3 and an advanced data analysis pipeline made possible a much deeper characterization of the N-termini proteome.

P-883.00

LOCATING POST TRANSLATIONAL MODIFICATIONS THROUGH THE INTERROGATION OF SHOTGUN PROTEOMICS

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The past decade has seen the development of a plethora of online proteomics resources in Arabidopsis reflecting multiple large-scale studies. These resources exist independently and lack a level of integration. The Multinational Arabidopsis Steering Committee, Proteomics (MASCP) has addressed this issue through the development of a proteomics aggregation portal, MASCP Gator (http://gator.masc-proteomics.org/).

The portal provides a summary of proteomics and protein information aggregated directly from ten online resources. The development of this portal has enabled us to develop a bioinformatics technique to identify likely regions of post-translational modifications in proteins of Arabidopsis. The ability to locate and identify post translational modifications experimentally by mass spectrometry is extremely challenging and there is a requirement for complementary techniques. Virtually all large-scale proteomics analyses in Arabidopsis have identified proteins with unmodified peptides.

Collectively, these data reveal modified regions of a protein as unmatched areas within a protein model. Using a recent large-scale N-linked glycosylation survey as a test set, we could demonstrate that unmatched regions represent modification hotspots in proteins. These sites can be further targeted for investigation and characterization. We have now developed a method to locate putative regions with modifications by exploiting mass spectral data in the public domain and are attempting to develop this into a functional portal for the assessment modifications in proteomic datasets.

P-884.00

STATISTICAL CONFIRMATION OF MALDI-TOF MASS SPECTRA WITH R AND MASSUP CAN BE USED TO DISTINGUISH BETWEEN BACTERIA

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Matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS) is a faster and more accurate method for identification of intact bacteria compared with conventional microbiology and/or molecular biology approaches. This technique is potentially applicable in diagnostic laboratories to distinguish and characterize bacterial species. Enterococcus spp. and Escherichia coli are commensal microorganisms from the gastrointestinal tract of humans and animals that are sometimes also major pathogens. The aim of this study was to characterize at the proteomic level isolates of E. coli and enterococci from wild birds of the Azores archipelago by MALDI-TOF MS.

Soluble proteins were extracted from intact cell cultures of 60 isolates of Enterococcus spp. and 60 isolates of Escherichia coli by an expedient method. After MALDI-TOF MS analysis of the proteins, the 1200 mass spectra obtained were compared to bioinformatics databases. A total of 215 distinct m/z were obtained, including the mass peak m/z 4428 ± 3, which is exclusively found in isolates of enterococci, and the peaks m/z 5379 ± 3 and 6253 ± 3, which are only detected in the spectra of E. coli isolates. With mass spectrum processing and statistical analysis with R and MassUp software, including principal component analysis (PCA) and clustering, it was possible to correctly distinguish between isolates of different enterococci species.

This is the first MALDI-TOF study that has made a clear distinction between isolates from different species of the genus enterococci from an animal source. The results of the proteomic analysis confirmed that these tools can be used to characterize whole bacterial cells. In the future, with an optimized protocol for obtaining intracellular proteins and the development of bioinformatics methods, it is likely that the mass peaks characteristic of antimicrobial resistance will be detected.

P-885.00

A STAND-ALONE VERSION OF MS AMANDA FOR INTEGRATION INTO PROTEOMICS WORKFLOWS

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Introduction and objectives

Protein identification by tandem mass spectrometry (MS/MS) is a core analytical tool in proteomics. Recent technological advances in instrument development, particularly high resolution mass spectra and enhanced mass accuracy, allow high-throughput identification of thousands of proteins in bottom-up proteomics studies. We aimed to develop a database search engine which can take advantage of the resolution and accuracy available in current MS/MS data sets. MS Amanda is capable to identify spectra derived from different fragmentation techniques (i.e., ETD, HCD, CID, or EThcD). To address requirements in high throughput analysis and enable incorporation in custom proteomic analysis pipelines we released MS Amanda Stand-Alone, a self-contained version of the free MS Amanda search algorithm.

Methods

The software is capable to take advantage of modern multiprocessor systems through multi-core support. MS Amanda efficiently uses the available system memory and automatically balances the workload over all available cores. MS Amanda Stand-Alone is implemented in C# (.NET 4.0). The current version of MS Amanda Stand-Alone is compiled for Microsoft Windows operating systems with upcoming support for Linux based on Mono.

Results and Discussion

Validation on publicly available and in-house generated high resolution data sets shows enhanced performance of MS Amanda compared to established database search algorithms. To provide user-friendly integration of MS Amanda Stand-Alone in workflows it can be configured via an XML settings file that features integration of UNIMOD and custom modifications. MS Amanda Stand-Alone is freely available as a download from the MS Amanda webpage (http://ms.imp.ac.at/?goto=msamanda) or integrated in PeptideShaker. Bundled with the Stand-Alone version we provide a detailed usage documentation to simplify integration into existing workflows.

Conclusions

MS Amanda offers a free and powerful alternative to established commercial database search engines.

P-886.00

PEPTIDE-CENTRIC DATABASE SEARCH ENGINES APPLIED TO DATA INDEPENDENT ACQUISITION UDMSE DATA

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Introduction and objectives

Data-independent acquisition techniques like HDMSE and UDMSE require protein-centric database search approaches, which are not optimally suited for the analysis of phosphopeptides or MHC class I ligands. Here, we analyzed the performance of state-of-the-art peptide-centric database search engines for the identification of pseudo-spectra derived from DIA data.

Methods

HeLa lysate was separated by SDS-PAGE and bands subjected to in-gel digestion. Next, samples of increasing complexity were generated by pooling defined numbers of bands. Samples were acquired by both DDA and DIA acquisition methods. Data were pre-processed by PLGS and searched by using multiple search engines (myrimatch, omssa, x!tandem, PEAKS, MASCOT, and PLGS). Additionally, we analyzed DDA and DIA data from purified MHC class I ligands and TiO2-purified phosphopeptides.

Results and discussion

We evaluated the performance of several peptide-centric database search engines for DIA data using deconvoluted pseudo-spectra generated by UDMSE experiments by utilizing a set of gradually more complex samples, which increases the degree of chimericy of the deconvoluted spectra. Analysis of the sample set revealed that sample complexity and therefore spectral chimericy influenced the different peptide-centric search engines to a different degree, likely due to the fact that the underlying identification algorithms penalize unassigned fragment ions differently. Identification rates highly differed between database search engines, probably due to different algorithm behavior in function of the number of fragment ions constituting a pseudo-spectrum, which is generally higher compared to standard MS/MS spectra obtained by DDA approaches.

Next, we analyzed the performance of the different search engines for non-tryptic peptides and purified phosphopeptides. In both cases, PEAKS outperformed PLGS, indicating that peptide-centric search engines can be successfully applied to DIA datasets.

Conclusions

State-of-the-art peptide-centric MS/MS database search engines can process HDMSE/UDMSE peptidomics data, enhancing DIA identification performance for phosphopeptide and non-tryptic samples.

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Topic 22: Computational proteomics. Data analysis and biostatistics

P-887.00
STATISTICAL METHODS IN CLINICAL PROTEOMIC STUDIES

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Clinical proteomics is a subject of systems biology that investigated large numbers of protein biomarkers associated with human disease. Like the other "omics", proteomics use systems biology techniques to identify proteome-wide markers simultaneously. Unlike genomics that has been established in decades, proteomics is still in its infancy.

The current biotechnologies have its limited power to discover all the existing 20,000s proteins from the human body. Biologists have not been able to understand the molecule functions of lots of those identified proteins. Statistical techniques become essential in proteomics research because systems biology generates a large amount of quantitative information in clinical proteomic studies investigating proteins' molecule activities. The complexities of clinical study and proteomic experiments also require statistical inputs to warrant a valid and unbiased proteomic study. This PhD research firstly proposed a new method to assess the reproducibility in clinical proteomic studies when a new device or new tissue is being used for a proteomic experiment. The reproducibility assessment utilizes a dimensional reduction technique and permutation method to make the assessment extend to a proteome-wise scale. It secondly proposed two optimal design algorithms and realized them via a R package to assist the multiple stage study designs through biomarker discovery to clinical utility.

The optimal design algorithms utilized a hybrid simulation annealing approach to finding the design parameters that achieve a maximal number of discoveries under the cost constraints. Finally, a multivariate multilevel model has been proposed for the analysis of proteomic data. The non-random missing data presented in proteomic mass spectrometric experiments were estimated under the Bayesian framework. The proposed analytical method was tested in a simulated study and used in two real life clinical proteomic studies.

P-888.00

ESTIMATION OF PROTEIN EXPRESSION PROFILES BASED ON PEPTIDE FEATURES AND QUANTIFICATION USING ISOBARIC LABELLING

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Isobaric labelling techniques such as iTRAQ and TMT allow for simultaneous absolute and relative protein quantification in different samples within a single run. This enables investigation of changes in protein expression across a series of time points or different sample conditions, which is crucial for the study of regulation processes in biological systems. The aim of this study is to reveal the underlying regulation profile of a protein based on its peptide profiles.

Measurements of label intensities are assessed at the peptide level and are subsequently combined to estimate the corresponding protein ratios. Generally, all peptides mapped to a protein are assumed to share the same expression profile, however, large variance heterogeneity is observed due to random and systematic errors.

Several options exist to infer protein ratios, such as computing the median, the mean, or the sum of the peptide intensities using all peptides or the top five with highest intensity. More sophisticated approaches weight peptides according to their intensity and to internal experimental variation or use replicate information.

So far, most approaches focus only on quantitative peptide information, while additional characteristics of peptides are available, which also reflect the overall reliability of a specific peptide and its measurements.

We propose a statistical data integration approach using peptide features as well as quantitative information. Features considered are the number of peptides and their profile similarity supporting a common protein, the number of unique and redundant peptides, scores, shared or modified state of a peptide. We evaluate specific peptide features based on empirical distribution functions and apply an iterative procedure to estimate the underlying protein profile within the peptide profiles. We evaluated our approach on an iTRAQ 8-plex experiment acquired on an LTQ Orbitrap Velos consisting of 9356 peptide spectrum matches with predefined protein fold-changes.

P-889.00

MZDB: A FILE FORMAT USING MULTIPLE INDEXING STRATEGIES FOR THE EFFICIENT ANALYSIS OF LARGE LC-MS/MS AND SWATH-MS DATASETS

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The analysis and management of MS data, especially those generated by data independent acquisition, exemplified by SWATH-MS, pose significant challenges for proteomics bioinformatics. The large size and vast amount of information inherent to these datasets need to be properly structured to enable an efficient and straightforward extraction of the signals used to identify specific target peptides. Standard XML based formats are not well suited to large MS data files, e.g., those generated by SWATH-MS, and compromise high-throughput data processing and storing.

We developed mzDB, an efficient file format for large MS data sets. It relies on the SQLite software library and consists of a standardized and portable server-less single-file database. An optimized 3D indexing approach is adopted, where the LC-MS coordinates (retention time and m/z), along with the precursor m/z for SWATH-MS data, are used to query the database for data extraction.

In comparison with XML formats, mzDB saves ~30% of storage space and improves access times by a factor of 2 fold up to even 2000 fold, depending on the particular data access. Similarly, mzDB shows also slightly to significantly lower access times in comparison with mz5. Both C++ and Java implementations, converting XML formats to mzDB and providing access methods, will be released under permissive license. mzDB can be easily accessed by the SQLite C library and its drivers for all major languages, and browsed with existing dedicated GUIs. The mzDB format can boost mass spectrometry data analysis, offering unprecedented performance in terms of efficiency, portability, compactness, and flexibility.

P-890.00

UNIPROT REFERENCE PROTEOMES FOR PROTEOMICS IDENTIFICATIONS

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The identification of peptides and proteins in MS-based proteomics experiments relies in searching protein sequence databases. Therefore, it is of paramount importance the provision of an up-to-date, stable and complete protein sequence database for a diversity of species.

UniProt provides a broad range of Reference protein data sets for a large number of species, specifically tailored for an effective coverage of sequence space while maintaining a high quality level of sequence annotations and mappings to the genomic and proteomics information.

A good example is the provision of a Reference proteome data set for human which consists of 20,259 canonical and 19,885 isoforms manually curated protein sequences and also includes translations from high-quality gene models in Ensembl and NCBI RefSeq, maintaining synchronized data with the CCDS project. Inclusion of the protein isoforms from these resources allows to complete the protein space for the human proteome with all protein sequences available in the public domain. Furthermore, most protein sequences in the UniProt human Reference set have been mapped to Reference genome in Ensembl and we are now providing the corresponding chromosomal and gene coordinates.

UniProt has also supplemented the set of currently provided manually curated human variants with a catalogue of novel Single Nucleotide Variants (SNVs or SNPs) from the 1000 Genomes and COSMIC projects for both UniProtKB/Swiss-Prot and UniProtKB/TrEMBL sequences. These variants have been mapped to UniProtKB sequences, including isoform sequences, through the corresponding Ensembl gene, transcript and protein identifiers, providing the chromosomal location with allele change and, where possible, a cross-reference to OMIM for the variant.

Here we present how MS based proteomics data and results deposited in the main public repositories are flowing into UniProt to enrich protein sequence annotations at the level of the evidence supporting the existence of a protein (isoforms and variant-containing sequences included).

P-891.00

ISOQUANT - AN INTEGRATED BIOINFORMATICS PIPELINE FOR EVALUATION AND REPORTING OF DATA INDEPENDENT (LC-MSE) LABEL-FREE QUANTITATIVE PROTEOMICS DATA

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Introduction and objectives

Recent generation high-resolution mass spectrometers supporting ion-mobility separation (IMS) combined with advanced acquisition schemes such as UDMSE enable the reliable identification and quantification of over 4,000 proteins in a single nanoUPLC-MS run. Large amounts of generated data induce new requirements for the data processing software. MSE/HDMSE/UDMSE data require processing by proprietary vendor software ProteinLynx GlobalSERVER (PLGS). Preprocessed data require further analysis to confidently identify and quantify regulated proteins, typically done by stepwise application of different dedicated algorithms and exporting to common data formats for result interpretation and sharing with the community. We present ISOQuant, an integrated open source software pipeline for in-depth evaluation and standardized reporting of MSE/HDMSE/UDMSE based label-free proteomics data.

Methods

Tryptic HeLa cell lysate was analyzed in MSE/HDMSE and UDMSE acquisition modes on a Waters Synapt G2-S mass spectrometer using 90 and 180 min gradients. PLGS was used for raw data processing including signal detection, peak picking, isotope and charge state deconvolution and database searching for peptide and protein identification. ISOQuant was used for downstream analysis.

Results and discussion

We developed a software tool for postprocessing evaluation of MSE/HDMSE/UDMSE based label-free proteomics data. ISOQuant provides automated access to PLGS data storage, exporting relevant information into a MySQL database and processing it in multiple steps, including non-linear retention time alignment, configurable density-based feature clustering, restrictive cluster annotation, false discovery rate filtering, protein homology filtering, and TOP3-based relative and absolute protein quantification. Using model datasets, we show that ISOQuant increases reliability and reproducibility of protein identification and quantification as well as simplifies data interpretation. Additionally, ISOQuant facilitates data sharing by exporting results to common report formats such as HUPO standardized mzIdentML. ISOQuant is freely available on www.isoquant.net.

Conclusions

ISOQuant enables automated high throughput analysis of MSE/HDMSE/UDMSE based label-free proteomics data, improves data quality and reproducibility.

P-892.00

NEW FUNCTIONALITY FOR THE TRANS-PROTEOMIC PIPELINE: TOOLS FOR THE ANALYSIS OF PROTEOMICS DATA

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High throughput LC-MS/MS is capable of simultaneously identifying and quantifying thousands of proteins in a complex sample; however, consistent and objective analysis of large datasets is challenging and time-consuming. Over the past eleven years, we have continually developed and provided improvements to the Trans-Proteomic Pipeline (TPP), an open source suite of tools that facilitates and standardizes such analysis. The TPP includes software tools for MS data representation, MS data visualization, peptide identification and validation, protein identification, quantification, and annotation, data storage and mining, all with biological inference. We present an overview of the TPP and describe newly available functionality.

We recently introduced several new features in the TPP: Support for Comet and MSGF+ results, and enhanced support for OMSSA, Myrimatch, and InsPecT, including a utility that fixes the pepXML result files. We include support for exporting TPP results into the mzIdent PSI format. We have improved the various scripts that assist in launching the TPP on clusters and on the cloud. We added a new decoy database generating utility. Many updates to the user interfaces, including a new protXML Viewer, a 'dashboard' view of all models derived by the various TPP tools, and an updated spectrum viewer, among others. We implemented support mzML files compressed with Numpress. Various security patches, bug fixes, and overall enhancements to the user interfaces.

All of the TPP software tools are available for download under an open source software license at tools.proteomecenter.org, and can be installed on including Microsoft Windows, UNIX/Linux, and MacOS X. Free email support for the installation and operation of these tools is also available through a popular, community supported listserver, as is a searchable knowledge base.

P-893.00

CONCEPT OF JAPAN PROTEOME STANDARD REPOSITORY/DATABASE (JPOST)

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Recent improvements of mass spectrometry have been causing a flood of MS data in proteomics. In order to facilitate sharing and reuse of the promising data sets, it is important to construct a public repository database being accessible and high quality. Recently, the ProteomeXchange consortium has been set up to provide a coordinated submission of MS proteomics data set to the main proteomics repositories. However, data transfer to current submission points of ProteomeXchange such as PRIDE in EU and PASSEL in US via the Internet from Asia is routinely very slow and highly troublesome.

In order to clear these problems, we decided to develop a new proteome repository and database called jPOST (Japanese ProteOme STandard repository/database) in the Japanese Proteomics Society Database Center. jPOST will consist of three layers of the data repository and databases. The primary layer is a data repository, which stores raw MS data sets. The secondary layer consists of distributed databases, which store several different types of processed, curated, and categorized data sets, with criterion of post-translational modifications, diseases, organisms, chromosomes, etc. The tertiary layer collects knowledge data that is integrated and mapped data in the secondary databases into a protein sequence.

In addition to constructing the repository and databases, we plan to disseminate the data using the Resource Description Framework (RDF) data model, which is a key technology of the Semantic Web. Because some major databases in lifescience such as UniProt, PDB, ChEMBL, and PubChem have already adopted to RDF, data stored in jPOST will be able to integrate with their data. jPOST aims to not only provide data repository and databases, but also provide an infrastructure for introducing proteomics data into the Semantic Web.

P-894.00

COLIMS: AN OPEN SOURCE LIMS SYSTEM TO AUTOMATE AND EXPEDITE PROTEOMICS DATA MANAGEMENT, PROCESSING AND ANALYSIS

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One of the first points of failure in the structured capture and dissemination of proteomics data and results is encountered at the level of local data management by research groups. Very often, no specialized software outside of a search engine and/or a quantification engine is used, and even if some form of aggregator or post-processing software is used, these existing (commercial or freely available) solutions lack structured, long-term data storage. Where some form of structured data storage does exist, conversion from the proprietary formats to files amenable to upload into public repositories such as PRIDE (notably the HUPO PSI's mzML, mzIdentML, mzQuantML) is sometimes missing.

Here, we present colims (http://colims.googlecode.com), an open source system to automate and expedite local data management, processing and analysis. A relational database such as MySQL or PostgreSQL is used as repository, containing the data structure for storing metadata, search input, identification and quantification results and user management. A single storage module is responsible for persisting search input and results, allowing the storage of large quantities of data in a controlled manner on a dedicated machine. Multiple users can connect simultaneously to colims with a desktop rich client to manage and browse stored proteomics projects. The clients communicate with the storage engine by means of a storage task module, enabling loose coupling and preventing possible data loss in case of network or system failures.

Colims currently supports import from MaxQuant and PeptideShaker (http://peptide-shaker.googlecode.com), two commonly used software packages for analyzing mass spectrometric datasets, with the latter supporting various search engines (X!Tandem, MS-GF+, MS Amanda and Mascot) through the SearchGUI (http://searchgui.googlecode.com) companion software. The use of controlled vocabulary terms for metadata facilitates the export into the ProteomeXchange public repositories. As a result, colims provides a key cog in the IT machinery in any proteomics research group.

P-895.00

SOFTWARE-SUITE FOR INTELLIGENT PROTEOME ANALYSIS AND BIOMARKER SEARCH ADAPTED TO AN AUTOMATED HIGH THROUGHPUT WORKFLOW

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Indroduction: After chromatographic proteome fractionation [1] thousands liquid sub-fractions may be produced and firstly characterized by spectrophotometry. For further analysis support by adapted software packages is required for faultless fraction selection, retrieval of selected fractions, reformatting, subsequent processing, mass spectrometry (MS), and final data attribution as well as to keep track of the lab´ objects during the workflows.

Methods: A software suite for proteome analysis has been developed and eval-uated, using big-data and business intelligence analyses.

Results and Discussion: The following modules and features are combined: sample inventory, automatic data interfaces, a data-warehouse, and several op-erational and analytical user views.
The inventory database locates lab objects and links their data with measured and context sample data (dates, volumes, processing times, temperature, spec-trophotometric and MS data). The interfaces automatically read in data directly from measurements or preprocessed results from third party software. The graphical user frontend visualizes laboratory logistics and data, and provides additional tools. So fraction selection, generation of sample lists for subsequent processing, comparison of results with various data types, and matching results with clinical data can be performed automatically by various eligible criteria. An ad-hoc reporting system is included consisting of filtering and sorting functions for all types of data as well as a cluster-analysis module.
Comparison and visualization are realized in versatile charts and reports pre-pared for data-export to third party systems. Accessibility is also guaranteed via extensibility-plugins and an API for new data interfaces or analyzing views. The technologies are also platform-independent (Windows, Linux, Macintosh).

Conclusions: Combining several data-sources and software tools in one central system is time-effective compared to manual processing and increases the quality of the analytical workflow and the reliability of data.

P-896.00

SIMULTANEOUS LOCALIZATION AND ASSIGNMENT OF DIFFERENT POST-TRANSLATIONAL MODIFICATIONS USING PTMRS

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Statistically correct quality parameters are a prerequisite for proteomics studies in both academic and pharmaceutical research. One of the current challenges in mass spectrometry is the correct localization of multiple post-translational protein modifications (PTMs) within a peptide. Recently, different software solutions have been developed, which associate PTM-site localizations with confidence values enabling their reliable assignment. Nonetheless, several commonly used tools are designed to estimate the localization probability for only a single type of PTM within a peptide. It is known that different PTMs can occur on a peptide, conveying disparate biological functions Peptide libraries were synthesized applying Fmoc chemistry. LC-MS/MS was performed on an LTQ Orbitrap Velos Pro ETD operated in positive ionization mode. MS/MS was performed via CID, HCD, ETD and EThcD.

The input of our novel tool, ptmRS, is the complete set of search engine-generated confident peptide-spectrum matches and the corresponding raw data. To accurately assess the site probabilities of a given peptide the software evaluates all possible position isoforms. Reporter ions can be used by ptmRS to limit the possible isoforms. Based on this, individual site probabilities are calculated permitting a straight-forward interpretation of a PTMs' locations within a peptide. The calculation is performed in an independent manner for all different types of PTMs. This constitutes an evident advantage over other approaches such as Mascot delta-score or Ascore.

To demonstrate the validity of our algorithm, we show the performance of ptmRS by an exemplarily analysis of a large synthetic peptide library. The library is derived from 95 seed sequences resulting in 47,740 unique peptides; each peptide was either acetylated, mono-, di-, trimethylated on one or two predefined positions. It could be shown that for a specific PTM a site probability cutoff of 99% resulted in a false localization equal or smaller than 1%.

P-897.00

MYMRM: A SOFTWARE TOOL FOR DESIGNING AD-HOC MRM EXPERIMENTS

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Discovery proteomics is performed by shotgun approaches and allows the identification and relative quantification of few thousands proteins from complex mixtures as cell lysates. However, due to a variety of reasons its reproducibility, specially at peptide level, is limited. Targeted approaches seem to be more robust and allow the performance of hypothesis-driven proteomics experiments, where only a set of proteins of interest are analyzed.

Selected Reaction Monitoring (SRM) performed in triple quadrupole-like instruments is the usual method for targeted proteomics, although similar methods, such as Parallel Reaction Monitoring (PRM), performed in Orbitrap instruments have been recently described. Any of these methods requires the selection of proteotypic peptides for each of the proteins of interest to be analyzed. Despite the existence of proteotypic peptides predictors and databases containing information about proteins and peptides identified in discovery proteomics studies, the most straightforward approach to develop a targeted method is to use the information obtained by shotgun experiments performed in the own lab. This communication presents a simple software tool to aid proteomic laboratories designing targeted proteomics methods for their own equipment by using the data of their shotgun experiments.

Shotgun data can be loaded as mzIdentML files and the application updates a database with the occurrences of the different peptides and fragments (if present) observed and their scores. In order to obtain precursor ions lists and transitions for targeted experiments, a complete fasta protein database and the list of the proteins of interest must be provided. The application selects the peptides unique to these proteins (not present in other proteins of the database) with the largest number of observations and best score, and then selects the precursor ions and the transitions (when available) most probable to be detected.

P-898.00

MQRUN — INTEGRATING MAXQUANT INTO A LINUX CLUSTER-BASED WORKFLOW SYSTEM

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OMICS technologies are becoming increasingly popular for large-scale data generation in biology and medicine. Recently, there have been major breakthroughs in the application of workflow systems (e.g. Galaxy, gUSE/WS-PGrade) towards automated analysis of biological high-throughput data. Mass spectrometry instrumentation, the core technology in proteomics, provides improved sensitivity and throughput for proteomics research. This increasing speed of data generation and the growing data amount require automated methods for data processing. MaxQuant is a widely used tool to analyze proteomics data. In its latest release it is operated using an interactive graphical user interface (GUI) on Windows and is not compatible with Linux, prohibiting easy access from the command line or as a library.

We propose mqrun — a wrapper to integrate MaxQuant workflows into a Linux environment. While previous work focused on the integration into Windows-based workflows, most clusters are Linux based. Making MaxQuant available from Linux broadens its applicability.

mqrun is implemented as a Python library that controls a MaxQuant instance on a remote Windows server. The configuration for MaxQuant is specified in a json file that aims to be easy for humans to edit and understand. The supplied json-schema can be used to generate HTML forms or GUIs to allow for integration into existing workflow solutions. The Windows machine can be virtualized on clusters or dedicated servers; only a shared directory between the Linux process and the Windows machine is needed.

We implemented tests to show mqrun reliably reports failures and crashes to the client.

With mqrun MaxQuant can be integrated into automated workflows on a cluster which allows for chaining with downstream analysis tools (e.g. R). The textual parameter file makes it easier to reproduce results. By taking advantage of cluster resources, many data analyses can be parallelized, increasing performance and throughput in proteomics research.

P-899.00

ORGANIZE & ANALYZE YOUR MASS-SPECTROMETRY DATASETS ACCORDING TO YOUR EXPERIMENTAL DESIGN WITH PROLINE.

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LC-MS/MS based proteomics evolved quickly in the past few years. It is now very common to produce 1 thousand of MS spectra and 10 thousands of MS/MS spectra per hour, leading to identification of thousands of peptides and proteins. In the same time, the proteomic studies have become more ambitious and as a consequence the experimental designs have become more complex, involving a larger number of samples deeply analysed using long chromatographic gradients.

To be efficiently processed, the amount of data generated by these studies requires efficient and user-friendly computational tools. Moreover such tools must take into account the experimental design to lead from a collection of analytical LC-MS runs to a curated identified and quantified proteomic dataset. In our opinion existing tools often do not address this issue and do not enable global analysis based on the different fractionation levels of a same experimental design. For these reasons, we develop "Proline", a program which can perform proteomics data computations and render obtained results in user-friendly graphical user interface (GUI). Proline provides algorithms for results validation (custom filters and target decoy analysis), merge and comparison of datasets, label-free quantification (spectral count and LC-MS). Mascot and OMSSA results are currently handled but the support of other ones may be quickly added. Proline is a client server application and the service based architecture as well as the use of long term proven relational database systems makes the application scalable from simple experiments on a single CPU to large studies on multiple servers.

Finally, two independent GUIs are available for launching and monitoring processing tasks and browsing results: Proline Studio has a tight integration with the user desktop environment and provides highly interactive components while Proline Web allows accessing the data remotely from a web browser and binds obtained results with online resources.

P-900.00

DIRECT QUATITATIVE ANALYSIS OF NATIVE HUMAN PEPTIDES IN COMPLEX SECRETOME SAMPLES

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Introduction

Native (poly)peptides released into body fluids contain highly relevant information as regulatory biomolecules with both diagnostic and therapeutic potential. A major analytical challenge in secretory peptide researches is the low abundance of the analyte proportional to the large volume of extracellular matrix proteins. The current analytical solution to this problem is to enrich the peptide fraction of a complex sample by physical removal of the most abundant proteins. Here we report a direct approach to quantitative analysis of endogenous peptides in complex secretomes, without removal of highly abundant background proteins.

Methods

A high-resolution mass spectrometric workflow combining database search with de novo sequencing was proposed to facilitate analysis of the secretome beyond the dominant tryptic fragments. After a first round of database searching, with the tryptic enzyme specificity, all confident matches were filtered out. The remaining data still contain a large amount of good-quality MS/MS spectra. A second round database search and de novo sequencing were performed without enzyme specificity. This allows the identification of endogenous peptides with high sensitivity and accuracy. Peptides were quantified with intensity-based label-free quantification.

Results

Samples were conditioned FCS-containing medium of a selected population of human T-cells, which were reduced, alkylated, fractionated over C4 RP-HPLC, digested with trypsin and run by LC-MS with an LTQ Orbitrap Velos. Given a complex two-dimensional LC-MS/MS dataset (>130 GB) of conditioned medium of two differently treated primary human cell cultures sampled at three time points, PEAKS was supplemented with the described novel data analysis workflow. This allows the identification of small endogenous peptides in the presence of a very busy background of predominant tryptic peptides, which made the former invisible in standard analyses. Label-free quantitation shows that these (poly)peptide profiles contain biomarkers for specific physiological or pathological conditions.

Conclusions

Direct analysis of endogenous peptides in complex secretomes

P-901.00

FROM DATA NORMALIZATION TO STATISTICAL SIGNIFICANCE: QUANTITATIVE PERFORMANCE OF LABEL FREE LC-MS PLATFORMS EVALUATED

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Introduction and Objectives

Quantitative label-free proteomics is rapidly gaining popularity due to its experimental simplicity and scalability, both as a fundamental discovery tool (shotgun) and as a verification technique (Selected Reaction Monitoring) [1]. Considering the relative computational complexity of analyzing label-free LC-MS data, monitoring and benchmarking of the process is key to reliable results. We here present a rigorous evaluation scheme, extended from previously presented methods [2].

Methods

A dataset designed to challenge current software tools was acquired by linear dilution of two sets of synthetic peptides with inverse concentrations, spiked into a complex background. Points along a theoretical dynamic range of five orders of magnitude were measured with several technical replicates in each. A quality control workflow, including statistical analysis to assess downstream impact of data processing, was developed.

Results and Discussion

Despite sound values of commonly used quality control metrics, the output of some software solutions displayed a deviation from the theoretical linearity, propagating to statistical analysis. Interestingly, the same deviation was found before and after alignment, indicating that it originated from systematic bias in the feature detection modules. Furthermore, although the feature alignment step increased the dynamic range of the shotgun data by one order of magnitude, the SRM data displayed a wider dynamic range.

Conclusions

To keep up with the rapid technological advancement of mass-spectrometers, constant improvement and evaluation of software tools is necessary to avoid making data analysis the next bottleneck of quantitative proteomics. We have highlighted some of the key issues that require consideration for accurate results and ultimately the identification of novel biomarkers.

P-902.00
SIMULATED LINEAR TESTS APPLIED TO QUANTITATIVE PROTEOMICS

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Introduction and objectives

Scientists in biomedical research are often interested in significant differences due to biological perturbation such as disease states or effect of treatment, while experimental proteomics data exhibits inherent technical variation. In discovery proteomics studies where the number of samples is typically small, technical variation plays an important role because it contributes considerably to the observed variation. Previous approaches have placed both technical and biological variations in tightly integrated mathematical models, which are difficult to adapt for different sources of technical variation. Our aim is to derive a statistical framework that allows the inclusion of a wide range of technical variability.

Methods

We propose a generic statistical model that allows for the inclusion of a variety of technical models. Our approach is based on linear modeling which supports a wide range of statistical designs including independent sample test, paired sample test and time course experiments. The central idea is to simulate likely data points from the technical distribution. The resulting test is therefore called the simulated linear test. We provide a generic and efficient method for model fitting. Devising a new test is then considered an engineering task, where most of the effort can be spent on obtaining an appropriate model for technical variation.

Results and Discussion

We demonstrate the applicability of the proposed approach by deriving a new significance tests for quantitative discovery proteomics for which missing values have been a major issue for traditional methods such as the t-test. We evaluate the results on three label-free (phospho) proteomics datasets with both spectral counting and ion-intensity based quantitation.

Conclusions

Our experimental results suggest that the simulated linear test is an attractive method for significance analysis of proteomics data.

Topic **23**

Human Proteome Project

OP095 - PROTEOGENOMICS DASHBOARD FOR THE HUMAN PROTEOME PROJECT

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The Human Proteome Project (HPP) aims to map the entire human proteome in a systematic approach. Two of the programs to achieve this goal are the Chromosome-based HPP (C-HPP), which characterize the human proteome on a chromosome-by-chromosome basis; and the Biology/Disease HPP (B/D-HPP) that provides a framework for the coordination of biology and diseased-based contributions. These projects specifically study the uncharacterized products for known protein coding genes, variants generated by alternative splicing and coding SNPs and also a comprehensive characterization of PTMs. In this work we have followed the strategy of the analog genomics projects like the Encyclopedia of DNA Elements (ENCODE) that provides a vast amount of data on experiments of different human cell lines and reports them in a intuitive, interactive web-based dashboard. We have therefore developed a proteomics-based dashboard named dasHPPboard that collects and reports the experiments produced by the HPP consortium.

A first logic approximation has been the integration of the data produced by the Spanish Chr-16 project including its proteogenomics approaches. Shotgun results are available with special emphasis on the identification of missing proteins. We have also processed the ENCODE and Human Body Map (HBM) transcriptomics data for the identification of those cell lines with high expression levels for protein coding genes, especially those classified as "missing" where no strong proteomic evidences are available, allowing the selection of cell lines or tissues to conduct the proteomics studies. We produce and allow downloading the alternative peptides databases derived from RNA-Seq data to be used for protein identification on the same cell line or tissue.

We expect the dashboard to be the central place of all experiments produced and collected by the C-HPP project, allowing the community to quickly explore and find the wide range of produced experiments. The dashboard can be freely accessed at: http://sphppdashboard.cnb.csic.es

P-903.00
CABA IN ISSUE IN NEPAL

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CABA (Children Affected by AIDS) need care home because they don't have their parents and family to care them. The official figures show that there are only 1627 CABA however it is estimated that there are 15000 CABA in Nepal. There is no official figure of the infected and affected children. According to the official figure, out of 1627, 800 have been provided with ART treatment and 50% are deprived of this facility.

I worked in the Saath Saath Project (SSP) of Family Health International (FHI) 360 from December 1, 2012 to January 30, 2013. This research was completed by interviewing the house of 5 CABA and 7 CABA family members in Chitwan. Similarly, in Parsa, 5 CABA and 3 CABA family members were interviewed. Focus Group discussion (FGD) was taken to get the information from CHBC/PP workers. In-depth interviews were taken in the case of DACC and District coordinators.

I have been interviewing individuals and understanding the context, in terms of the stigma and discrimination attached to being an HIV infected person. The project ends in 2014 and so far, the qualitative findings show that HIV/AIDS infected/affected people need a lot of care and attention both by the society as well as the government.

P-905.00

TERMINI ORIENTATED PROTEOMICS-HUMAN AMINO TERMINOME (TOP-HAT) FOR THE HUMAN PROTEOME PROJECT: N TERMINI AND NA-ACETYLATION STATUS DIFFERENTIATE STABLE CLEAVED PROTEIN SPECIES FROM CLEAVAGE REMNANTS

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A goal of the Chromosome-centric Human Proteome Project is to identify all human protein species. With 3,844 proteins annotated as "missing" this is challenging. Moreover, proteolytic processing generates new protein species with characteristic neo-N termini that are frequently accompanied by altered half-lives, function, interactions and location. The extent and rate of isoform expression, protein modification, or speciation, depends on cell type, location, stimulus and human developmental stage.

Monitoring in vivo proteolytic cleavage of a protein not only observes speciation but also obtains proof for the expression of missing proteins. Proteolysis alters the protein sequence and results in neo-N termini and hence novel semi-tryptic N-terminal peptides upon tryptic digestion in bottom-up proteomics. Some of these N terminal peptides exhibit beneficial m/z, ionization and fragmentation properties over their fully tryptic counterparts, rendering these peptides and cognate proteins more amenable to mass spectrometric identification. In our HPP project known as Termini Orientated Proteomics-Human Amino Terminome (TOP-HAT) we aim to analyze less commonly studied or accessible cells by high throughput N-terminomics using terminal amino isotopic labelling of substrates (TAILS)1 in order to identify rare cell-restricted or developmental-stage restricted expression of proteins and their speciation. Recently we have determined the N terminome of human erythrocytes, platelets, placenta, gingiva, dental pulp and B lymphocytes identifying hundreds of tissue specific proteins including 6 missing proteins in erythrocytes and 18 in platelets. N-terminal acetylation occurring cotranslational can be distinguished from post-translational acetylation occurring after protein cleavage.

Based on N terminal acetylation specificity patterns we described a new stabilizing N-end rule for processed protein termini, which discriminates novel protein species from degradation remnants and that is distinct from the destabilizing N-acetylation N end rule.

P-906.00
SIZE OF HUMAN PROTEOME: EXAMPLE OF CHR 18

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In spite of the fact that the international Human Proteome Project (HPP) was launched in 2010, the size of the human proteome, which would require estimates of the number of protein species (proteome width) and the number of copies of an individual protein in a biosample (proteome depth), has not been determined.

The proteome width for master (canonical) proteins can be estimated as the number of expressed proteins detected in biosample, although this number is limited by the number of protein-coding genes in the human genome (~21 000). We calculated the minimal number of human proteins according data from NextProt about average number of AS(2), SAP(17) and PTM(12) modifications per gene. Width of complete human proteome estimated as about 2 million of protein species. For chromosome 18, selected for the Russian part of Chromosome-Centric Human Proteome Project (C-HPP), we can expect ~25 000 different protein species.

Experimental estimates of proteome width and depth are dependent on the sensitivity level of proteomic technology. To demonstrate the dependence of the proteome width and depth on sensitivity level of proteomic technology (SRM), the distribution histogram was obtained on the basis of data about copy numbers of Chr18 encoded proteins. This distribution is also bell-shaped, with a maximum of 108 copies for plasma proteins and 105 copies for liver and HepG2 cells. Our results for chr18 were extrapolated to human proteome; about 87% of human proteome width for master proteins in blood plasma possible detected and quantitated by current technology. For liver tissue and HepG2 cell this estimate is 78%.

P-907.00
PROTEOMIC EVALUATION OF LOW- AND HIGH-FLUX HEMODIALYSIS

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The principal mechanisms of blood depuration by hemodialysis are diffusion, convection and adsorption. Hemodialysis may be performed using low-flux (LF) membranes which remove only small molecules , or high-flux (HF) membranes with remove also bigger molecules. Aim of the this study was to assess, by means of proteomic techniques, the relevance of convection and adsorption of proteins with different membranes.

Methods: Seventeen patients treated with LF polysulphone and with HF membranes Triacetate; Helixone; Polyamide.
Proteomic analysis.30 min after the beginning of the dialysis a sample of ultrafiltrate fluid was analyzed by means of biochemical and proteomic techniques. At the end of the dialysis we assessed the adsorption of proteins on the different dialytic membranes. The proteins in the ultrafiltrates and those eluted from the membranes were analyzed through SDS PAGE, 2DE, and MALDI-TOF.

Results: Biochemical analysis. A very high removal of small molecules was demonstrated, which was similar with all the membranes. The removal of B2M was high with HF membranes and insignificant with LF membrane. The removal of bigger molecules (myoglobin and BNP) was higher with triacetate than with the other HF membranes. Proteomic analysis. No convection of small proteins (LMWP) in the ultrafiltrate fluid was found through LF polysulfone. Polyamide and helixone allowed the convection of LMWP. In the ultrafiltrate of triacetate a higher amount of different LMWP was demonstrated. Proteins with different MW were found in the eluates from the different membranes. In particular, albumin and LMWP were demonstrated on the inner part and inside the triacetate membrane. Much more than with the other membranes.

Conclusions: Proteomic technology allows a better understanding of mechanisms of blood depuration of the different membranes.

P-908.00
PROTEOME OF THE HUMAN FULL TERM PLACENTA
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Introduction and objectives: The success or failure of a pregnancy depends on formation of the placenta. Placenta is a transient structure that supports the fetus nutritionally and metabolically. Placentation is a dynamic process and proper migration of the fetal trophoblast cells through endometrium is a key step in placental formation. Several different biological and haemostatic processes are involved in regulation of a normal placentation. Identification of placental proteins can profoundly impact on the prediction of fetal outcome and treatment efficacy. Despite the major role of the placenta in pregnancy, interestingly only few studies have been published regarding the human placental proteome. Thus, the present study aimed to investigate and expand the total human placental proteome.

Methods: In the present two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) method was used for separation and Matrix-assisted laser desorption/ionisation time-of flight mass spectrometry (MALDI-TOF/TOF) technique for identification of the full term human placental proteins.

Results: In analysis of the coomassie blue stained 2D gels, 550 protein spots (with % intensity greater than 0.01) were observed. 121 distinct out of 550 protein spots were successfully picked and identified, among them 21 are unique to the present study and are newly introduced and added to the human placental proteome.

Conclusion: In conclusion the data of the present study expanded the human placental proteome and would be useful for future placental related disorders studies.

P-909.00

THE HUMAN DIABETES PROTEOME PROJECT (HDPP): 13TH HUPO WORLD CONGRESS IN MADRID

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Diabetes is a major health concern today and will continue to be an important cause of morbidity and mortality in the future. Large research efforts have been made to better understand and act upon this complex pathology and its complications. Along these lines, the Human Diabetes Proteome (HDPP) consortium was created during the 11th HUPO meeting in Boston. The consortium gathers worldwide experts in the field of diabetes to generate and leverage data sets addressing molecular mechanisms leading to diabetes, and to understand the dysfunctions induced by, for example, elevated levels of glucose and fatty acids.

These multi-platform-derived molecular data sets are expected to deliver systems-level insights into diabetes-associated cellular changes. The partners involved in the HDPP represent a broad scope of diabetes-related topics, including research on both type 1 and type 2 diabetes at molecular, organelle (mitochondrial), cellular (e.g. beta cells), organ (e.g. pancreas, liver, kidney) and organism (human, rodent) level. Within HDPP distinct valuable datasets have already been collected, which will be concatenated with the help of bioinformatics.
HDPP is part of the B/D-HPP initiatives, and has published a list of 1350 proteins expected to be important in diabetes (www.hdpp.info). The list was extended during the second year with the creation of 2 sub-sets of 100 and 25 proteins, including potential biomarkers for diabetes and associated pathologies.

The consortium has also built the largest human proteomic islet database, using the two most extensive studies published yet, and created a list of more than 6400 islet-associated proteins, which is expected to serve as a reference for future studies. All the work performed during the second year within the consortium will be presented in the HDPP workshop at the 13th HUPO congress in Madrid.

P-910.00
THE HUMAN SURFACEOME

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Introduction and objectives

The human surfaceome is defined as the repertoire of plasma membrane residing proteoforms with an exposed, extracellular domain. These proteoforms, alone or in concert as proteotype, enable but also limit cellular communication with the microenvironment, reflect the cellular differentiation state and indicate functional capacity. Despite biological relevance, a considerable part of the surfaceome's constituents remain unknown. On top of that, the surfaceome composition on different cell types, as well as the relative and absolute quantities of its proteoforms are mostly unknown. Here we approach the specification of the human surfaceome from a protein-centric computational perspective, based on proteomic evidence in combination with machine-learning algorithms.

Methods

Cell Surface Capture (CSC) technology enabled the generation of the Cell Surface Protein Atlas (CSPA) consisting of ~1000 experimentally verified human cell surface exposed proteins. Machine-learning algorithms like random forests and logistic regression were employed to bioinformatically define the human surfaceome.

Results and Discussion

Based on a unique experimental surfaceome resource, the CSPA, we derived a generic computational model of surfaceome proteins. The model revealed sequence motifs, domains and structural features that are predictive for cell-surface proteoforms. Application as a classifier to the entire human proteome predicted an extended repertoire of cell-surface protein candidates, which was further curated and refined using publicly available resources. This human surfaceome encompasses a minimum of 2500 proteins. Using the resource to filter transcriptomic datasets of various cell types leads to the assumption that the surface proteotype of a cell encompasses a minimum of 1000 different proteins, of which more than a hundred are CD annotated.

Conclusions

The human surfaceome reference set enables the informed filtering of proteotranscriptomic datasets for comparative biomedical analysis. The number of proteoforms residing in the plasma membrane is higher than generally thought, requiring further experimental tools for validation and functional analysis.

P-911.00

SUBCELLULAR FRACTIONATION ANALYSIS OF PLACENTAL TISSUE MEMBRANE PROTEINS FOR IDENTIFICATION OF MISSING PROTEIN.

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Introduction and objectives

To completely understand human disease and biological process, we have to study not only genomics but also focus on proteomics as cellular level. For this reason, International project which named as chromosome based human proteome project(C-HPP) has established and it regards as a solution that solve a gap between gene and protein. One of important object of C-HPP is finding proteins which has uncertain origin that named as "Missing proteins". Missing proteins defined as which has weak or no mass spectral evidence.

Placenta is organ that appears temporary but it containing lots of biological information. It also regards as a sample that represent of Chr.13 and related various pregnancy disease like preeclampsia. And recently there were some study revealed membrane proteins which has trans-membrane domain were containing lots of missing proteins. Thereby, we study missing proteins by using subcellular fractionation method with placental tissue.

Methods

Samples were analyzed by optimized method as stepwise centrifugation, 1DE GEL and high pH reverse phase LC fractionation then Orbitrap mass spectroscopy. In data processing step, with in-house tool for extracting protein sequences which can easily verify whether identified proteins have trans-membrane domains (TMDs) or not by membrane search engine (SOSUI).

Results and Discussion

With such sample preparation conditions and methods, we were able to identify more than 340 membrane proteins present in placenta. And now we find missing proteins from placental membrane protein list and also focus on nucleus and cytoplasmic proteins that by-product of membrane extraction step.

Conclusions

This study with subcellular fractionated sample analysis is one of efficient tool for find missing proteins and it will be a valuable resource of finding disease biomarkers, treatment and C-HPP achievement.

P-912.00

SIMULTANEOUS TARGETED ANALYSIS OF FIVE OSTEOARTHRITIS BIOMARKER CANDIDATES RELATED TO CHROMOSOME 16 BY LIQUID CHROMATOGRAPHY-MULTIPLE REACTION MONITORING MASS SPECTROMETRY

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Introduction and objectives. The aim of this project is framed within the global Human Proteome Project initiative (HPP). We are focused on the global study of proteins that are encoded by chromosome 16, as members of the Spanish consortium. Within this consortium, our group specifically aims to identify new markers useful for diagnosis and prognosis of rheumatic diseases.

Methods. Samples of cartilage, chondrocytes and serum from donors suffering osteoarthritis (OA) and healthy controls were used. Proteins from the different OA samples were quantified and digested with trypsin. The peptide mixtures were separated and analyzed by nano-LC coupled to a 5500 QTRAP mass spectrometry, using the MRM methodology. Co-elution of at least three MRMs per peptide was monitored and at least 2 peptides per protein were chosen. The specificity of the precursors was validated by the generation of MS/MS spectra, which were launched against a human database to confirm the identity of the peptides using the protein Pilot software.

Results. Five proteins codified in the chromosome 16 were analyzed. These proteins which were previously identified as putative OA biomarker candidates in shotgun proteomics experiments performed by our group: Haptoglobin, Fructose-bisphosphate aldolase A, Insulin-like growth factor-binding protein complex acid labile subunit, C-type lectin domain family 3 member A and matrix metallopeptidase 2 (gelatinase A, 72kDa gelatinase, 72kDa type IV collagenase). MRM methods were developed to evaluate the usefulness of these proteins as diagnostic and prognostic biomarkers of OA. Data analysis was performed using the skyline software for method refined and optimization, and Multi-Quant softwareTM for relative quantitation.

Conclusions. Development of MRM methods for the analysis of Chromosome 16-encoded proteins is one of the objectives of the Spanish HPP consortium. Furthermore, our work has provided a valuable tool for the screening of sera from patients suffering rheumatic diseases.

P-913.00

SERUM BIOMARKERS OF ERYTHROPOIETIN RESISTANCE: ESTABLISHING AN OPTIMAL MULTIDIMENSIONAL LIQUID CHROMATOGRAPHY WORKFLOW COMPATIBLE WITH HEXAPEPTIDE-BEAD LIBRARY AND ISOBARIC PEPTIDE TAGGING REAGENTS

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Current approaches to managing anemia in end-stage renal disease (ESRD) patient populations receiving dialysis include the use of erythropoiesis stimulating agents (ESA). Substantial difficulties exist for predicting each patient's response to ESA dosing. We hypothesized those candidate serum biomarkers that reflect the response to ESA's existed in the low abundant serum proteome. We developed a strategy identify these candidate biomarkers involving enrichment of low abundant proteins using a hexapeptide-bead library (Proteominer, BioRad), and isobaric tagging reagent (TMT, ThermoFisher) to allow for semi-quantitative LC-MS methods to establish candidate biomarker lists. The integration of these two workflows required integration of a Filter Aided Sample Preparation (FASP) to minimize the effects of strong denaturants (needed to recover the protein sample from the beads) on tryptic digestion, isobaric tagging reagents and down-stream LC-MS analysis.

Here we present the results of the application of an optimized serum biomarker discovery workflow for comparison of six ESRD patient samples receiving ESAs to manage their anemia. This method integrated serum proteome enrichment, FASP digestion, peptide isobaric tagging, and then used these samples to compare first dimensional separation by strong cation exchange (SCX)chromatography versus high pH reversed phase (RP)chromatography (seven fractions each) within a multi-dimensional-LCMS analysis. A preliminary analysis demonstrates the successful application of this method by identification of greater than seven hundred serum proteins using a decoy database strategy, a two peptide rule, and Peptide Prophet to minimize false discovery rates from the combined 2DLCMS analysis. Of 206 proteins common to the SCX and high pH RP experiments, 53 proteins were identified as differentially abundant between the two patient groups.

Within these proteins, neither the SCX or high pH RP LC method demonstrating a significant methodological benefit based on observed protein identifications. These observations may change with increased 1-dimension fractionation or with use of other chemical tagging reagents.

P-914.00

DEVELOPMENT OF IMPROVED SUBCELLULAR FRACTIONATION PROCEDURES OF THE PLACENTAL MEMBRANE PROTEINS FOR DISCOVERING DISEASE BIOMARKERS AND MISSING PROTEINS

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To better understand the function of genes and proteins involved in human disease and biological process, the gene-centric approaches have been adopted recently to produce human proteome parts list. For this reason, the International Consortium of the Chromosome-Centric Human Proteome Project (C-HPP) has been established in 2012 and now well positioned as part of HPP. Given that one of the important tasks of C-HPP is to identify "Missing proteins" which are known to have weak or no mass spectral evidence.

We were interested in exploring membrane proteins present in placenta of both normal and preeclampsia (PE) patients to profile membrane proteins involved in PE and identify missing proteins. To this end, membrane protein samples of both normal and PE patients were analyzed by the in-house proteomic platform which includes a stepwise centrifugation, 1D gel, high pH reverse phase LC fractionation and Orbitrap MS analysis. With an improved data processing strategy, some specific protein sequences were extracted and then used for verifying those proteins having trans-membrane domains (TMDs) without the use of membrane search engine (SOSUI). Using the label free quantitative analysis and bioinformatics quantitative programs (Scaffold and Sieve), we were able to identify more than 340 placental membrane proteins with high confidence among which 28 proteins are differentially expressed in PE tissues. Identified also was one missing protein and 13 other proteins which are known to be secreted into plasma when crossed checked with human plasma databases (Peptide Atlas and Human plasma proteome).

Currently, a scale-up fractionation and proteomics analysis are now underway to search for more missing proteins and PE-specific membrane proteins in the placenta. (This work was supported by the International Consortium Project from the Ministry of Health and Welfare [HI13C2098 to YKP]).

P-915.00

GENOMEWIDEPDB V 2.0: UPDATE ON THE TRANSCRIPTOMIC AND PROTEOMIC EXPRESSION DATA WITH ALTERNATIVELY SPLICED PRODUCTS LAYERED IN A GENOME-WIDE MANNER

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To promote our efforts on production and managements of both proteomic and genomic parts list, important components of the international Chromosome-centric Human Proteome Project (C-HPP), we previously developed a gene-centric proteomic database called GenomewidePDB, which integrates proteomic data of proteins encoded by human chromosomes and contains the experimentally identified proteins that are present in normal human placenta tissue. With a rapid progress in proteomic analysis of human tissues, cell lines and bio-fluids, it was necessary for us to make update on various information deposited in this DB.

Given that this DB was designated to provide the insight of protein¡¯s function with their partners (e.g., surrounding genes or interacting partners) and also designated to expandable to all chromosome encoded proteins through various samples, we made updates on the transcriptomic and protein expression profiles according to different type of samples in addition to the number of proteins deposited in this DB. Furthermore, alternatively spliced products of each gene were newly integrated into this GenomewidePDB v2.0 by taking recent data from both NCBI UniGene EST profile and Human Protein Atlas.

Thus, this newly updated database will provide more information on identifying protein isoforms and other RNA resources that will be useful for the comprehensive studies of genome-wide proteomic mapping.

P-916.00

UNRAVELING THE PROTEOME OF NUCLEUS BASALIS OF MEYNERT

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The nucleus basalis of Meynert (NBM) is composed by a population of large cholinergic neurons that send their axons to the cortex, the olfactory bulbs, and the amygdala. Although it has been suggest that the degeneration of NBM underlies the cognitive decline observed in neurological dementing diseases, the proteome present in NBM are yet to be determined. In this work, we have used anatomical and peptide fractionation strategies coupled to nanoLC-MS/MS to identify the proteome present in the human NBM. Using the Triple TOF 5600 mass spectrometer, we have identified 2775 proteoforms corresponding to 2750 unique proteins.

Using functional analysis and data mining of MS-generated proteomic data, we found great diversity of proteins involved in a plethora of neuronal processes and functional categories. This protein compilation has also been classified according to their chromosomal origin. Interestingly, NBM mass spectrometric analysis has provided evidence of 9 human proteins that are currently annotated as "missing" proteins in NeXtProt.

These are expressed by seven different chromosomes and were identified by at least two unique peptides, providing useful information to the chromosome-centric human proteome project (C-HPP).

P-917.00

IDENTIFICATION PROTEINS OF CARDIOVASCULAR SYSTEM IN HEALTHY SUBJECTS' URINE DURING "DRY" IMMERSION

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«Dry» immersion (DI) is widely used model in gravitational physiology to simulate microgravity effects. It accurately reproduces cardiovascular, sensory-muscul and other changes with development rate and intensity close to those observed in flight. Body fluids redistribution and afferent inputs alteration induce the changes in the functional state of cardiovascular system. A urine proteome of 14 healthy men aged 21 to 29 years was studied before (B-8 and B-2), during (DI 2, DI 4, DI 5) and after (R+3 and R+8) five days of DI.

The median part of the second morning urine void was used. Peptide mixtures from urine samples were analyzed, after trypsin digestion, by liquid chromatography-mass spectrometry instrument Maxis 4G (Bruker Daltonics GmbH, Germany) and time-tag strategy. To identify the proteins related to cardiovascular system, it was used TiGER database and ANDSystem containing the lists of proteins and biological processes. From a totality of 238 proteins identified, it were revealed 9 proteins related to cardiovascular system. The detection rate of these proteins in the samples was characterized by different dynamics during the experiment. For some proteins, the dynamics depended on kidney functions modified by DI. For the others, the change in dynamics reflected a development of endothelial dysfunction. Most of urine proteome changes were characterized by both the very rapid onset and quickly recovery within return to normal conditions.

P-918.00

BIOLOGICAL PROCESSES IN COSMONAUTS AFTER LONG DURATION SPACE FLIGHTS BY STUDYING URINE PROTEOME

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We attempted to describe biological processes based on changes in the proteome composition of the cosmonauts' urine. The protein composition in urine samples of 15 Russian cosmonauts (male, aged of 35 up to 51) performed long duration flight missions (from 169 up to 199 days) on the International Space Station (ISS) was analyzed. Urine samples were collected before launch and on the first and seventh days after landing, from the second morning void (space experiment «Proteome»).

As a control group, urine samples of 12 back-up cosmonauts were analyzed. This group is epidemiologically identical to the cosmonaut cohort. Sex, age and protocol of investigation were the same to the prime crew cosmonauts. Sample preparation was performed via liquid chromatography-mass-spectrometry. Liquid chromatography-mass-spectrometry was performed on a nano-HPLC Agilent 1100 system (Agilent Technologies Inc., USA) in combination with a LTQ-FT Ultra mass spectrometer (Thermo Electron, Germany). 294 proteins were detected. 34 of these proteins were specific for the first post-flight day. Using BiNGO tool software we found 63 overrepresented processes on the first day after landing ($p < 0.05$ with consideration of Benjamini-Hochberg correction for multiple comparisons). 9 processes were identified on the first day after landing, which were related to renal function and water-electrolytic balance. 3 processes were related with cardiovascular system adaptation to the Earth's gravity, and 9 processes were associated with lipid peroxidation, regulation of reactive oxygen species and metabolic process.

Thus, it was possible to identify proteins participating in physiological processes which provide adaptation to microgravity and readaptation to the ground conditions. The verification process over represented identified by examining urine proteome for physiological phenomena at the system level.

P-919.00

HIGH-THROUGHPUT EVALUATION OF ANTIBODIES ON WESTERN BLOT WITHIN THE HUMAN PROTEIN ATLAS PROJECT

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The Human Protein Atlas (HPA) project aims to map the human proteome using an antibody based proteomics approach, and has over the last eleven years produced antibodies towards more than 80% of all human proteins. The Immunotechnology group within the project is responsible for the generation and purification of all antibodies produced, as well as for testing them in Western blot experiments. In a high-throughput workflow all antibodies are validated with a Western blot setup using human protein lysates from cell lines (RT-4 and U-251 MG), plasma, as well as whole tissue lysates from liver and tonsil. To date, over 40 000 Western blots have been analyzed and 30% has been given a supportive score.

In order to improve validation success rates, antibodies giving a non-supportive result in this platform are re-evaluated in another Western blot setup, using over-expression lysates (VERIFY-Tagged AntigenTM, OriGene Technologies, Rockville, MD). This approach was implemented recently and has been utilized on more than 3000 antibodies scored as non-supportive and has so far a success rate of 82%. To further enhance the quality of validation, a third Western blot assay has been set up, using the technique of short interference RNA (siRNA).

Here, each antibody is tested against two cell lysates where siRNA has been used to down-regulate or knock-out the expression of the target protein in the U2OS cell line. A cell culture treated with scrambled siRNA is used as control. These efforts are part of the goal towards a first draft of the Human Protein Atlas.

P-920.00

INFLUENCE OF EXPERT TEST WITH LARGE RADIUS CENTRIFUGE ROTATION ON URINE PROTEOM OF COSMONAUTS

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Medical studies conducted to withstand stresses during rotation in the large radius centrifuge with a direction of the vector overloads chest-back (+ Gx) at an angle 78-80 ° (angle between the vector of the total overload and the longitudinal axis of the body).

Conducted two rotations in one day with an interval of 5-10 minutes between rotations: the value of overload chest-back (+ Gx) 4,0 units with the duration of exposure to the "step" for 60 seconds, and 8.0 units - for 30 seconds. Chromatography-mass spectrometry analysis of urine samples collected prior to the test in the centrifuge and after it, carried out on a system consisting of chromatograph Agilent 1100 GC (Agilent Technologies Inc., Santa Clara, USA) and hybrid mass spectrometer LTQ-FT Ultra (Thermo, Bremen, Germany).

After exposure to moderate overloads + Gx direction, below the maximum limits of human tolerance minute speed of diuresis was increased (p

P-921.00

CELL TO GEL WORKFLOW: A STREAMLINED PROTEOMICS WORKFLOW FOR MINIMIZING SAMPLE PREPARATION AND MAXIMIZING PROTEIN SAMPLE UTILIZATION.

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In an effort to streamline proteomics workflows, reduce reagent consumption and maximize the number of proteins available for analysis, we have developed a streamlined procedure for rapid proteomics analysis. Traditionally, the preparation of cultured cells for proteomics analysis involves harvesting, lysis, clarification of cell material, and protein assays before proceeding to the first steps of analysis. These processes are labor intensive and inefficient. We have developed a rapid and easy proteomics analysis protocol that reduces not only the number of cultured cells required, but reduces the number of preparation steps as well.

3T3 cells were cultured in a 48-well plate, and were simply lysed in place using Bio-Rad SuperShot lysis buffer with DNAse treatment. After lysis and DNAse treatment, samples were removed from the wells and were immediately processed for western blot analysis beginning with SDS-PAGE Electrophoresis using Bio-Rad TGX StainFree gels. Following electrophoresis, the proteins were then transferred to a PVDF-based membrane and immunodetection with anti-alpha-tubulin antibody was performed.

We have demonstrated a fast and easy sample preparation protocol for proteomics sample preparation and analysis. Furthermore, we have developed a standard curve for the number of cells required for immunodetection-based analysis methods.

Additionally, this protocol takes advantage of the total protein signal obtained via Bio-Rad Stain-Free technology. The total protein signal obtained by Stain-Free technology is a key component in the Bio-Rad V3 Western Workflow, and is the basis for accurate, reliable and quantifiable western blotting results. Nonetheless, this technique is also compatible with other analytical techniques, including but not limited to 2D-Electrophoresis, Mass-Spectrometry and ELISAs.

P-922.00

PROTEOMIC BIOMARKER ANALYSIS OF MATERNAL SERUM FOR IDENTIFICATION OF PRETERM LABOUR

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Introduction:

Spontaneous Preterm birth (sPTB) remains a major clinical dilemma with an incidence of 7% to 11% and current diagnostic procedures and interventions have not reduced the rate of this problem. Understanding sPTB ($<$ 37 weeks gestation) is difficult due to heterogeneities associated with multitudes of risk factors and pathophysiological pathways. Opportunities to intervene in a timely fashion to avoid poor pregnancy outcome are therefore limited.

We aim to identify biochemical markers that demonstrate the different pathogenetic mechanisms (inflammatory/infectious, hormonal, vascular, stress related, extracellular matrix degradation–related, and genetic) of sPTB. Using proteomic and molecular techniques, we identified a short list of candidate maternal serum proteins that are present in relative abundance or absence in preterm birth pregnancies compared to controls.

Objectives:

To improve our understanding of the biochemical changes seen in preterm birth
To develop an accurate predictive test that would allow effective intervention to reduce neonatal mortality and morbidity

Methods:

This cross-sectional study included patients with spontaneous preterm labour and intact membranes who delivered before 37 weeks of gestation and those who delivered at term. Maternal serum sample were collected from women attending routine antenatal clinic at 11-12 weeks of gestation at Royal Prince Alfred Hospital, Sydney, Australia. Proteomic profiling was performed using 2 - dimensional gel electrophoresis and mass spectrometry.

Results and Discussion: The proteomic approaches employed in this study identified distinct sets of proteins that were deferentially abundant in serum of women delivering preterm compared to those found in the serum of women with who delivered at term. Conclusion: Further characterization of these markers in a larger cohort of subjects may provide basis for new tests for the early, non-invasive positive prediction of sPTB.

P-923.00
ANTIBODY RANKER

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P-924.00

MITOCHONDRIAL PROTEOME OF CYBRID CELLS HARBOURING A TRUNCATIVE MITOCHONDRIAL DNA MUTATION IN RESPIRATORY COMPLEX I

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Transmitochondrial cytoplasmic hybrids (cybrids) are an established model system to reveal the effects of mitochondrial DNA (mtDNA) mutations on cell metabolism excluding the interferences of a different nuclear background and the mutation threshold associated to pathologic phenotype (1). The m.3571insC mutation in MTND1 gene of respiratory complex I (CI) is commonly detected in oncocytic tumors, in which it causes a severe CI dysfunction leading to energetic impairment when present above the 83% mutation load (2). To assess whether the energetic deficit may alter the mitochondrial proteome, OS-78 and OS-93 cybrid cell lines bearing two different degrees of the m.3571insC mutation (78% and 92.8%, respectively) and control cybrids bearing wild-type mtDNA were analyzed.

Mitochondria from cybrid cell lines were prepared by differential centrifugation and mitochondrial proteins were separated by two-dimensional electrophoresis. Three preparations for each cell lines were analyzed. Differentially expressed proteins were identified by nano ESI MS/MS mass spectrometry and validated by western blot. Proteomic analysis revealed the presence of differentially expressed proteins only in cybrids bearing the mutation level above the threshold (OS-93). In particular, the level of pyruvate dehydrogenase E1 chain B subunit, of lipoamide dehydrogenase, the enzyme component of pyruvate and 2-oxoglutarate dehydrogenase complexes and of lactate dehydrogenase B was reduced. When OS-93 cybrid cells were grown in galactose medium, a metabolic condition that forces cells to use respiration, a significant decrease of the pyruvate dehydrogenase complex activity was found.

In conclusion, the almost homoplasmic m.3571insC mutation in CI causing a mitochondrial energetic impairment perturbs cellular metabolism leading to a decreased steady state level of components of very important mitochondrial NAD-dependent dehydrogenases.

P-925.00
QUANTITATIVE HUMAN TEARS PROTEOME IN SHORT-TERM DECORATIVE CONTACT LENS WEAR

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Introduction and objectives
Human tear is regarded as multilayers of aqueous containing vast proteins. Many studies have suggested biomarkers found in tear in diseased states. Since decorative contact lens has gained popular, the complications of pigmented contact lens are of great concern. This study aimed to apply mass spectrometry to study the quantitative human tears protein changes in decorative contact lens compared with clear contact lens.

Methods
This is a randomized, controlled, single masked study where 25 healthy subjects were recruited. The color contact lens was worn randomly on either eye; the other eye wore clear contact lens as a control. Tears were collected after 1 hour and 7 hour. The samples were pooled and digested for dimethyl labeling. The Protein Identification and quantification were performed using TripleTOF 5600+ system fitted with Nanospray III source. The MS/MS spectra are searched against the database using Paragon algorithmTM in the ProteinPilot software. Differentially expression ($\geq$20% fold change) with at least two peptides (P-value $\leq$0.05) were screened using the Pro Group™ Algorithms.

Results and Discussion
According protein assay, no significant differences were found in the total protein concentrations. Over 100 proteins could be identified in either treatment or control and their protein profiles were found to be similar. Most of them are responsible for catalytic activity, binding. Eight proteins were found significantly up-regulated in the first hour of decorative CL wear. However, some of them were found down-regulated after seven hour wearing. Most of them are responsible for protective mechanism such as Anti-microbial and immune response.

Conclusions
We demonstrated the rapid protein changes in abundance in short-term decorative contact lens wear compared with traditional clear contact lens of the same design. The pigments embedded in decorative contact lens could be one of reasons to stimulate protein changes in human tears.

P-926.00

PROTEOMIC ANALYSIS OF THE SPERM CELL: TOWARDS THE COMPLETE SPERM CELL PROTEOME AND IDENTIFICATION OF PROTEINS INVOLVED IN MALE INFERTILITY

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Introduction and objectives:Alterations in sperm function are responsible for male infertility, a common condition present in about half of infertile couples. However, not all proteins in the sperm cell are known at present and most of the protein alterations associated with male infertility remain to be identified. Our objectives are to identify novel proteins which will contribute towards the completion of the human sperm proteome and to detect proteins related to male infertility.

Methods: Whole sperm cells as well as sub-cellular fractionations have been applied coupled with shotgun LC-MS/MS. Quantitative alterations in chromatin proteins potentially present in asthenozoospermic infertile patients as compared to controls have been investigated through tandem mass tag (TMT) differential proteomics and protein identification through LC-MS/MS.

Results and Discussion: LC-MS/MS of isolated sperm cell nuclei or isolated sperm tails has allowed us to identify 403 and 1049 proteins respectively. The majority of these proteins are novel providing new insights into the sperm function (de Mateo et al. 2011, Proteomics 11:2714–26; Amaral et al. 2013, Mol Cell Proteomics 12:330-42). Compilation and detailed revision of the human sperm proteins described thus far has allowed the generation of a catalogue of 6198 proteins (Amaral et al. 2014, Hum Reprod Update; 20:40-62). By calculating the mean of the ratios of all Reactome pathways identified, we estimate that approximately 78% of the sperm proteins have already been identified and that the complete human sperm proteome is composed of >7500 different proteins. Application of TMT differential proteomics to study proteins involved in sperm motility failure allowed the identification of over 1000 proteins with 80 differential proteins detected. Interestingly, our results revealed that many of these proteins are post-glycolytic enzymes, supporting the idea that other metabolic pathways also contribute to fuel and regulate sperm motility.

P-927.00

MEASURING SUCCESS A PRIORI? THE REGULATION OF RESEARCH BY TRANSLATIONAL TENSIONS

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Introduction and objectives: We are conducting a case study of proteomics science as one of three studies in a larger research project focused on the translation of scientific knowledge into applied health interventions. Our objectives are to engage with scientists working in proteomics in order to understand their experiences in communicating the impact of their work to clinical practitioners, policy makers, and research funding bodies.

Methods: To date we have conducted participant observation at several large proteomics conferences including three HUPO congresses, and have conducted semi-structured interviews with key scientists working in the field in Canada, the US, and Australia. Our qualitative data is coded using the NVivo analysis system to identify central themes and how they are connected.

Results and Discussion: Based on the data collected to date, we have identified several tensions that regulate the interaction between practitioners of proteomics science, and the professional sectors that "use" the research generated, evaluate its impact, and thus have an impact on the type of knowledge generated: clinical practices, health policy makers, and funding bodies. This poster will present these key tensions and the questions they raise (illustrated by examples from our data):
1) science push vs. user pull: which is more likely to improve health and healthcare?
2) curiosity-driven vs. funder-driven: which way of framing research will result in high-impact discoveries?
3) big science vs. small science: how is success measured and compared across different scales?

Conclusions: Our findings suggest a tendency for a misalignment between the assumptions of those who produce proteomics science, and those who assess and identify its impact. The key tensions illuminated by our research could help inform a more productive, strategic discussion between the producers and users of scientific knowledge.

P-928.00

URINE PROTEOMICS FOR THE DISCOVERY OF NON-INVASIVE DIAGNOSTIC BIOMARKERS FOR PEDIATRIC DISEASES

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Many childhood diseases are surprisingly difficult to diagnose; thus, even mundane disease such as appendicitis are associated with up to 20% false diagnosis rates. To overcome these diagnostic limitations and to facilitate non-invasive diagnostics based on objective molecular patterns, the Steen laboratory in collaboration with the Emergency Medicine Department at Boston Children's Hospital has established a urine proteomics platform, with a special emphasis on acute inflammatory, infectious and traumatic conditions.

The initial urine proteomics studies were based on laborious fractionation and subsequent GeLC/MS strategies requiring significant amount of instrument time. The more recent urine proteomics studies are now filter-aided sample processing-based significantly decreasing the processing time from days to hours. In addition, with the adaptation of state-of-the-art instrumentation (e.g. Q-Exactive and/or TripleTOF 5600) there is no need for prefractionation any longer.

The first urine proteomics studies have resulted in biomarker candidates for acute pediatric appendicitis and Kawasaki disease. The former is surprisingly difficult to diagnose resulting in yearly ~10,000 unnecessary appendectomies in children in the U.S. alone; the latter currently does not have an objective diagnostic test. The identified urinary biomarker candidates - confirmed in >375 and >100 samples, respectively - surpass the performance of any currently available diagnostic modality. With the adaptation of FASP/FASP-like workflows and state-of-the-art instrumentation, the sample amount, processing time and instrument time requirements were reduced by >90% without compromising the results. These advancements in combination with recent data independent acquisition routines allows performing urinary biomarker discovery studies in much larger sample cohorts even on urine samples of limited availability.

Urine proteomics has successfully identified diagnostic biomarkers for appendicitis and Kawasaki disease. Recent improvements in the methodology now enable such urine proteomics studies with as little as 200 ul of urine thereby extending their applicability to preemies, neonates and infants, where sample volume is very limited.

P-929.00

DEVELOPMENT OF MS-ASSAYS FOR HUMAN CANCER: TOWARDS THE CANCER PROTEOME

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The central theme of the human biology and disease-human proteome project (B/D-HPP) in cancer (Ca-HPP) is to 1) identify cancer-specific target proteins or protein modifications, 2) develop and 3) disseminate assays for target protein validation and clinical application. The use of mass spectrometry (MS)-based platform such as selected reaction monitoring (SRM) has become an increasingly popular method for quantitative analysis of target proteins.

It has been shown that the use of synthetic peptide standards and isotope dilution allows reproducible identification and accurate quantitation of proteins in multiple laboratories possible. Therefore, the MS-based assays, once developed, can be easily transferred and used in other laboratories. However, the development of MS-based assays is often limited to a specific cancer type in each laboratory, and efforts may be duplicated with different laboratories developing the same assays. Acceptance of high throughput development of MS assays for proteins or protein modifications has been limited to date due to the difficulty in establishing these assays and the easy availability of the assays compared to the traditional antibody approach. We propose to coordinate and facilitate international efforts to target cancer proteins for each cancer type. During this HUPO meeting, Ca-HPP would like to bring together investigators from a number of different areas including cancer target selections, assay developments for targeted proteomics using SRMs, and target distribution.

By working together, we can create a synergistic effort to work with a library of cancer protein targets, develop sensitive and specific assays, and make these assays widely available to investigators from biological or clinical laboratories. We will further discuss the procedure for selection of target proteins for each cancer type or involved in the disease molecular mechanism, the strategy for assay development and validation, quality control, and procedure and materials needed for transferring the established assays to new laboratories.

P-930.00

MITOCHONDRIAL PROTEOMICS INVESTIGATION OF A CELLULAR MODEL OF IMPAIRED DOPAMINE HOMEOSTASIS, AN EARLY STEP IN PARKINSON'S DISEASE PATHOGENESIS

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Impaired dopamine homeostasis is an early event in the pathogenesis of Parkinson's disease. Generation of intracellular reactive oxygen species consequent to dopamine oxidation leads to mitochondrial dysfunction and eventually cell death. Nevertheless, an experimental Parkinsonism may be induced in animal models by MPTP administration. MPTP is oxidized to 4-methylphenylpyridinium (MPP+) that enters dopaminergic neurons and induces mitochondrial dysfunction. The mitochondrial proteome of the SH-SY5Y human neuroblastoma cell line was investigated by the combination of two orthogonal proteomic approaches, two-dimensional electrophoresis and shotgun proteomics, in order to highlight the specific pathways perturbed by the increase of intracellular dopamine, in comparison with those perturbed by MPP+.

Proteins altered by MPP+ did not completely overlap with those affected by dopamine treatment. In particular, the MPP+ target NADH dehydrogenase [ubiquinone] iron-sulfur protein 3 was not affected by dopamine together with 26 other proteins. Enrichment analysis of proteins altered by MPP+ revealed a strong effect on protein folding, energy production and programmed cell death as well. The list of proteins altered by MPP+ treatment was then employed to build an enriched network based on physical interactions. Proteins related to glycolysis, response to unfolded proteins, apoptosis, protein folding, nucleosome assembly and DNA replication, regulation of transcription, translational elongation, ATP synthesis and anion transport were found to be significantly enriched. Eventually, the interaction network displayed a main hub, represented by HSP60, guiding the mitochondrial response to MPP+ treatment.

The orthogonal approach highlighted the fragmentation of some mitochondrial proteins, suggesting an alteration of the mitochondrial protease activity. Pathway and disease association analysis of the proteins affected by dopamine revealed the overrepresentation of the Parkinson's disease and the parkin-ubiquitin proteasomal system pathways and of gene ontologies (GO) associated to generation of precursor metabolites and energy, response to topologically incorrect proteins and programmed cell death.

P-931.00

CHARACTERIZATION OF CANCER-ASSOCIATED PROTEINS RELATED TO CHROMOSOME 16

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Within the Human Proteome Project (HPP) initiative, the Human Proteome Organization (HUPO) launched a project that specifically addresses biological problems, disease-oriented, in collaboration with expert biologists. This is known as the Biology and Disease initiative (B/D-HPP). The B/D-HPP aims to prepare tools, assays and reagents for studying the proteins of relevance in specific diseases. The B/D-HPP has defined a number of selected diseases for study. The Spanish B/D-HPP initiative has adopted Chromosome 16 (Chr 16) for study and selected cancer among its priorities.

Cancer-related proteins constitute the top biofunction represented in Chr 16. A large number of proteins (over 200) have been associated to different types of cancer, among them 182 to colorectal cancer. However, most of these proteins are largely unknown and uncharacterized, except for some proteins related to the epithelial-mesenchymal transition (EMT) like E-cadherin and other members of the cadherin family. EMT is a key process in cancer progression, invasion and metastasis. Together with these EMT proteins, there are a number of other proteins related with cell cycle, proteases, metallothioneins and other proteins highly mutated in cancer. Following the recommendations of the B/D-HPP guidelines we have generated a target list with these cancer-related proteins in order to define relevant assays and reagents for these targets.

Our objective is to define proteotypic peptides for each of these proteins and to set-up specific SRM conditions for the identification and quantification of these proteins in samples from different types of cancer, with particular emphasis on plasma and other biofluids detection. Initial results will be presented and discussed.

P-932.00
DISECTING CHROMOSOME 16 PROTEOME

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Introduction. The Chromosome 16 Consortium is integrated in the global initiative Human Proteome Project that aims to develop an entire map of the proteins encoded following a gene-centric strategy (C-HPP) to make progress in the understanding of human biology in health and disease (BD-CHPP). The structure of the consortium involves 15 groups that organize in four working sections namely, SRM and protein sequencing, antibody and peptide standard, clinical healthcare and biobanking and bioinformatics. Methods. A combination of methods including protein expression and purification, peptide synthesis, shotgun and targeted proteomic approaches as well as bioinformatics has been used to characterize the chr16-associated proteome.

Results. Upon cell fractionation a significantly improved coverage of chr16 was achieved. Methods for targeted detection of chr16 proteins were developed and recombinant missing proteins were expressed to develop SRM methods for their detection in complex biological matrices. In addition to MIAPE extractor tool, bioinformatics strategies have been implemented to comprehensively report chr16 proteome knowledge, to annotate the missing proteome according to intrinsic properties of missing proteins and the cell/tissue specific gene expression patterns. Moreover, the SpHPP B/D pipeline has been defined and lists of potential driver proteins were proposed for cardiovascular, rheumatoid and infectious diseases, obesity and liver and cancer.

Conclusions. A global overview of the current state of the SpHPP chr16 project will be provided in the poster.

P-933.00

THE SPANISH BIOLOGY/DISEASE INITIATIVE WITHIN THE HUMAN PROTEOME PROJECT

Juan P. Albar[1], Oriol Bachs[1], Francesc Canals[1], Ignacio Casal[1], Fernando Corrales[1], Manuel Fuentes[1], Concha Gil[1], Javier Muñoz[1] Fernando Vivanco[1] Francisco Blanco[1]
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The Spanish Chromosome 16 Consortium is integrated in the global initiative Human Proteome Project, which aims to develop an entire map of the proteins encoded following a gene-centric strategy (C-HPP) in order to make progress in the understanding of human biology in health and disease (B/D-HPP). Chromosome 16 contains many genes encoding proteins involved in the development of a broad range of diseases, which have a significant impact on the health care system. The Spanish HPP consortium has developed a B/D platform with five programs focused on selected medical areas: cancer, obesity, cardiovascular, infectious and rheumatic diseases. Each of these areas has a clinical leader associated to a proteomic investigator with the responsibility to get a comprehensive understanding of the proteins encoded by Chromosome 16 genes.

Based in the genes located in the chromosome 16, each medical area will focus on one to three specific diseases in order to acquire improved capabilities for early detection and diagnosis, therapeutic development and monitoring and personalized healthcare programs: colon cancer, brain cancer, breast cancer, obesity, atherosclerosis, candidiasis, rheumatoid arthritis and osteoarthritis. The clinical research essential to validate the proposed biomarkers on each medical area will be carried out using the national clinical networks of the Carlos III National Institute of Health (ISCIII), such as the CIBER (Network of Biomedical Research Center) on obesity (CIBEROBN) and different RETICS (Thematic Networks of Cooperative Health Research): Cardiovascular Network (RIC), Cancer National Network (RTICC), Infectious Pathologies Network (REIPI) and National Network of Inflammation and Rheumatic Diseases (RIER).

As the quality of biological samples is a essential to obtain high confidence results in the HPP project, the Spanish National Biobank Network (also belonging to the ISCIII) has been involved in the collection and storage of all selected biological fluids and tissues that are needed to carry out clinical research in the B/D-HPP platform, and will be the provider of clinical samples to the project.

P-934.00
TARGETING PROTEINS OF CHROMOSOME 16

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Introduction. Detection and quantification of chromosome 16 proteins in biological matrices by SRM procedures is one of the major goals of the SpHPP chr16 Consortium. Targeted methods were previously defined for 49 out of the 840 chr16 proteins in different cell lines and the coverage has been now increased to 20% of chr16 protein set.

Methods. SRM methods have been developed using Skyline and tested on different triple Q instruments. Initial analyses were performed on Jurkat extracts and verified in alternative cell lines.

Results. A collection of 150 chr16 proteins previously identified within our consortium by a shotgun approach was selected to develop robust SRM detection and quantitation procedures. The advantages of using our high resolution MS data from unsupervised experiments to consolidate targeted methods and the different workflows involving distinct instruments were evaluated. Similar results were obtained across different platforms and non-significant differences in performance were observed between peptides from our shotgun experiments or from other sources including Peptide Atlas and MRM Atlas.

Conclusions. We report new progress on chr16 proteome coverage using targeted MS methods. Moreover, the benefits of using previously generated shotgun MS data to facilitate the detection of missing proteins are discussed.

P-935.00

SUBCELLULAR FRACTIONATION ENHANCES CHROMOSOME 16 PROTEOME COVERAGE

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The Human Proteome Organization (HUPO) launched the Human Proteome Project (HPP) initiative based on two different approaches, a chromosome-centric approach and a biology/disease approach. Within the chromosome-centric approach, the 24 human chromosomes have been adopted by teams from 21 different countries. Chromosome 16 (Chr 16) was awarded to a Spanish consortium integrated by 15 proteomic groups working coordinately under the umbrella of the Spanish Proteomics Institute, ProteoRed-ISCIII.

Five work packages have been organized to cover protein expression, SRM/MRM, protein sequencing and characterization, bioinformatics and clinical applications. Here, we will summarize recent advances in the sequencing and characterization of Chr 16 proteins. To this end, several cell lines representing major cell lineages were initially selected: MCF7 (epithelial breast cancer), CCD18 (colon myofibroblasts), Jurkat (T lymphocytes) and Ramos (B lymphocytes from Burkitt´s lymphoma). Basically, cells were lysated with CHAPS/urea and extracts were separated and in-gel digested based on a 1D-SDS-PAGE-LC-MS/MS workflow and analyzed mainly with LTQ-Orbitrap-Velos or Triple-TOF (Jurkat) instruments. Data reporting was submitted to the ProteoRed MIAPE web repository. Using that approach, our consortium mapped the 41.4% of Chr 16 proteins. After checking the last version of NextProt (25.02.14) and evaluation of the results, we observed an underrepresentation of membrane and nuclear proteins in our initial results. To overcome that bias we decided to follow a subcellular fractionation strategy to enrich the extracts in nuclear and membrane proteins of CCD18 and MCF7.

In addition, we included HEK293 cells and platelets in the analysis as many of the undetected proteins were more abundant in these two cell types. In the poster, we will discuss the different fractionation approaches and the improvements obtained in protein coverage of Chr16.

P-936.00

TRANSCRIPTOMIC PROFILING TOWARDS THE LOCALIZATION OF THE MISSING PROTEINS

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[2]Consortium of C-HPP (Chr-16)

Experimental evidence for the entire human proteome has been defined in the Human Proteome Project (HPP) context using proteomic experiments stored in neXtProt database. The complexity of the detection of proteins using shotgun and targeted proteomics has led the research community to the integration of transcriptomics and proteomics landscapes using high-throughput technologies. In particular, the definition of an appropriate strategy to detect the subset of human proteins without experimental evidence has become one of the overriding objectives in the chromosome-centric study of the proteome.

We have developed a bioinformatics analysis pipeline to predict the probability of a missing protein to be expressed in a biological sample based on several features: 1) sequence characteristics of the protein coding genes, 2) protein domains associated with the missing proteins, 3) pathways and biological functions enriched in the set of the missing protein coding genes and 4) the probability of a certain gene to be expressed in a transcriptomic experiment. We have analyzed more than 3400 microarray experiments corresponding to three different biological sources: cell lines, normal tissues and cancer samples. A gene classification based on the gene expression profiles has distinguished between ubiquitous, non-ubiquitous, not-expressed and missing protein coding genes. These gene sets present important differences in cellular localization and biological processes in which they are involved. Besides this, a different pattern of tissue specificity for missing protein coding genes is reported between cell lines, normal and cancerous samples. This fact underlines the importance of evaluating the biological sample where the detection of missing proteins is more feasible.

P-937.00
MISSING PROTEINS IN CHROMOSOME 16. SPANISH HPP

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Introduction. According to neXtProt database, there are proteins that have not evidence at protein level, called "Unknown proteins". The Spanish HPP consortium has developed a specific protocol for unknown proteins, in order to get proteomic information for the MRM method, which is based on the expression of recombinant proteins in a cell free translation system (IVTT).

Methods. From the DNASU plasmid repository we got the corresponding clones for unknown proteins available in the pANT7_cGST vector (cell-free expression vector with GST tag). SRM methods were developed using Skyline and tested on expressed proteins produced using IVTT system. Peptides for the MRM were chosen among that that matched the settings: no trypsin misscleavages, 7-25 aminoacids in length, excluding that containing Met, Trp and Cys. Transitions between 400m/z and 1250 m/z were selected among the fragments of 2+ and 3+ precursors. All the peptides selected for MRM were tested to be proteotypic against the in house database used.

Results. Unknown proteins were detected by MRM (when possible, at least three peptides with three transitions each one) and identified by IDA (at least one MS/MS spectrum matched). In total, we analysed 29 proteins available in the human expression clone collection and developed the corresponding MRM methods (as well as the methods for 11 unknown proteins developed the last year). The approach used to study the unknown proteins allows us to obtain the MRM data (peptides and transitions) necessary for the detection, identification and quantification of these proteins in complex mixtures, and can be applied to any set of proteins.

Conclusions. We report our progress in chr16 missing proteins knowledge using targeted MS methods. Moreover, we are ready to test these methods in biological samples to get the proof of concept of our approach on the study of the human missing proteins

P-938

MULTIPLEXED AND SENSITIVE PROTEIN QUANTITATION OF FRACTIONATED HUMAN PLASMA BY MRM/MS WITH ISOTOPICALLY LABELED STANDARDS

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Introduction and Objectives
Absolute quantitative techniques are emerging as a popular tool in the biomedical and clinical proteomic fields for determining endogenous concentrations and obtaining disease-relevant information. A powerful approach for biomarker verification involves multiple reaction monitoring/mass spectrometry (MRM/MS) with stable isotope-labeled standards (SIS). Due to current method's limits in peptide multiplexing and detection sensitivity, we have developed a new strategy for expanded comprehensiveness and depth of quantitation in complex plasma samples.

Methods
Human plasma (Bioreclamation) was subjected to simple sample pre-treatment before tryptic proteolytic digestion and the addition of 1035 SIS peptides (synthesized in-house via Fmoc chemistry). Peptide mixtures were extracted and fractionated by 2D LC, using alkaline (ammonium hydroxide, pH 10) and acidic (formic acid, pH 3) eluents. The latter was interfaced to a triple quadrupole mass spectrometer (Agilent 6490) for dynamic MRM. Development involved optimization and interference testing, with quantitation of control and unknowns conducted on 13 concatenated fractions.

Results and Discussion
After preliminary optimization, technical replicate analysis of our novel 2D LC fractionation method revealed high robustness (average CVs of 10% for signal and 0.1% for retention time) and good quantitative performance. It enabled 256 plasma proteins (634 interference-free peptides) to be quantified across an 8 order-of-magnitude concentration range (15 mg/mL to 450 pg/mL). This represented a 2 order of magnitude enhancement in the quantitative depth of a fractionation-free assay and allowed 41 additional moderate abundance proteins to be quantified through improved separation efficiency. A subset panel is to be interrogated shortly by 2D LC-MRM/MS for blood-borne factors of skin transplant rejection.

Conclusions
We have developed a novel, 2D LC fractionation approach with MRM detection and SIS peptides for improved depth and breadth of plasma protein quantitation. The method is robust and sensitive, while having the multiplexing capability required for subsequent analyses of protein disease panels.

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OP017 - THREE DIMENSIONAL ANALYSES OF PTM PATTERNS IN LARGE SCALE PROTEOMIC DATA

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Introduction and Objectives

Protein post-translational modifications (PTMs) have a substantial impact on cell biology, and the description of their characteristics and distributions is an active field of research. We present a tool to describe the relative positions of protein post-translational modification sites in three-dimensional space within large data sets. This presents a 'real world' view of the positioning of PTMs, allowing assessment of overall trends and the likelihood of interaction between modification categories and sites, giving a deeper understanding of PTM interplay on a high throughput basis.

Methods

The program is written in Perl and functions to remove redundancy from PTMomic datasets, while simultaneously mapping the locations of all PTMs relative to all other PTMs on a 2D and 3D level. Data used for analysis was generated from enrichments and pulldowns targeting phosphorylation, sialylation, ubiquitination, acetylation, and lysine trimethylation, as well as being extracted from large scale datasets available online.

Results and Discussion

Applying this analysis has revealed that there are significant patterns in PTM distributions on a proteome scale which are visible when taking account of 3D position. That is, PTMs are distributed such that there consistently exists a trend for 3-dimensional proximity, even when looking at thousands of sites over an entire proteome. This suggests that PTMs function to a substantial degree in concert with each other, and has implications for our understanding of the manner in which PTMs are used.

Conclusions

These analyses have shown associations between PTMs on a 3D level, which suggests that there may be a functional role for this proximity, as repeatedly suggested in the literature. These associations have been observed from cell culture models and intact tissue samples across different species. We believe this may represent a snapshot of the 'PTM code', an information carrying code based on proteins and PTMs.

OP018 - DISCOVERING THE GLUTEN FINGERPRINT: A SMALL STEP TOWARDS THE QUANTIFICATION OF GLUTEN IN PROCESSED FOODS

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Coeliac disease affects 1% of the EU population. Gluten refers to a very complex group of proteins present in cereals, that are insoluble in water and to which some individuals are intolerant. European Commission Regulation 41/2009 established a requirement for using the labels "gluten-free" (lower than 20 mg kg-1) and "very low gluten" (equal or lower than 100 mg kg-1) on food products. In support of this regulation an accurate, robust, sensitive and specific method for gluten quantification is needed to instil confidence in affected consumers. Currently an antibody ELISA method based on the R5 Mendez method is recognised by Codex and accepted by AOAC International as the official method for gluten quantification. However, the main drawbacks of the current ELISA methods are the lack of comparability and accuracy of measurement results which have been attributed to a lack of reference materials. Here, we propose a proteomic approach to identify the proteins and peptides present in GluVital™ wheat gluten in order to detect potential candidates to develop an MS-based quantification method.

A combined discovery strategy was followed to obtain a potential peptide marker list. The protein and peptide set was identified through a screening SRM method (Xevo TQS, Waters) in combination with untargeted LC–MS/MS analyses (Synapt G2, Waters) of trypsin and chymotrypsin-digested gluten. SRM assays were developed based on experimentally obtained MS/MS on sequences downloaded from the FARRP database (www.allergenonline.org), which contains peer-reviewed sequences from wheat that elicits coeliac disease. LMW/HMW glutenins, alpha, beta, gamma and omega gliadin proteins containing known toxic/immunogenic sequences were identified. The advantages and disadvantage of gluten quantification via ELISA and MS-based approaches will be discussed.

This proteomic approach will help to determine a "gluten fingerprint" or set of peptide markers for the development of an SRM method to quantify gluten in cereal-containing food products.

OP019 - CELL-CELL COMMUNICATION AND SIGNALING IN COLON CANCER

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Cancer-associated fibroblasts (CAFs) are stromal components of tumors which communicate with other stromal and tumor cells through direct cell-cell contacts or paracrine signaling. The latter process is strongly modulated by stromal proteases, which either degrade or create stable cleavage products with often altered functionality. One of the major agents in cellular crosstalk in cancer is fibroblast activation protein−α (FAP−α), a cell surface type 2 dipeptidyl peptidase, specifically expressed by CAFs. In this study we elucidate the role of FAP−α in shaping colon cancer microenvironment and its effect on tumor biology.

The project included proteomic analysis of cell lines by high resolution liquid chromatography-tandem mass spectrometry, microarrays as well as cell characterization assays and co-culture systems mimicking tumor entity. The studies comprised of: (a) a quantitative proteome comparison of cell-conditioned media derived from colon CAFs expressing altered levels of active and inactive FAP-α, (b) analysis of CAFs gene expression and biology in respect to differential FAP-α expression and activity, (c) investigating the impact of CAFs exhibiting differential FAP−α activity on colon cancer cells by analysis of their adhesiveness, motility, as well as proteome and transcriptome when co-cultured with CAFs.

Quantitative profiling of CAFs' secretome showed more than 1600 proteins consistently identified, including matrix proteins, chemokines, and growth factors were changed in abundance. In N-terminomic analysis strong preference towards cleavage after proline was observed. In addition, we report new putative FAP−α substrates. CAFs co-culturing with cancer cells led to tremendous changes in gene expression and proteome composition, which linked to altered activation of multiple signaling pathways in cancer cells. The latter resulted in increased adhesiveness, migration capacity and proliferation of cancer cells. Moreover, increased FAP-α activity enhanced these effects.

We showed that CAFs communicate with cancer cells by matrix remodeling and FAP-α, plays a pivotal role in this crosstalk.

OP020 - PROGNOSTIC EVALUATION OF 15 AUTOANTIBODY-TYPE SPECIFIC COLORECTAL CANCER BIOMARKERS IDENTIFIED THROUGH PROTEIN MICROARRAYS

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Colorectal Cancer (CRC) is one of the deadliest cancers in developed countries. Early diagnosis and therapy personalization would improve its survival rates. Detection of autoantibodies directed to Tumor Associated Antigens (TAAs) through ELISA provides a reliable alternative to current screening methods.

We aim to validate previously discovered biomarkers and evaluate their role in disease evolution using a well documented population. By probing protein arrays, we identified MAPKAPK3, PIM1, MST1/STK4, TRIM21, AKT1, IRAK4, PRKCH, PAK1, PRKD2, GTF2B, HCK and EDIL3 as CRC-specific TAAs. When discovered within the same array, TAA's showed high correlation.

From E.coli BL21 DE3, we purified full-length human TAA's through IMAC. Ninety-five CRC patients' sera with up to 10 years survival data were surveyed for autoantibody response by ELISA. Survival differences between groups were detected through log rank test. Predictive ability was determined by logistic regression and ROC curves. TAA redundancy was examined through Pearson Coefficient.

Regarding survival role, we observed different behavior among biomarkers: while high levels of EDIL3, GTF2B, HCK, IRAK4, p53, PIM1, SRC and STK4 showed to improve survival odds, presence of AKT1, FGFR4, MAPKAPK3, Trim21 and PAK1 indicated poor survival odds. AKT1 was associated exclusively to shorter recurrence (DFS) while EDIL3, HCK, IRAK4 and TRIM21 only to mortality (OS). By using logistic regression, we found that combining p53, IRAK4, Trim21 and SRC we were able to discriminate between early (Dukes A and B) and late (C and D) cancer stages with an AUC of 0.8231. Surprisingly, high levels of autoantibodies to these TAAs indicated an early stage, suggesting a process of immunotolerance to cancer.

Although proteomic studies help us to discover biomarkers of high interest, their validation in well documented cohorts is necessary to determine their clinical role. An exhaustive statistic analysis will evaluate their utility in a clinical setting, allowing for further development.

OP021 - TOP-DOWN VENOMICS - HIGH RESOLUTION MASS SPECTROMETRY AS A FAST AND ACCURATE TOOL FOR THE PROFILING OF SNAKE VENOMS

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Snake venoms are complex mixtures of bioactive peptides and proteins. A deep understanding of the composition of venoms is of high importance not only for exploring their enormous potential as sources of pharmacological novelty, but also to fight the dire consequences of snakebite envenoming. In the last decade several bottom-up proteomic strategies to explore venom proteomes have been developed. Drawbacks of these approaches are the co-elution (HPLC), low mass resolution (SDS-PAGE), or impaired quantification ability (2DE) of venom components. On the other hand, shotgun proteomics of whole venom or IDA protocols involving electrophoretic bands containing mixture of proteins may result in difficulties assigning all toxin isoforms.

To overcome these problems, we developed an LC-MS approach for the rapid characterization of snake venoms, which combines mass profiling of native and reduced venom components and online top-down MS/MS using CID and HCD.

Here, we present results on the profiling and relative quantification capabilities of top-down MS for elapid snake toxins. This is the first time, top-down mass spectrometry has been applied for the in deep characterization of snake toxins.

The proteome quest
to understand
biology and disease

October 5 - 8, 2014

Centro de Convenciones Norte IFEMA

Topic **25**

HUPO Young Investigator

OP041 - PROTEOMIC ANALYSIS OF INFLAMMATORY BOWEL DISEASE BIOPSIES IDENTIFIES PROTEINS THAT SEGREGATE CROHN'S DISEASE FROM ULCERATIVE COLITIS IN PEDIATRIC PATIENTS

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Introduction and objectives
Inflammatory bowel disease (IBD) affects over 230,000 Canadians with 10,000 additional cases diagnosed each year, ¼ of which are pediatric patients. IBD is comprised of Crohn's disease (CD) and ulcerative colitis (UC). Diagnosis is made by biochemical parameters and endoscopy evaluation, the latter of which has an accuracy of only 60-74%. Pediatric IBD patients tend to have more aggressive and extensive disease than adults, making diagnosis and differentiation between CD and UC more challenging. Accurate diagnosis of CD or UC is critical to ensure early and appropriate therapeutic intervention; to this end, we sought to identify proteins that differentiate between diseases and their severity.

Methods
Colon biopsies were obtained from pediatric patients at the time of diagnosis and prior to therapeutic intervention. Using a super-SILAC-based approach, the proteomes of over 100 pediatric non-IBD control, CD, and UC patient biopsies were compared. ANOVA, t-test, linear regression, and principle component analysis were applied to identify proteins that are specific to each disease state. Paired comparisons of proteomes from patient biopsies obtained from non- or inflamed areas were employed to identify additional biomarkers of disease severity.

Results and Discussion
1949 proteins were accurately quantified from the patient biopsies; 50% of these were found to be significantly different between patient groups by ANOVA. 296 proteins were determined by t-test to be significantly different between CD and UC patients; principle component analysis of resulted in segregation of control, CD and UC patient groups.
Proteins identified to have the greatest affect on group segregation include both novel and known IBD biomarkers, including S100A9 and carcinoembryonic antigen.

Conclusions
Combining the proteins identified in this study for use as biomarkers will enable for differential diagnosis of IBD subtypes by less invasive techniques to permit for appropriate therapeutics.

OP042 - CALPAIN-DEPENDENT CLEAVAGE IN HIGHLY ACTIVATED PLATELETS REVEALED BY QUANTITATIVE N-TERMINAL CHAFRADIC: ALTERATIONS IN SCOTT SYNDROME

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Introduction: The Scott syndrome is a rare bleeding disorder, associated with a mutation in anoctamin-6 (TMEM16F). When stimulated with strong agonists, causing a prolonged rise in cytosolic Ca2+, syndromatic platelets are deficient in exposure of procoagulant phosphatidylserine and do not form membrane blebs. The latter response is considered to be a consequence of calpain-dependent degradation of the membrane actin cytoskeleton.

Methods: Washed blood platelets from healthy control donors and a Scott patient were activated with the strong, Ca2+-mobilizing agonists, convulxin/thrombin or ionomycin. Neo N-terminal peptides, e.g. formed by calpain cleavage, were quantitatively assessed, using our ChaFRADIC strategy.

Results/discussion: Stimulation of control or Scott platelets with thrombin alone resulted in only limited formation of neo N-terminal peptides, as determined by ChaFRADIC. In contrast, stimulation of control platelets with convulxin/thrombin or ionomycin markedly increased the formation of neo N-terminal peptides from multiple cytosolic proteins, 48 of which were identified as potential calpain substrates. The protein list showed an enrichment of protein kinases and cytoskeletal and membrane raft proteins (GO terms); and it partly overlapped with a literature list of 97 calpain substrates. Stimulation of Scott platelets with convulxin/thrombin or ionomycin did not result in phosphatidylserine exposure or membrane bleb formation. Similar neo N-terminal peptides were generated, although quantitative profiles differed between Scott and control platelets. After convulxin/thrombin stimulation in Scott platelets we detected diminished cleavage in proteins such as TMEM40, cavin-2, and Src-kinase. Western blot analysis confirmed delayed cleavage of Src-kinase in Scott platelets in a calpain-dependent way.

Conclusion: We combined N-terminal ChaFRADIC and iTRAQ labeling to compare proteolytic cleavage between platelets from healthy donors and Scott patients. Our findings indicate that Ca2+-dependent membrane blebbing is not the result of random cleavage of structural or cytoskeletal proteins, but rather is linked to specific, likely calpain-dependent cleavage patterns that associate with phosphatidylserine exposure.

OP043 - CHEMICAL PROBES FOR STUDYING CELL-TO-CELL COMMUNICATION IN PATHOGENIC BACTERIA

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The opportunistic pathogen Pseudomonas aeruginonsa has been shown to utilise quorum sensing to coordinate activation and repression of many unlinked genes in response to cell density. P. aeruginonsa uses small signalling molecules, which are synthesised continually throughout cell growth, to determine cell density. Once a critical threshold concentration of these molecules has been reached, binding to a specific receptor occurs resulting in synchronised changes in gene expression.

PQS, a quorum sensing signalling molecule used by P. aeruginonsa, and its biosynthetic precursor HHQ have been shown to bind the PqsR receptor. While PQS and HHQ bind PqsR, it is believed that they also interact with additional proteins regulating both virulence factor production and biofilm formation leading to infections that are difficult to eradicate. The aim of this work is to use a chemical proteomic approach to identify these additional proteins.

We present the results of chemical proteomic studies in which immobilised PQS and HHQ probes have been used to identify unknown macromolecular biological targets of the two molecules. The design and synthesis of second-generation photoaffinity-based PQS and HHQ chemical probes for use in vivo will also be presented and the results from both proteomic studies are compared. Preliminary studies using these probes have been the first to identify that PQS directly binds MexG, a component of the MexG-OpmD efflux pump, which has previously been linked to quorum sensing, antibiotic susceptibility and virulence.

In conclusion, as well as improving the current understanding of bacterial communication these studies also have the potential to uncover new drug targets for combating antibacterial resistance.

OP044 - PROTEOGENOMIC ANALYSIS OF AN ASIAN MALARIA VECTOR – ANOPHELES STEPHENSI

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Introduction and Objectives

Anopheles stephensi is a major malaria vector in Asia accounting for 12% of the malaria cases from this region. Sequencing of whole genome of an Indian strain of An. stephensi has recently been completed. We carried out a global proteomic and transcriptomic analysis of An. stephensi to annotate protein-coding regions in the genome.

Methods

Multiple tissues and developmental stages were dissected from laboratory grown mosquitoes. Proteins extracted were subjected to multiple fractionation methods and proteomic analysis on LTQ-Orbitrap Elite and Velos mass spectrometers using HCD fragmentation. MS/MS data was searched against protein databases, six-frame translated genome databases and three-frame translated transcript databases of An. stephensi. RNA-seq analysis was performed using the Illumina's HiSeq platform. iTRAQ-based quantitative proteomic analysis of midgut of female mosquitoes fed with Plasmodium berghei infected blood meal was carried out to identify proteins that could be potentially useful in developing transmission blocking vaccines.

Results and Discussion

We established protein-coding evidence for over 9,000 predicted genes in An. stephensi. Using proteogenomic analysis, we also identified more than 75 novel protein coding regions and revised annotation of 180 predicted genes. N-terminal acetylated peptides allowed us to confirm predicted translation start sites for 1,124 annotated genes and identification of novel start sites for an additional 1,430 genes. In addition, we identified several alternatively spliced isoforms supported by transcriptomic and proteomic evidence. Using quantitative proteomics approach, we have identified elevated expression of several proteins in the midgut of An. stephensi that are induced in response to malaria parasites.

Conclusions

We present the first global integrated proteomic and transcriptomic map of An. stephensi. This effort resulted in comprehensive annotation of protein-coding regions in An. stephensi. We also identified several midgut proteins that are potentially useful in the development of transmission-blocking vaccines to control malaria transmitted through An. stephensi.

OP045 - UNRAVELING TRKA SIGNALING IN NEUROBLASTOMA USING A QUANTITATIVE MS-BASED APPROACH

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Introduction and objectives

Neuroblastoma (NB), a cancer of the sympathetic nervous system, comprises the most common extracranial tumor in childhood. NBs with favorable prognoses frequently express high levels of the receptor tyrosine kinase TrkA, and these tumors have a propensity to either differentiate or regress, but the mechanisms responsible for these intriguing fates are still not completely understood.

Methods

We used a quantitative mass spectrometry (MS)-based proteomics approach to unravel TrkA signaling associated with NGF-induced neurite outgrowth in a NB cell line, SH-SY5Y-TR-TrkA with tetracycline-inducible expression of TrkA. Using stable isotope labeling by amino acids in cell culture (SILAC) samples were generated to study signaling dynamics in terms of interactome, phosphoproteome and proteome changes. All samples were analyzed by high-resolution LC-MS/MS on a Q-Exactive mass spectrometer and processed with MaxQuant software.

Results and Discussion

Phosphoproteomics identified and quantified more than 10,000 phosphorylation sites and classification according to their temporal dynamic profiles led to 7 distinct clusters. With a focus on sustained signaling clusters and inhibitor-based targeting of active protein kinases we identified several kinases with importance to NGF-induced neurite outgrowth. In the dynamic interactome we identified several known and also novel dynamic interactors of TrkA. Among them, the signaling adaptor GAREM2 was validated by western blotting. Furthermore, we identified 447K and 775K as TrkA ubiquitination sites. Interestingly, we also identified an E3 ligase not previously linked to TrkA signaling. Currently, we are focusing on the potential of this E3 ligase to influence TrkA ubiquitination and stability as well as neurite outgrowth.

Conclusions

Quantitative proteomics is a powerful tool to unravel signaling dynamics in NB. The combined analysis of the NGF-TrkA interactome, phosphoproteome and proteome changes associated with neurite outgrowth has a potential as valuable resource to better understand TrkA signaling and ultimately to expand the biomarker repertoire for NB patients.

Topic 26

Late Break
Abstracts

P-1.00

2D PROTEOME EXPRESSION PATTERN IN ORAL SQUAMOUS CELL CARCINOMA OF EXTRACTS FROM AGRIMONIA PILOSA LEDEBROOT

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Oral squamous cell carcinoma (OSCC) is known as the most common malignancy and a major cause of worldwide cancer mortality. Oral squamous cell carcinomas (OSCCs) are characterized by a marked tendency for local assault, so the identification of agents inhibiting the inceptionand progression of OSCC has recently gained notice. Here, we found that Agrimonia Pilosa Ledeb Roots Extract(ARE) inhibited cell proliferation in YD-10B OSCC cells. In this study, we have followed proteomic approach to investigate the effects of ARE treatment in YD-10B OSCC cells. 2D based quantitative proteome studies showed that among totally quantifiable many proteins and bioinformatic analysis further revealed that those quantifiable proteins were mainly involved in multiple metabolic and enzyme-regulated pathways as anti-cancer drugs. Most of identified proteins that were differently altered in ARE treated cells were involved in regulating the many biological processes in the human body.

P-2.00

DIMETHYL LABELING AFFECTS RECOVERY OF SINGLY PHOSPHORYLATED PEPTIDES IN TIO2 CHROMATOGRAPHY

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Introduction: Chemical dimethylation is a reliable method for stable isotope labeling in quantitative proteomics1. Many studies showed adequate performance of dimethyl labeling on the proteome level; however, its performance was never systematically assessed at phosphoproteome level. Here we investigated the influence of dimethyl labeling on the phosphopeptide recovery after TiO2 enrichment.

Methods: On-column dimethyl labeling (light) or mock treatment was performed on tryptic HeLa digests, followed by five consecutive rounds of TiO2 chromatography. Enriched phosphopeptides were pooled and loaded onto C18 stage tips. The unlabeled phosphopeptide fraction was differentially (heavy) labeled. Labeling efficiency reached ?95% for both fractions. Phosphopeptide samples were either analyzed separately (light and unlabeled), or mixed 1:1 (light and heavy) and analyzed by nanoLC-MS/MS on an LTQ Orbitrap Elite mass spectrometer. Two technical replicates were performed and all raw MS/MS spectra were processed together using the MaxQuant software suite.

Results and Discussion: TiO2 chromatography of dimethylated phosphopeptides lead to approximately 15% less identified peptide evidences and 10% less phosphopeptide evidences compared to unlabeled fractions. However, the overall numbers of phosphorylation events were similar between labeled and unlabeled, because dimethylation predominantly affected recovery of singly phosphorylated peptides, while multiply phosphorylated and acidic peptides were slightly enriched. This was also confirmed in quantitative analysis, where ratios of 1911 non-redundant phosphorylat