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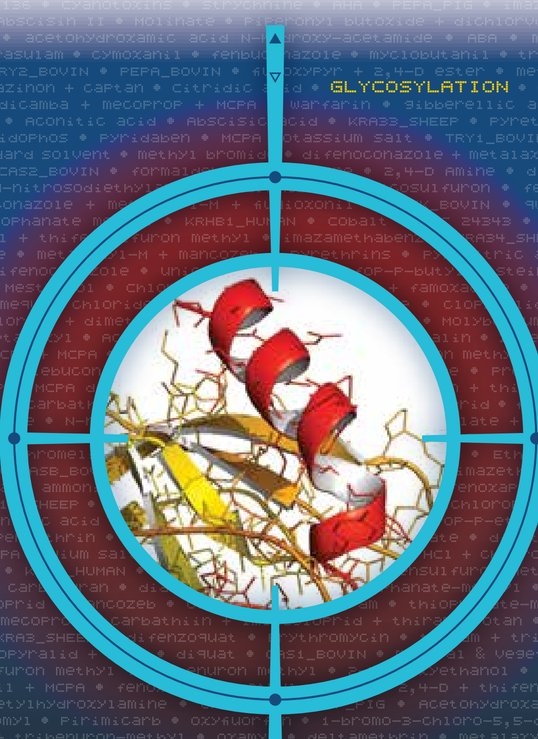

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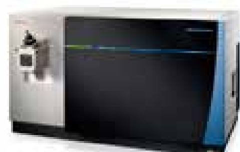

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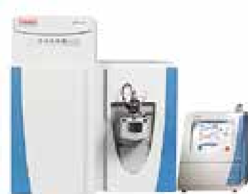

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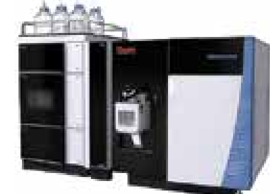

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PLENARY SESSIONS

PL01: OPENING PLENARY SESSION

PL01: OPENING PLENARY SESSION
SUNDAY, SEPTEMBER 27, 2015 – 18:00 – 19:30

PL01.04 Population Proteomics: Embracing Genomic Variability

Ruedi Aebersold
ETH Zurich, Institute for Systems Biology, Zurich, Switzerland

Abstract: The presentation will discuss the development of highly reproducible and high throughput proteomics techniques to do comparative analyses on a large number of samples. It will also discuss the use for these techniques to quantify the proteome of populations of cell, or animals with defined genomes to determine how genotypic variation is translated into the proteome and how proteome variation affects phenotypes. Examples from yeast strains, flies and medically relevant examples from mouse strain collections will be presented.

PL01: OPENING PLENARY SESSION
SUNDAY, SEPTEMBER 27, 2015 – 18:00 – 19:30

PL01.05 Opening Up New Areas of Drug Discovery with High Quality Research Tools

Aled Edwards
Structural Genomics Consortium (SGC), University of Toronto, Toronto, ON, Canada

Abstract: Although the proteomics community has been developing quantitative assays for most proteins in the human genome, most of the literature concerning human genes and proteins remains focused on the proteins that were the focus of attention in the 1990's. Part of the resistance to move into new areas is due to the lack of high quality research reagents for the novel proteins. Dr. Edwards' project has been focused on developing and disseminating high quality antibodies and chemical inhibitors for the proteins that regulate epigenetic signalling, and he will speak about how these efforts have contributed to the launch of many clinical trials.

PL02: PLENARY SESSION 2

PL02: PLENARY SESSION 2
MONDAY, SEPTEMBER 28, 2015 – 11:10 – 13:00

PL02.01 Quantitative Proteomics in Biology, Chemistry and Medicine

Steven A. Carr
Proteomics Platform, Broad Institute, Cambridge, MA, United States of America

Abstract: A new era of quantitative biology enabled by mass spectrometry based proteomic technologies has arrived. We can now define the content, relative abundance, modification states and interaction partners of proteins in a dynamic and temporal manner on a near-global basis in organelles, whole cells and clinical samples, providing information of unprecedented detail. At the Broad Institute we are developing and applying these technologies in a wide array of studies including defining the subcellular locations of proteins in health and disease, connecting cancer genotype to molecular phenotype, unraveling the basis of the innate-immune response, identifying the mechanism of action of drug-like molecules and to discover and verify protein biomarkers of disease. I will present the results of several recent studies that convey a sense of the breadth and depth of application of modern proteomics to biology and medicine.

PL03: PLENARY SESSION 3

PL03: PLENARY SESSION 3
MONDAY, SEPTEMBER 28, 2015 – 17:30 – 18:15

PL03.01 A New Chapter in Liver Physiology & Pathology Is Being Written with Big Data

Fuchu He
The Academy of Military Medicine Science, Beijing, China

Abstract: With the rise of the different types of omics and high-throughput sequencing technologies, life science is ushered into a new era, the era of big data. Over the last 18 years, we have been continuously generating both genomic and proteomic data on liver, the vital organ of human body. Several datasets were built including liver transcriptome and proteome, liver protein-protein interaction map, and GWAS of human hepatocellular carcinoma. These datasets helped us reveal a novel network that regulates tumor suppressor p53, especially at the decision point between cell cycle arrest and apoptosis, identify susceptible loci in HCC, and establish rules of proteome organization. As technologies are advancing, lifeomics data are pouring out on unprecedented scale and speed. Today, terabyte or even petabyte scale-data of mass specand DNA/RNA-seq analyses are being generated routinely. It will be a daunting task to manage, to navigate through, and to mine these massive datasets. However, it is believed that the era of big data will bring in new thinking in life sciences and present new opportunities in research. Our experience with the liver datasets should be illuminating in that regard.

PL04: PLENARY SESSION 4

PL04: PLENARY SESSION 4
TUESDAY, SEPTEMBER 29, 2015 – 09:15 – 10:00

PL04.01 Metabolomics - An Important Piece in the 'Omics Puzzle

Ute Roessner
The University Of Melbourne, The University of Melbourne, Victoria, VIC, Australia

Abstract: Metabolomics is a vast growing 'omics tool in all biological sciences combines analytical chemistry and computational biology aiming to identify and quantify as many metabolites as possible in biological tissues and fluids. With the advent of high throughput transcriptomics and proteomics, metabolomics has become an important member of any systems biology approach aiding the generation of more complete pictures of an organism under investigation. This presentation will discuss the importance of metabolomics in biology and biomedicine, and its advantages to investigate health and disease.

PL05: PLENARY SESSION 5

PL05: PLENARY SESSION 5
TUESDAY, SEPTEMBER 29, 2015 – 17:30 – 18:15

PL05.01 Using Mass Spectrometry to Understand Cystic Fibrosis as a Protein Misfolding Disease

John R. Yates III
Chemical Physiology, The Scripps Research Institute, La Jolla, United States of America

Abstract: A component to understanding biological processes involves identifying the proteins expressed in cells as well as their modifications and the dynamics of processes. Several major technologies, but especially mass spectrometry, have benefited from large-scale genome sequencing of organisms. The sequence data produced by these efforts can be used to interpret mass spectrometry data of proteins and thus enables rapid and large-scale analysis of protein data from experiments. Advances in multi-dimensional separations as well as mass spectrometry have improved the scale of experiments for protein identification. This has improved the analysis of protein complexes, and more complicated protein mixtures. Quantitative mass spectrometry can be used to study biological processes such as protein-protein interactions, development or the effects of gene mutations on pathways. Recent studies on the loss of function mutant form of the Cystic Fibrosis Transport Regulator (DF508) as it progresses through the folding pathway will be presented. Through the study of protein-protein interactions and modifications that regulate maturation of CFTR, we are beginning to understand the critical interactions regulating pathways for export or destruction.

PL06: PLENARY SESSION 6

PL06: PLENARY SESSION 6
WEDNESDAY, SEPTEMBER 30, 2015 – 09:15 – 10:00

PL06.01 Deciphering Functional Proteomes in the Human Protein Atlas - Organelles, Substructures and the Cell Cycle

Emma Lundberg
Science for Life Laboratory, KTH - Royal Institute of Technology, Stockholm, Sweden

Abstract: Dr. Lundberg will talk about her work in deciphering functional proteomes in the Human Protein Atlas project. Partly she will cover the work on characterizing the tissue specific proteomes as recently published in Science. She will describe how an integrative-omics approach, combining spatial protein expression data on a single cell level with RNA-seq patterns across all tissues, allow us to identify groups of functionally related proteins. Representing for instance organelles such as mitochondria, substructures such as nuclear domains, the cytokinetic bridge and a small uncharacterized rod-like cytoplasmic structure.

In addition she will for the first time present their major effort of characterizing the cell-cycle dependency of the human proteome and quantify changes both in expression levels and spatial distribution in non-perturbed asynchronous cells with single cell resolution. This effort is based on the development of novel methodology for computational image analysis and we aim to redefine the spatiotemporal aspects of the human cell cycle in unprecedented detail. Already preliminary data show that over 3000 proteins show cell cycle dependent variations in expression. A much higher number than reported in any transcriptome based study of the cell cycle, indicating that post-translational regulation is highly important. This detailed characterization of the human cell cycle proteome may increase our current understanding of cell biology and provide novel implications for cancer diagnostic in terms of markers for proliferation and distinct cell cycle positions.

CONCURRENT SESSIONS WITH ABSTRACT AND KEYNOTE PRESENTATIONS

CS01: CANCER PROTEOMICS

CS 01: CANCER PROTEOMICS
MONDAY, SEPTEMBER 28, 2015 – 11:10 – 13:00

CS01.03 Proteomic Characterization of Pancreatic Ductal Adenocarcinoma

Sheng Pan, Ru Chen, Teresa Brentnall
University of Washington, Seattle, WA, United States of America

Introduction and Objectives: Pancreatic ductal adenocarcinoma (PDAC), which accounts for ~90% of pancreatic malignancies, is an aggressive and devastating disease characterized by its poor prognosis and resistance to chemotherapy. Currently there is no widespread screening in place for pancreatic cancer, even for moderate or high-risk groups of patients. Proteomic characterization of PDAC and its precursor lesion – high-grade pancreatic intraepithelial neoplasia (PanIN3) would provide essential information to facilitate the development of biomarkers for early detection of this disease. The objective of this study is to reveal proteome alterations and associated molecular events implicated in PDAC. **Methods:** Quantitative proteomics and glycoproteomics were applied to global profiling of PDAC and PanIN3 tissues collected from PDAC and PanIN3 patients in comparison to diseased non-cancerous and healthy controls. A targeted proteomic assay was developed to interrogate selected protein candidates in plasma for biomarker development. **Results and Discussion:** Many of the differential proteins identified in pancreatic cancer tissue and it's precursor lesion, PanIN3, involves signaling interactions between the ductal epithelium and the extracellular matrix. These proteins orchestrate PDAC tumor growth, migration, angiogenesis, invasion, metastasis, and immunologic escape, underscoring the importance of the tumor microenvironment in promoting pancreatic cancer progression. The differentially expressed proteins also mirror the histological and functional changes in the pancreatic cancer. The glycoproteomic study revealed a roster of aberrant glycoproteins, with an increased activity of N-glycosylation implicated in several cancer pathways. Targeted analysis of a group of selected protein candidates revealed that metalloproteinase inhibitor 1, gelsolin and lumican were significantly increased in the blood of early stage pancreatic cancer cases. **Conclusion:** The profound changes in the PDAC proteome were driven by a variety of complex, multifaceted molecular events implicating in pancreatic tumorigenesis and disease progression. The comparison of tissue and plasma proteomes suggested the feasibility of detecting cancer-associated signals in blood.

Keyword: proteomics, mass spectrometry, pancreatic cancer

CS 01: CANCER PROTEOMICS
MONDAY, SEPTEMBER 28, 2015 – 11:10 – 13:00

CS01.04 Proteolytic Processing in the Progression of Low-Grade Astrocytomas to Glioblastoma Multiforme

Zon Lai[1], Eva Keller[1], Martin Biniossek[1], Joerg W. Bartsch[2], Oliver Schilling[1]
[1]*Insitute of Molecular Medicine and Cell Research, Freiburg, Germany,* [2]*Department of Neurosurgery/Lab, Marburg, Germany*

Introduction and Objectives: Glioblastoma multiforme is the most aggressive World Health Organization grade IV brain tumor. With median survival rate of less than 15 months, glioblastoma accounts for more than half of all neoplasm of the brain. While therapies for affected patients consist of maximal resection of the affected area followed by extensive radiochemotherapy, clinical prognosis remains poor due to resistance to these treatments. The present study investigates the regulation of key proteins as well as proteolytic cleavage events in non-malignant tissues of the brain, early stages from astrocytomas (grade I, II and III), and glioblastoma grade IV. **Methods:** Multiplexed analyses of extracted proteins from clinically dissected human tissues were carried out using isotopic labeling followed by two-dimensional liquid chromatography tandem mass spectrometry. To elucidate proteolytic processing in astrocytomas and GBM tissues, we applied multiplexed Terminal Amine Isotopic Labeling of Substrates (TAILS) to globally assess cleavage events in disease progression. **Results and Discussion:** A total of 5609 non-redundant proteins were identified in the comparison of glioblastoma and non-malignant tissues, many of which are known to be involved in the up regulation of proteolysis and the down regulation of neuronal functions such as synaptic transmission and neurological system processes. Comparative analyses had also identified a total of 10,227 protein N-termini in glioblastoma and non-malignant tissues, and approximately 5300 non-redundant protein N-termini in the comparison of low grade astrocytomas and GBM tissues. Differential cleavage processes were identified in a number of extracellular matrix proteins including actin, tubulin, glial fibrillary acidic protein and microtubule-associated proteins. **Conclusion:** Taken together, our study provides an in-depth mass spectrometry based profiling of proteolytic events in the progression of early stages of astrocytomas to glioblastoma.

Keywords: glioblastoma, cancer, proteolysis, astrocytoma

CS 01: CANCER PROTEOMICS
MONDAY, SEPTEMBER 28, 2015 – 11:10 – 13:00

CS01.05 Autoantibody Profiling of Glioma Serum Samples Using Human Proteome Arrays

Sanjeeva Srivastava[1], Shabarni Gupta[1], Parvez Syed[1], Aliasgar Moiyadi[2]
[1]*IIT Bombay, Mumbai, India,* [2]*ACTREC, Mumbai, India*

Introduction and Objectives: Glioma is known to be one of the most common CNS tumors and has been associated with heterogeneity and poor prognosis. Early diagnosis and identification of the underlying sub-categories in which glioma is segregated is crucial for therapeutics. The immune system identifies certain aberrant proteins against which it produces auto-antibodies, which can serve as putative early diagnostic markers using minimal invasive techniques. **Methods:** Protein microarrays has been utilized to screen the auto-antibodies on Human Proteome Arrays harboring more than 17,000 full length human proteins. Students t-test with $p<0.05$ and fold change 1.5 fold gave us a list of significantly dysregulated proteins. SVM was used to deduce sets of classifier proteins, which could help distinguish various diseased and healthy cohorts.

Results and Discussion: We have deduced sets of 10 classifier proteins from among statistically significant proteins, which could help distinguish healthy from Glioma grade II, III and IV, with 88, 89 and 94% sensitivity and 87, 100 and 73% specificity. IGHG1, PQBP1, EYA1 and SNX1 were significantly dysregulated across all grades. Further subtype analysis of GBM revealed a protein NEDD9 which could be a putative prognostic marker depending on the location of tumor with respect to the sub ventricular zone of the brain. Apart from the pathways commonly associated with tumorigenesis, we found, enrichment of immunoregulatory and cytoskeletal remodelling pathways which influences cell adhesion and chemotaxis. **Conclusion:** This is the first investigation, which encompasses a comprehensive screening of 17,000 human proteins to screen the autoantibody response in glioma patients across all grades. The panels of these putative classifier proteins would help diagnose glioma to the extent of identifying the grade or the sub-catergory, which would help in therapeutic interventions which otherwise is a challenge due to the heterogeneity presented by the disease. The biochemical alterations also provide us understanding into the pathobiology of the disease.

Keyword: Glioma, Autoantibody, Microarray

CS 01: CANCER PROTEOMICS
MONDAY, SEPTEMBER 28, 2015 – 11:10 – 13:00

CS01.06 Large-Scale Proteomic Characterization of Ovarian High-Grade Serous Carcinoma

Zhen Zhang[1], Hui Zhang[2], Li Chen[1], Bai Zhang[1], Jianying Zhou[1], Shisheng Sun[2], Lijun Chen[1], Punit Shah[3], Stefani Thomas[1], Paul Aiyetan[3], Yue Wang[4], Lori Sokoll[1], Yuan Tian[5], Le-Ming Shih[6], Akhilesh Pandey[7], Michael Snyder[8], Douglas Levine[9], Dan Chan[1]

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Introduction and Objectives: Large-scale multi-omics profiling of clinical specimens offers a unique resource for the understanding of the molecular basis of disease mechanisms and clinically relevant phenotypic variations. **Methods:** We analyzed the proteomes of 122 tumor tissue samples from ovarian high-grade serous carcinoma (HGSC) patients that have been previously characterized by the Cancer Genome Atlas (TCGA). The samples were selected based on patient homologous repair deficiency (HRD) status using TCGA genomic data. In addition to the characterization of the ovarian HGSC proteome, the generated data were integrated in analysis with existing TCGA and other genomic data to identify molecular changes associated with HRD and other phenotypic subtypes of ovarian HGSC. **Results and Discussion:** With rigorous experimental design and protocol development, the generated data demonstrated high analytical precision. In 10 embedded/co-randomized repeats of a single quality control sample, more than 7,200 proteins (out of >10,000 identified) were quantified in at least 9 repeats. Among these proteins, the mean and median coefficients of variation (CVs) of protein quantitation were 10.7% and 8.7%, respectively, with 81.4% of the proteins had a CV < 15%. Furthermore, over 93% of the proteins had an estimated standard error < 10%, a level of analytical precision comparable to many other genomic assay platforms including RNA-seq (e.g., this was the criteria of "precise" used in a recent SEQC/MAQC-III Consortium report assessing RNA-seq technology). Through integrated proteogenomic analysis, we identified a number of interesting co-regulated proteomic modules that could potentially provide insight into key characteristics of ovarian cancer such as chromosomal instability and functional mechanisms of HRD in ovarian cancer. **Conclusion:** In summary, we have generated a comprehensive proteomic characterization data for ovarian cancer with extremely high analytic precision. This dataset, together with the existing TCGA genomic data, provides a unique resource for the cancer research scientific community as evidenced by our preliminary analysis results.

Keywords: CPTAC, TCGA, Proteogenomics, Ovarian cancer

CS 01: CANCER PROTEOMICS
MONDAY, SEPTEMBER 28, 2015 – 11:10 – 13:00

CS01.07 Coupling an EML4-ALK Centric Interactome with RNAi Screen to Identify Sensitizers to ALK Inhibitors

Guolin Zhang[1], Hannah Scarborough[2], Jihye Kim[2], Andrili Rozhok[2], Ann Y. Chen[3], Lanxi Song[4], Bin Fang[5], Richard Z. Liu[6], John Koomen[7], James Degregori[2], Eric Haura[8]

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Introduction and Objectives: EML4-ALK gene rearrangements in lung cancers define a subgroup that is hypersensitive to ALK tyrosine kinase inhibitors (TKI). However, responses are partial and resistance is nearly universal. We hypothesized an ALK centric protein interactome could provide insights into ALK signaling and identify protein targets that synergize with ALK TKI. **Methods:** We combined label free quantitative phosphoproteomics using anti-phosphotyrosine antibody enrichment and tandem affinity precipitation (TAP) combined with LC MS/MS to identify the EML4-ALK signaling components and measure signaling perturbation by crizotinib, an ALK TKI. Multiple database searching tools were combined to retrieve evidenced protein-protein interactions from databases and generate an EML4-ALK integrated network. Network analysis tools were used to predict predominant signaling sub-networks underlining the network. Cell viability analysis along with shRNA library screening against entire network was employed to identify subsets of ALK network proteins that affect ALK TKI sensitivity. **Results and Discussion:** We identified 487 unique phosphotyrosine sites (pTyr) (310 proteins) from H3122 cells harboring EML4-ALK translocation and sensitive to ALK TKI. Of those, 68 pTyr sites (48 proteins) and 68 pTyr sites (58 proteins) were increased or decreased by Crizotinib respectively (p<0.05, fold change>1.5). Four junction pathways and 57 pathways were activated and inactivated by crizotinib. The EML4-ALK physical interacome contained 169 proteins and was constructed to bridge phosphotyrosine proteins. An integrated EML4-ALK signaling network composes of 462 proteins with 4410 edge connections was identified. A shRNA screen identified 5 proteins whose loss-of-function sensitized H3122 cells to two ALK TKIs (alectinib and crizotinib). **Conclusion:** Our results provide deep insights into signaling proteins and networks driven by EML4-ALK in lung cancer cells and were able to drive a focused RNA interference screen to identify proteins involved in ALK TKI sensitivity. Additional validation experiments are underway for the RNAi screen and updated results will be presented.

Keywords: lung cancer, quantitative proteomics, eml4-alk signaling network, ALK TKI

CS01.08 MicroRNAs Upregulated in Colorectal Cancer Metastasis Target Multiple Overlapping Proteins

Ignacio Casal[1], Sofia Torres[1], Irene Garcia-Palmero[1], Ruben A. Bartolomé[1], Beatriz Escudero-Paniagua[1], Maria Jesus Fernandez-Aceñero[2], Maria Fernandez-Lucendo[1]
[1]*Cellular And Molecular Medicine, Centro de Investigaciones Biológicas, Madrid, Spain,* [2]*Pathology, Hospital Clinico, Madrid, Spain*

Introduction and Objectives: MicroRNAs (miRNAs) are small regulatory noncoding RNA molecules that regulate target mRNAs by interacting with their 3' untranslated region. Aberrant miRNA expression has been observed in cancer. Here, we analyzed and compared miRNA expression profiles between colorectal cancers cells with different metastatic properties (highly metastatic KM12SM and SW620 colorectal cancer cells with poorly metastatic KM12C and SW480) and their target proteins. **Methods:** For the miRNA screening, we used Taqman array microRNA cards containing 756 specific probes for human miRNAs. For the proteomic analysis, protein extracts were trypsin-digested. Then, peptides were iTRAQ-labeled and fractionated with OFFGEL. Each fraction was analyzed by duplicate in a LTQ-Orbitrap Velos. Data analysis was carried out with Proteome Discoverer v1.4, Percolator and MASCOT. **Results and Discussion:** A total of 40 miRNAs were identified to be differentially expressed between KM12SM/KM12C and SW620/SW480 cancer cell lines, being mir-424* remarkable overexpressed in metastatic colorectal cell lines. To identify and characterize proteins associated with colon cancer metastasis mediated by mir424* and other deregulated miRNAs, we investigated the differences in the protein expression profile of conditioned medium from KM12C and KM12SM transfected with miRcontrol and KM12C overexpressing several deregulated miRNAs. In total, 1448 proteins were identified in the conditioned medium using. After applying a fold-change ≥ 1.5, we found 179 proteins deregulated (121 downregulated and 58 upregulated) in conditioned medium common in KM12SM and KM12C overexpressing different miRNAs. An extensive overlapping of target proteins was found among the different miRNAs. In silico analyses of deregulated proteins in the secretome of metastatic cells showed a major abundance of proteins involved in cell adhesion, ECM-receptor interaction and focal adhesion. Among others we can mention CDH17, Villin1 or IDH2. This significant number of overlapping proteins suggests a convergent action of different miRNAs to target identical proteins relevant in metastasis. **Conclusion:** Different miRNAs target identical proteins relevant in metastasis.

Keywords: miRNAs, iTRAQ, colorectal cancer, metastasis

CS 01: CANCER PROTEOMICS
MONDAY, SEPTEMBER 28, 2015 – 11:10 – 13:00

CS01.09 Identification of Cancer-Associated HLA Antigens as Targets for Soluble TCR-Based Immunotherapy

Geert P. Mommen, Ricardo J. Carreira, David Lowne, Alex S. Powlesland, Bent K. Jakobsen
Research, Immunocore, Abingdon, United Kingdom

Introduction and Objectives: HLA class I molecules presenting peptides derived from cancer-associated antigens are attractive targets for immunotherapy using high affinity soluble TCRs. Here, we present the development of mass spectrometry-based workflows on the Orbitrap Fusion for the discovery and validation of HLA peptides derived from cancer-associated proteins. **Methods:** HLA class I-peptides were isolated from cancer cell lines by affinity purification, fractionated by reversed phase liquid chromatography (LC) and analysed by LC-MS/MS using the Orbitrap Fusion. MS/MS spectra were searched against the human proteome using PEAKS software and filtered to 5% FDR. Decision-guided acquisition methods were developed for identification of singly charged HLA class I peptides and the target identification of cancer-associated peptides. **Results and Discussion:** To improve the identification of HLA peptides, we have developed an advanced acquisition method for the Orbitrap Fusion. Singly charged HLA class I peptides represent a considerable fraction of the HLA class I ligandome. Using decision-guided identification, singly charged HLA-peptides can now be identified with a similar identification success rate as those for multiple charged peptides. About 20,000 peptides can be routinely identified from a single cancer cell line, representing almost 7,000 proteins. To further advance the identification of cancer-associated HLA antigens, we have implemented a target identification approach in our discovery workflow to maximise the information gained from each single experiment. This method relies on the bioinformatic prediction of candidate peptide antigens and the development of selected reaction monitoring (SRM) assays using heavy labelled peptide standards. MS/MS spectra are acquired by data dependant analysis (i.e. discovery) using the Orbitrap analyser, while the Ion Trap is operated in SRM mode for the parallel quantification of a cancer-associated peptides present at the attomole level. **Conclusion:** Mass spectrometry-based identification of HLA presented peptides from cancer-associated proteins require advanced acquisition methods.

Keywords: T-cell receptors, Mass spectrometry, Ligandome, Human leukocyte antigen

CS 01: CANCER PROTEOMICS
MONDAY, SEPTEMBER 28, 2015 – 11:10 – 13:00

CS01.10 Protein Expression Profiling of the Chemoresistant AML Proteome

Elise Aasebø[1], Marc Vaudel[1], Maria Hernandez-Valladares[1], Olav Mjaavatten[1], Øystein Bruserud[2], Frode S. Berven[1], Frode Selheim[1]
[1]*Biomedicine, University of Bergen, Bergen, Norway,* [2]*Internal Medicine, Haukeland University Hospital, Bergen, Norway*

Introduction and Objectives: Acute myeloid leukemia (AML) is an aggressive hematopoietic cancer of the myeloid lineage, often associated with chemoresistance and poor outcome due to leukemia relapse in the first hematological remission. There is need for better risk stratification of patients, especially regarding who would benefit from allogeneic stem cell transplantation when the therapy is used early during first remission after standard intensive chemotherapy. As drug resistance is one major determining factor of the success of a given therapy, we aimed at profiling the chemoresistant proteome of AML patients to provide insights into the mechanisms underlying resistance and to identify prognostic biomarkers for chemoresistance. **Methods:** To obtain accurate protein quantification we applied the so-called super-SILAC approach to samples from 12 AML patients at two stages of the disease: (1) time of diagnosis and (2) during leukemia relapse - which reflects the chemoresistant proteome. This approach involved adding an isotope labeled internal standard to each patient sample, cell lysis, SDS-PAGE protein separation, protein digestion and LC-MS/MS analysis on an LTQ Orbitrap Elite. **Results and Discussion:** The protein abundance ratio between each patient and the internal standard enabled relative quantification of approximately 3000 proteins in the AML patient blast cells proteome. Comparative analysis of the primary and chemoresistant proteome in the 12 patients revealed significantly altered expression of sever-

al hundred proteins, including proteins previously described as altered during relapse. Proteins involved in immune defense processes and migration were significantly higher expressed in the primary proteome, while proteins involved in transcription, splicing and mitochondrial processes were higher expressed in the chemoresistant proteome. **Conclusion:** Protein expression profiling of the proteomes revealed potential prognostic biomarkers and pathways involved in the development of chemoresistance, which can provide valuable information about disease progression and outcome, and serve as guidance for the choice of therapy.

Keywords: quantitative proteomics, Acute myeloid leukemia, chemoresistance, super-SILAC

CS02: NEUROLOGICAL DISORDERS

CS 02: NEUROLOGICAL DISORDERS
MONDAY, SEPTEMBER 28, 2015 – 11:10 – 13:00

CS02.01Proteomic Analysis of Synaptosomes Isolated from a Huntington Mouse Model: A Cautionary Tale

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Abstract: Presynaptic nerve terminals or synaptosomes were isolated from the cortex and striatum of a mouse knockin model of Huntington's disease (HD) containing the huntingtin protein (Htt) with an expanded polyQ repeat. Surprisingly, synaptosomes from the knockin mouse showed no bioenergetic defects compared to wildtype, despite known behavioral and cognitive differences. To assess the possibility that a selective loss of synaptosomes from the 175Q Htt knockin mice might explain this lack of bioenergetic phenotype, we employed an in vivo stable isotope labeling strategy in mice (SILAM) to assess recovery efficiency of synaptosome-specific protein markers. We first isolated synaptosomes from Htt 175Q/175Q knockin mice and WT controls using standard Percoll-gradient centrifugation. These preparations were then subjected to various bioenergetics measurements using a Seahorse respirometer. However, no statistically significant dysfunction was found in 175Q synaptosomes. While there are several potential reasons for this, we considered the possibility of a selective loss of defective synaptosomes during Percoll gradient isolation, leading to a possible discordant comparison of a subpopulation of relatively normal and more fragile synaptosomes. To investigate this hypothesis, we used a SILAM mouse diet protocol with light and heavy lysine chow for WT and Htt- 175Q mice. Our SILAM mouse protocol consisted of four pairs of WT and 175Q HD mice whereby one mouse group was fed chow containing ^{13}C6-lysine (30 day) and the other was fed normal ^{12}C6-lysine. The cortex and striatum were recovered, and a total mixed homogenate was generated for each of the WT/175Q pairs (per tissue type) prior to synaptosome preparation. Mass spectrometric analysis was carried out after tryptic digestion of proteins in both whole tissue homogenates (after mixing) as well as their corresponding synaptosomal preparations. After 30 days of feeding, the SILAM heavy and light (H/L) ratios ranged from 0.15 to 0.5 depending on protein isotope label incorporation. A set of previously reported synaptosome specific proteins, including synapsin-1 and 2, synaptophysin, synaptosomal associated protein 25, and syntaxin-binding protein 1 were carefully examined in the linked data sets. Indeed, a relative loss of between 15-20% of the expected isotope amounts for these latter proteins in the 175Q animals (synaptosomes vs. homogenates) was observed, even when the experimental protocol was repeated with a second cohort where the lysine isotopic labels were reversed. To increase sensitivity and robustness of these measurements, we developed PRM-MS assays to obtain additional data from lower abundant subtype-specific synaptosomal markers, such as the vesicular glutamate transporter-1, a marker for glutamatergic neurons. Our studies suggest a selective loss of synaptosomes is occurring or, alternatively, an aberrant neuronal redistribution of synaptosome-selective markers in the 175Q animals, either of which has important consequences when interpreting and comparing data from isolated synaptosomes. The selective loss of synaptosomes in our HD mouse model during synaptosome isolation has to be accounted for during experimental design of proteomics and other studies, or when interpreting and comparing data from isolated synaptosomes. The selective loss of synaptosomes during sample workup may have ramifications for other studies of this type.

Keywords: Synaptosomes, Mass spectrometry, Isotope labeling, Huntingtons Disease

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CS02.02 Employing Proteomics to Unravel the Molecular Underpinnings of Schizophrenia

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Abstract: Schizophrenia is likely to be one of the most complex human disorders to be characterized from the clinical to molecular aspects. This complexity is the result of an intricate combination of genetic and environmental components that influence brain functions since pre-natal neurodevelopment. Proteomics have been contributing to understanding the biochemical basis of schizophrenia at the cellular and tissue level through the identification of differentially expressed proteins and consequently their biochemical pathways. In addition, mass spectrometry-based proteomics have identified and precisely quantified proteins that may serve as biomarker candidates to prognosis, diagnosis, and medication monitoring in peripheral tissue. Data produced by proteomic investigation will be approached here, focusing on postmortem brain tissue and peripheral blood serum and plasma. This information is providing an integrated picture of the biochemical systems involved in the pathobiology, and has suggested potential biomarkers. These proteins warrant potential targets to alternative treatment therapies to schizophrenia.Schizophrenia is likely to be one of the most complex human disorders to be characterized in all aspects, from clinical to molecular. This complexity is the result of an intricate combination of genetic and environmental components that influence brain functions since pre-natal neurodevelopment. Proteomics have been contributing to understanding the biochemical basis of schizophrenia at the cellular and tissue level through the identification of differentially expressed proteins and consequently their biochemical pathways. In addition, mass spectrometry-based proteomics have identified and precisely quantified proteins that may serve as biomarker candidates to prognosis, diagnosis, and medication monitoring in peripheral tissue. Data produced by proteomic investigation will be approached here, focusing on postmortem brain tissue and peripheral blood serum and plasma. This information is providing an integrated picture of the biochemical systems involved in the pathobiology, and has suggested potential biomarkers. These proteins warrant potential targets to alternative treatment therapies to schizophrenia.Schizophrenia is likely to be one of the most complex human disorders to be characterized in all aspects, from clinical to molecular. This complexity is the result of an intricate combination of genetic and environmental components that influence brain functions since pre-natal neurodevelopment. Proteomics have been contributing to understanding the biochemical basis of schizophrenia at the cellular and tissue level through the identification of differentially expressed proteins and consequently their biochemical pathways. In addition, mass spectrometry-based proteomics have identified and precisely quan-

tified proteins that may serve as biomarker candidates to prognosis, diagnosis, and medication monitoring in peripheral tissue. Data produced by proteomic investigation will be approached here, focusing on postmortem brain tissue and peripheral blood serum and plasma as well as pre-clinical models. This information provides an integrated picture of the biochemical systems involved in the pathobiology, and suggests potential biomarkers. These proteins warrant potential targets to alternative treatment therapies to schizophrenia and may contribute to innovative translational strategies.

Keywords: psychiatry, Schizophrenia, Translational Medicine, brain

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CS02.03 Aggregation of Physiological and Parkinson- Synucleins Revealed by Ion Mobility-MS and HDX- MS

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Introduction and Objectives: Many cellular processes are based on the formation and dynamics of multi- and supramolecular protein assemblies, and several diseases, previously thought to be unrelated, are characterised by “misfolded” protein aggregates. Chemical structures and reaction pathways of pathophysiological aggregates are poorly characterised at present. “Soft-ionisation” mass spectrometry (MS), such as HPLC-electrospray-MS, is often unsuitable to direct analysis of reaction pathways and intermediates in aggregation. Here we report ion mobility-MS (IM-MS) as a powerful tool for analysis of protein aggregation due to its concentration-independent gas phase separation capability, while HDX-MS has been shown as an effective approach to identify interaction structures in oligomerisation -aggregation. **Methods:** Although αSyn and βSyn have a sequence homology of 62%, βSyn has a different triplet sequence (70-72), VFS, and is devoid of aggregation. The fragmentation of αSyn motivated us to examine mutants with modified aggregation propensity. We designed several αSyn mutants with substitutions of three to six aa residues within the Aggregation domain. All mutant synucleins were prepared by expression in E.Coli and purified by HPLC. Proteins were separated by SDS-PAGE and analyzed by MALDI-MS. **Results and Discussion:** Applications of IM-MS to the in vitro oligomerization of αSyn enabled the identification of hitherto unknown degradation and aggregation products. Time- dependent studies of the oligomerization-aggregation of αSyn provided evidence of autoproteolytic fragmentation by cleavage at V71/T72. Corresponding recombinant αSyn(72-140) showed substantially higher aggregation and neurotoxicity compared to the intact protein. Online bioaffinity-MS and IM-MS enabled top-down studies in vivo from brain homogenate. Applications of affinity-MS using epitope-specific αSyn-antibodies were used the characterization of oligomers and interactions in vivo. Specific mutations of the (70-72) triplet and affinity-MS provided breakthrough results, showing that mutation of VFS(70-72) from physiological ßSyn into αSyn completely abolished neurotoxic aggregation. **Conclusion:** Ion mobility- and affinity-MS are powerful tools for the molecular elucidation of structures and intermediates of α-synuclein aggregation.

Keyword: Pathophysiological and physiological synucleins; ion mobility-MS; affinity-MS; HDX-MS

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CS02.04CSF Proteome Resource (CSF-PR) as a Tool for Proteomics Biomarker Discovery in Multiple Sclerosis

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Introduction and Objectives: There is a growing demand for biomarkers for the neurodegenerative disease multiple sclerosis (MS), for diagnosis, treatment guiding, disease prediction and prognosis, and to learn more about the pathogenesis underlying the disease. Cerebrospinal fluid (CSF) is a suitable material for finding biomarkers for MS and other disorders affecting the central nervous system. We saw the need to create a map of the CSF proteome and share this information via a freely available online repository of qualitative and quantitative mass spectrometry based proteomics experiments performed on CSF. This will make it a lot easier to navigate the published literature and perform comparisons between datasets. **Methods:** We have made an online resource, called CSF-PR (CSF Proteome Resource), containing an overview of the neurologically healthy CSF proteome, represented by a pool of CSF from 21 neurologically healthy donors. The methods used prior to LC-MS/MS were SDS-PAGE protein separation, mixed-mode peptide fractionation and glycopeptide enrichment. We have also started to investigate published and available quantitative data from existing studies on CSF and plan to extend the resource to include this information in the near future. **Results and Discussion:** CSF-PR is available at http://probe.uib.no/csf-pr and contains LC-MS/MS identification information for over 3000 CSF proteins, including protein distributions on an SDS-Gel and information about over 1000 glycopeptides from over 500 glycoproteins. The resource will soon also contain data from various published quantitative studies in CSF, mainly biomarker studies for MS or other neurological disorders. CSF-PR can be used to navigate the CSF proteome to select signature peptides for targeted quantification. The quantitative data available in CSF-PR is a valuable resource for all researchers working with CSF and neurological disorders. **Conclusion:** Mass Spectrometry data from proteomics studies in CSF collected at one place in a uniform format is a great asset for the field of CSF research, especially for biomarker discovery studies.

Keywords: proteome mapping, biomarkers, cerebrospinal fluid, Resource

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CS02.05 Investigating TDP-43-Mediated Neurodegeneration by Mass Spectrometry

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Introduction and Objectives: There is evidence that TAR DNA-binding protein 43 (TDP-43), its hyper-phosphorylated, ubiquitinated and cleaved forms lead to protein mislocalization and aggregation, contributing to frontotemporal dementia and Alzheimer's disease pathogenesis. It has been difficult to assess the potential of TDP-43 as an in vivo prognostic and diagnostic biomarker of neurode-

generation as characterization of TDP-43 in biofluids has been largely accomplished via qualitative and semi-quantitative immunometric assays. Our objective is to develop a clinical-grade mass spectrometric (MS) method to identify and quantitate TDP-43 isoforms in human biofluids in order to explore the relationship with disease pathogenesis. **Methods:** We have designed a quantitative multiple reaction monitoring (MRM) assay for detection and quantitation of human TDP-43 isoforms. **Results and Discussion:** Currently, we have developed MRM transitions for measuring TDP-43 and C-terminal fragment TDP-25, through targeted selection of tryptic peptides, with optimized declustering potentials and collision energies. We have also evaluated different protein digestion conditions (denaturants, reducing agents and trypsin type) as well as the digestion profile. Detection sensitivity of our assay currently reaches the low fmol range. As the concentration of TDP-43 in plasma and cerebrospinal fluid is estimated to be in the picomolar range, we are evaluating additional enrichment steps, including immunoprecipitation and protein/peptide fraction techniques. Our quantitation strategy involves an external calibration curve and isotopically-labelled internal standards. **Conclusion:** We have completed an evaluation of optimal protein digestion conditions and developed a MRM method for detecting and quantitating TDP-43 and TDP-25. Analytical and clinical performance of our assay will be assessed using plasma and cerebrospinal fluid specimens from the biobank of the University of British Columbia Hospital, Clinic for Alzheimer's Disease and Related Disorders.

Keywords: Clinical proteomics, MRM, biomarkers, Neurological disorders

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CS02.06 Mitochondrial Networks Reveals Novel Components Associated with Neurological Disorders

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Introduction and Objectives: Due to the integrative role of mitochondria in diverse cellular processes, mitochondrial dysfunction is emerging as a causative factor in a wide range of human diseases, including neurodegenerative disorders (NDs) such as Alzheimer's and Parkinson's diseases. This question requires the elucidation of protein-protein interaction networks spanning mitochondrial and non-mitochondrial proteins, which to date are only sparsely mapped in neuronal cells. Here, I will describe a just completed study focused on a selected subset of 200 ND-linked mitochondrial proteins (MPs) through which new molecular insights were gained into the complex clinical presentations of NDs. **Methods:** As part of this effort, we have affinity purified the targeted ND-linked MPs expressed at endogenous levels using protein-specific antibodies in mammalian non-neuronal and differentiated neuronal cells, and identified interactors of the purified proteins with an Orbitrap Elite mass spectrometer. MP complexes were then identified by applying rigorous confidence scoring procedures followed by graph clustering, and characterized both through novel experiments, and by intersecting complementary datasets. **Results and Discussion:** By taking this systematic, integrated look at inter- and extra-mitochondrial protein function, our analysis captured both previously known interacting proteins, as well as several new associations, including components of oxidative stress and mitochondrial fission required for axonal integrity that have not yet been reported. **Conclusion:** This work expands our understanding of how ND-linked mitochondrial processes function at a systems level and how mitochondrial pathways are organized with other mitochondrial pathways in neuronal cells, revealing new insights into MP function and targets for further research into novel treatments of NDs.

Keywords: Neurodegenerative disorders, Fission, Mitochondrial proteins, Mass spectrometry

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CS02.07 Quantitative Proteomics Analysis of Human Autoptic Pineal Glands to Study Autism Spectrum Disorders

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Introduction and Objectives: Melatonin is synthesized in the pineal gland during the night and is involved in various physiologic functions, including sleep induction, circadian rhythm regulation, and immune response. Abnormal melatonin signaling has been reported as a risk factor for medical conditions as diverse as diabetes mellitus and circadian rhythm, and psychiatric disorders including autism spectrum disorders (ASD). ASDs are a group of pervasive neurodevelopmental disorders that are characterized by qualitative impairments in reciprocal social interaction and communication, as well as restricted, repetitive and stereotyped patterns of behavior, interests and activities. It has been reported that patients with ASD present a low melatonin level, but the underlying cause of this deficit and its relationship to susceptibility to ASD remains unclear. Furthermore, previous studies have reported deleterious mutations of the ASMT gene, encoding the last enzyme of melatonin synthesis, in several individuals with ASD, and subsequently, causing a deficit in melatonin production. **Methods:** In this study, we aimed to investigate the molecular mechanisms behind melatonin deficiency in humans and in particular in individuals with ASD using mass spectrometry (MS)-based label-free quantitative proteomics. To study the differential protein expression, we compared pineal glands of ASD patients from regular autopsies with known time of death to healthy control individuals. **Results and Discussion:** In total, approximately 5,400 proteins were accurately identified and quantified by nano-LC-MS/MS on a Q Exactive mass spectrometer. To further dissect the changes in the proteome, a dedicated statistical analysis was performed, and the data were compared in terms of: day versus night for the time of death, ASD versus healthy individuals. A circadian analysis of the protein profile has also been drawn up. Interestingly, ours results show clear circadian regulation of some proteins. Several proteins were also differentially expressed in patients with ASD compared with controls. **Conclusion:** These results will be further validated using biochemical methods.

Keywords: autism spectrum disorders, High resolution mass spectrometer, Melatonin, Label-free quantification

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CS02.08 Development of Cerebrospinal Fluid Signatures of Lewy Bodies and Neuronal Loss

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Introduction and Objectives: Their presence of Lewy bodies along with loss of specific nerve cells, that make dopamine in a region of the brain called the substantia nigra, have been associated with onset and progression of Parkinson's disease. Our objective was to develop a protein abundance signature that can detect the presence of Lewy bodies and associated neuronal loss, with the ultimate goal of developing a clinical test for diagnosis of Parkinson's disease and related disorders. **Methods:** We have designed a study comprised of 17 subjects with high density of Lewy bodies in substantia nigra paired to controls matched by age, sex and number of other neuropathologic covariates. As a first step we have applied a label-free LC-MS/MS approach to discover proteins associated with the presence of Lewy bodies directly in the brain tissue. As a second step the selected protein set was measured in the cerebrospinal fluid (CSF) of the same subjects using combination of selected reaction monitoring (SRM) targeted proteomics approach and a more sensitive involving sample pre-fractionation (PRISM)-SRM. **Results and Discussion:** The Lewy body presence is associated with the ratio of two hormone peptide related proteins (AUC = 0.69) while neuronal loss associated with a broader group of ratios of peptides derived from neuron-specific proteins to the ones that are likely originating from blood (AUC = 0.72). The derived protein signatures are currently validated on an alternative cohort of Parkinson's disease patients and matching controls. Robustness of the biomarker signatures and the actual clinical or neuropathological features they are most strongly correlating are the questions for follow-up investigation. **Conclusion:** We have developed preliminary CSF protein abundance signatures associated with the key neuropathologic features of the Parkinson's disease: Lewy bodies and neuronal loss in the substantia nigra. The signatures are under validation in CSF samples from an alternative cohort involving Parkinson's disease patients and matching controls.

Keyword: parkinsons, biomarkers, srm, mrm

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CS02.09 Analyzing Laser Microdissected Neuromelanin Granules from Human Post Mortem Substantia Nigra

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Introduction and Objectives: Analyzing neuromelanin (NM) granules from human substantia nigra is mainly interesting because of two reasons: first, the role of the pigment, protein and lipid complex in dopaminergic cells is still unknown. Second, it is still unknown, why NM granules containing dopaminergic neurons in the substantia nigra are widely affected in Parkinson's disease. A closer look at NM granules is interesting to get further insights in the pathogenesis of Parkinson's disease. **Methods:** In the past, most proteomic studies were performed on whole-brain lysates, without differentiation between the specific cell types of the brain or subcellular compartments within the cells [1], thus low abundant proteins were masked. For in-depth analysis, NM granules have to be isolated with high purity. We developed a laser microdissection based method for NM granules isolation both from neurologically normal cases with subsequent mass spectrometry based proteomic analysis. **Results and Discussion:** Our analysis revealed deeper insights in the NM granules genesis: combined data from our studies and previously published data suggest, that NM granules develop by an endolysosomal pathway similar to multivesicular bodies. Additionally, this could explain why alpha-synuclein redistributes to NM granules in Parkinson's disease. As our cohort was limited, application of this method to a bigger cohort and to post mortem tissues from Parkinson diseased cases is planned. These studies could allow deeper insights in the role of NM granules in healthy and diseased ageing. **Conclusion:** The aim is to identify the function of NM granules in healthy ageing and Parkinson's disease. Sensitivity and specificity of modern proteomics combined with laser microdissection allows identification of proteins from minimal amounts, and will help deepen our knowledge of disease-specific dysfunctional processes. [1] Plum, S., et al., Proteomics in neurodegenerative diseases: Methods for obtaining a closer look at the neuronal proteome. Proteomics Clin Appl, doi: 10.1002/prca.201400030

Keywords: Neuromelanin, Laser microdissection, Parkinson's disease

CS03: GLYCOMICS IN BIOLOGY AND DISEASES

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CS03.01 Glycomics-Assisted Glycoproteomics: Deciphering the Complexity

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Abstract: Glycoproteomics means different things to different people. Researchers in proteomics often limit their analysis to identifying the previously glycosylated protein sites by identification of the deglycosylated peptides, and tend to neglect the importance of conjugated glycan structures in proteomics workflows. Glycomics researchers on the other hand, can now routinely determine the detailed N- and O- linked glycan structures released globally from highly complex protein mixtures, albeit in a protein- and site-unspecific manner. True glycoproteomics, the system-wide study of the heterogeneity of glycans attached to the different sites on specific proteins, remains technically challenging. This talk will, through various examples of the analysis of purified and complex mixtures of glycoproteins, show how the combination of mass spectrometry based glycomics, together with glycopeptide analysis and the development of bioinformatics data analysis tools, can assist the further development of glycoproteomics technologies. Depending on the question being asked, complementary protein glycosylation information is currently necessary to enhance our understanding of the structure and function of this ubiquitous protein modification.

CS03.02 Evolving Approaches for Separation and Tandem MS of Disease-related Glycans and Glycoconjugates

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Abstract: Infectious and congenital diseases, aging and undefined disorders affect lives in both developed and undeveloped countries. Nevertheless, incomplete understanding of the relevant molecular pathways still limits diagnostic efficiency and/or the rational design of therapies. Advances in analytical methodology, coupled with the rapidly growing power of bioinformatics, now make the exploration of these phenomena more approachable, not only for proteomics, but also for parallel and intersecting fields. With respect to the diagnosis and treatment of sporadic and chronic conditions that involve glycan-regulated or affected pathways, mass spectrometry developments in both pre- and post-ionization separations and in dissociation methods are providing critical tools that facilitate investigation of diseases at the molecular level, by enabling qualitative and quantitative determinations of post-translational modifications of proteins, differentiation among isomeric glycans, and structure-specific information on intermolecular interactions and turnover, that have the power to define subtleties previously so fearsome that they were often not considered approachable. Current development projects in our Center include ion mobility-mass spectrometry, two-dimensional imaging, glycoform definition, and software approaches that facilitate site-specific determinations of post-translational modifications. For data analysis, we are using a combination of commercial, public and home-built tools and software., e.g., Scaffold (Proteome Software), Progenesis LCMS (Nonlinear Dynamics), Proteome Discoverer (Thermo-Fisher), Mascot (Matrix Science), the Trans Proteomic Pipeline (ISB), and STRAP PTM (in-house). The lecture will focus on opportunities for emerging methods to address difficult challenges in health care. Acknowledgement: These investigations are supported by NIH grants P41 GM104603, S10 RR025082 and S10 OD010724 and NIH contract HHSN268201000031C.

Keywords: tandem mass spectrometry, ion mobility spectrometry, ExD dissociation, glycobiology

CS03.03 N-Acetylglucosaminyltransfease III (GnT-III) Is a Novel Drug Target for Alzheimer's Disease

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Introduction and Objectives: Beta-site APP cleaving enzyme-1 (BACE1) is an essential aspartyl protease in central nervous system (CNS) for the generation of amyloid-β (Aβ) peptide, and is a major druggable target for Alzheimer's disease (AD). However, BACE1 inhibitors could also affect several physiological functions of BACE1, which is a matter of concern. Here, we show that BACE1 is functionally modified with bisecting N-acetylglucosamine (GlcNAc), a product of N-acetylglucosaminyltransferase- III (GnT-III) which is highly expressed in brain. **Methods:** We have developed mutant mice lacking GnT-III (Mgat3) with high expression of Aβ. The amount of Aβ, BACE1 activity and its localization, and cognitive function in the above mice were examined. We also analyzed AD patient samples **Results and Discussion:** We demonstrate that AD patients have higher levels of bisecting GlcNAc on BACE1. Glycan analysis of purified BACE1 from mouse brains showed that BACE1 N-glycans contain many bisecting GlcNAc glycans structures. Analysis of GnT-III (Mgat3) KO mice with high expression of Ab revealed that cleavage of Aβ-precursor protein (APP) by BACE1 is reduced in these mice, resulting in a decrease in Aβ plaques and improved cognitive function. The lack of this modification directs BACE1 to late endosomes/lysosomes where it is less co-localized with APP, leading to accelerated lysosomal degradation. On the other hand, BACE1 catalytic activity did not change. Furthermore, other BACE1 substrates, CHL1 and contactin-2, are normally cleaved in GnT-III-deficient mice, suggesting that the effect of bisecting GlcNAc on BACE1 is selective to APP and that GnT-III inhibitors have fewer side effects. Considering that GnT-III-deficient mice remain healthy, GnT-III may be a novel and promising drug target for AD therapeutics. **Conclusion:** An inhibitor against GnT-III would be a likely drug target for AD.

Keywords: bisecting GlcNAc, GnT-III, Alzheimer's disease, BACE1

CS03.04 N- and O-Glycomics on Formalin-Fixed Paraffin-Embedded (FFPE) Clinical Specimens by PGC-LC ESI-MS/MS

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Introduction and Objectives: The composition of a cell's glycome depends on numerous factors such as age, health or disease state making glycosylation an interesting biomarker target. Nevertheless, the availability of well-defined clinical specimens in sufficient numbers often represents a serious obstacle in glyco-biomarker analyses. Here we present a PGC-nanoLC ESI-IT MS/MS based approach for the in-depth glycomic profiling of both, N-and O-glycans starting from single FFPE tissue sections as thin as 3 μm. **Methods:** Fresh and FFPE conserved tissue specimens were prepared from the very same hepatocellular carcinoma (HCC) patients. (Glyco) proteins were extracted from 3-5 μm thick FFPE sections, immobilised on PVDF membranes and N-glycans and O-glycans sequentially released. The reduced N- and O-glycans were analysed by porous graphitized carbon (PGC) nanoLC ESI-MS/MS, and data analysed by semi-automated structure identification via spectral matching and relative quantitation of individual structures within a single experiment. **Results and Discussion:** More than 90 individual N- and 14 O-glycans were successfully isolated from unstained and hematoxylin/eosin (HE) stained FFPE tissue samples obtained from routinely taken clinical preparations of HCC and control liver tissues. Overall no qualitative FFPE- or staining induced glycan structure alterations were identified. However, the relative amounts of complex-type, diantennary N-glycans carrying one or more α2,3 linked neuraminic acid residues were increased in FFPE preparations when compared to frozen tissue of the very same origin and disease state, indicating that similar preparation methods are mandatory when using FFPE material. Laser capture micro-dissection allowed selective isolation of histopathology cross-checked HCC/non-HCC cells and N- and O-glycomic profiles were obtained from as low as 1000 cells, still revealing distinct and significant glycosylation al-

terations of paucimannosidic N-glycans in the HCC diagnosed tissue. **Conclusion:** These results provide novel opportunities for studying disease-associated protein N- and O-glycosylation on the microscopic scale and emphasise the potential of FFPE tissues in future glyco-biomarker studies from clinical specimens.

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CS03.05 Identification of Biomarkers from Oral Cancer Patient Serum by Glycoproteomic Approaches

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Introduction and Objectives: Oral cancer is the tumor grows on the lips, tongue, cheeks, floor of the mouth, hard and soft palate, sinuses, and pharynx. The high prevalence and mortality rate of oral cancer makes it important to investigate new biomarkers for the surveillance of high-risk population. We previously identified several potential glycoprotein and N-glycan tumor markers from oral cancer cell lines and patient serum. We found that the mRNA levels of FUT8 was higher in oral cancer cell than in normal cells. High levels of core-fucosylated N-glycans were observed on the oral cancer cell surface. In addition, the proportions of bi-, tri- and tetra-antennary N-glycans were significantly increased in oral cancer patients. The N-glycan structures also showed high sensitivity, high specificity, and high AUC values (>0.8) for cancer patients. Here, we further explored the serum glycoproteins of normal (20, healthy volunteers) and oral cancer patients (60, obtained from tissue bank of NCKUH) by glycoproteomic analysis. **Methods:** Serum was lyophilized and resuspended in buffer followed by AAL (Aleuria aurantia lectin) lectin column purification. The purified core-fucosylated serum proteins were separated by electrophoresis and stained. After compared the protein bands on gel between normal with patient serum, bands showed significant expression differences were collected, subjected for in-gel digestion, and protein identification on a Mass-spectrometry. **Results and Discussion:** Several glycoproteins were identified, and then verified by western blotting (serum) and immunohistochemical staining (tissue array). **Conclusion:** In the future, we are going to investigate the relationships between the identified glycoproteins with oral cancer progression and metastasis.

Keywords: Oral Cancer, core-fucosylation, Glycoproteomics

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CS03.06 MALDI Imaging Mass Spectrometry of Glycans on Formalin-Fixed Paraffin-Embedded Ovarian Tumours

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Introduction and Objectives: Ovarian cancer is the most fatal gynaecological malignancy in adult women with a five-year overall survival rate of 30%. The most widely used clinical biomarker to diagnose ovarian cancer is a heavily glycosylated protein called CA125, but lacks specificity and sensitivity. Glycomic and glycoproteomic profiling studies have reported extensive glycosylation pattern alterations in ovarian cancer. Therefore, further investigation of these patterns may unearth novel biomarkers for early stage diagnosis. A novel method for investigating tissue-specific N-linked glycans was recently developed by our group on formalin-fixed paraffin-embedded (FFPE) murine kidney. Matrix-assisted laser desorption/ionization (MALDI) imaging mass spectrometry (IMS) spatially profiles glycoforms in tissue-specific regions, while through liquid chromatography electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) the corresponding glycol compositions are structurally characterized. **Methods:** Regions of interest (e.g. tumour, stroma, adipose and necrotic) were isolated from FFPE late stage patients (n=3) with serous ovarian cancer. N- and O-linked glycans were structurally characterized through enzymatic peptide-N-glycosidase F (PNGase F) release of N-glycans, followed by β-elimination of O-glycans. The released glycans were analyzed through porous graphitized carbon liquid chromatography (PGC-LC) and collision induced negative mode fragmentation analysis. The glycan repertoire identified through this analysis was further used to spatially profile the location and distribution of N-glycoforms on FFPE ovarian cancer sections. **Results and Discussion:** High resolution MALDI-IMS revealed tissue-specific N-glycoforms, for example, high mannose glycans were predominantly expressed in the tumour region while complex glycans were significantly abundant in the underlying stroma. Therefore, tumour and non-tumour tissue regions established clear demarcation based on their N-glycoform distributions. **Conclusion:** MALDI-IMS and LC-ESI-MS/MS were used as complementary techniques to generate high resolution images and structural information of tissue-specific N-glycoforms. Furthermore, O-glycoforms were characterized for the first time in FFPE ovarian cancer patients. Application of this method enabled the first steps in the glycomic quest for the Holy Grail: an early stage diagnosis biomarker.

Keywords: MALDI imaging, Glycans, Ovarian cancer, Mass spectrometry

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CS03.07 Lectin RCA-I Specifically Binds to Metastasis-Associated Cell Surface Glycans in TNBC

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Introduction and Objectives: Triple-negative breast cancer (TNBC) patients often face a high risk of early relapse characterized by extensive metastasis. Previous works have shown that aberrant cell surface glycosylation is associated with cancer metastasis, suggesting that altered glycosylations might serve as diagnostic signatures of metastatic potential. To address this question, we took TNBC as an example and analyzed six TNBC cell lines, derived from a common progenitor, that differ in metastatic potential. **Methods:** We used a microarray with 91 lectins to screen for altered lectin bindings to the six TNBC cell lines. **Results and Discussion:** Using the lectin microarray, we found that the bindings of RCA-I to TNBC cells are proportional to their metastatic capacity. Tissue microarray experiments showed that the intensity of RCA-I staining is positively correlated with the TNM grades. The real-time cell motility assays clearly demonstrated RCA-I inhibition of adhesion, migration, and invasion of TNBC cells of high metastatic capacity. Additionally, a membrane glycoprotein, POTE ankyrin domain family member F (POTEF), with different galactosylation extents in high/

low metastatic TNBC cells was identified by LC-MS/MS as a binder of RCA-I. **Conclusion:** We discovered RCA-I, that bound to TNBC cells to a degree that is proportional to their metastatic capacities, found that this binding inhibits the cell invasion, migration, and adhesion, and identified a membrane protein, POTEF, that may play a key role in mediating these effects. These results thus indicate that RCA-I specific cell surface glycoproteins may play a critical role in TNBC metastasis and that the extent of RCA-I cell binding could be used in diagnosis to predict the likelihood of developing metastases in TNBC patients.

Keywords: lectin microarray, metastasis, glycan, Triple negative breast cancer

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CS03.08 Glycan Analyses of Murine Plasma and Lung Membrane to Find Biomarker Candidate for COPD

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Introduction and Objectives: Chronic bronchitis and pulmonary emphysema are features of chronic obstructive pulmonary disease (COPD). Biomarker discovery is essential for the diagnosis and treatment, especially at the stage of exacerbations of COPD. **Methods:** We analyzed glycan structures on plasma glycoproteins and lung cell membrane glycoproteins in four group model mice which mimics COPD. Emphysema model group: administration of elastase to break down elastin in the extracellular matrix. Inflammation model group: administration of LPS. Exacerbation model group: after administration of elastase, LPS was given to stimulate inflammation. Treatment model group: before administration of elastase, glycosaminoglycans (GAGs) were given. Proteins in the plasma were separated on SDS-PAGE, and transferrin (TF) and alpha1-antitrypsin (A1AT) were subjected to in-gel digested with trypsin. Glycans on the tryptic peptides were analyzed by LC-ESI MS. Proteins in cell membrane of the lung tissue were dotted on PVDF membrane to release glycans with PNGaseF. The released glycans were analyzed by LC-ESI MS. **Results and Discussion:** In the inflammation model group and the exacerbation model group, increases in trisialo-biantennary glycans and decreases in fucosylated glycans of TF and A1AT were observed as compared to those in the non-treated control group. In the treatment model group, decreases in the trisialo-biantennary glycans were observed compared with those in non GAG-treated group. It was suggested that LPS-induced inflammation might influence the structures of glycans in the two glycoproteins. These glycan changes were well correlated with the number of inflammatory cells in the bronchoalveolar lavage fluid of each model group. In the emphysema model group, increase in a2-6 sialo-biantennary glycans in lung cell membrane were observed as compared to those in other groups. **Conclusion:** Changes of trisialo-biantennary glycans and fucosylated glycans of TF and A1AT in plasma and a2-6 sialo-biantennary glycans of lung cell membrane would be biomarker candidates for the COPD.

Keywords: COPD, glycan, biomarker, glycoprotein

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CS03.09 Confident, Automated and Quantitative N-Glycoproteomics Analysis in Exosome Samples

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Introduction and Objectives: Site-specific characterization, quantification of glycopeptides and glycoforms increases the biological relevancy tied to glycan distributions across different biological states. However, challenges in global LC-MS glycopeptide distribution profiling remain in processing the data. This is due to diluted MS signal, glycopeptide co-elution, increased charge state distribution and density profiles. We present a novel software, Pinnacle and workflow, incorporating high resolution mass spectrometry data to perform robust glycan distribution profiling. We combine evidence from isotopic fidelity of parent ion, high resolution HCD/CID/ETD product ions to build a comprehensive glycopeptide lists, and score them using a multivariate scoring system. The results demonstrate exhaustive identification, quantification and low FDR determination. **Methods:** Tryptically digested recombinant human EGFR protein and glycopeptides from human exosomes originating from three different Human T-Cell Lymphotrophic Virus -1 disease states were analyzed on Orbitrap Fusion. All data was processed using Pinnacle software that performs qualitative scoring on MS and combined MS/MS data while quantifying all precursor data. **Results and Discussion:** Data processing in Pinnacle was evaluated with glycopeptides from recombinant EGFR to determine overlap with published data. The results were stratified in Pinnacle based on a multi-variant scoring system for rapid evaluation. A strong overlap was observed with published glycosites and glycans while maintaining a low FDR. Data analysis of the exosomal glycopeptides demonstrated similar performances for identification and glycan distributions. This workflow was applied to HTVL-1 samples from different disease states. The results demonstrated five key exosomal proteins showing similar glycan patterns across disease states. **Conclusion:** Confident glycopeptides identification should take into account all scoring attributes including MS1, MS/MS and retention time data. Combining these, Pinnacle utilizes unique scoring system to significantly increase sensitivity and selectivity for identification of more glycoforms/peptide to create a complete profile of the state of modification at the protein level. Pinnacle also performs relative quantification for global differential expression analysis.

Keywords: Glycoproteomics, informatics, Glycopeptides, exosome

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CS03.10 Development of a Method for Large-Scale Analysis of the Site-Specific Glycomes of Glycoproteins

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Introduction and Objectives: Generally, a glycoproteomics approach means a large-scale identification of “de-glycosylated (formerly glycosylated) peptides” and glycosylated sites. A single glycosylation site has multiple different glycans in natural, and the glycome alterations associ-

ated with pathological events such as carcinogenesis and fibril formation are shown to be a useful indicator (biomarker) of the diseases. Therefore, we developed a new method to identify the site-specific glycomes of glycopeptides identified as de-glycosylated peptides by an existing glycoproteomic technology, IGOT-LC/MS method (Kaji et al. 2003, Nat Biotechnol.). **Methods:** Glycopeptides were enriched by HILIC from a protease digest of glycoprotein(s). An aliquot of the obtained glycopeptide mixture was applied to the IGOT method to identify a series of glycopeptide core sequences. Another aliquot of the mixture was analyzed by LC/MS using a high accuracy LTQ-Orbitrap spectrometer for acquisition of masses (m/z) of glycopeptides. Based on the mass-chromatographical features, the signals of glycopeptides were selected from the all detected signals as clusters using in-house software. Then, the core sequences and their glycan compositions were assigned by matching the theoretical masses of the actually present core peptides and observed masses of the glycopeptides considering the masses of glycan portion. **Results and Discussion:** We applied this method to the analyses of glycopeptides prepared from cultured cells and mouse tissues, and large numbers of glycopeptides and their glycan compositions could be assigned by the method. **Conclusion:** The newly developed our technology will serve as a tool for comprehensive elucidation of site-specific glycan heterogeneity and for the glycan structural verification of disease glycobiomarker molecules.

Keyword: Glycoproteomics, Glycoprotein, Glycome, Biomarker

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CS04.01 Types of Tumors: Imaging the Difference

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Abstract: The chemical complexity of biological surfaces is highly dynamic and subject to local changes in response to a changing environment. This chemical heterogeneity is a particular important parameter when considering treatment of diseases such as cancer. It is this inconceivably complex heterogeneity that makes tumors so difficult to treat as no single therapy targets all permutations of phenotypes and environment precisely. This implies that to make truly personalized tumor therapy reality a diagnostic method is needed that unveils the spatial and molecular complexity of tumor tissue. The molecular complexity on the genome, proteome and metabolome level all needs to be taken into account. A multimodal analytical approach is needed. The distribution of several hundreds of molecules on the surface of complex (biological) surfaces can be determined directly in a single imaging MS experiment. This enables molecular pathway analysis as well as the role of the different molecular signals during tumor development. The changing behavior under influence of a drastically changing chemical or biological environment is studied in model systems. State-of-the-art molecular imaging mass spectrometry has evolved to bridge the gap between different disciplines such as MRI, PET, fluorescence imaging and histology. The combination with tools from structural biology makes it possible to perform imaging experiments at length scales from cells to patients. In this lecture we will discuss the development and application of new MS based chemical microscopes that target biomedical tissue analysis in various diseases as well as other chemically complex surfaces. We will demonstrate how to elucidate the way in which local environments can influence molecular signaling pathways on various scales. The integration of this pathway information in a surgical setting is imminent, but innovations that push the boundaries of the technology and its application are still needed. The imaging MS community is driving translational molecular imaging research and these needed developments rapidly forward. This lecture will highlight fundamental research, innovative developments, and unique applications in multimodal imaging MS. Results already demonstrated that rapid, direct tumor phenotyping is now reality and pointing the way towards personalized diagnostic and therapy.

Keywords: Imaging mass spectrometry, tissue proteomics, Molecular Imaging

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CS04.02 Imaging Mass Spectrometry: Molecular Microscopy for Biological and Clinical Research

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Abstract: MALDI Imaging Mass Spectrometry (IMS) produces molecular maps of peptides, proteins, lipids and metabolites present in intact tissue sections. It employs desorption of molecules by direct laser irradiation to map the location of specific molecules from fresh frozen and formalin fixed tissue sections without the need of target specific reagents such as antibodies. Molecular images of this nature are produced in specific m/z (mass-to-charge) values, or ranges of values. Thus, each specimen gives rise to many hundreds of specific molecular images from a single raster of the tissue. In a complementary approach where only discrete areas within the tissue are of interest, we have developed a histology-directed approach that integrates mass spectrometry and microscopy. Thus, mass spectra are collected from only selected areas of cells within the tissue following laser ablation and analysis. We have employed IMS in studies of a variety of biologically and medically relevant research projects. One area of interest is the molecular mapping of molecular changes occurring in diabetes in both a mouse model and in the human disease. Major molecular alterations have been recorded in advanced diabetic nephropathy involving both proteins and lipids. Other applications include developmental studies of embryo implantation is mouse, assessment of margins in renal cancers as well as that in other organs, and neurodegenerative disease. Molecular signatures have been identified that are differentially expressed in diseased tissue compared to normal tissue and also in differentiating different stages of disease. These signatures typically consist of 10-20 or more different proteins and peptides, each identified using classical proteomics methods. One such application described is that concerning the differentiation of benign skin lesions from melanomas. Our PIMS (Pathology Interface for Mass Spectrometry) will be demonstrated. In addition, Imaging MS has been applied to drug targeting and metabolic studies both in specific organs and also in intact whole animal sections following drug administration. This presentation will also describe recent technological advances both in sample preparation and instrumental performance to achieve images at high spatial resolution (1-10 microns) and at high speeds so that a typical sample tissue once prepared can be imaged in just a few minutes. Applications will include the use of MS/MS, ultra-high mass resolution, ion mobility, and ion accumulation devices for IMS. Finally, new biocomputational approaches will be discussed that deals with the high data dimensionality of Imaging MS and our implementation of 'image fusion' in terms of predictive integration of MS images with microscopy and other imaging modalities.

CS04.03 Phospholipid MALDI Imaging MS Stratification of Colorectal Cancer Liver Metastasis Clinical Biopsies

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Introduction and Objectives: Colorectal cancer (CRC) is the third most common cause of cancer death worldwide. Although the primary tumor can be controlled with surgery, death is the result of distant metastases. Almost all patients with CRC develop resistance to treatment despite the presence of an initial response, highlighting the need to develop novel technologies for tumor assessment. To address the need for accurate tumor assessment, we analyzed a cohort of human CRC liver metastasis samples by phospholipid MALDI imaging MS. **Methods:** IMS data was acquired at 100 µm resolution on each tissue section (n=40). Data analysis was performed in the R environment. Extracted sample peak data was submitted to k-means clustering and these results correlated to histological staining. IMS segments highly correlated to relevant histological features were extracted and the peak data compared to identify the major contributing PLs. PLS discriminant analysis was trained using a subset (n=12) of samples and used to predict histologies. **Results and Discussion:** IMS PL data sets trained with histopathological information were able to correctly predict the tissue topography of new samples implementing PLS-DA multivariate classification using several tissue environments. Classified topographies were validated with conventional stainings and Ki67 immunostaining. Lipid signatures were developed from the topographies by pinpointing 20 individual lipids best delineating histological features through area under ROC curve analysis. Among the important lipids for classification are PCs, LPCs, PIs, SMs, and PEs. **Conclusion:** As expected, the different histologies were better defined when the classifiers were applied to the IMS data acquired at higher spatial resolution. A secondary classification was performed on the predicted histologies using clinical factors including tumor grade and therapeutic response to determine relevant markers and potential for prognostic and diagnostic prediction. Predicted IMS histologies were quantified by their total area of the tumor and compared to pathologist assessment of the stained sections.

Keywords: Imaging mass spectrometry, Lipids, Colorectal cancer liver metastasis, Histology

CS04.04 Optimizing MALDI-MS Based Virtual 2D Gel Method for Protein Characterization

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Introduction and Objectives: Virtual 2D gel/MS is a top-down approach combining first dimension of 2D gel electrophoresis with rapid mass analysis by MALDI-MS. Protein samples are first separated by high resolution isoelectric focusing (IEF) using immobilized pH gradient (IPG) gels. The proteins are then desorbed directly from the gel by MALDI. Replacing the second dimension of 2D-PAGE with MALDI imaging has the advantages for characterization of post-translational and post-transcriptional modifications, membrane proteins, and small open readings frames with high sensitivity and throughput. We have improved the methodology to increase ion signal intensities and reproducibly produce high quality aerogels for mass spectrometry analysis. **Methods:** Protein samples from cell lysates were rehydrated with IPG gels overnight prior to isoelectric focusing using a Multiphor II electrophoresis apparatus. The focused gels were briefly washed in a 1:1 acetonitrile:water solution. Sinapinic acid matrix was then applied to the gel. The gels were imaged by a Bruker Autoflex MALDI TOF/TOF and analyzed by the Bruker FlexImaging software. Acquired images were analyzed for masses of interest and the corresponding proteins were identified by in-gel digestion on duplicate gels. **Results and Discussion:** Among our many optimization approaches to the IPG gel matrix application, the most effective one so far has been soaking washed IPG gels in 1:3 acetonitrile:water with 0.1% trifluoroacetic acid with saturated sinapinic acid overnight. Using this improved protocol, whole cell lysates from Escherichia coli and Methanosarcina mazei have been examined and a broad mass range of proteins with good ion signals have been identified including proteins larger than 50 kDa. **Conclusion:** This novel virtual 2D gel/MS method can mass-analyze intact proteins after gel electrophoresis and provide complementary information to the bottom-up proteomic approaches.

Keywords: MALDI-MS, Virtual 2D Gel/MS, protein characterization

CS04.05 Peptide and Glycan 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 and Glycan 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 and PNGase F Glycan 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 or Glycan Imaging Mass Spectrometry could be able to distinguish if patients have metastasis by analysing the primary tumour using FFPE tissue from large patient cohorts. **Conclusion:** The potential for tryptic peptide and glycan imaging on FFPE tissue micro arrays as a diagnostic tool for the metastasis of gynaecological cancers will be discussed on data from FFPE endometrial and vulvar tumour cancer tissue from patients with and without metastasis.

Keywords: Glycan Imaging, Peptide Imaging, FFPE tissue, tissue microarrays

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CS04.06 MSI and Proteomic Studies of Rat Spinal Cord Injury: Caudal Segment for Possible Therapy Target

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Introduction and Objectives: Spinal cord injury (SCI) belongs to currently incurable disorders of the CNS. Primary damage and axonal disruption is followed by progressive cascade of secondary deleterious reactions. Although axonal regeneration is initiated, it is quickly repressed due to severe inflammation, lack of trophic support and inhibitory environment. **Methods:** A balloon-compressive technique was used to produce SCI at thoracic Th8-9 level in adult rat. We have taken advantages of proteomic technology to screen and identify proteins in each spinal cord segment-derived conditioned medium (CM), to better understand protein composition changes along the rostral-caudal axis after SCI at 3, 7, 10 days. Temporal-spatial MALDI Imaging and tissue microproteomics were undertaken to specify the molecular environment. In-vitro and in-vivo tests were realized with CM or stem cells secretome to investigate primary microglial cells activation by chemotaxis assay, M1/M2 polarization and confocal imaging. **Results and Discussion:** We showed using quantitative proteomic and physiological assays that 3 days after SCI, the factors produced by the cells are different according to the spatial localization along the lesion axis. The study was further extended spatially to the whole spinal cord and temporally (3, 7 and 10 days after lesion). At the rostral part R1, the factors secreted are anti-inflammatory and neurotrophic whereas the secreted factors from the caudal segment C1 contain pro-inflammatory, necrotic factors. On the contrary, C2 and C3 segments present the same patterns than R2 and R3 segments. Tissue MALDI MSI and microproteomic studies followed by shot-gun analyses pointed out the presence of anti-inflammatory factors in rostral segments whereas in caudal segments neurites outgrowth factors, inflammatory cytokines and chondroitin sulfate proteoglycan are overexpressed. **Conclusion:** These data established spatiotemporal evolution and indicate that we can initiate regenerative processes in the disconnected caudal segments if trophic and inflammatory inhibitors factors are added and if bridge is formed on either side of the lesion.

Keywords: Spinal cord injury, proteomic, MALDI imaging

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CS04.07 Protein Profiling of Brain Ischemia by MALDI-Imaging-Mass-Spectrometry

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Introduction and Objectives: MALDI Imaging-Mass-Spectrometry is a powerful technique that allows the visualization of protein distribution along a tissue without labeling. Our aim is to study the distribution of proteins along mouse brain after an ischemic insult and to identify those relevant proteins. **Methods:** We occluded the middle cerebral artery (60min) of C57BL/6J mice (n=4) with 24h of reperfusion. We obtained brain slices for MALDI-TOF analysis. ROIs were defined on infarct and healthy contralateral regions. The obtained mass spectra were analyzed by ClinProTools. We used PCA to select relevant m/z peaks and analyzed their capacity of discrimination by ROC curve analysis. Each m/z distribution was analyzed by FlexImagin3.0. To identify the protein related to each m/z we developed bottom-up (tricine-gel fractionation and nanoLC-MS/MS (HR Orbitrap)) and a top-down (RP- HPLC and MALDI-ISD) approaches using mouse brain extracts. The identifications were confirmed by immunohistochemistry. **Results and Discussion:** We identified 102 m/z with different abundances between the infarct and healthy contralateral regions ($p<0.05$). We selected 21 m/z by PCA and obtained their corresponding ion distribution maps. Thirteen of them were found increased in the infarct region and 4 m/z showed a discrimination higher than 90% when infarct was compared with contralateral. Three of these peaks appeared significantly increased in the healthy contralateral region when compared with infarct, with high abundance in the striatum, the cortical and subcortical region respectively. The fourth m/z was significantly increased in the infarct and was absent in other healthy brain areas, with the anterior commissure as the only exception. After bottom-up/top-down approaches we identified 14 proteins that matched the MALDI-identified m/z. **Conclusion:** We identifed several m/z peaks with different abundances between infarct and contralateral regions of mouse brain by MALDI-Imaging-Mass-Spectrometry. These proteins might represent potential biomarkers or therapeutic targets of brain ischemia.

Keywords: MALDI-Imaging-Mass-Spectrometry, brain ischemia, biomarkers, transient MCAO

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CS04.08 Multiplexed Imaging of Biomolecules in Tissues by MALDI-MS

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Introduction and Objectives: MALDI-MS imaging determines the spatial location and abundances of biomolecules in situ. Usually, only a limited number of biomolecules can be imaged from a single tissue. We have developed new methods for increasing the number of metabolites and proteins that can be imaged by LDI-MS **Methods:** Ten hydroxyflavones were screened for on-tissue metabolite detection by MALDI-MS. Tissues were cryosectioned and mounted on ITO-coated glass slides. Matrix coating assisted by an electric field (MCAEF; patent pending) was performed by applying optimized static electric fields during matrix coating. LDI-MS was then performed with a 12-Telsa FTICR or a MALDI-TOF/TOF. Biomolecules were identified by combining accurate mass measurements, MALDI- or LC-MS/MS, and metabolome or proteome database searches. **Results and Discussion:** Eight of the ten hydroxyflavones were found to be useful MALDI matrices for lipid imaging, with the best being two penta-OH flavones (quercetin and morin). The quercetin matrix without MCAEF allowed the imaging of 212 and 544 lipids imaged in rat brain (+ ion) and porcine adrenal gland (+/- ion), respectively, by MALDI-FTICR/MS (compared to the detection of ~150 lipids with standard matrices without MCAEF). Using MCAEF-LDI-FTICR/MS with quercetin and 9-aminoacridine in the positive and negative modes, respectively, >800 lipids and 51

other metabolites in human prostate cancer tissues were localized, a 90% increase in the number of compounds imaged. Approximately 400 compounds displayed unique distributions in the cancerous or non-cancerous regions of the tissues. Using sinapinic acid, MCAEF-(+)LDI/TOF MS was able to image 242 peptides/proteins in the same cancer tissues, with 110 showing unique or distinct distributions in the cancerous region. MCAEF-LDI/FTICR MS for the whole-body imaging of tadpoles showed organ-specific lipid distributions associated with a thyroid hormone treatment. **Conclusion:** This work has demonstrated the imaging of >800 metabolites and 240 proteins by LDI-MS, which is a breakthrough in the number of molecules detected with MALDI imaging.

Keywords: MALDI imaging, quercitin, MCAEF, cancer imaging

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CS04.09 Applying Mass Spectrometry Imaging for the Acceleration of Drug Discovery and Development

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Introduction and Objectives: In drug discovery and development, the investigation of the localization of drug is essential for the understanding of absorption, distribution, metabolism and excretion (ADME). In addition, the optimal properties of Pharmacokinetics/Pharmacodynamics (PK/PD) and Toxicokinetics- Toxicodynamics (TK/TD) is key for the new generation of targeted treatment. Autoradiography (ARG) is traditionally used to determine the localization of the drug in the tissue. However, the synthesis of the radiolabeled drug is an expensive and frequently time-consuming process. In addition, it can take several weeks of exposure time to develop radiographic images of sufficient sensitivity, why mass spectrometry imaging (MSI) is a powerful alternative and complement to ARG. **Methods:** Twenty-six CNS and Cardiovascular drug products were investigated on 10-μm-thickness cryosections of rat brain and liver, prepared using cryomicrotome (Leica Microsystems). Mass spectrometric analyses were performed on a MALDI LTQ Orbitrap XL (Thermo Fisher Scientific) at a spatial resolution of 30-200 μm. **Results and Discussion:** The chemical/physical properties of these drugs in relation to laser-desorption ionization on target plate, in liver,- and brain- tissue environment were investigated at drug concentrations of 0.003-300 pmol. Each tissue section was imaged within 4 hours after application of the drug. The ion images of the drugs were easily obtained directly from tissue in comparison to the time-consuming processes of ARG. This data indicated that MSI is an imaging technique, absolutely comparable to ARG, and favorable due to the cold compound analysis, and the ability to identify metabolism mechanisms. **Conclusion:** Our data clearly indicate that MSI is a simple and powerful technology platform to provide drug compound property information within organ tissue compartments as well as within disease area localizations, providing a better understanding of ADME, PK/PD and TK/TD. Therefore, MSI is a crucial technology for the decision making in drug discovery and development.

Keyword: *Mass Spectrometry Imaging, drug, autoradiography, localization*

CS 04: IMAGING MASS SPECTROMETRY
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CS04.10 MALDI - MS Imaging Reveals Calreticulin Overexpression in Penile Cancer

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Introduction and Objectives: Penile cancer (PeCa) corresponds to 2.1% of Brazilian men's malignant tumors. In the present study, it was our objective to provide molecular information regarding the differential protein expression in PeCA, and look for potential diagnostic, prognostic and/or predictive biomarkers. MALDI Mass Spectrometry Imaging (MSI) experiments based on predetermined target were perform to assist in this effort. Calreticulin (CRT), an endoplasmic reticulum resident protein, plays a critical role in multiple cellular functions, including protein folding, calcium homeostasis and regulation of transcriptional activity of the target genes The CRT seems to be involved in tumor progression; however, the molecular mechanisms involved in this process is still unclear. **Methods:** We use the 2D gel-based proteomics to isolate this protein from PeCa whole tissue extract and subject to MALDI TOF TOF analysis. On tissue digestion followed by MALDI Mass Spectrometry Imaging (MSI) analysis were perform, in fresh-frozen tumor and normal margins, to evaluate the spatial distribution of CRT in the tissue section. All experiments were conducted on an Autoflex MALDI-TOF/TOF, using FlexControl software to supervise the analysis and FlexImaging 4.0. **Results and Discussion:** The MALDI TOF TOF data integrated with MALDI-MSI visually resulted in an overexpression of CRT in tumor sample, revealed by the molecular images. It was the first time that CRT overexpression was related to PeCa. **Conclusion:** This preliminary data combined with histopathological analysis might represent the basis for further studies on the characterization of PeCa and for the development of biomarkers with significant clinical value.

Keywords: 2D gel, penile cancer, MALDI imaging, Calreticulin

CS05: PROTEOMICS AT PHARMA

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CS05.01 Application of Proteomics Technologies in the Pharmaceutical Industry

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Abstract: Proteomics technologies can be used in several key aspects of modern drug development: • to better understand disease diversity/subtypes • to understand the differences between patients • to identify the best drug targets • to provide biomarkers and diagnostic tests • to provide better, safer and more efficacious treatments I will present a few examples in this context and especially that mass spectrometry is becoming truly quantitative. One example will be chemical proteomics which is a mass spectrometry (MS)-based, affinity chromatography approach that uses immobilized small molecules as bait to capture and identify interacting protein complexes from an entire proteome. This technique has been successfully applied to kinase inhibitors, for exercises such as exploring their mode of action, and on natural products for target identification. A new area is targeted quantitative proteomics using the SRM approach. I will show examples where we were we applied a 250 plex quantitative assay in neuronal stem cells. Another example will be the discovery of biomarkers and the validation of them in clinical samples – what are the hurdles to overcome?

Keywords: drug development, biomarkers, Chemoproteomics, SRM

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CS05.02 Automated MRM - Applied to Quantification of Circulating Proteins Associated with Insulin Sensitivity Improvement following Bariatric Surgery

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Abstract: Targeted quantification of proteins using mass spectrometry based approaches are emerging as a core technology in the biomedical and clinical fields for addressing health-related queries. A significant challenge to standardization in MRM-based proteomic workflows is the variability caused by the multistep, highly manual nature of conventional sample preparations. If MRM based protein quantification is to enjoy widespread use in the clinic, then the technique must be standardized and automated for more precise protein quantification.To address such issues, an automated sample preparation workflow based on a robotic platform was developed, leading to the robust quantification of human plasma proteins.

It is well documented that the glycemic control is restored in diabetic patients after surgical treatment for morbid obesity as a consequence of the long term weight loss [1-3]. Interestingly, with metabolic surgical procedures that combine reduction of gastric volume with a rerouted digestive process, such as biliopancreatic diversion (BPD), improvement of insulin sensitivity generally occurs already within days after surgery [4]. In a clinical study, twenty morbidly obese male subjects scheduled for BPD were included and plasma samples were collected before, at the day and at several time points after surgery. Unbiased proteomics was performed on patient samples from two time points (before/after surgery) with the aim to identify proteins regulating insulin sensitivity. The automated and targeted MRM platform was subsequently used to verify the discovery findings. Quantification of a 21-plex protein panel was performed at nine time points during the first week after surgery, that enabled the monitoring of changes in protein concentrations. Preliminary data will be presented and discussed.

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CS05.03 Screening of Drug-Induced Protein Expression - Quantifying Cytochrome P450 Enzymes and Transporter

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Introduction and Objectives: One central issue in pharmacokinetics and -dynamics is the induction of drug metabolizing enzymes and transporter by drugs and drug candidates. The expression of such proteins can be increased by the factor 100 after drug administering. As a consequence the drug availability can drastically lowered. The quantification of these proteins can be assessed by mass spectrometry-based immunoassays with highest specificity. **Methods:** 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 and transporter. These antibodies are applied in an immunoaffinity step prior and quantified by high-resolution parallel reaction monitoring. Thus we are 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, tissue and tumors. Currently, assays targeting 23 ABC/ SLC transporters are under development. **Results and Discussion:** The established assays were used to analyze the CYP450 induction of 450 FDA-approved drugs. Primary hepatocytes from three donors 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. **Conclusion:** The established set-up allows to screen the induction effects of pharmaceutical compounds. Valuable hepatocyte material can be cultured in 96-well plate format and directly analyzed by ms-based immunoassays. The data-sets might allow predictive conclusions about novel drug candidates and their behavior in terms of CYP –induction in future.

Keywords: Cytochrome P450, Transporter, MS-based Immunoassay, Toxicology

CS05.04 Development of a Protease Inhibition Assay Based on Targeted Top down Quantitation

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Introduction and Objectives: Developing target-specific protease inhibitors has been a challenge in drug discovery, partly due to limited options of characterizing enzymatic inhibition when using endogenous protein substrate. A bottom-up proteomic approach using stable isotope-labeled substrate is typically required for accurate MS quantification. Yet when assessing protease inhibitor potencies it's preferable to measure the amount of uncleaved substrate. Also, digestion results in the loss of critical combinatorial PTM information. We demonstrate that targeted top-down quantitation using Tandem Mass Tags (TMT) can provide accurate quantitation of uncleaved substrate while retaining MW information. **Methods:** Various inhibitor concentrations were incubated with protease and substrate. TMT reactions were optimized from standard protocols. Samples were run on a Waters RPLC coupled to an Orbitrap Elite MS. LC-MS/MS data analyses were performed using ProSight PC and PepFinder. **Results and Discussion:** Calibration curves were generated using substrate of known concentration either with (reporter-ion ratios) or without (XIC peak area) TMT-label . Labeled substrates resulted in <10% error in quantitation versus >30% for unlabeled. Interestingly, even when an accurate precursor mass was undetectable, quantitation via the reporter-ion was unaffected. This was attributed to the higher S/N of the MS/MS liberated reporter-ion. Both amine-reactive and cysteine-reactive TMT yielded excellent linearity in quantitation at tested substrate concentrations. The cysteine-reactive labeling appeared to be more selective than amine-reactive TMT which resulted in undesired O-acylation of tyrosine residues. Finally, the method was applied to IC50 determination for several protease inhibitors. **Conclusion:** Top-Down substrate quantitation was successfully performed and enabled protease inhibitor IC50 evaluation. We continue to reduce O-acylation of tyrosine labeling via de-esterification or kinetic control to improve precursor spectrum homogeneity. This method could be broadly applied to biochemical assays in which accurate intact protein quantitation is necessary.

Keywords: protein quanittation, Targeted proteomics, Top down, drug development

CS05.05 The Proteome of Human Cells Is Altered by Toxins of Clostridium Dificile

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Introduction and Objectives: The anaerobe Clostridium difficile is one of the most common nosocomial pathogens and triggers antibiotic-associated gastrointestinal infections ranging from mild diarrhea to life-threatening pseudomembranous colitis. Toxins TcdA and TcdB are the two major virulence factors of C. difficile that specifically glucosylate and inactivate small GTPases. The consequences are reorganization of the cytoskeleton, loss of cell-cell contacts, and finally cell death. **Methods:** Comprehensive proteome analyses were conducted using data-dependent shotgun proteomics and human epithelial cell lines that had been treated with wild type or mutant toxins. Protein quantification was done by the SILAC technique. Long (24 h) and short term (5, 8 h) effects of TcdA and TcdB on Caco-2 and Hep-2 cells were analyzed. Proteins were analysed by gel-LC-MS and results were verified by western blot and MRM analyses. **Results and Discussion:** Wild type toxins induced considerable changes in the protein profile of epithelial cells. In case of TcdA more than 800 proteins of the over 6000 identified proteins were altered in their abundance. Regulated proteins were primarily involved in regulation, metabolic processes, endocytosis, organelle function, cell-cycle and cytoskeleton organisation. Glucosyltransferase deficient TcdA induces only changes after short incubation periods. After treatment with TcdB 183 of 5320 identified proteins were affected. These proteins are involved in signal transduction, cytoskeleton organisation, cell-cycle and cell death. The mutant TcdB revealed a pyknotic effect, which is glucosyltransferase independent but triggered the response of 129 proteins. Besides known targets of TcdA, glucosylation was additionally identified in Rap1(A/B), Rap2(A/B/C), Ral(A/B), and (H/K/N)Ras which had not been identified as TcdA targets before. **Conclusion:** This proteome analysis demonstrates that clostridial glucosylating toxins affect several cellular processes that have not been considered before.

CS05.06 ISDetect: Rapid, Semi-Automated Protein Terminal Characterization by Mass Spectrometry

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Introduction and Objectives: Confirmation of protein identity is essential for the production of protein reagents and novel biotherapeutics. Edman degradation remains the gold standard for such work, despite its significant limitations in speed, inoperability with blocked N-termini, and its inability to examine C-terminal sequence. Although MALDI in source decay (MALDI-ISD) mass spectrometry is a flexible alternative that alleviates these issues, its adoption as a high-throughput method of protein terminal characterization is hampered by the lack of a broadly applicable methodology to analyze MALDI-ISD data. Here we describe ISDetect, a robust software tool for automated protein terminal sequence verification. **Methods:** ISDetect utilizes MALDI-ISD mass spectrometry of intact proteins to generate fragment ladders from both termini. After a noise reduction step, the resulting spectrum is probed for sequence tags corresponding to the putative protein sequence. These tags are then extended to match all theoretical / observed ion pairs, and the resulting data are scored using a binomial model. **Results and Discussion:** ISDetect was validated by its application across a wide array of purified protein samples. In total, 5,916 high confidence ISD results were obtained from 2,991 analyses. ISDetect identified the N-terminus, C-terminus, or both termini for 1,593 of 1,787 (89%) different proteins analyzed, including 876 C-terminal sequences undetectable using Edman chemistries. Its sequence tag scoring approach also easily identified terminal modifications, including pyroglutamate and acetylation. ISDetect also provided superior speed to Edman degradation (5 minutes vs. 12 hours). Lastly, the method was also validated in depth by its application to interpret activity-based enzyme probes and recombinant proteins. **Conclusion:** ISDetect provides a robust, high-performance solution for the rapid confirmation of protein terminal sequences which has been validated over thousands of real-world samples. It provides a flexible, performant, and statistically-validated alternative to many cumbersome Edman degra-

dation workflows.

Keywords: high-throughput, maldi in source decay, edman degradation, computational approaches

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CS05.07 Label-Free Method for Profiling Human Liver Enzymes: Validation with QconCAT

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Introduction and Objectives: Xenonbiotic and drug-metabolizing enzymes (DME's) are involved in the bioconversion of xenobiotics including drugs, synthetic chemicals and environmental pollutants into inactive or active compounds. In pharmacological therapy, bioconversion can either lead to detoxification or activation of the drug, which has implications on treatment effectiveness and toxicity. Quantitative profiling of the drug-metabolizing sub-proteome can be used in the characterization of liver drug metabolism profiles in individual patients which can be a major step towards stratified or personalized medicine. **Methods:** Label-free quantification of approximately 70 drug-metabolizing enzymes in three individual human livers was carried out using a UPLC-nanoAcquity liquid chromatography system (Waters) attached online to a Synapt G2-Si HD quadrupole-traveling wave-ion mobility-time of flight mass spectrometer (Q-TWave-IMS-ToF-MS/MS) (Waters) with an LC program of 70 minutes. A range of cytochrome P450 and uridine 5'-diphosphate glucuronosyltransferase (which are the main drug-metabolizing enzymes in the liver) were quantified previously using QconCAT SRM assays [1] and were used to externally validate the label-free quantification. **Results and Discussion:** The label-free approach showed a high level of precision and reproducibility. The concentrations of the liver enzymes quantified using the two methods (untargeted vs. targeted proteomics) were shown to agree (R^2 = 0.50, Rs = 0.80, $p < 0.0001$***, n = 38 enzymes, three individual livers). A range of 70 drug-metabolizing enzymes were quantified. **Conclusion:** The label free methodology described here can be used for the global profiling of enzymes and transporters in different biological in vitro and in vivo systems relevant to pharmacology. [1] Achour B, Russell MR, Barber J, Rostami-Hodjegan A (2014) Simultaneous quantification of the abundance of several cytochrome P450 and uridine 5'-diphospho-glucuronosyltransferase enzymes in human liver microsomes using multiplexed targeted proteomics. Drug Metab Dispos 42(4): 500-510.

Keywords: Drug-metabolizing enzymes, Human liver, Label free proteomics, QconCAT

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CS05.08 Phosphoproteomics in Drug Discovery – Application to Cancer Drug Resistance

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Introduction and Objectives: Phosphoproteomics approaches are being used to support drug discovery throughout the pipeline, from unravelling basic disease mechanisms through compound profiling all the way to the discovery of biomarkers in patients. Here we describe the application to the elucidation of resistance mechanisms to an inhibitor of the PI3K pathway. **Methods:** Not applicable.
Results and Discussion: The PI3Kpathway is hyperactivated in many cancers, including 70% of breast cancers. Pan- and isoform-specific inhibitors of the PI3K pathway are currently being evaluated in clinical trials. In order to anticipate potential molecular mechanisms of resistance to the p110α isoform-selective inhibitor BYL719, we developed resistant breast cancer cell lines and assessed the concomitant changes in cellular signaling pathways using unbiased phosphotyrosine proteomics. We found an increase in IGF1R, IRS1/IRS2 and p85 phosphorylation in the resistant lines. Co-immunoprecipitation experiments identified an IGF1R/IRS/p85/p110β complex that causes the activation of AKT/mTOR/S6K and stifles the effects of BYL719. Pharmacological inhibition of members of this complex reduced mTOR/S6K activation and restored sensitivity to BYL719. **Conclusion:** Our study demonstrates that p110β confers resistance to BYL719 in PIK3CA mutant breast cancers. This provides a rationale for the combined targeting of p110α with IGF1R or p110β in patients with breast tumors harboring PIK3CA mutations.

Keywords: signaling, phosphoproteomics, cancer, PI3K pathway

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CS05.09 Monkey, Dog, Rat and Men - Safety Biomarker across Species

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Introduction and Objectives: There is an unmet need in pharmaceutical industry for reliable assays to detect organ toxicity induced by drug candidates. This is evidenced by the fact that from 175 drugs pulled from the market after approval in the last 50 years one third was withdrawn due to liver and/or kidney damage. During the drug development process, mandatory tests need to be carried out in different animal species, such as rodents, dogs, and non-human primates to exclude organ toxicity. To date such studies are mainly dominated by time-consuming, difficult-to-reproduce histological analyses. Protein-based tests for determining drug-induced liver injury (DILI) and kidney injury (DIKI) in humans are currently being clinically validated by consortia such as SAFE-T or PSTC. We are complementing these efforts by validating these biomarkers in animal models, such as monkey, dogs, and rodents, using MS-based immunoassays. **Methods:** Proteins in species' urine or plasma are enzymatically digested into peptides. Synthetic isotope-labelled reference peptides are added in known concentrations. Multi-specific antibodies (TXP-antibodies) targeting common C-terminal amino acid motifs present in peptides originating from potential protein biomarkers are used to enrich endogenous and isotope-labelled peptides followed by quantitative analyses using nanoLC-Parallel Reaction Monitoring-MS. The use of our group-specific TXP-antibodies allows protein analysis of specimen across species using the same antibody for the same assays. **Results and Discussion:** We developed MS-based Immunoassays applicable for screening plasma and urine for liver (DILI) and kidney (DIKI) injury protein biomarkers across 5 species; human, cynomolgus, mouse, rat and dog. We investigated the potential DIKI biomarkers aquaporin 2, podocin, synaptopodin, kidney-injury molecule 1 and osteopontin in urine samples

from toxicity studies of in cynomolgus monkeys, rodents and humans. **Conclusion:** Applying our MS-based immunoassays using group-specific TXP-antibodies allows the quantification of biomarkers in bio-fluids of all pharma industry-relevant model organisms. Our results strongly support the development of translational DIOI-biomarkers working across species.

Keywords: Safety Biomarker, MS-based Immunoassay, Animal Models, SISCAPA

CS06: NEW TECHNOLOGICAL ADVANCES IN PROTEOMICS

CS 06: NEW TECHNOLOGICAL ADVANCES IN PROTEOMICS
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CS06.01 Imaging Mass Cytometry: A Novel Imaging Modality to Visualize Dozens of Biomarkers in a Targeted and Simultaneous Manner in Tumor Samples

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Abstract: Each year approximately 1.1 million new cases of breast cancer are diagnosed and about 0.3 million women worldwide die from this disease. Tumor metastases, relapse, and resistance to therapy are the main causes of death in breast cancer patients. Communication between heterogeneous cancer cells and normal cells in the so-called tumor microenvironments (TME) drives cancer development, metastasis formation, and drug resistance. To understand the TME and its relationship to clinical data, comprehensive investigation of the components of the microenvironment and their relationships is necessary. We recently invented a novel imaging modality based on mass cytometry, called imaging mass cytometry (IMC) that enables this type of study. In IMC, tissues are labelled with antibodies that carry pure metal isotopes as reporters. Theoretically 135 antibodies can be visualized simultaneously; in practice, we routinely quantify 44 markers. The antibody abundance is determined using laser ablation coupled to inductively coupled plasma mass spectrometry.

Here we show the results of the analysis of hundreds of breast cancer samples by IMC. To extract biological meaningful data and potential biomarkers from this dataset, we developed a novel computational pipeline for the interactive and automated analysis of large-scale, highly multiplexed tissue image datasets. Our analysis revealed a surprising level of inter- and intra-tumor heterogeneity and diversity within known human breast cancer subtypes as well as the stromal cell types in the TME. Furthermore, we identified cell-cell interaction motifs in the tumor microenvironment that correlated with clinical outcomes. In summary, our results show that IMC provides targeted, high-dimensional, subcellular resolved images of tissue samples. The identified spatial relationships among complex cellular assemblies have potential as biomarkers. We envision that IMC will enable a systems biology approach to diagnosis of disease and will ultimately guide treatment decisions.

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CS06.02 Advances in Proteomics Based upon Ion Mobility in Structures for Lossless Ion Manipulations

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Abstract: Mass spectrometry (MS)-based proteomics measurements are having profound impacts on broad areas of biological research, as well as related areas that include metabolomics, lipidomics and glycomics. More and more, advances in the quality, resolution, and the speed of e.g. polypeptide and protein separations are arguably as important as mass spectrometric developments in improving the sensitivity and coverage of proteomics measurements. Both liquid phase, e.g., using liquid chromatography, and increasingly gas phase ion mobility separations, respectively, provide a basis for increasing the quality of proteomics measurements, such as the completeness of protein coverage. While these capabilities are challenged by very small sample sizes, the recent development of more efficient nanoelectrospray ion sources and MS interfaces has helped enable ultra-sensitive measurements. Progressively more advances involve gas phase ion manipulations that are conducted between the ion source and m/z analyzer. These manipulations include: ion transport through regions of elevated pressure, trapping, reactions (both ion-molecule and ion-ion), and mobility-based separations. This presentation will discuss the utility of ion mobility separations for proteomics applications, and describe new developments based upon long path length Structures for Lossless Ion Manipulations (SLIM) that enable very fast high resolution separations, as well as the use of other gas phase ion manipulation approaches having broad utility for facilitating MS analysis capabilities. The SLIM developments will be discussed with regard to their sensitivity, measurement throughput, and their utility for both broad (proteome-wide) and targeted quantitative measurements. The presentation will conclude with consideration of pending developments enabled by SLIM and their potential impacts for mass spectrometry-based proteomics.

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CS06.03 Methods to Increase Reproducibility of a Multi-Analyte MRM MS Assay for Analysis of Plasma Samples

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Introduction and Objectives: Xpresys® Lung [Sci Transl Med 5, 207ra142 (2013); J Thorac Oncol 10, 629 (2015)] is the first multi-analyte MRM MS proteomics assay to be offered in a regulated clinical laboratory. Xpresys® Lung analyzes plasma samples with the intended use of rescuing patients with benign lung nodules from unnecessary invasive procedures. We present here methods to control both pre-analytical and analytical variation in routine clinical testing over time. **Methods:** Plasma samples were analyzed using immunoaffinity-based protein depletion coupled with MRM-MS. Proteins were quantified using InteQuan [Clin Proteomics 12, 3 (2015)]: (1) Stable isotope-labeled internal standard peptides were spiked into samples after protein digestion to control variation in post-digestion procedures; (2) The abundance of six carefully selected endogenous proteins were used to normalize the abundance of proteins of interest to minimize systematic variation in pre-analytical and analytical procedures. Both positive controls and negative controls (blank) were analyzed along with clinical samples to monitor the integrity of analytical procedures. Furthermore, a sophisticated quality control matrix was developed to assess data quality per experimental batch. **Results and Discussion:** Xpresys® Lung was tested on a human plasma standard sample over six months. Despite of major changes in experimental settings (e.g., different operators, different depletion columns, major instrument repairs, different sample loading volumes, different reagent lots), the five diagnostic proteins were measured with high precision: FRIL (12 ng/ml, CV 15.9%), COIA1 (35 ng/ml, CV 16.1%), ALDOA (250 ng/ml, 11.0%),

LG3BP (440 ng/ml, CV 8.6%), and TSP1 (510 ng/ml, 20.3%). The CV of the classifier score was 24.5% (mean 0.294, SD 0.072) in the study and 14.9% (new lot, mean 0.339, SD 0.050) in commercial testing over six months. **Conclusion:** We demonstrated that Xpresys® Lung is reproducible and robust over time. Our methods can be informative for others to develop similar multi-analyte MRM MS proteomics assays.

Keywords: multiple reaction monitoring, Plasma or serum analysis, multi-analyte proteomics assay, Clinical proteomics

CS06.04 PALM (Pulse Azidohomoalanine Labeling in Mammals): Tissue Analysis of Newly-Synthesized Proteins

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Introduction and Objectives: We developed PALM (Pulse Azidohomoalanine Labeling in Mammals) analysis to identify and quantify NSP from rodent tissues by mass spectrometry. Azidohomoalanine (AHA) is modified methionine that is accepted by the endogenous methionine tRNA and insert into proteins in vivo. The objectives were 1) to test the safety of azidohomoalanine(AHA) in whole animals 2) define the time required for adequate AHA incorporation for MS analysis and 3) develop new tools to quantitate NSP in animal tissues. **Methods:** A special rodent diet was developed where methionine was replaced with AHA. Mice were given the PALM diet for different time periods and multiple tissues were tested for incorporation of AHA with immunoblot analysis and MS analysis using high resolution mass spectrometers. To quantify the NSP, a heavy biotin-alkyne was synthesized. To test this novel quantitation tag, differences in NSP in liver tissue induced by the transgenic removal of liver kinase B1(LKB1) were quantified. Finally, to the improve NSP quantitation pipleline, we investigate the utility of a novel compound, heavy-AHA (H-AHA). **Results and Discussion:** Mice were placed on the PALM or control diet and no differences in gross behavior, physical appearance or weight were observed between the mice on the two diets. By immunoblot analysis, AHA proteins were detected at 2 days in all tissues, but there was a significant increase at 4 days and a smaller increase at 6 days. With brain tissue from a mouse on the PALM diet for 4 days, thousands of AHA peptides were identified by MS analysis. To demonstrate usefulness of the heavy biotin alkyne tags, LKB1 KO and one WT livers were analyzed demonstrating excellent trechnical and biological reproducibility. Finally, H-AHA allows mixing of samples prior to extensive sample prepration for reduce systematic errors in quantitation. **Conclusion:** In conclusion, PALM analysis allows the global identification and quantification of NSP in mammalian tissues.

Keywords: azidohomoalanine, pulse labeling, newly synthesized proteins, quantitation

CS06.05 Extending the Reach of Data Independent Acquisition

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Introduction and Objectives: 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 variable width Q1 windows can improve peptide detection and increase sample coverage. Many labs are now using DIA to perform larger scale quantitative proteomic experiments with solid reproducibility on 1000s of proteins in complex matrices. As this technique increasingly proves to be a solid tool for biomarker research, larger sample sets are being analyzed, driving the need for further investigation of workflow improvements for throughput and robustness. **Methods:** Here microflow LC was investigated in combination with SWATH™ acquisition on a number of complex matrices, to assess depth of coverage and robustness relative to current nanoflow strategies. The MS analysis was performed on a TripleTOF® System equipped with an Eksigent nanoLC 425 with microflow modules. **Results and Discussion:** A number of column diameter and lengths were explored, along with different gradient times and sample loads, to understand the workflow options in this flow regime. Optimization of SWATH™ acquisition settings for each chromatographic condition was done, to fully understand impact on results. Key workflow recommendations have been established to provide researchers additional strategies for performing large scale, higher throughput SWATH™ acquisition studies. **Conclusion:** SWATH™ acquisition coupled with microflow chromatography provides an additional workflow options to researchers with higher throughput and robustness needs. When more sample is available to move to the higher flow rate regime, very high reproducibility is achievable with faster run times, while still achieving reasonable depth of coverage.

Keywords: SWATH, microflow, Chromatography

CS06.06 Rapid and Automated Quantitation of Candidate Disease Biomarkers in Mouse Plasma and Tissues

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Introduction and Objectives: MRM-MS with stable isotope-labeled standards (SIS) is a powerful strategy for precisely quantifying proteins in biological samples. However, only a limited number quantitative MRM assays have been developed for the mouse model. Various methodologies under the MRM-with-SIS-peptide approach were therefore systematically evaluated towards the quantitation of disease-associated protein panels in mouse plasma and various tissues, with the quantitation of 200 mouse plasma proteins being our short-term goal. **Methods:** Towards our objective, an expanding library of >800 proteotypic peptides (corresponding to >450 mouse proteins) were selected using our in-house developed PeptidePicker software. These peptides were synthesized, purified, and characterized in their isotopically labeled state, then optimized for improved detectability. Targets were subsequently evaluated against several methodological variations using mouse plasma and

tissues (namely heart, lung, and brain). With automation in mind on the AssayMap Bravo platform, the final method involves urea-based sample denaturation (instead of our conventional deoxycholate surfactant) prior to overnight tryptic digestion, SIS peptide addition, and UHPLC/MRM-MS on an Agilent 6490 triple quadrupole. To qualify the targets for quantitation, rigorous detectability and interference testing was performed. **Results and Discussion:** The interference testing resulted in 264 qualified peptides (corresponding to 174 proteins) in mouse plasma. Quantification of these qualified peptides using standard curves and a concentration-balanced SIS mixture is currently underway and will ultimately be performed with analytical replicates to determine reproducibility. In parallel, mouse tissues have been evaluated, with 424 peptides (corresponding to 270 proteins), 398 peptides (257 proteins), and 354 peptides (227 proteins) currently qualifying in mouse lung, heart, and brain tissue, respectively. **Conclusion:** Mouse is the most widely used animal model in health-related research, and the development of sensitive, highly multiplexed quantitative MRM assays, such as this one, should prove useful in advancing the mouse protein biomarker pipeline so that deeper questions in systems biology can be addressed.

Keywords: mouse biosamples, multiple reaction monitoring (MRM), proteins, quantitation

CS 06: NEW TECHNOLOGICAL ADVANCES IN PROTEOMICS
MONDAY, SEPTEMBER 28, 2015 – 14:30 – 16:20

CS06.07 It's Easy: Ultra-High Resolution Separations for the Complete Analysis of the Yeast Proteome

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Introduction and Objectives: Yeast is one of the most important model organisms. First, the genetic manipulation of yeast is easy and cost effective. Second, there are genes in yeast and mammals that encode similar proteins, providing the opportunity to learn about human genes and protein functions from the study of yeast homologs. Finally, understanding protein dynamics and protein turnover during the lifetime of the yeast is very useful for the understanding of biological mechanisms in living systems. We have developed a single-shot proteomic workflow utilizing ultra-high resolution chromatography in combination with Orbitrap mass spectrometry detection that allows the dynamic study of the yeast proteome in a streamlined fashion. **Methods:** Small-scale (50 ml) fermentations were carried out in triplicate in 5% malt extract over 48 hours. Cells were collected at 5 different time points. A modified Mass Spec Sample Prep Kit for Cultured Cells (Pierce, Rockford IL) was used to prepare the proteomic samples. Peptide digests were analyzed by LC-MS/MS analysis on a Q Exactive HF mass spectrometer. Database searching was performed using Proteome Discoverer 2.0 with an in-house built database. **Results and Discussion:** Our analyses resulted in more than 4100 proteins identified, which matches very closely to the number of estimated proteins expressed by yeast at a given time point of its life cycle. Correlation among runs is above 94% allowing very accurate and sensitive relative quantification. As expected, three different yeast development phases could be differentiated. Firstly, the onset of the fermentation with osmotic stress activated, secondly, the middle phase with an increased amino acid metabolism and finally the last phase, where nutrient depletion occurred. **Conclusion:** This work provides the methodology and theoretical framework necessary to conduct straightforward proteome-wide analyses of the biologically important yeast model organism under different conditions, and may also open the door to better methodos for expressing therapeutically important human proteins in yeast.

Keywords: Full Proteome Coverage, Yeast Proteome, Orbitrap, UPLC

CS 06: NEW TECHNOLOGICAL ADVANCES IN PROTEOMICS
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CS06.08 Enhanced Trypsin Digestion for Improved Biomarker Sensitivity and Peptide Identification

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Introduction and Objectives: Use of suboptimal trypsin digestion methods makes biomarker analysis artificially challenging. A comparison of multiple methods as well as the development and implementation of a simplified overarching strategy resulted in dramatic improvements of sensitivity and peptide generation as presented here. **Methods:** A comparison was made of sequencing grade trypsin, additive/solvent enhanced digestion, and elevated temperature methods. These methods were applied to apolipoproteins, thyroglobulin and C-reactive protein in purified forms and complex matrices. Samples were analyzed using a Thermo Velos Pro IonTrap and TopTen as well as SRM based detection methods. **Results and Discussion:** Based on our screening and a survey of the literature we focused on three peptides corresponding to thyroglobulin, four to C-reactive protein, and one peptide to each Apo A, B100, C-II, C-III, and Apo-E. For each individual protein of interest, samples were prepared by adding 2ug of target to each well of a thermophilic immobilized enzyme containing 150µL digest buffer and plasma (2-50uL), then incubated at 70°C and 1400rpm for 2 hours or less.For each target the digestion method above was compared to established methods from the literature. For the three peptides associated with thyroglobulin the immobilized enzyme give a 2, 17 and 1400 fold increase in signal respectively. For three of the four peptides associated with C-reactive protein a 12, 14 and 21 fold increase in signal was observed with the fourth peptide only observed using the immobilized enzyme. For peptides associated the apolipoproteins use of an immobilized enzyme give equivalent results for all peptides. **Conclusion:** The thermally stable immobilized trypsin enabled the use of various temperatures to increase the speed and quality of results, while offering the ability to remove the need of agents that can harm the digestion process.

Keyword: trypsin, digestion, biomarker, proteomics

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CS07.01 Discovery of Brain Biomarkers and Their Translation to Clinical Settings

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Abstract: There is currently few brain biomarker that can add utility to the classical clinical and imaging parameters. This is expecially true for the diagnosis, prognosis and treatment follow-up of acute brain inhuries. Re-

search in the last decades have unravelled a number of potential candidates that have shown preliminar good performance including glial fibrillary acid protein (GFAP), neuron specific enolase (NSE), and S-100ß. However, none of them are used routinely in clinical settings due to a lack of sensitivity and/or specificity. Neuroproteomic has the great potential to highlight better candidate biomarkers. Here we will present the strategy that was carried out to discover, verify and validate very specific intent-of-use markers in the fields of traumatic brain injury, stroke and subarachnoid hemorrhage.

Keywords: brain, biomarkers, stroke, traumatic brain injury

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CS07.02CPTAC Omics Data Combined with TCGA Omics Data Produces a Unified Snapshot of Tumors

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Abstract: The NCI Clinical Proteomic Tumor Analysis Consortium (CPTAC) program was established to identify and quantitate proteins that derive from alterations in cancer genomes. CPTAC, which involves a national consortium of researchers who apply state-of-the-art standardized proteomic workflows to genomically-characterized tumors (such as those from The Cancer Genome Atlas, TCGA), is now revealing new biological insights that help us better to understand the proteogenomic complexity of cancer. This seminar will highlight how deep proteomic analysis in combination with deep genomic analysis produces a unified understanding of tumor biology, and discuss the public resources (data, assays, reagents) being developed for future studies by the cancer research community.

Keyword: cancer, proteogenomics, translational, omic

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CS07.03 Serum Diagnostic Glycoprotein Biomarkers for Esophageal Adenocarcinoma

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Introduction and Objectives: Incidence of esophageal adenocarcinoma (EAC) has been rising and prognosis remains poor. Economic blood test screening for disease progression in Barrett's esophagus (BE) patients may facilitate early diagnosis and intervention. Here, we investigated the potential of glycosylation changes of serum proteins as biomarker candidates to distinguish healthy, BE and EAC phenotypes. **Methods:** Glycosylation changes of serum glycoproteins between healthy, BE and EAC patient groups were measured by lectin affinity in independent discovery and verification cohorts. The discovery phase (n=29) used a panel of 20 lectins for pull-down and tandem mass spectrometry to identify and quantify bound proteins. 41 protein candidates and 6 lectins were selected for verification against 60 independent samples using targeted mass spectrometry. The verified candidates were assessed for the effect of confounding factors, reflux frequency, obesity, smoking history and alcohol consumption, using a total of 39 disease-free population samples as the healthy comparison group. **Results and Discussion:** This study discovered and verified 45 glycoforms as candidate serum markers, as measured by lectin affinity. The top candidate for distinguishing healthy from BE was NPL lectin-reactive Apolipoprotein B-100 (P value = 0.0231; AUROC = 0.71); BE vs EAC, (AAL lectin-reactive complement component C9 (P value = 0.0001; AUROC = 0.85); healthy vs EAC, EPHA lectin-reactive gelsolin (P value = 0.0014; AUROC = 0.80). A careful assessment of possible confounder covariates led to 40 glycoforms as potentially useful serum diagnostics, amongst which the combination of 8 glycoforms showed an improved AUROC of 0.94 to discriminate EAC from BE. **Conclusion:** This preclinical study confirmed the potential of serum glycoprotein changes as biomarkers for diagnosis of Barrett's metaplasia and associated adenocarcinoma. The 40 verified candidate glycoforms will need to be validated in a larger cohort. We envisage the potentially rapid translation of the final validated markers by using lectin-immunoassays with existing antibodies.

Keywords: Cancer diagnosis, Glycoproteomics, Biomarker discovery

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CS07.04 Plasmatic RBP4 and GFAP as Biomarkers to Differentiate Ischemic from Hemorrhagic Stroke

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Introduction and Objectives: Differentiate ischemic stroke (IS) and intracerebral hemorrhage (ICH) at the prehospital setting could achieve reductions in time to acute therapies, therefore improving outcome. Opposite to current approaches based on expensive tools such as portable CT scans, our aim was to identify biomarkers to differentiate acute IS from ICH, with the purpose of building a panel easily applicable in the prehospital setting to accelerate stroke treatment. **Methods:** We screened a library of 177 proteins (SearchLight) with plasma samples from IS (N=36, pooled in 9 pools of 4 samples each) and ICH (N=10) collected <6h after symptoms onset. Proteins with a p-value<0.01 between IS and ICH were selected for a first replication in new individual samples (N=16/group) measured by simple or multiplex ELISA. Proteins overcoming this phase (p-value<0.05), entered into a second replication (N=38 IS, N=28 ICH) together with already known markers (GFAP, RAGE). Apart from basic statistical analyses, discrimination between IS and ICH patients when biomarkers were added to clinical information was assessed by means of AUC and integrated discrimination improvement (IDI) index. **Results and Discussion:** Among 177 proteins, PEDF, APOB100, RBP4, VEGF and SHBG differentiated between IS and ICH strokes in the discovery phase. This was confirmed in the first replication for PEDF, APOB100 and RBP4. In the second replication, high RBP4 levels in IS maintained significantly, together with high GFAP levels in ICH. Cut-off points with high specificity for both biomarkers were entered into a model with hypertension, age, initial severity and gender, increasing the discrimination between IS and ICH in a 29% and reaching an AUC of 0.875. **Conclusion:** If replicated in larger cohorts, this panel containing RBP4 and GFAP combined with other biomarker candidates, might be specific enough to be translated to the clinical setting and to help in hyperacute treatment decision-making in stroke.

Keyword: biomarker, protein array, stroke

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CS07.05 Neutrophil Extracellular Traps in Ulcerative Colitis - A Proteome Analysis of Intestinal Biopsies

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Introduction and Objectives: Inflammatory bowel diseases, including Ulcerative colitis affects 2.5–3 million people in Europe. The lifelong conditions significantly reduce the quality of life of the patients, and compose a significant economic burden for society. The etiology of these diseases remains incompletely explained. We, therefore, performed the most thorough proteome-based study of ulcerative colitis colon tissue, based on number of analyzed proteins, and have for the first time observed neutrophil extracellular traps in ulcerative colitis, detected by proteomics and verified by microscopy. **Methods:** We performed a comparative proteome analysis of non-inflamed mucosal colon biopsies from 10 patients with ulcerative colitis and 10 controls. The proteome of the biopsies were characterized by high throughput label-free quantitative proteomics, and the biopsy histology was analyzed by microscopy. **Results and Discussion:** We quantified 5,711 different proteins in the colon biopsies. Forty-six proteins had a statistically significant difference in abundance between the ulcerative colitis colon tissue and controls. Eleven of the proteins with increased abundances in the ulcerative colitis biopsies were associated with neutrophils and neutrophil extracellular traps. The abundance of calprotectin and lactotransferrin in the tissue correlated with the degree of tissue inflammation as determined by histology. The findings were validated by microscopy where an increased abundance of neutrophils within the mucosal tissue was found. Additionally, we confirmed the presence of neutrophil extracellular traps by extracellular DNA in the ulcerative colitis colon tissue. **Conclusion:** We found an increased abundance of several proteins hitherto not associated with innate immunity and neutrophils in non-inflamed mucosal colon biopsies from ulcerative colitis patients. The increased abundance of these antimicrobial compounds points to the stimulation of the innate immune system in the etiology of ulcerative colitis. Our findings demonstrate that even though remission has been achieved on the surface of the ulcerative colitis colon tissue, a chronic condition is still present within the morphologically normal tissue.

Keywords: neutrophil extracellular traps, proteomics, microscopy, Ulcerative colitis

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CS07.06 Targeted Discovery of Subtype-Differentiating Biomarkers for Early Diagnosis of Breast Cancer

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Introduction and Objectives: Time of diagnosis of breast cancer (BC) is the single most important factor determining disease outcome. Moreover, breast cancer is a molecularly heterogeneous and the different BC subtypes notably include ER+, HER2+ and triple negative (TN) types that are treated as distinct diseases with the two latter being rarer, but more aggressive. A blood-based early diagnostic for cancer is desirable, but will need to address (i) breast cancer heterogeneity; (ii) measure biomarker proteins at very low concentration to identify tumors as they are still small. We established the antibody colocalization microarray (ACM) that can measure 108 proteins with an LOD of 0.6 pg/ml (=35 fM). Here, using the ACM, we profiled the blood of controls and BC patients to identify candidate biomarkers. **Methods:** Individuals with or without a suspicious mammogram were recruited and serum samples collected at the time of the mammogram, biopsy or before surgery from healthy women including (a) normal controls (29) and (b) women with benign lesions (30), and cancer patients, (c) ER-/HER2- (31,TN), (d) ER-/HER2+ (28), (e) ER+/HER2+ (34) and (f) ER+/HER2- (48). 35 µL of sample diluted 1:3 and 1:50 was analyzed with the ACM and R/Bioconductor. **Results and Discussion:** Using the healthy cohort as reference, we found 7 proteins differentially expressed compared to cancer patients, and when comparing healthy vs BC subtypes, 0 for ER+HER2- or ER-HER2+, 3 for ER+HER2+ and 4 for ER-HER2-. Concordant with these results, when considering all ER- disease (both HER2+ and TN) 7 proteins were identified, but different from the full cohort. **Conclusion:** These results indicate that (i) changes in protein concentration in the blood as result of BC development may be detected and (ii) different BC subtypes show different profiles. By considering BC subtypes it may be possible to establish blood-based BC diagnostics, and distinguish the most aggressive lesions (HER2+ and TN).

Keywords: biomarkers, Subtype specific, Breast cancer, Early diagnosis

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CS07.07 Quantitative Proteomic Analysis of Microdissected Oral Epithelium for Cancer Progression

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Introduction and Objectives: Predictive biomarkers are urgently needed for the detection and progression of oral cancer. The objective of this study was to discover oral cancer biomarkers with high sensitivity and specificity by using quantitative proteomics approaches coupling with specific study design. **Methods:** Morphologically malignant, epithelial dysplasia, and adjacent

normal epithelial tissues were laser capture microdissected (LCM) from 19 patients and used for proteomics analysis. Total proteins from each group were extracted, digested and then labelled with corresponding isobaric tags for relative and absolute quantitation (iTRAQ). Labelled peptides from each sample were combined and analyzed by liquid chromatography-mass spectrometry (LC-MS/MS) for protein identification and quantification. Selected candidate protein biomarkers were verified by immunoassays. **Results and Discussion:** In total, 500 proteins were identified and 425 of them were quantified by iTRAQ based quantitative proteomic approaches. When compared with adjacent normal oral epithelium, 17 and 15 proteins were consistently up-regulated or down-regulated in malignant and epithelial dysplasia, respectively. Half of these candidate biomarkers were discovered for oral cancer for the first time, which were promising candidates for further translational research. Protein Cornulin was initially confirmed in tissue protein extracts and further validated in saliva samples. Myoglobin and S100A8 were pre-validated by tissue microarray, which could differentiate cancer patients from normal control and dysplasia subjects. The molecular mechanisms of these biomarkers for the early detection of oral cancer were analyzed and discussed. **Conclusion:** These data collectively demonstrated that the proteomic biomarkers discovered through this strategy are potential targets for oral cancer detection and progression as well as salivary diagnostics.

Keywords: Oral epithelium, Laser capture microdissection, cancer progression, biomarkers

CS07.08 Method Comparison of an Immuno-MALDI Plasma Renin Activity (PRA) Assay with Two Clinical Methods

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Introduction and Objectives: Primary aldosteronism (PA) is by far the most common form of secondary hypertension accounting for approximately 10% of all hypertensive patients. Screening with plasma renin level is crucial, as early diagnosis is associated with cardiovascular risk reduction and better patient outcome. While conventional immunoassays are available to measure PRA, the recent introduction of an iMALDI-based approach for PRA determination has drawn attention of clinical laboratorian. The aim of this study is to validate the iMALDI PRA assay against two clinical methods using patient samples. **Methods:** 140 patient samples were collected at the Jewish General Hospital in Montreal for comparison on four different instruments. For diagnostics purposes, PRA were determined using Diagnostics Biochem Canada (DBC) enzyme-linked immunosorbent assay (ELISA) in our clinical laboratory. PRA determination by iMALDI was done by two different MALDI-TOF instruments (Bruker Microflex LRF and AB Sciex 4800) with an Agilent Bravo automated liquid handling system. 40 of the 140 clinical samples were also analyzed using clinically employed LC-MS/MS (AB Sciex) method. Bio-Rad EQAS external quality control results were also correlated with all methods. **Results and Discussion:** iMALDI PRA assay showed high-throughput capability of analyzing up to 360 samples per run. It also revealed strong correlations with two currently employed clinical methods for PRA determination. Regression analyses showed R-squared values of ≥ 0.92 and ≥ 0.97 when compared to the clinical DBC ELISA and LC-MS/MS assay respectively. On different MALDI TOF instruments (Bruker Microflex and AB Sciex 4800), iMALDI PRA assay showed excellent robustness by yielding an R-squared value of ≥0.99. **Conclusion:** Clinical implementation of the high-throughput iMALDI PRA assay will still require studies of reference ranges, and cost-effectiveness, but through optimization of reagents, protocols, robotic systems, and software, the entire iMALDI platform for PRA determination has been automated into a robust, and user-friendly diagnostic platform applicable for use in clinical laboratories.

Keywords: Primary Aldosteronism, Renin, Clinical proteomics, iMALDI

CS07.09 Targeted Quantitation of Human Gastric Fluid Proteins for Gastric Cancer Detection

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Introduction and Objectives: Gastric fluid is a potential source of gastric cancer (GC) biomarkers becauseof its anatomic proximity to the site of disease. We have previously highlighted that the intrinsicproperties of human gastric fluid presents significant challenges for biomarker discovery andvalidation using conventional protein-centric approaches. To overcome these difficulties, we usedpeptide-centric approaches to identify potential GC biomarkers and developed MRM-based assays fortargeted protein quantitative analysis. **Methods:** Gastric fluids obtained from GC patients and non-cancer controls were analyzed usingiTRAQ® method to identify potential biomarkers. We then developed standard-flow LC-MRM/MSassays to quantify and evaluate the clinical utility of candidate biomarkers. **Results and Discussion:** Using two markers as examples, our MRM analysis showed that GKN1 was down-regulated (p<0.01) and TFF3 was up-regulated (p<0.01) in GC patients, consistent with our iTRAQ® results.ROC analysis revealed a sensitivity and specificity of 76.1% and 90.3% for GKN1 and 64.4% and61.3% for TFF3. The combination of GKN1 and TFF3 showed improved sensitivity and specificity of85.9% and 80.6% respectively, compared to each marker alone. **Conclusion:** We identified several candidate GC biomarkers in human gastric fluid using iTRAQ®approach. We developed MRM-based assays for 2 candidate biomarkers as examples, for multiplexedmonitoring in human gastric fluid using standard-flow LC-MS. We found that the combination ofGKN1 and TFF3 is potentially useful for early detection of GC. Our study highlights the applicationof LC-MRM/MS for targeted quantification of disease markers in human body fluids and its potentialclinical utility.

Keywords: Gastric Fluid, iTRAQ, multiple reaction monitoring (MRM), Gastric cancer

CS07.10 Correlation of Gene and Protein Tissue Expression for Treatment Decisions in Early Stage Lung Cancer

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Introduction and Objectives: Early stage lung cancer can successfully be treated by surgical resection. Chemotherapy after surgery can provide additional survival benefit for patients at risk of relapse and sensitive to the therapy. Reliable prognostic and predictive biomarkers would identify these patients. Here, we investigate correlations between mRNA and protein abundances in tissue as prognostic and predictive biomarkers for early stage lung cancer. **Methods:** We performed whole transcriptome sequencing (RNA-seq) in parallel with multidimensional LC-MS/MS protein analysis in forty-four early stage non-small cell lung cancer tissue samples. Each cohort consists of equal numbers of patients with disease free survival (good prognosis) or tumor recurrence (lower prognosis) after treatment. Half of the patients received chemotherapy after surgery. Bayesian models were applied to quantify and score relation between mRNA and protein abundance among groups of patients. **Results and Discussion:** Parallel transcript and protein abundance was available for more than two thousand genes. mRNA and protein abundance correlated poorly in the cohort (spearman correlation, ρ= 0.38). This was reflected in the groups of disease free (ρ= 0.35) and disease recurrent patients (ρ= 0.39). We investigate this correlation difference among the two groups to identify candidate biomarkers. Utilizing Bayesian models we observed over one hundred genes with differential transcript-to-protein abundance between the two groups. Among them were previously proposed lung cancer biomarkers such as ALDH7a1 or MUC1. Applying predictive modeling we identified an initial pool of 39 candidate biomarkers prognostic for early stage lung cancer. A subset of which indicates additional predictive potential, separating patients benefitting from chemotherapy after surgery. **Conclusion:** We explored relations between mRNA and protein abundance in tissue as prognostic and predictive biomarkers in early stage lung cancer. Integrated gene-transcript and protein-phenotype analysis in tissue presents potential for clinical treatment decisions in early stage lung cancer.

Keywords: treatment decision, lung cancer, protein expression, mRNA

CS08: PROTEOMICS AND CELL IMMUNITY

CS08.02 Heterogeneity in the Immunopeptidome Impacts on the Outcome of Human Disease

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Abstract: Our ability to characterise the products of antigen processing and presentation is now unprecedented with great advances in the qualitative and quantitative analysis of the immunopeptidome. The combination of efficient chromatographic separation and high resolution ultrafast LC-MS/MS allows us to appreciate the impact of the heterogeneity of peptide processing, antigen post-translational modification and the influence of drugs and metabolites on the outcome of disease at the molecular level. I will discuss examples of how heterogeneity in antigens and their processing impacts on the composition of the immunopeptidome and how this then relates to disease outcome. Such studies provide definitive quantitative data to understand the mechanism of human autoimmune diseases like Type 1 Diabetes and Rheumatoid arthritis and susceptibility to pathogens such as Salmonella and Human Immunodeficiency Virus (1-7). 1. N. L. Dudek et al., Constitutive and inflammatory immunopeptidome of pancreatic beta-cells. Diabetes 61, 3018-3025 (2012). 2. J. L. Dunne, L. Overbergh, A. W. Purcell, C. Mathieu, Posttranslational modifications of proteins in type 1 diabetes: the next step in finding the cure? Diabetes 61, 1907-1914 (2012). 3. P. T. Illing et al., Immune self-reactivity triggered by drug-modified HLA-peptide repertoire. Nature 486, 554-558 (2012). 4. N. P. Croft et al., Kinetics of Antigen Expression and Epitope Presentation during Virus Infection. PLoS Pathog 9, e1003129 (2013). 5. S. W. Scally et al., A molecular basis for the association of the HLA-DRB1 locus, citrullination, and rheumatoid arthritis. J Exp Med 210, 2569-2582 (2013). 6. R. B. Schittenhelm, T. C. Lim Kam Sian, P. G. Wilmann, N. L. Dudek, A. W. Purcell, Revisiting the arthritogenic peptide theory: Quantitative not qualitative changes in the peptide repertoire of HLA-B27 allotypes. Arthritis & Rheumatology 67, 702-13 (2014). 7. N. P. Croft et al., Simultaneous quantification of viral antigen expression kinetics using data-independent mass spectrometry. Mol Cell Proteomics 14, 1361-72 (2015).

Keywords: HLA, immunopeptidome, antigen presentation

CS08.03 Global Proteogenomics Analysis of Polymorphic Human MHC Class I-Associated Peptides

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Introduction and Objectives: The impact of genomic polymorphisms on the repertoire of peptides presented by major histocompatibility complex class I (MHC I) remains largely unknown though it has important implications in the development of effective cancer immunotherapy. In the treatment of leukemia, the use of adoptive immunotherapy is relatively rudimentary and is hampered by the variable anti-hematological cancers (HC) activity of allogeneic hematopoietic cell transplantation,

and the risk of a devastating autoimmune response. The enhancement of anti-HC activity for immunotherapy relies on the identification of patient-specific minor antigens (MiHAs) that can be targeted by primed T cells. Here, we present a novel approach that combines genomic, transcriptomic and mass spectrometry-based proteomic data to profile MHC I peptides from human B-cells and identify minor antigens (MiHAs) that result from non-synonymous nucleotide polymorphisms. **Methods:** B-lymphocytes from 13 individuals expressing the HLA-A*02:01 and/or B*44:03 alleles were used to generate personalized protein databases using in silico translation of genome/transcriptome sequencing data. MHC-I peptides isolated from B-cells by mild acid elution were analyzed by LC-MS/MS on a LTQ-Orbitrap Elite and searched against individual and human reference databases using Mascot. **Results and Discussion:** Our high throughput quantitative proteomics platform identified more than 35000 MHC I peptides from all B cells, of which 16173 peptides were assigned to HLA A0201 and B4403 alleles (Binding affinity < 1200 nM). Approximately 1% of these MHC I peptides were identified as MiHAs, and 36 shared optimal features required for adoptive immunotherapy of HC. **Conclusion:** In a context where finding suitable targets is arguably the most important obstacle to cancer immunotherapy, our work uncovers MiHAs that can be used for treatment of practically all HLA-A*02:01;B*44:03 patients with HCs. Further extension of this strategy to three or four additional HLA haplotypes would allow treatment of almost all patients.

Keywords: cell immunity, MHC I peptides, Proteogenomics, quantitative proteomics

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CS08.04 Characterization of Regulatory T Lymphocytes Using Label-Free Quantitative Proteomics

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Introduction and Objectives: 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. Here, we present a large-scale quantitative proteomic study that allowed to define a specific “signature” of the Treg sub-population of T lymphocytes, and identify some candidate proteins that may contribute to their suppressive function. **Methods:** Sub-populations of Treg and conventional T lymphocytes (Tconv) were sorted by flow cytometry with high purity, and global proteomic analysis was performed by single-run nanoLC-MS/MS on a fast-sequencing Q-Exactive 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, starting from low-numbers of primary cells. Label-free quantitative methods based on MS signal analysis were implemented for the comparison of protein expression profiles in Treg versus Tconv, as well as in AP-MS studies on particular candidates. **Results and Discussion:** Besides “historical” proteins that characterize Treg, our study identified numerous new proteins that are significantly up- or down-regulated in Treg versus Tconv. By knocking-down or over-expressing some of them, we could further evidence their functional importance in Treg through in-vitro co-culture tests or animal models of autoimmune pathologies. We further investigated the molecular mechanisms underlying the function of Themis, a protein particularly under-expressed in Treg, and previously proposed as a new target gene that could participate in the pathogenesis of immune diseases. Using affinity purification coupled to mass spectrometry analysis and label-free quantification, we identified the Themis interactome, leading to new insights in the mechanisms of this important regulator of T cell receptor signaling. **Conclusion:** Label free quantitative methods were used in both expression and interaction proteomic studies, and allowed to better characterize the Treg cell lineage, and decipher the molecular mechanisms underlying the potential role of Themis in these cells.

Keywords: label free quantification, regulatory T lymphocytes, autoimmunity, Interactome

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CS08.05 Proteomic Characterization of the Antigen-Specific Antibody Repertoire Elicited by Vaccination

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Introduction and Objectives: Vaccination is the most effective means of protecting against infectious disease, however certain pathogens have proven challenging for vaccine development. Understanding how vaccines elicit an effective immune response, and why some vaccines fail, is crucial to the development of new vaccines. While it is clear that the majority of vaccines convey protection by stimulating B cells to produce a diverse repertoire of antigen-specific antibodies, the mechanisms governing this process are not well understood. Single B cell analysis and next-generation sequencing (NGS) technologies have become invaluable tools for investigating the cellular response to vaccination, but a comprehensive delineation also requires characterization of the monoclonal antibodies (mAbs) comprising the serological response. By integrating transcript- and protein-level repertoire analyses using methodologies recently developed by our laboratory, we can now determine the identity, temporal dynamics, relative concentrations, and functions of antigen-specific mAbs in a complex repertoire, allowing deconvolution of the polyclonal antibody response in serum. We have applied our methods to the study of three different human vaccines - tetanus, polio, and influenza - providing unprecedented insight into the complexity and functional dynamics of the serological repertoire.
Methods: not applicable
Results and Discussion: Analysis of the trivalent 2011-2012 Flu-Zone seasonal influenza vaccine exemplifies the capabilities of antibody profiling. By characterizing the individual IgG repertoires specific to each of the three viral strains in the vaccine (H1/California, H3/Victoria, and Influenza B/Brisbane), we identified strain-specific and cross-reactive antibodies. Recombinant expression and analysis of H1/H3 cross-reactive antibodies revealed generally high affinity (nanomolar) for both components. Interestingly, pre-existing antibodies (detected prior to vaccination) were more likely to be cross-reactive, while newly emerging antibodies tended to be more strain-specific.
Conclusion: This method provides the first extensive molecular-level description of the serological repertoire, demonstrating the potential of antibody profiling for the evaluation and rational engineering of future vaccines endowed with higher and broader protective efficacy.

Keywords: Serological repertoire, Vaccine response, Antibody profiling, Influenza vaccine

CS08.06 HLA Ligandomics Drives Immunotherapies Based on High Affinity Soluble TCRs

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Introduction and Objectives: HLA complexes present peptides derived from cancer-associated proteins to the immune system that can be used as targets for cancer immunotherapy. Here we present 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. **Methods:** The selection of peptide targets is based on differential expression in cancerous tissues by combining data from multiple sources, including: i) proteomic identification of HLA peptides presented by cancer cell lines and tissues; ii) proteomic evaluation of relative expression levels in cancerous and normal tissues; iii) expression frequency in cancer 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 used to generate peptide-HLA specific T-cells by T-cell cloning, and the TCRs isolated from these cells are then engineered into soluble molecules (mTCRs) through the incorporation of a stabilising disulphide bond. Finally, 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. **Results and Discussion:** 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 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 I/IIa trial. **Conclusion:** Mass spectrometry-based peptidomics has enabled the identification of HLA presented peptides that can be used to develop potent immunotherapeutics for cancer treatment.

Keywords: Mass spectrometry, immunopeptidomics, Human leukocyte antigen, T-cell receptors

CS08.07 An Open-Source Computational and Data Resource to Analyze Digital Maps of Immunopeptidomes

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Introduction and Objectives: The repertoire of peptides presented by human leukocyte antigen (HLA) molecules to the immune cells is referred to as the HLA peptidome/immunopeptidome and is of great importance for understanding the immune system and for the development of next-generation vaccines against infectious diseases and cancers. The reproducible measurement of the immunopeptidome is therefore of great biological and clinical importance. In this study, we aimed at conducting a worldwide, community-based effort to generate standardized HLA allele-specific peptide assay libraries that could be further used for the robust quantitative analysis of HLA peptidomic data generated by SWATH mass spectrometry. **Methods:** HLA peptides were isolated from cells by immunoaffinity chromatography. Fifteen PBMC samples, five cell lines and 15,000 synthetic HLA peptides were used to build the libraries. SWATH maps of HLA peptidomic data were acquired in five international laboratories and were analyzed using the OpenSWATH and the Skyline software. **Results and Discussion:** To date, the pilot repository of standardized HLA peptide assay libraries contain a total of 222,487 transitions for 26,652 unique peptides and were stored by class and allele in the SWATHAtlas database. Our data indicate that up to 81% of HLA peptides present in an assay library can be extracted from a quantitative digital SWATH map. The R^2 value for SWATH quantification was 0.982 from technical replicates. The dynamic range of peptides quantified in different cell types was about 3-4 orders of magnitude. **Conclusion:** We demonstrated the feasibility of an international effort to build standardized HLA allele-specific peptide assay libraries, which were used to extract quantitative information from digital SWATH maps acquired in different sites. The workflow and the computational and data resources presented in this study is a first step towards highly reproducible and quantitative MS-based measurements of HLA peptidomes across many samples and could therefore be greatly beneficial in the design of personalized immune-based therapies.

Keywords: human leukocytes antigen, immunopeptidome, targeted mass spectrometry, SWATH/DIA

CS08.08 Insights into Immune Responses to Commensal Bacteria through MHC Class II Antigen Quantification

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Introduction and Objectives: The immune system plays a major role in protecting hosts from pathogen infection and maintaining tolerance in the gut. When the balance between the gut microbial ecosystem and immune homeostasis are disrupted, individuals can develop Inflammatory Bowel Disease (IBD). Pathophysiology resulting in loss of immune tolerance and exaggerated inflammatory responses in IBD patients is not well understood. To help elucidate how commensal antigens shape the host response, we have developed a quantitative proteomic workflow that facilitates bacterial antigen identification from mouse dendritic cells (DCs). Within the gut, DCs engulf bacterial antigens, and proteolyze them into peptides that are presented by cell surface major histocompatibility (MHC) class II complexes. Bacterial antigen presentation by DCs shapes the nature of the T cell response. We executed our workflow on WT and Atg16l1-deficient murine DCs to identify regulated bacterial and mouse antigens.

Methods: We cocultured WT and Atg16l1-deficient murine DCs with listeria for 10 minutes and 6 hours in biological duplicate and immunopurified their MHC II-peptide complexes. MHC class II-associated peptides were then labeled with iTRAQ4 reagents and analyzed in two iTRAQ 4-plex experiments on a Q Exactive HF. The data were searched against both mouse and listeria databases with Spectrum Mill. The log transformed ratios of the validated peptides were compared to identify peptides that were significantly up or down-regulated among each condition. **Results and Discussion:** A total of 52 unique listeria antigens and 3,671 unique mouse antigens were identified across all experiments. We identified sets of bacterial and mouse antigens that were regulated upon listeria treatment, including a set of bacterial antigens that play a key role in listeria pathogenesis. Additionally, we observed an increased presentation of lysosomal proteins in the Atg16l1-deficient DCs when compared to WT. **Conclusion:** Quantitative proteomics enables MHC class II-associated antigen characterization and furthers our understanding of how bacterial antigens dictate host responses.

Keywords: quantitative proteomics, immune responses to commensal bacteria, Inflammatory Bowel Disease, MHC class II antigens

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CS08.09 Multi-Omics to Examine Proteolytic Cleavage, Expression and Abundances in Macrophage Differentiation

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Introduction and Objectives: Macrophages are dynamic immune cells responsible for a variety of tasks in the body including clearance of foreign molecules/cells by phagocytosis, recruitment of other immune cells by chemokine signaling, antigen presentation, tissue repair and mediation of inflammation. Different subpopulations of macrophages are responsible for the variety of biological processes described, and their differentiation by chemokine stimulation results in distinct phenotypes that can have pathogen-killing or wound-healing phenotypes. THP1 monocytes were activated with phorbol 12-myristate 13-acetate (PMA) into macrophage-like cells (M0) and differentiated them with interferon gamma into macrophage type M1 (killer phenotype) and with interleukin 4 into macrophage type M2 cells (wound healing phenotype). We have performed multi-omics techniques to characterize the differentiation response in these distinct cells with particular emphasis on proteolytic processing. **Methods:** Gene expression data was gathered utilizing an RNA microarray developed in our laboratory targeted against 389 proteases and their inhibitors in humans (CLIP-CHIP) in these three subpopulations of macrophages. Additionally, macrophage cell lines were cultured with isotopically labeled amino acids and whole proteome extracts prepared for global-scale protein quantification using SILAC (stable isotopic labeling of amino acids in culture), isoelectric fractionation and high-speed, high-sensitivity LC-MS/MS using an LTQ Orbitrap Velos. **Results and Discussion:** A total of 1421 proteins were quantified across all macrophage populations – one of the largest proteome characterizations of these immune cells to date. Furthermore we performed terminal amine labeling of substrates (TAILS) to characterize protease substrates between macrophage types and correlated them to their protein abundances quantified by SILAC. This degradome dataset revealed 519 peptides as potential cleavage substrates. **Conclusion:** We present a systems-biology approach to the characterization of macrophage differentiation and reveal some key genes involved in pathogen killing such as the cytosol aminopeptidase LAP3, responsible for trimming of antigenic peptides in MHC presentation and dipeptidyl peptidase II in M1 cell maturation.

Keywords: TAILS, SILAC, degradomics, macrophage differentiation

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CS08.10 Proteomics Analysis of Tumor Associated Macrophages-Derived Exosomes

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Introduction and Objectives: As major players in the tumor microenvironment, tumor-associated macrophages (TAMs) can provide a favorable microenvironment to promote tumor development and progression. Exosomes seem to be a main class of vesicles involved in cell-cell communication by transporting bioactive molecules between cells. To survey the proteins in exosomes derived from TAMs and understand their functions to tumor cells, we constructed a TAM cell model based on a macrophage cell line, initiated quantitative proteomics to analyze protein responses in exosomes related with TAMs induction. **Methods:** We induced a macrophage cell line, Ana-1, with conditioned medium from tumor cells to construct the TAM model, which was estimated by mRNA abundances of several cytokines using Real-time PCR. Exosomes were prepared from the conditioned media of Ana-1 and TAMs with Exo-kit, respectively. Exosome proteomics implemented through iTRAQ and Q-Exactive LC-MS/MS. **Results and Discussion:** The TAM model was well established with M1/M2 mixed type, and the exosomes from TAMs and Ana-1 might behave significantly different. On the basis of MS/MS signals, Mascot search resulted in a total of 2453 proteins in macrophages exosomes. Comparison among our data and three other exosome datasets demonstrated that exosomes from different cells might share some similar functions due to high protein overlap rates among them. On the other hand, approximate 40% of the exosomal proteins in macrophage were uniquely identified and enriched in the RNA processing and ribosome biogenesis, suggested that macrophages-derived exosomes might exhibit a distinct cell-type dependent feature. Based on a stringent criterion (fold change>1.5 and $p<0.05$), a total of 115 differential proteins were defined in exosomes between TAMs and Ana-1. Interestingly, thirteen 20S proteasome subunits were up-regulated and 19 ribosomal proteins were down-regulated in exosomes from TAMs, indicating that TAMs could release the exosome with enhancement of proteolytic activity and weakened RNA binding capacity. **Conclusion:** Macrophage-derived exosomes exhibited a cell-type dependent feature.

Keywords: Tumor associated macrophages (TAMs), Exosomes, Tumor microenvironment

CS09: CHEMICAL PROTEOMICS AND DRUG DISCOVERY

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CS09.01 Chemical Proteomics Based Target Identification of Small Molecule towards Translational Studies

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Abstract: The interaction between the small molecule and its target protein is the key to understanding the cellular mechanism by which the small molecule acts. However, discovering the on- or off-target of small molecule is often the most challenging and time-consuming step. Chemical proteomics has played as a key research engine to identify direct interacting proteins and to explore mechanisms of action of small molecules towards functional and translational applications. A number of methodologies including conventional affinity chromatography using labeled small molecules as well as recent target identification methods of label-free small molecules such as Drug Affinity Responsive Target Stability, Stability of Proteins from Rates of Oxidation, Cellular Thermal Shift Assay, and Thermal Proteome Profiling have been developed and applied to identify the direct binding proteins of small molecules. This interaction information of small molecule and target protein facilitates structure based better drug development and functional annotation of target protein, respectively. Furthermore, integration of MALDI-MS imaging technology with chemical proteomics will enable to validate the interaction of label-free small molecule with target protein in tissue that harnesses the preclinical studies of small molecules in respect with their efficacy, toxicity, and pharmacokinetics. In this presentation, recent advances of chemical proteomics for target identification of small molecules towards functional and translational applications will be presented by introducing our case studies.

Keywords: Chemical Proteomics, small molecule, Target Identification, Drug discovery

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CS09.02 An Integrated Proteomics Strategy for Drug-Proteins Interactions and Compound Mode of Action

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Abstract: Mass spectrometry-based proteomics has become an indispensable tool in phenotypic as well as target-based drug discovery. One key area of application is in the context of target identification and elucidation of mode of action of a small molecule drug candidate. Chemoproteomic approaches enable the characterization of compound-protein interactions at proteomic scale. They can aid in the identification of both efficacy and off-targets of bioactive compounds such as hits from phenotypic screens and can provide important information about cellular target engagement. In addition, the integration of proteome-wide studies of compound-induced changes in post-translational modifications such as phosphorylation and ubiquitination as well as global changes in protein levels can provide valuable complementary information to help drive a drug discovery effort. These approaches can help understand the modulation of pathway mechanisms that lead to a compound-induced phenotype and identify pharmacodynamic markers for monitoring compound activity in preclinical models and their translation to the clinical setting. Various chemoproteomics-based approaches are available: These include non-covalent small molecule affinity chromatography-based chemoproteomics, covalent variants thereof using for example photoaffinity-labeling, as well as a number of protein stability-based approaches including the Cellular Thermal Stability Assay (CETSA) in combination with quantitative proteomics. These approaches differ not only with respect to requirements such as tool compound generation as well applicability to living cells, but importantly can provide complementary information including coverage of protein classes and definition of direct vs. indirect interactions. A combined use of these approaches can therefore allow for a more comprehensive analysis of compound-proteins interactions. Several examples for characterization of compound-protein interactions will be presented and the relative merits of the various approaches will be discussed, demonstrating the integrated application of proteomics in the context of small molecule drug discovery at Novartis.

Keyword: chemical proteomics, integrated proteomics, compound mode of action, drug discovery

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CS09.03 Target Selectivity of Clinical JAK Inhibitors in Peripheral Blood Monocytes

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Introduction and Objectives: 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 potency and selectivity. **Methods:** To improve the kinase coverage, we investigated a series of promiscuous kinase inhibitors and present a new bead matrix which captures more than 350 kinases from human cell lysates. Furthermore, the sensitivity improvements of recent MS technology in conjunction with a miniaturization of the pull-down procedure enabled a dramatic down-scaling of the assay input material which now permits the use of primary human cell material. **Results and Discussion:** Monitoring small molecule affinities to kinases from primary patient material would allow testing of kinase engagement in a simple in vitro assay to reveal variabilities between individuals. As a proof-of-concept, we determined target selectivity of the clinical BCR-ABL inhibitor Dasatinib for peripheral blood mononuclear cells (PBMCs) from different donors. The ability to perform kinase inhibitor screening from relevant primary cell material enabled us to profile a series of preclinical, clinical and marketed JAK kinase inhibitors in a pooled human PBMC lysate. We demonstrate target selectivity within the JAK family as well as across the kinome. Interestingly, although the majority of these inhibitors are highly specific towards the tyrosine kinase branch, off-targets are identified from nearly all kinase families underpinning the need to profile kinase inhibitors against the complete kinome. **Conclusion:** Here, we present the combination of an improved chemoproteomic bead matrix and a miniaturized workflow which allows the quantitative profiling of kinase inhibitors against a large proportion of the kinome from primary human samples.

Keywords: kinase inhibitor, JAK kinase, Chemoproteomics

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CS09.04 Proteomics Tools to Predict Nanoparticles Targeting and Uptake Capability

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Introduction and Objectives: Nanotechnologies hold enormous potential to revolutionize the field of nanomedicine and targeted drug delivery. How to systematically evaluate which properties from a new nanomaterials could improve the performance from previous formulation? Here, we will present two methods that could address: i) if a nanoparticle can reach the target cell; ii) if a nanoparticle can be uptake by the target cell. The final goal of this project is to integrate several methods based in lab-in-a chip and proteomic analysis that could offer a platform to assist the safe by design principle for new engineered nanoparticle with application for biomedicine. **Methods:** Nanomedicines contain signals for targeting that should be exposed. We applied a quantitative analyze of the surface of the protein corona to predict the targeting and the evolution of the corona to predict the uptake capability. **Results and Discussion:** By using high resolution mass spectrometry (LTQ Orbitrap Velos Pro-ETD), we characterized the evolution of the protein corona formed according to different NP sizes (10 and 30 nm), concentration (25 and 100 µg/mL) and trafficking through different intra- and extracellular media and compared with the surface protein corona. Our results show that the quantitative analysis of the surface of the protein corona could provide sufficient information to determine if the targeting signal included in a nanoparticle would be exposed after environmental interaction and processing. The nature of protein corona can also help to predict the uptake capability and be used as an early warning of toxicity. **Conclusion:** Those proteomic-based methods could offer a shortcut strategy for the assessment of new nanomaterials for nanomedicine at an early stage of development. The final goal of this project is to contribute to the efficient implementation of nanomaterials for medical applications.

Keywords: nanoparticle, quantitative proteomics, surface proteomics, nanomedicine

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CS09.05 Selectivity Profiling of 200 Clinical Kinase Inhibitors Using Chemical Proteomics

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Introduction and Objectives: More than 200 small molecule kinase inhibitors are in clinical trials, of which 30 have been approved for use in humans so far. Because of their chemical structure, kinase inhibitors may not only act on its intended target, but may also target other proteins. This promiscuity creates both problems and opportunities. In this study, we have systematically mapped the target space of 200 clinical kinase inhibitors using chemical proteomics. **Methods:** The Kinobeads technology features broad spectrum small molecule kinase inhibitors immobilized on Sepharose beads for affinity enrichment of the kinome from cell line or tissue lysates. Combined with quantitative mass spectrometry, this technology allows for the analysis of more than 200 human protein kinases in a single experiment. **Results and Discussion:** Competitive Kinobead profiling of all commercially available molecules currently in clinical trials resulted in a matrix, identifying the druggable kinome, new opportunities for existing drugs as well as other off-targets of these drugs. For example, we engage in preclinical studies for repurposing Cabozantinib from its approved use as a thyroid cancer treatment to the treatment of AML based on its potent inhibition of FLT3. An unexpected discovery is the identification of Ferrochelatase (FECH) as common off target of kinase inhibitors. FECH inhibition can be directly linked to the observed side effect of photosensitivity under Vemurafenib therapy **Conclusion:** This work highlights the ability of chemical proteomics for the unbiased identification of a drug's target spectrum leading to a better understanding of the mechanism of action of cancer drugs and shedding light on their side effects. The matrix is valuable for drug repositioning, design of combination therapy or identification of potential toxic side effects. Importantly, it also expands the repertoire of tools in chemical proteomics and drug discovery.

Keywords: Chemical Proteomics, Kinobeads, selectivity profiling, kinase inhibitors

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CS09.06 High-Throughput Detection of Endogenous Protein Targets Bound by Bioactive Small Molecules

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Introduction and Objectives: Identification of protein targets of drugs is important to understand the mechanism of actions and unwanted side effects of drugs. The objective of the study is to develop a nonbiased method to identify endogenous cellular protein-small molecule interactions to maximize therapeutic potential of drugs. **Methods:** Target identification by chromatographic co-elution (TICC) and optimized thermal shift (TS) methods were combined by use of SILAC to establish the high-throughput platform. The shift ratios of the heavy and light proteins were used to identify targets stabilized by drugs, and chromatographic profiles of proteins and drugs were evaluated to investigate binary protein-target interactions. **Results and Discussion:** Three positive controls including DHFR, endogenous protein Hsp90 and FabI showed clear thermal shift ratio in the presence of their inhibitors(MTX, radicicol, and triclosan), indicated that the method could be used for monitoring endogenous protein-ligand interactions. Then, to further test the method performance, 62 anti-cancer drug pools were used and 28 of the drugs showed a clear shift in chromatographic profiles in the presence of protein, with 15 compounds exhibiting multiple binding peaks suggestive of distinct protein engagements. 10 known protein targets corresponding to >40 drugs were identified by thermal shift ratios. Of 17 novel targets identified by the method, histone H4 was selected and successfully validated by recombinant protein. **Conclusion:** While the method reported here showed relatively high hit rates for high binding-affinity targets, some interactions were missed which were readily discovered by TICC. Multiple binding peaks were observed for one of the third of the drugs tested by TICC, and the abundance of most of these proteins likely exceeded 5 nM based on corresponding estimates of the amount of associated small molecule. Therefore, we conclude that the combination of TS and TICC methods can increase overall hit rates, forming the basis of a more effective strategy for routine drug target characterization.

Keywords: Thermal shift, TICC, Mass spectrometry, Chemical Proteomics

CS09.07 An R Package for the Analysis of Thermal Proteome Profiling Experiments

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Introduction and Objectives: The fact that proteins complexed to ligands tend to become more resistant against heat-induced unfolding is utilized by the recently developed cellular thermal shift assay (CETSA) and isothermal dose response (ITDR) approaches to assess target engagement in living cells [1]. Combining the concepts of CETSA and ITDR with multiplexed quantitative mass spectrometry we recently demonstrated that large scale unbiased thermal proteome profiling (TPP) of human cells enables the comprehensive identification of a drug's direct and indirect targets and appraisal of drug efficacy in situ [2]. We developed the freely available Bioconductor package "TPP" to perform bioinformatic analysis of quantified proteins from TPP experiments and identify proteins that upon drug treatment show a significant change in their thermal stability. [1] Molina et al Science 2013 [2] Savitski et al Science 2014 **Methods:** The TPP package efficiently processes protein quantification data for thousands of proteins from one or several TPP experiments either corresponding to the relative amount of protein across different temperatures (TPP-TR) or the proteins' apparent stabilities across different compound concentrations at a fixed temperature (TPP-CCR). It performs cross-experiment normalization, fitting of melting- and dose response curves, determination of melting points and pEC50 values and statistical considerations tailored to TPP experiments. It reports all relevant protein information, curve parameters and significance estimations as well as visualizations of the melting- and dose response curves. **Results and Discussion:** TPP analysis of cells treated with panobinostat results in reproducible, significant change in thermal stability of the drug's cognate targets. A novel finding is a significant stoichiometric effect on TTC38 already at low panobinostat concentrations. **Conclusion:** With the TPP package we provide the required functionality to infer and statistically assess direct and indirect effects of protein ligand binding from TPP experiments on a proteome wide scale to the community.

Keywords: Thermal Proteome Profiling, Bioinformatics, target engagement, CETSA

CS09.08 Chemical Proteomics for Development NDPKA/Nm23-H1 Activator

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Introduction and Objectives: Nm23-H1 acts as tumor metastasis suppressor through its Nucleotide diphosphate kinase (NDPK) activity. We identified novel modulator of Nm23-H1, NAA001, for developing tumor metastasis inhibitor which activates NDPK activity of Nm23-H1. To get insight into the underlying processes involved in metastasis suppression via NDPK activation, we performed proteomic approaches that were analysis of phosphor-proteome, and 2DE-based proteomics. **Methods:** To investigate the influence of NDPK activator to breast cancer cell line, MDA-MB-231, we examined metastasis suppression of MDA-MB-231 by NAA001. We observed that NAA001 influenced cell morphology, and invassiveness of MDA-MB-231 cells by matrigel invasion assay. In order to elucidate molecular determinanrs responsible for NAA001 control for metasitasisness phenotypes, we observed phospho-proteome influenced by treatment of NAA001. Using two dimensional difference gel electrophoresis in combination with LC-MS/MS, we identified target proteins influenced by NAA001 treatment. **Results and Discussion:** We examined whether NAA001 activates NDPK activity of Nm23-H1 in vitro, and in vivo. NAA001 activated hexamer form of Nm23-H1, and suppressed invasion of MDA-MB-231 breast cancer cell line. To examine whether suppression of invasiveness by NAA001 is targerting Nm23-H1, we observed active Rac1 which is one of cellular target of Nm23-H1. Rac1 was inactivated by NAA001, and this was only observed in control siRNA not but in knocking down Nm23-H1. To get insight into the underlying processes involved in metastasis suppression via NDPK activation, we performed 2DE based proteomics. We identified 16 proteins pots in defferential 2DE with LC-MS/MS. In the phospho-proteome research, we identified novel targets of NAA001. **Conclusion:** In this study, we identified novel Nm23-H1/NDPK activtor for metastasis inhibitor, and varified that NAA001 targets Nm23-H1. Proteomic approach revealed novel pathway of NAA001(and/or)Nm23-H1, and the specific stratagies of metastasis inhibitor therapies.

Keywords: Nm23-H1/NDPKA, Chemical Proteomics, Metastasis suppressor

CS09.09 Serine Hydrolase Activities in Urine from Patients Undergoing Cardiac Bypass Surgery

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Introduction and Objectives: Acute kidney injury (AKI) is a frequent consequence of cardiac surgery with 17% of patients developing mild AKI. AKI is associated with increased hospitalization, mortality, and chronic kidney disease or loss of function. Cardiac-surgery associated AKI is felt to be secondary to ischemia-reperfusion injury. Due to the abbreviated time during injury induction, we hypothesized that the pathophysiology may be related in part to enzyme activation rather than changes in protein composition. We questioned specifically whether members of the serine hydrolase family might be involved in AKI as these enzymes perform a broad range of metabolic and proteolytic functions potentially involved in pathological processes. **Methods:** We used a fluorophosphonate based fluorescent probe to examine the quantitative and qualitative changes in serine hydrolase activities in patients undergoing cardiac bypass surgery. Specifically we sought to identify those activity changes that occur during the surgery and ultimately to ascertain if any of these correlated with the development of AKI observed following surgery. A cohort of cardiac surgery patients (n=350) was established with sequential urines collected before, during and after surgery. Serine hydrolase activity was assessed in a nested, case-controlled cohort with AKI defined as a serum creatinine rise >50%, and non-AKI as a serum creatinine rise <10%. **Results and Discussion:** SDS-PAGE separation of probe-labeled samples revealed that there were marked but highly variable individual specific quantitative and qualitative changes in activity throughout the course of ischemia-reperfusion injury. The identities of the active enzymes are being determined by sequential affinity purification, peptide separation by liquid chromatography and analysis by tandem mass spectrometry.

Conclusion: This data offers the first detailed profiling of the natural history of urinary enzymatic changes during cardiac surgery. It also offers a possible basis for comparing the compositional and kinetic differences in enzyme activities in AKI and non-AKI patients.

Keywords: Fluorophosphonate, Cardio bypass surgery, Acute Kidney Injury (AKI), Serine hydrolases

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CS09.10 MALDI-MSI Analysis of a Small Molecule and Its Target Protein Interaction on Tissues

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Introduction and Objectives: The objective of our research is to discover new bioactive small molecules with cell-based screening and mode of action study. Furthermore, we performed the target validation for demonstrating the drug-target interaction in vivo level using MALDI-MSI(MALDI-mass spectrometry imaging) and IF(immunofluorescence) staining. This approach will provide new information on elucidating the pharmacokinetic and pharmodynamic properties of bioactive small molecule and its target protein on xenografted tumor tissues. **Methods:** Endothelial cell-based screening assay with 300 natural plants library, viability assay(chemotoxicity test), in vitro angiogenesis assay(tube formation assay, chemo-invasion assay), in vivo CAM(chicken chorioallantoic membrane) assay, Western blotting, hVEGF ELISA, Tumor cytokine invasion assay, DARTS(DrugAffinityResponsiveTargetStability)assay,MALDI-MSI(ThermoFisher Scientific, MALDI LTQ Orbitrap XL), IF(Immuno-Fluorescence) staining. **Results and Discussion:** In previous studies, a natural small molecule, YCG185, was identified as an anti-angiogenic agent in vitro and in vivo. Additionally, YCG185 significantly decreased the expression levels of HIF1-α and its target gene, VEGF. Furthermore, we demonstrated that YCG185 directly bound to VR receptor and specifically suppressed not only the phosphorylation of VR receptor but also its downstream signaling, ERK and Akt. To further deconvolute and validate the mode of actions of YCG185, we focused on elucidating the pharmacokinetic and pharmodynamic properties with identifying the localization of YCG185 and its target receptor on xenografted tumor tissues using MALDI-MSI(MALDI-Mass Spectrometry Imaging) and IF (Immuno-Fluorescence) staining. As the result, the localization of MALDI-MSI of YCG185 is highly correlated with IF staining of VR receptor on xenograft tumor tissues, suggesting that YCG185 binds to its target receptor in vivo. **Conclusion:** This study could be a novel platform to understand the pharmacokinetic properties of small molecules with free-labeled or non chemical modification and provide novel insights into deciphering interaction of a drug and its targets even on tissues.

Keywords: IF (Immuno-Fluorescence) staining, Anti-angiogenesis, small molecule, MALDI-MSI (MALDI-Mass Spectrometry Imaging)

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CS10.02 Interrogation of the Dynamic Role of Linker Histone Modifications in Cancer

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Abstract: Histones are evolutionarily conserved proteins responsible for condensation, organization and regulation of the DNA within the nuclei of all eukaryotic cells. The nucleosome core particle is made up of DNA wrapped about a protein octamer comprised of two copies of each core histone H2A, H2B, H3 and H4 and the linker histone H1 is responsible for the connection and stabilization of the nucleosome. The functional role played by the core histones (H2A, H2B, H3, H4) has long been recognized as a topic of importance to cancer research. However, there is an urgent need to understand the functional relevance of the linker histone H1 and its variants. The combinations of H1 modifications produce a diverse catalog of H1 proteoforms, each of which is hypothesized to rationalize a unique functional chromatin landscape. Specifically of interest to our lab is the contribution of each H1 proteoform to chromatin binding dynamics and regulation of chromatin associated factors. To date, only a small number of linker histone PTMs has been functionally annotated. Our labs are currently proficient at reliably identifying modifications at specific sites, but this information has little value if we do not understand the functional consequences of these modifications. We aim to characterize H1 proteoforms and gain a more complete understanding as to how H1 post-translational modifications maintain normal gene activity. To achieve the objectives we employ a top-to-bottom proteomic approach to characterize linker histones in cancer cell lines.

Keywords: FT-ICR, phosphorylation, cancer, histone

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CS10.03 Accelerating Epigenetic Cancer Drug Discovery Using a High Throughput Histone Analysis Platform

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Introduction and Objectives: Deregulation of histone modifications can result in various diseases including cancer. We have developed a global chromatin profiling platform based on targeted mass spectrometry to quantitatively measure roughly 50 distinct modifications focusing primarily on histone H3. In this presentation, we will overview the platform and use several examples to illustrate its performance and impact on drug discovery. **Methods:** To facilitate profiling of >100 samples, we have developed a plate based method to parallel process histone samples for LC-MS analysis. In total, 50 peptides are measured on QExactive. Data processing is semi-automated using Skyline. We have evaluated the platform using a variety of samples ranging from cell lines to tissues and found it very robust and reproducible. This allows us to take advantage of a collection of histone profiles generated by the Broad Institute as a part of the Broad-Novartis Cancer Cell Line Encyclopedia (CCLE) collaboration. **Results and Discussion:** The comprehensive data generated by the plat-

form enables global view of the effects of drug treatment, and permits systematic investigation of acquired drug resistance, drug mode of action, to facilitate compound selection, and the identification of biomarkers. In one example, MS data revealed that the EZH2 mutants retained substrate specificity of their predecessor complexes, but became refractory to biochemical inhibition by EZH2 inhibitor. The results suggest that the resistance is developed by co-operation between the EZH2 WT and mutants to promote tumorigenesis. Further, we integrated this data set with recently published CCLE data where >100 cell lines have been profiled. The results confirmed similarity of epigenetic profiles of mutant cells annotated as loss of function and compound based inhibition. Other examples include identification of distal safety and PD biomarkers. **Conclusion:** In summary, the high throughput global histone profiling platform has provided data important for understanding biology, compound profiling, and selection, and for PD marker discovery and measurements.

Keywords: Histone profiling, Epigenetics, Mass spectrometry, cancer drug discovery

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CS10.04 Identification of Cofactors Influencing Smyd1's Histone Methyltransferase Activity via ChIP-MS

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Introduction and Objectives: Smyd1, a myocyte-specific histone methyltransferase, was originally identified as a necessary regulator of cardiac development. More recently we have shown that Smyd1 also regulates pathologic cell growth and gene expression changes during the development of adult heart disease. Regarding its enzymatic activity, Smyd1 has been shown to methylate histone H3 on lysine 4, in vitro—an established mark of gene activation. However, we have confirmed that Smyd1 represses transcription at several genomic loci in the cardiomyocyte. Despite these seemingly dichotomous findings, very little is known regarding Smyd1's methyltransferase activity in vivo. In this study we have utilized chromatin immunoprecipitation and mass spectrometry (ChIP-MS) to identify the proteins and post-translational modifications in complex with Smyd1 on chromatin to determine the factors influencing its binding to specific regions of the genome and those modulating its enzymatic activity. **Methods:** Adenoviral-mediated expression of FLAG-tagged Smyd1 was carried out in isolated myocytes in the presence and absence of hypertrophic stimuli. Macromolecules were crosslinked, chromatin was MNase digested and Smyd1-bound nucleosome complexes were immunoprecipitated via the FLAG epitope. Isolated proteins were trypsin digested, identified by mass spectrometry, and analyzed using label-free quantitation to determine enrichment of proteins and post-translational modifications. **Results and Discussion:** Our analyses identified several nucleosome remodelers (Rbbp4, Nap1l1) and transcriptional co-repressors (Ctbp1, Tbl1xr1) as novel Smyd1 interactors and characterized a unique pattern of post-translational modifications on Smyd1-bound nucleosomes defined by enrichment in histone H3 K37 di-/tri-methylation, and R40 and K79 mono-methylation and a void of mono-/di-methylation and acetylation on K9 and K14, respectively. **Conclusion:** These experiments begin to define the active Smyd1 complex on chromatin and elucidate the molecular components influencing its methyltransferase activity and gene targeting in the myocyte. In addition, this data provides key insights into understanding how Smyd1 regulates molecular and phenotypic changes in the heart during the development of heart disease.

Keyword: Smyd1, ChIP-MS, Chromatin remodeling, Heart failure

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CS10.05 Modification-Specific Chromatin Proteomics: Phosphorylated RNA Polymerase II Associated Proteins

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Introduction and Objectives: The transcription of protein-coding genes in eukaryotes is tightly regulated by the cooperative action of numerous chromatin associated protein factors. Our knowledge of the complex mechanisms that control RNA polymerase II (RNAP II) progression through the transcriptional cycle is still limited. We investigate the role of protein phosphorylation in RNAP II function by using optimized chromatin-interacting protein mass spectrometry (ChIP-MS). We determined the protein composition of chromatin regions associated with RNA polymerase II phosphorylated at two different residues in the C-terminal domain (CTD). **Methods:** HeLa cells were incubated with cross-linking reagent (15 min, 2% formaldehyde) and chromatin was extracted and sheared by sonication. RNAP II – associated chromatin regions were immunoprecipitated using antibodies specific to distinct phosphorylated RPB1 CTD serine residues and isolated using magnetic protein A/G beads. The isolated proteins were subjected to on-bead trypsin digestion and then analyzed and sequenced by LC-MS/MS (Thermo Q Exactive HF) and quantified by a label-free approach (Proteome Discoverer or Progenesis). Proteins that exhibited more than three fold enrichment in precipitated chromatin preparations as compared to input chromatin were considered to be specifically associated with the targeted chromatin regions. **Results and Discussion:** We identified >300 proteins, including >30 novel candidate proteins that specifically associated with CTD phosphorylated RNAP II chromatin. These included subunits of RNAP II associated complexes, such as DSIF, P-TEFb, PAF, PTW/PP1 and FACT, RNAP II CTD-binding factors, histone modifiers and numerous components of the spliceosome. Among the novel candidates we identified several uncharacterized putative transcription factors implicated in human diseases. **Conclusion:** A gel-free ChIP-MS workflow was optimized and applied for the first detailed modification-specific proteomic characterization of chromatin regions associated with phosphorylated RNAP II. More than thirty novel candidate proteins were found to be specifically associated with transcribed chromatin, including several that are interesting in disease biology.

Keywords: RNA polymerase II, Transcriptional regulation, Chromatin proteomics

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CS10.06 In Gel Derivatization for Histone PTMs Analysis in Arabidopsis Thaliana

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Introduction and Objectives: Post-translational modifications (PTMs) on histone are highly correlated with genetic and epigenetic.Mass spectrometry (MS) has developed to be an optimal tool for PTMs analysis. Derivatization of histones with chemicals has been widely used in histone PTMs analysis. However, biological histone samples are not always prepared with high purity, as an alternative approach, we utilized NHS-propionate for in gel histone derivatization to reach a broad application. **Methods:** MALDI-TOF and LC-MRM are used for evaluating derivatization efficiency and reproducibility of in gel derivatization method. **Results and Discussion:** The reproducibility of this method was val-

idated through analyzing rhistone H3. Using this in gel derivatization method, we succeeded to quantitatively profile the histone PTMs from Arabidopsis with selective knock down of CLF (clf-29) and the original parental (col), which indicated the high degree of specificity of CLF on H3K27me3 in Arabidopsis. **Conclusion:** We have proved that the derivatization of histones with NHS-propionate can also be expansively applied in gel system. This in gel derivatization method performs comparable efficiency in a reproducible manner with those conducted in solution.Biological samples can be proceeded for in gel derivatization to quantify histone PTMs without any purification steps, tapering off the time greatly and avoiding wastage during sample preparation.

Keywords: MRM quantification, Arabidopsis, in gel derivatizion, histone PTMs

CS10.07 Iron Induces Histone Deacetylation in the Human Pathogen Trichomonas Vaginalis

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Introduction and Objectives: Iron enhances virulence expression and pathogenesis of the human pathogen Trichomonas vaginalis. We have previously shown that iron overloading triggers immdeiate signal transduction via the activation of cAMP biosynthesis and protein kinase A to phosphorylate a Myb3 transcription factor for its rapid nuclear influx. Since cAMP and the protein kinase A might have multiple downstream targets, we figured that iron might cause tremendous changes in cell physiology of the parasite. Thus we wish to elucidate global roles of iron overloding in the parasite, **Methods:** A phosphoproteomics approach was taken to address the significance of iron overloading in Trichomonas vaginalis. Cells depleted of iron were repletewith iron for 5 min and total lysates or isolated nuclei were prepared for a solution-based trypsin digestion. The peptides were dimethyl-labeled and phosphopeptides were enriched with IMAC. The resulting samples were analyzed by liquid chromatography-coupled tandem mass spectrometry. We also purified histones from nuclei for a gel-based proteomics analysis. **Results and Discussion:** We found that iron might activate phosphorylation of a histone deacetylase, (HDAC) and also deaceylation of the histone H3 at K9. Overall changes in posttranslation modifications in histones are being investigated. To our knowledge, the roles of iron in regulation of epigenetics have great implications on how iron regulate virulence expression and pathogenesis of the human pathogen. The significance will be discussed in greater detail once our study is completed. **Conclusion:** Iron causes tremendous changes in cell physiology, possibly through its effect on epigeneitic regulation.

Keywords: histones, Trichomonas, iron, HDAC

CS10.08 AP-MS and BioID Generates Comprehensive Interactome for Chromatin-Associated Protein Complexes

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Introduction and Objectives: The analysis of protein-protein interactions by affinity purification coupled to mass spectrometry (AP-MS) has been successfully used to analyze interactions for soluble protein complexes. Specific cellular compartments, including chromatin, have been more difficult to profile by standard AP-MS approaches, due to their limited solubility under conditions that maintain protein-protein interactions. An alternative approach to identify interactors is proximity biotinylation (BioID), in which a protein of interest is fused to a mutated biotin ligase that activates exogenous biotin, allowing for the covalent biotinylation of proteins in the vicinity of the bait. This enables the use of harsh lysis conditions to recover the biotinylated proteins, offering potential for the analysis of chromatin-associated proteins. **Methods:** In this study, we compare AP-MS and BioID for the identification of protein-protein interactions by stably expressing FLAG or BirA* tagged histones and Mediator complex proteins in Flp-In T-REx HEK293 cells. Furthermore, we explore the background signal associated with the exogenous expression of BirA* constructs to allow for the identification of true interactors. **Results and Discussion:** Both interactome mapping approaches recovered known interactors associated with histones H2B, H3 and with the mediator complex. However, we observed only a small overlap in interaction partners between the two approaches, with BioID showing a larger interactome than AP-MS while still allowing for the purification of prey proteins of lower cellular abundance. **Conclusion:** AP-MS and BioID were both effective in capturing a fraction of the known interaction partners for a given bait protein. However, our results demonstrate that these two approaches are complementary in providing a global view of the interactions established by chromatin-associated proteins.

Keywords: Affinity purification coupled to mass spectrometry, Chromatin, protein-protein interactions, Proximity biotinylation

CS10.09 Comparison and Combination of Search Engines to Discover and Characterize Identifications and PTM Signatures in Biology

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Introduction and Objectives: As the mass spectrometry advances, the correct identification of peptides and proteins as well as the biological post-translational modifications (PTMs) is becoming more critical. In this study, we established a workflow-based database searching method for a HeLa lysate and histone PTMs using the Proteome Discoverer software platform. Multiple search algorithms were compared and used in combination to provide an increase in the number of confidently identified peptides with PTMs. **Methods:** The Hela and histone data files were searched using SequestHT, Mascot, Byonic and MS Amanda as part of the Proteome Discoverer 2.0 platform, and MaxQuant with the Andromeda search engine. A FDR of 1% at the peptide level was used to filter the results. **Results and Discussion:** We compared SequestHT, Mascot, Byonic, and MS Amanda for searching both lightly-modified and heavily-modified proteins. For unmodified or lightly-modified samples, Byonic is superior for peptide and protein identifications, in part due to the 2D FDR capability. Se-

questHT outperformed other search engines in terms of searching speed. When it came to heavily-modified proteins like human histones, the performance was more variable amongst the different search engines. For complete PTM characterization, the best results were obtained not with a single search engine, but with multiple combined searches. We recommend using several search engines in combination to improve the coverage for each modification, a unique capability within Proteome Discoverer 2.0. Another benefit of searching with multiple search algorithms in Proteome Discoverer is reduction in total search time. We performed multi-threaded searches on three engines in parallel, followed by a consensus report on three processing results. This strategy shortened the overall searching time by 20% without compromising the results. **Conclusion:** A flexible and efficient search strategy for peptide and histone PTM characterizations.

Keywords: flexible, histone, PTM, efficient

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CS10.10 Monitoring Histone PTM Dynamics in Anti-Cancer Drug Resistant Cells Using MRM

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Introduction and Objectives: Dynamic modification of histone plays fundamental roles in gene regulation and proteins involved in the modifications have been prime targets for the development of anti-cancer drugs. To investigate dynamic modulation of the histone modifications associated with anti-cancer drug resistance, we have established a quantitative MRM assay for monitoring molecular crosstalk between the site-specific modifications on core histones. In conjunction with unique cell surface phenotype changes in cancer stem cell-like cells (CSCs) derived from MDA-MB453 along with altered signaling pathways conferred anti-cancer drug resistance, we analyzed the dynamic changes of the core histone PTMs in CSCs and compared to its counterpart cell line. **Methods:** Isolated core histones (60) by the acid extraction method were digested with trypsin and then analyzed by a Q-Exactive. The multiplexing MRM assay was performed on an Agilent 6490 QQQ MS with 63 MRM transitions for 21 target peptides. For statistically significant quantification, MSstats analysis was carried out for translating chromatographic peak areas into log2 values and data normalization. **Results and Discussion:** After isolation of core histones, we carried out in-solution digestion followed by LC/MS analysis and identified 15 histone-PTM peptides of core histones as MRM target peptides. Additionally, we included 6 known histone-PTM peptides which were not detected in the profiling data. With pre-determined 63 MRM transitions of 21 histone-PTM peptides, we carried out a multiplexing MRM experiment in triplicate for CSCs and NCSCs and observed significant abundance changes in 11 histone-PTM peptides (p-value ≤0.01, fold change ≥1.5). **Conclusion:** In this study, we have established a quantitative MRM assay that enabled the measurement of the dynamic changes of histone-PTMs in CSCs. Although our preliminary data show possible clues how CSCs epigenetically regulate core histone tails associated with anti-cancer drug resistance, further studies are required to verify key regulatory histone modifications in other anti-cancer drug resistant cell lines.

Keyword: histone PTMs, anti-cancer drug resistance, Multiple Reaction Monitoring

CS11: HOT TOPICS

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CS11.01 Exploring Mitosis Dynamics by Combining Data Dependent and Data Independent Strategies

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Introduction and Objectives: Proteins abundances span in the proteome at vastly different ranges; yeast proteome, for example, is estimated to fluctuate from fewer than 50 to more than 10^6 molecules per cell. Many of these molecules, including cell cycle proteins, are present at levels not readily detectable by traditional data dependent proteomic methods (DDA). Moreover, the DDA runs are plenty of missing values among the replicates due to the stochastic nature of the method, thus hampering the robustness of the analysis. We designed a robust workflow named Missing values Monitoring (MvM) to quantitate low abundant proteins; in particular, we focused on the quantitation of cell cycle proteins in mitosis versus G1 phase in S. cerevisiae. **Methods:** We compared yeast arrested in mitosis upon MAD2 overexpression versus yeast arrested in G1 phase by alpha factor using a "one shot" label free analysis. We overcome the limitations of shotgun approach implementing DDA with data independent (DIA) workflow. After a first shotgun analysis, we derived the list of all identified cell-cycle related proteins and, for the proteins measured in all runs, we quantified them with high confident DDA quantitation. For the proteins poorly quantified among the replicates, we supplied the missing values with DIA using the library previously generated in DDA analysis. **Results and Discussion:** By DDA we identified 2903 proteins. Among them, cell cycle related proteins are the major components of the missing values-rich proteome. Using the MvM workflow, we doubled the number of quantified cell cycle related proteins and we did a comprehensive screening of proteins involved in spindle mitotic checkpoint, pheromone regulation and DNA damage checkpoint statistically regulated in mitosis or in G1 phase. **Conclusion:** We obtained successful quantitation for cell cycle related proteins till ~50 molecules per cell comparable to an SRM based method, demonstrating the potential of MvM workflow to quantitate dynamically regulated proteins.

Keywords: quantitative proteomics, Data Independent Analysis, Cell Cycle, Mitosis

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CS11.02 Sportomics: Building a New Concept in Metabolic Studies and Exercise Science

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Introduction and Objectives: Advances in biochemical and biophysical methods have enabled us to better understand cells and organisms. For more than a decade, we have used alternative approaches to understand metabolic responses to physical stress. In addition to classic laboratory studies (cell and animal models), we have used elite athletes and sports to examine metabolic stress. In 2011, in an analogy to other "-omics" sciences, we proposed the concept of Sportomics to mimic the real challeng-

es and conditions that are faced during sports training and competition. **Methods:** Sportomics is non-hypothesis-driven research on an individual's metabolite changes during sports and exercise. Focusing mostly in proteomics and metabolomics (also other "-omics" approaches) Sportomics centers on sports as a metabolic challenge. Our study is holistic and top-down; we treat the data systematically and have generated a large computer-searchable database. We also propose that in-field metabolic analyses are important for understanding, supporting and training elite athletes. **Results and Discussion:** Sportomics is a useful tool for managing athlete training and performance. We showed that collecting and analyzing physiological data during training could provide important information on an athlete's clinical condition and performance. Here we are going to show how Sportomics is helping in preparing world-class athletes for the forthcoming Games. Thus, using Sportomics protocols, we can better understand the metabolic changes that are induced by exercise and sports. **Conclusion:** We believe that this approach can fill a methodological gap between systems biology and translational medicine similar as a bench to the field approach.

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CS11.03 How to Unravel the Brain Proteome

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Introduction and Objectives: We recently published the first version of the human tissue proteome based on antibody profiles and RNAseq data. These data generated new perspectives to investigate tissue specific proteomes as well as histological and subcellular location of proteins important for organ functions. The brain has many specialized functions mediated by specific proteins expressed in selective cells. Identifying these proteins, their regional, cellular and subcellular distribution will reveal molecular components involved in normal brain physiology and could contribute to identify pathomechanisms involved in neurological disorders. **Methods:** Here we identified brain-enriched genes based on expression in 32 different organs including cerebral cortex and explored the regional and cellular distribution of genes in human and rodent brain. **Results and Discussion:** By comparing the transcript profile for cerebral cortex to 31 other normal tissues we defined a certain set of genes as brain-enriched. This set includes well-known and novel brain related proteins mainly expressed by neurons, astrocytes and oligodendrocytes. In contrast to widely expressed genes, the majority of brain-enriched genes are associated with brain specific processes and are often only expressed in a single cell type. When analyzing the transcriptome a set of genes remained missing. Because the brain is heterogeneous both in function and gene expression we searched for expression evidence in other more extended brain related genomic databases. We were able to link some of our "missing" genes to other tissues? eg. we identified several proteins exclusively expressed in the pituitary (POU1F1, TRHR and TSH) and confirmed this on protein level using antibodies. To complement our data on the human brain proteome, we started exploring protein expression in mouse brain using our extensive collection of HPA antibodies. This allowed us to extend our protein atlas providing valuable information on distribution in the brain as well as cell type and sub cellular location. **Conclusion:** not applicable

Keywords: IHC, RNAseq, Protein Atlas, brain proteome

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CS11.04 High-Throughput Detection of Protein Targets Bound by Bioactive Small Molecules

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Abstract: The routine detection of ligand-target interactions is important for characterizing the mechanism of action of bioactive small molecules, but unbiased screening approaches remain elusive. Here, I report a high-throughput experimental platform for systematic target identification that combines complimentary protein thermal stabilization and chromatographic co-elution assays to monitor the physical association of bioactive small molecules with cellular proteins. I will demonstrate the versatility and effectiveness of this hybrid approach for detecting both known and unexpected targets of diverse compounds, including anti-microbials and anti-cancer drugs.

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CS11.05 CPTAC Assay Portal: A Public Repository for Well-Characterized Quantitative Targeted Assays

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Introduction and Objectives: Targeted MS techniques, like multiple reaction monitoring (MRM) and parallel reaction monitoring (PRM), hold tremendous promise for transforming proteomic research through reproducible, standardized quantification of peptides and proteins. Although these techniques have taken hold in the proteomics community, the benefits have not yet been fully realized by the wider biological and clinical research communities. This is largely due to dispersal of assay information throughout the literature, with no established standards for characterization of assay performances and no public database of analytically validated assays and standard operating protocols (SOPs), which are critical for standardizing and harmonizing proteomic results. **Methods:** To address this, the Clinical Proteomic Tumor Analysis Consortium (CPTAC) has launched an Assay Portal (http://assays.cancer.gov) to serve as a public repository of well-characterized proteomic assays and bring together clinicians or biologists and analytical chemists to answer hypothesis-driven questions using targeted, MS-based assays. **Results and Discussion:** Assay content is accessed through queries and filters to find assays to proteins involved in specific cellular pathways, protein complexes, or chromosomal regions. Assays are displayed by mapping peptides relative to features of interest (e.g. sequence domains, isoforms, SNPs, and PTMs). Characterization data (linked through Panorama, https://panoramaweb.org) are available for each assay, to evaluate performance and download SOPs and other files. To ensure sufficient assay characterization, a guidance document describing the minimal data required for inclusion in the CPTAC Assay Portal is available for download. This guidance document provides a list of experiments that will help potential downstream users of assays feel more confident that investing time, money, and energy into adopting and deploying the assays will be beneficial. The CPTAC program

will be adding hundreds of characterized assays over the next 2 years, and the portal will soon be open for contributions from the community at large. **Conclusion:** The CPTAC Assay Portal is a repository for highly characterized targeted assays.

Keywords: Targeted proteomics, NCI CPTAC, quantification

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CS11.06 BatMass: A Software Platform for Visualization and Analysis of Raw MS Data and Processed Results

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Introduction and Objectives: Any study utilizing mass-spectrometry begins with processing of raw data from the instruments. Runs need to be checked for quality of mass-spec measurements and LC/MS features need to be identified before something useful can be done with the data. This is a critical step often overlooked as it's tedious – how would one easily check performance of an MS instrument or validate that the feature finding algorithm settings were adequate? BatMass offers a simple solution with its visualization capabilities, allowing for quality control of raw measurements at a single glance. **Methods:** not applicable
Results and Discussion: BatMass has two aspects to it: first it serves as a tool in itself for visualizing raw mass spectrometry data and processing results, secondly it is intended to be a platform for developers, working with mass-spec data. Our package provides a set of visualizations for traditional MS data as well as emerging novel SWATH-like acquisition strategies. The package provides standard spectrum and chromatogram (viewers, but the most powerful is the 2D map viewer. Unlike commonly implemented in other software, it does not bin the data, allowing to viewing in high resolution, which is required to quickly assess the quality of an LC/MS run; scan-to-scan mass accuracy stability, average elution time for less abundant ions are made clearly visible. Feature finding results can be overlaid on top of spectrum and 2D plots, as well as can be peptide or metabolite identification results. With 2D map tools it is also easy to check quantitation results. Instead of using extracted ion chromatograms, which is error prone, mapping IDs back to raw data in 2D gives you a clear view. Viewers can be linked together to allow quick navigation between feature-finding or identification results and raw data (spectra and LC/MS regions in 2D). **Conclusion:** not applicable

Keywords: LC/MS, java, Mass spectrometry, Data Visualization

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CS11.07 Visual and Intuitive Access to Repository Data

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Introduction and Objectives: Although few people think of their biological hypotheses as a massive assortment of data files, most scientists end up staring at such a spreadsheet for an unfortunately long time. Importantly, relatively few tools exist for viewing data within the familiar context of biological processes and pathways. We introduce PNNL's Biodiversity Library and Plugin which allows users to directly access a large repository of proteomics data for 112 organisms including Human to simplify the targeted proteomics assay design. **Methods:** The Biodiversity Plugin exposes curated MS/MS data from 112 different organisms. Human data is additionally subdivided by tissue type. Protein coverage of each organism is ~80% of functionally annotated proteins. The plugin is written in C# .NET using a WPF graphical user interface and a SQLite database backend. The interface allows users to select an organism and KEGG pathways, and then displays the KEGG pathway map highlighting proteins observed in the Library. To transfer protein and peptide data to Skyline, the plugin generates a custom FASTA file containing all selected proteins. Peptide data (including spectra and chromatograms) are also exported to Skyline. **Results and Discussion:** By facilitating data access within the context of familiar pathways and networks, the Biodiversity Plugin is able to reduce development time required for targeted proteomics experiments. This data layered on KEGG pathway images give users a biologically relevant visual interface to interpret and manipulate data in a more intuitive way. The plugin also enables a higher quality experiment design because peptides are based on real MS/MS data and not predictions. **Conclusion:** The Biodiversity Plugin is a useful tool for streamlining and simplifying the design of SRM experiments and is freely available to the public at omics.pnl.gov/software.

Keywords: SRM, KEGG, visualization, software

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CS11.08 SuperQuant: A Data Pre-Processing Algorithm for Increasing Quantitative Proteome Coverage

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Introduction and Objectives: Correct interpretation of fragmentation spectra obtained from co-isolated precursors is one of the severe challenges of shotgun proteomics. SuperQuant is a methodology that expands on the complementary ion pair matching approach of identifying co-isolated peptides. Here we investigate if SuperQuant allows for additional quantitative information to be obtained from MS[1] type quantitative measurements. **Methods:** Dimethyl-labeled HeLa cell lysate with artificially constructed inter-channel ratio – 10:4:1 (L:M:H) was analyzed without pre-fractionation using a Dionex Ultimate 3000 nano-LC coupled to an Orbitrap Fusion mass spectrometer. Analysis was performed with survey and fragmentation scans recorded with high mass accuracy mass. Isolation windows of 1, 2 and 4 Th were used. An algorithm for identification and extraction of co-isolated peptide fragments was implemented in C# and compiled as node to be used with Proteome Discoverer 2.x. **Results and Discussion:** The extent of co-isolation was tuned by varying the isolation window. Each non-targeted peptide supported by at least 3 ion pairs was considered co-isolated and used to generate a virtual spectrum. On average 1.3, 2.5, and 15.3 additional spectra were generated per input spectrum using 1, 2, and 4 Th, respectively. SuperQuant workflow was capable of identifying more peptides and PSMs at all significance levels. The optimal performance of SuperQuant was observed at the 2 Th isolation width, resulting in 35% more peptide identifications (FDR < 0.01). Despite the hampered quantification of co-isolated peptide ions, due to lower isotopic cluster intensity and possible interferences with other ions in MS[1] spectrum, the ratio of quantified to identified peptides was 49% for non-processed data and 42% for SuperQuant data. Su-

perQuant allowed for 15% additionally quantified peptides. The median coefficient of variation of protein quantitation between replicates was comparable for SuperQuant data (6.7%) and non-processed data (5.9%). **Conclusion:** Deeper quantitative proteome analysis can be achieved by SuperQuant pre-processing of shotgun proteomics data.

Keywords: data pre-processing, chimeric spectra, quantitation, shotgun proteomics

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CS11.09 New Functionality for the Trans-Proteomic Pipeline: Tools for the Analysis of Proteomics Data

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Introduction and Objectives: 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 twelve 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. **Methods:** We recently introduced several new features in the TPP: ProteoGrapher is a new tool for visualizing ProteinProphet files in the context of Gene Ontology terms. Improved and more accurate calculation of ProteinProphet probabilities for proteins in complex groups. Models 'dashboard' page now includes support for displaying decoy analysis results, as well as ASAPRatio pvalue analysis. Enabled wavelet signal processing for ASAPRatio quantitation. Many updates to the user interfaces, including the new protXML Viewer, the 'dashboard' view of TPP models, and the Petunia user interface. Updated versions of Comet, Lorikeet, and ProteoWizard. Improved the build and deployment system, and bug fixes and improvements to the user interfaces. We also plan on integrating a novel SWATH analysis toolset on our next release. **Results and Discussion:** 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. **Conclusion:** We describe the new functionality of an open-source analysis software suite for tandem mass spectrometry data.

CS12: METABOLOMICS AND METABOLOMIC DISEASES

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CS12.02 Using Metabolomics to Investigate Pulmonary Diseases in Human Cohorts and Animal Models

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Abstract: The NIH Common Fund has created six regional resource cores to increase the capacity for metabolomic research for NIH-funded projects. At UC Davis, the West Coast Metabolomics Center integrates more than 25 mass spectrometers and 5 NMR instruments in six laboratories, focusing on glycomics, complex lipids, eicosanoids and lipid mediators, imaging, primary metabolism and identification of unknowns. In addition, integration with genomic data including pathway mapping and statistics is part of research advancements and fee-based services. The presentation will highlight novel informatics tools how to interrogate mass spectrometry-based metabolomics data, from compound identification to quality controls. A range of protocols are being used and will briefly discussed with respect to coverage, robustness, and best cases of use. These tools include GC-TOF and GC-QTOF MS as well as QTRAP targeted metabolomics and SWATH-type data independent LC-QTOF and TripleTOF MS/MS experiements. Currently, over 25,000 samples per year are analyzed in our service core, in addition to ranges of studies in our research laboratories. We will show how metabolomic data from lung tissues, blood and heart tissues of smoke-exposed rats can be integrated with physiological and biochemical data for interpreting how aged and diluted environmental tobacco smoke affects different parts of metabolism in a time-response study. A second study shows that even lungs of rat fetuses get metabolically impaired when dams get exposed to secondhand smoke during pregnancy. In a third, most recent study, we demonstrate how novel blood-based biomarkers enable diagnosis of lung cancer in smokers up to one year before subjects present to the clinic, potentially giving more time for treatment for these individuals. When adding a blood based proteomic biomarker, sensitivity and selectivity in blinded validation studies reaches AUC for ROC curves of better than 0.81, enabling clinicians and primary physicians to recommend subjects at risk to receive low-dose CT lung scans, the current gold standard for diagnosing lung cancer.

Keyword: data independent analysis, lung cancer, lipidomics, exposome

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CS12.03 The Unanticipated Complexity of Bile Acid Pools in Human and Mouse

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Introduction and Objectives: Bile acids (BAs) play key roles in human physiology but also have a wide range of cytotoxicity which is associated with pathology. Identification and quantitation of BAs have the potential for diagnosis, prognosis and management of multiple diseases and cancers. We previously developed a new UPLC-MS/MS method for simultaneous quantitation of 50 BAs in blood (Han, et al. Anal Chem, 2015). In this presentation, we describe the use of

this method, plus a combined UPLC-MS[2] and UPLC-MS[3] approach for the expanded profiling of >100 BAs in a variety of biological samples. **Methods:** Human or mouse blood, liver, bile, urine, and fecal samples were studied. The untargeted detection of potentially new BAs was performed by reversed-phase UPLC/multiple-reaction monitoring (MRM)-MS on a triple-quadruple instrument using class-specific MRM transitions of known BAs. This was complemented by UPLC-high-resolution (HR) MS[2] and neutral loss- and precursor ion-triggered MS[3] on an LTQ-Orbitrap, with or without the use of carboxyl group-specific chemical derivatization. **Results and Discussion:** Using class-specific UPLC/MRM-MS detection and the UPLC/HR-MS[2] and -MS[3] analyses, >100 BAs, including 30 phase II metabolism products (sulfates and glucuronides) and 15 tetra-OH BAs, were detected. Sulfates and glucoronides of the unconjugated and glycine-conjugated mono- and di-OH BAs were the main forms of the phase II metabolites in human and mouse, and had higher abundances in urine samples. Secondary BAs and their oxidized keto- and oxo-species dominated the BA compositions in the fecal samples. UPLC/scheduled MRM-MS following untargeted detection was used to simultaneously quantitate all of the known and putative BAs in the various human and mouse samples, with low-femtomole analytical sensitivities, high precision (CV<11%), and high accuracy (80% to 114%). **Conclusion:** This work revealed an unanticipated complexity of the BA pools in human and mouse with many potentially new BAs being quantified for the first time.

Keywords: bile acids, metabolic profiling, UPLC/MRM-MS, phospholipid depletion

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CS12.04 Digestomics: A New Paradigm for Investigating Pathogenic Diseases in Humans

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Introduction and Objectives: A pathogen's nutritional requirements are one of the first, and most critical, barriers to establishing an infection. Host proteins represent a convenient source of biosynthetic precursors and can provide an essentially limitless energy supply to pathogens. Understanding the role that proteolysis plays in infectious disease is a promising new area of research that offers a fresh perspective on host-pathogen dynamics. We have developed a new analytical workflow and custom software tools to investigate this topic in diverse pathogenic organisms. Here, we demonstrate both the power and challenges in applying this new label-free peptidomics strategy through a comparative analysis of malaria and Babesia infections. **Methods:** The strategy we developed integrates existing proteomics tools–high-resolution nano-flow UHPLC-MS/MS and Mascot peptide searches–with quantitative analyses of extracted ion chromatograms in the MAVEN metabolomics software package. Integrating these approaches, and mapping the results onto host and pathogen genomes, has necessitated significant custom software development using the R statistical software environment. **Results and Discussion:** Our new analytical workflow and software tools allows us to construct qualitative and quantitative maps of complete proteolytic cascades. We show how these digestion maps can be used as comprehensive phenotypes for monitoring the metabolic activity of pathogens, investigating the action of drugs, and elucidating selective pressures in host-pathogen interactions. **Conclusion:** Digestomics is a powerful new approach for investing complex host-pathogen dynamics. It provides a unique window into how many pathogens support the nutritional requirements and may provide a new platform for drug discovery.

Keywords: Metabolomics, peptidomics, Infection, Malaria

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CS12.05 Exploring S-(1,2-Dichlorovinyl)-L-Cysteine (DCVC) Nephrotoxicity in the Rat Using UPLC-MS

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Introduction and Objectives: A targeted UPLC-MS/MS method for the accurate quantification of amino acids for metabolic phenotyping, combined with untargeted reversed-phase and HILIC UPLC-MS profiling, is presented in order to generate a comprehensive metabolic picture of the metabolism of the potent nephrotoxin S-(1,2-dichlorovinyl)-L-cysteine (DCVC). Such metabolite profiling studies are envisioned to help in the diagnosis and treatment of kidney disease, streamlining the identification of toxic biomarkers and offering potential treatment targets. **Methods:** Untargeted analysis: Urine and serum samples from male Sprague-Dawley rats administered with S-(1,2-dichlorovinyl)-L-cysteine (DCVC) intraperitoneally at a low (6 mg/kg) or a high dose (60 mg/kg) were analysed by reversed-phase and HILIC-UPLC-MS on an Acquity UPLC system connected to a Xevo G2 QToF mass spectrometer in positive and negative electrospray (ESI) modes. For amino acid analysis, protein removal was performed using three volumes of methanol containing 0.1 % FA with subsequent derivatisation with 6-aminoquinolyl-n-hydroxysuccinimidyl carbamate prior to reversed-phase LC-MS/MS analysis. **Results and Discussion:** The effects of DCVC on urinary and blood metabolite profiles at several time points post administration were investigated and compared with control animals. Unsupervised principal components analysis (PCA) showed separation between urine metabolite profiles from dosed and control rats, with control and pre-dose samples clustering together and dosed samples showing distinct separation in a time dependent manner. Changes in amino acid excretion in high dose urine samples 24 and 48 hr post dose were highlighted and subsequent amino acid quantification showed a significant increase in amino acid concentrations, suggesting that reabsorption by the renal tubule was impaired, compatible with renal injury. Amino acid concentrations returned to normal 72 hr post dose, suggesting that necrosis was accompanied by a regenerative process in surviving tubular epithelial cells, and this was supported by histopathology. **Conclusion:** Metabolite profiling and amino acid quantification of biofluids was performed to investigate the nephrotoxic effects of DCVC.

Keywords: Metabolomics, Amino acid quantification, Nephrotoxicity, UPLC-MS

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CS12.06 A Lipidomic Analysis of Cancer Cells

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Introduction and Objectives: Lipids are essential cellular components and energy sources of living organisms that fulfill multiple distinct yet critical roles in cellular function. Multiple diseases prevalent nowadays resulting largely from disorders of lipid homeostasis including diabetes, obesity, atherosclerosis, and cancer. None-

theless, changes of lipid species have not, to date, been used as a prognostic factor or therapeutic target of cancer. In particular, the exact roles of lipid alteration in cancer progression remains elusive. **Methods:** Shot-gun lipidomic analysis was applied to three hepatocellular carcinoma (HCC) cell lines with different metastatic potentials (Hep3B<MHCC97L<HCCLM3). GC-MS spectra and MRM analysis were used to verify the quantitative changes of palmitic acyl. Effects of palmitic acid treatment on cell proliferation and invasion were measured by CCK-8 exprериments and transwell assays while in vivo assays were carried out for tumor growth and metastasis. The fluidity of cellular membranes was directly examined by a quantitative fluorescence microscopy technique and glucose levels in culture media were measured using the Glucose Uptake Colorimetric Assay Kit. **Results and Discussion:** Using shot-gun lipidomic analysis, we observed that the metastatic abilities of HCC cell lines were negatively related with palmitic acid (PA) accumulation. Both in vitro and in vivo experiments revealed that palmitic acid incubation or feeding specifically reduced cell proliferation, impaired cell invasiveness, and suppressed tumor growth in mouse xenograft models with negligible toxicity. Preliminary mechanism study implied that the antitumor effect of palmitic acid may be mediated by decreasing the membrane fluidity and limiting the glucose uptake of cancer cells. **Conclusion:** These data collectively herald the potential of using PA as diagnostic marker for aggressive HCCs and open an opportunity for treating advanced HCCs by targeting the altered PA metabolism.

Keywords: palmitic acid, glucose utilization, Lipidomics, Hepatocellular carcinoma

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CS12.07 Plasma Metabolomic Profiling of Diabetic Retinopathy

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Introduction and Objectives: Diabetic retinopathy (DR) is the most common microvascular complication of diabetes and the leading cause of visual impairment in working-age adults. DR can be caused by both Type 1 and Type 2 diabetes. Diabetic patients often develop DR despite appropriate control of systemic risk factors, suggesting the involvement of other pathogenic factors. This study aims to identify a plasma metabolic signature of DR which could be resolvable from diabetes without DR. **Methods:** Samples were selected from banked plasma collected as part of the Singapore Indian Eye Study. Forty samples were selected from diabetic participants with moderate non-proliferative DR (level 43 on the ETDRS scale) or worse in at least one eye. Another forty samples were selected from retinopathy-free diabetic participants with comparable diabetes duration as controls. We performed gas chromatography-mass spectrometry-based global metabolomic profiling of plasma samples. **Results and Discussion:** A total of eighteen metabolites were found to be correlated with the plasma metabotype of DR. 1,5-Gluconolactone, 2-deoxyribonic acid, gluconic acid and urea were found to be robust markers of DR even with adjustments for metabolic risk factors and confounding kidney disease. 2-Deoxyribonic acid is a novel metabolite marker that is not curated in major metabolic pathway databases. The pentose phosphate pathway and galactose metabolism are the most significantly perturbed pathways from our list of DR metabolite markers. **Conclusion:** The identification of metabolite markers for DR provides insights into new pathogenic pathways for this microvascular complication and holds translational value in DR risk stratification and the development of new therapeutic measures.

Keyword: Metabolomics, Diabetic Retinopathy, Metabolic Profiling, GC-MS

CS 12: METABOLOMICS AND METABOLOMIC DISEASES
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CS12.08 LC-MS/MS Quantitation of Malondialdehyde (MDA) as a Biomarker of Oxidative Stress in Human Plasma

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Introduction and Objectives: Oxidative stress is a feature of many health conditions including neurodegenerative diseases, cardiovascular disease, diabetes, cancer, and acute tissue injury. There is therefore substantial interest in analytical methods for the sensitive and reliable measurement of oxidative stress biomarkers such as malondialdehyde (MDA), a low molecular weight aldehyde that results from lipids peroxidation in biological systems. Our objective is to develop and validate a new liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for precise and accurate quantitation of MDA in human body fluids. **Methods:** 3-Nitrophenylhydrazine (3NPH) was used for chemical derivatization of MDA under acidic reaction conditions to form a stable derivative that favored positive-ion electrospray ionization (ESI)/MS. A UPLC-quadrupole time-of-flight instrument was used to assess the product of the derivatizing reaction, which was optimized for reaction temperature and duration. Multiple-reaction monitoring (MRM) transitions for UPLC-MRM/MS quantitation were optimized through direct infusion of the derivative on an AB 4000 QTRAP triple-quadrupole MS. To achieve precise and accurate measurements, ^{13}C6-3NPH was used to produce a stable isotope-labeled analogue of the derivative. A reversed-phase C18 column was used for chromatographic separation with isocratic elution and an acetonitrile-water-formic acid mobile phase. **Results and Discussion:** The stable-isotope labeled internal standard used in this method compensated for the matrix effects in ESI. The analytical method was validated to determine its sensitivity, selectivity, precision and accuracy in human plasma. The method demonstrated high sensitivity with lower limits of detection and quantitation of 15 fmol and 60 fmol (on-column amounts) respectively, a wide linear (R^2 ≥0.999) range of >1000-fold concentrations, and good intra- and inter-run CVs (0.999). **Conclusion:** An LC-MS/MS method with ^{12}C/^{13}C-labeled chemical derivatization was developed for quantifying MDA as a biomarker of oxidative stress in blood. This method may offer higher sensitivity and throughput than existing techniques for quantitating MDA, making it uniquely suitable for translation to clinical applications.

Keywords: oxidative stress, biomarkers, quantitative metabolomics, Mass spectrometry

CS 13: REGENERATIVE MEDICINE AND STEM CELLS
TUESDAY, SEPTEMBER 29, 2015 – 11:10 – 13:00

CS13.01 Proteome Meets Genome: Dynamics of Chromatin Organization in Embryonic Stem Cells

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Abstract: One of the key questions in genome biology is to understand how transcription of every single gene in the genome is regulated, and how this depends on cellular context. While the core transcriptional machinery is known to consist of specific and general transcription factors, it is increasingly clear that their activity and determinants to regulate transcription in a gene-specific manner is modulated by a great variety of accessory proteins. This is particularly clear in ES cells where the 'master' transcription factors Oct4, Sox2 and Nanog (OSN) regulate pluripotency aided by an expansive yet still not fully characterized network of regulatory proteins. To study such networks of DNA-bound proteins we have developed 3 novel and complementary methods to characterize the composition and dynamics of chromatin in a global and locus-specific manner. These allow us to study protein interactions around OSN but they can be generically applied essentially to any other systems. The first approach aims to specifically isolate and identify the collective set of proteins that associate with the genome (SICAP, Selective identification of chromatin-associated proteins). In this way we could demonstrate that chromatin in stem cells consists of >5000 proteins, the composition of which depends on pluripotency state. In the second approach we combine SICAP with ChIP to identify the proteins that co-localize with a bait protein on DNA. This has enabled us to identify the protein network around the pluripotency facors Oct4, Sox2 and Nanog, recapitulating and extending the core circuitry of pluripotency. The third approach entails the identification of proteins binding to a single genomic locus of interest (TiGr, Targeted isolation of genomic regions). Applied to the promoter region of Nanog, we have identified many of the known proteins involved in transcriptional regulation of this pluripotency gene, as well as many novel candidates. This includes several proteins that differentially bind to this region in stem cells obtained in distinct pluripotency states. Since all three methods are highly generic, they will prove powerful tools for the discovery of proteins conferring functionality to chromatin.

Keywords: Chromatin, stem cells, pluripotency

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CS13.02 Direct Conversion of Stem Cells Cells by Changing Protein Dynamics

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Abstract: A holy grail of proteomics is to identify the cellular locations of all proteins. Several studies have sought to identify protein locations systematically, but an even larger task is to map and validate which of these proteins dynamically translocate as well as interact in development and disease (the translocalizome, the interactome). We developed the novel algorithm for predicting condition-specific subcellular locations of the gene coding proteins at genome-wide level using only limited and condition-unspecified known locations. With systems biological mRNAs analysis of human stem cells using this method, the key target genes and their coding proteins which involved in maintaining pluripotency and differentiation process were predicted. Molecular biological experiments for target genes and proteins as well as novel protein function validation methods such as FCCS (Fluorescent Cross Correlation Spectroscopy), PLA (Proximity Ligation Assay), molecular imaging, gene transcription will be demonstrated for visualizing the validation process of key mechanisms. Finally, artificial translocation and interaction of novel protein complexes induced direct conversion of human stem/cancer cells will be introduced.

Keywords: stem cell, conversion, protein location, protein interaction

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CS13.03 Identifying Key Stem Cell-Associated Proteins in Normal Blood Development and Leukemia

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Introduction and Objectives: Hematopoietic stem cells (HSC) are the origin of all cell lineages in the blood hierarchy, which, through a line of cell divisions and differentiations, mature into progenitor and fully differentiated cells. Evidence suggests acute myeloid leukemia (AML) to also be hierarchically organized, with leukemia stem cells (LSC) at the apex. LSC are quiescent and insensitive to chemotherapy targeting actively dividing cells, essentially causing patient relapse as they reconstitute the tumor hierarchy post-treatment. The global protein landscapes defining the various sub-populations in humans are largely uncharacterized, especially in terms of protein signaling networks distinguishing HSC and LSC from each other and their respective progenitor populations. By combining animal models and quantitative proteomics, this work aspires to define and functionally validate key proteins that confer the unique phenotypic features of HSC/LSC sub-populations. **Methods:** Flow Cytometry is used to sort the phenotypically defined populations, both within the human blood hierarchy and functionally validated sub-populations from primary AML samples. Using highly optimized single-reaction-chamber digestion protocols, we are able to obtain broad proteome coverage out of 100,000 cells (coverage >8,000 proteins on Orbitrap Fusion), essentially overcoming a major obstacle that precluded this work thus far in humans. Using label-free quantitation, we derive quantitative protein maps of the various sub-populations. **Results and Discussion:** Computational modeling of the proteomics data uncovered key proteins that were enriched in the HSC- and LSC-containing subpopulations, which are deemed potential regulators of stemness. Additionally, we identified LSC-specific proteins, thereby providing potential targets for therapeutic intervention that would spare HSC. Through functional validation of these protein targets using genetic gain- or loss-of-function assays, we are firmly establishing their role in maintaining stemness of HSC/LSC populations in vivo. **Conclusion:** In the long term, this work will likely improve our understanding and methods of targeting hematopoietic diseases arising from early hematopoietic progenitors, such as leukemia, anemia and immune disorders.

Keywords: Xenografts, Cancer Stem Cells, Hematopoiesis, Leukemia

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CS13.04 Super-SILAC Mix for Quantitative Proteomics of Bone Marrow-Derived Mesenchymal Stem Cells

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Introduction and Objectives: Bone marrow-derived mesenchymal stem cells (BM-MSC) are responsible for, among others, the regeneration of the bone tissue and, consequently, alterations in their homeostasis and

osteoblastic differentiation ability can underlie several bone disorders. BM-MSC constitute an attractive option for cell/tissue-based therapies due to their potential to multilineage differentiation, self-renewal and their easy accessibility. However, it has been reported that their regenerative capacity decreases with age, though the molecular characters explaining this decline remain unclear. Here, we constructed a SILAC-labeled mix that can be used as a standard for the study of the bone differentiation process within different patient-derived human BM-MSC. **Methods:** The osteoblastic differentiation of an immortalized human BM-MSC cell line was carried out stimulating the cells with a combination of EGF, dexamethasone, b-glycerophosphate and vitamins C and D. A label-free proteomic approach was followed to accurately quantify proteome changes within BM-MSC along the differenciation process. Among the analyzed time-points, we selected the most representative ones to generate a super-SILAC spike-in standard applicable to BM-MSC. Thus, BM-MSC were grown in heavy SILAC media and then differentiated, harvested at the selected stages and mixed in equal proportions. **Results and Discussion:** Our protocol proves to successfully induce the osteoblastic differentiation of BM-MSC, reproducing its early, intermediate and late phases. The comparison of the label-free proteomes through Principal Components Analysis (PCA) reveals a discreet number of subgroups during the differentiation in terms of proteome changes. So as to include the largest diversity in the super-SILAC mix, we selected the stages showing the most distinct protein expression profile. The super-SILAC was spiked-in as an internal standard to quantify bone differentiation-related proteome differences in patient-derived BM-MSC. **Conclusion:** Our super-SILAC approach proves successful for the investigation of patient-derived human BM-MSC.

Keywords: Bone Marrow-derived Mesenchymal Stem Cells (BM-MSC), quantitative proteomics, super-SILAC, Osteoblastic Differentiation

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CS13.05 Pathological Variability of Motor Neuron Disorders in Patent-Derived iPSC Using SWATH-MS

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Introduction and Objectives: Motor neuron (MN) disorders are life threatening with no cure or standard of treatment. Here, we focus on spinal muscular atrophy (SMA), a MN disorder diagnosed in young children, and amyotrophic lateral sclerosis (ALS) that, in contrast, has a mean age of onset at the age of 55. Developing new therapies for complex diseases such as MN disorders requires elucidation of underlying changes that define the true pathological signaling events from biological variability of individual genetic backgrounds and other environmental/technical factors. **Methods:** Analysis of both iPSCs and MN cell cultures were performed on 12 patient-derived lines of ALS, SMA and control individuals. Sources of variation were tested by analyzing individual sample collections originating from 3 separate cell culture plates at 4 different passages. Lysates were performed using SDS and sonication. Peptide libraries from 12 iPSC and differentiated MN were created to either iPSCs or MNs by basic-reverse phase collection of 30 fractions from pooled tryptic digests and IDA-MS analysis. All SWATH acquisition files were acquired on the Sciex triple TOF 6600. **Results and Discussion:** We report proteomic changes among thousands of quality protein identifications and pathways implicated among disease and control lines. From these we are able to make distinctions among protein expression profiles that are reproducibly stable verses those that were highly variable. By defining these subsets we have begun to establish internal sample-specific quality control measures. These experiments will result in the identification of disease-specific markers from potentially hundreds of implicated pathways that can be targeted simultaneously in high-throughput diagnostics to determine the state and degree of organ and cellular health during the development of patient therapies. **Conclusion:** SWATH-based proteomic analysis of multiple human iPSC lines with various quality control sample collections helps distinguish between environmental and biological background changes in proteomic profiles to better elucidate MN disease-specific signatures of ALS and SMA.

Keywords: iPSC, Motor Neuron Disorders, SWATH

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CS13.06 Identification of Novel Protein Signatures for Subtypes of Breast Cancer Stem/Progenitor Cells

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Introduction and Objectives: There is evidence to suggest that perturbation in stem cells or their progenitors within the normal human breast tissues gives rise to cancer stem cells. Many panels of surface markers including $CD44^{high}/CD24^{low}$, aldehyde dehydrogenase ($ALDH^{high}$), and differentiation markers like MUC-1 are currently being used independently or in combination to characterize cells with stem-like features. However, there is lack of universal utility of these markers with different breast cancer subtypes and their relationship to the normal mammary gland cells. Therefore, there is a need to develop more objective novel tissue-specific biomarkers that may be universally adapted. Quantitative proteome analysis can potentially be useful as a means of unsupervised artificial intelligence algorithm for objective classification and stratification of biological samples. This study aims to identify novel protein biomarkers specific to normal and malignant breast stem cells by comparing the protein fingerprints of stem/progenitor cells from normal and breast cancer tissues as well as different subtypes of breast cancer cell lines for their expressions of stem cell phenotypic protein profiles. **Methods:** Cells from normal human breast tissues, human breast tumors, and cells from different subtypes of breast cancer cell lines (SK-BR-3, MDA-MB-468 & MCF-7) were Flow Cytometry-sorted using combinations of surface markers. The phenotypic characteristics of these cell populations were subsequently evaluated by Waters Synapt G2- label-free quantitative Nano-LC-MS-MS expression analysis. **Results and Discussion:** Some of the more than 800 so far identified differentially expressed proteins between cells representative of each of the breast cancer subtypes are membrane/stem cell-related proteins including CEACAM, S100A6, ASXL1, FGFR1, KRT8, RECQL, and RUVBL2. **Conclusion:** We have used expression proteomics as quality control and biomarker discovery tool in stem cell research. Potential applications of some of the identified proteins towards standardization of cultures and reproducible cell differentiation into tissue specific cell lineages for clinical applications are being validated.

Keywords: Proteomics Stem Cell Biomerker, Expression Proteomics, Stem

-Progenitor Cells, Breast cancer

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CS13.07 Systematic Identification of Single Amino Acid Variants in Glioma Stem Cell Lines

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Introduction and Objectives: According to the neXtProt (release 2015-01-01) current status of the human proteome annotation is 20,061 proteins with ~30% of the protein-coding genes still lacking evidence at the protein level. Additionally, ~1,500,000 known single nucleotide polymorphisms (SNPs) create a large pool of single amino acid variant (SAV) containing missing proteins. In this work we attempt to routinely identify missing proteins and use them for phenotyping glioma stem cell (GSC) lines. **Methods:** Thirty-six GSC lines provided by M.D. Anderson Cancer Center were analyzed using nanoLC-MS/MS (Orbitrap Elite, Thermo). Each sample was run in triplicate. MS (.raw) data files were searched against a combination of the UniProt Human database (release June 2014) with all available through neXtProt (release 2014-07-01) SAV-containing sequences together with a contaminant database using PEAKS 7 (BSI). Identified peptides were filtered based on their spectrum matching score and unique existence in human proteome based on the BLAST analysis. Corresponding non-variant peptides were matched to the genome (ensemble release 70) to determine their genomic position. RNA-Seq data, also provided by M.D. Anderson Cancer Center, was screened for each genomic region using SAM tools and translated to amino acid sequence to validate the peptide. All matches/variants were accounted. **Results and Discussion:** We confidently identified 1,022 SAV-containing peptides in the proteomic data from 36 cell lines. However, 241 of them were found to be non-unique/homologues. Based on RNA-seq data, a few SAV transcripts were heavily based toward the variant form. Among those, the known cancer-associated genes nestin and tenascin were found with the variant evidence in COSMIC database. Other germline variants were identified in glioblastoma-related proteins. We believe that a subset of SAVs may be novel targets or biomarkers for GSCs, while ones that are present only in a fraction of the cell lines and cluster together on the heatmap could be cancer phenotype specific. **Conclusion:** Not applicable

Keywords: transcriptomics, single amino acid variant, proteomics, Proteogenomics

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CS13.08 Extracellular Matrix Quantification: Defining the Scaffolding of Regenerative Medicine and Disease

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Introduction and Objectives: Changes in ECM composition and architecture have been shown to mediate cell proliferation, differentiation, and growth, serving as a critical component of both disease process and the development of organ scaffolds for tissue engineering efforts. However, traditional methods used to characterize the protein composition of native, acellular, and diseased tissues fail to accurately quantify ECM proteins. Thus, we aim to provide a method to quantify ECM proteins from tissues for accurate protein level comparison between heterogeneous tissues in order to enhance our understanding of regenerative medicine and disease. **Methods:** Human and mouse lung samples were sequentially solubilized using a hypertonic detergent (cellular fraction), a strong denaturant (soluble ECM), and chemical digestion (insoluble ECM). We developed Stable Isotope Labeled (SIL) QconCATs representing ECM targeted and cellular proteins. SIL polypeptides were added to protein fractions prior to trypsin digestion and peptide quantification was obtained using a LC-SRM assay. This quantitative assay was employed for ECM protein comparisons between (1) native & acellular mouse lungs and (2) used to characterize the regional distribution of ECM between human normal, acellular and Idiopathic Pulmonary Fibrosis (IPF) lungs. **Results and Discussion:** We developed a method consisting of 561 SIL-labeled peptides representing 216 ECM-targeted and cellular proteins with species specificity across commonly used mammal models. Initial experiments that have resulted in (1) defining the protein composition of whole organ scaffolds being developed for regenerative medicine (2) and characterizing the regional distribution (upper/lower lobe pleura/parenchyma, vasculature, and airway) of ECM composition in normal and IPF human lungs. **Conclusion:** Our approach allows for robust and accurate quantification of protein levels of the ECM using LC-SRM mass spectrometry. The accurate characterization of ECM proteins should help advance our understanding of disease process, and provide a readout for tissue engineering that can be correlated with functional outcome to drive further development.

Keywords: extracellular matrix, QconCAT, targeted mass spectrometry, Tissue Engineering

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CS13.09 Proteome-Scale Analysis of Transcription Factors in Liver Regeneration

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Introduction and Objectives: Liver is one of the few adult organs that have capability of complete regenerating in response to cellular injury from tissue resection, toxins, and hepatitis. Transcription factors play central roles in this process. However, large-scale analysis of transcription factors in liver regeneration was ill known. The prosperity of proteomic researches in recent years greatly stimulates the interests in uncovering mechanism in physiology and pathology. **Methods:** We have developed an affinity reagent named concatenated tandem array of the consensus TFREs (catTFRE) that permits identification of endogenous TFs at the proteome scale. In this study, we employed catTFRE approach to monitor regulation of TFs in liver regeneration induced by 70% partial hepatectomy. Comprehensive bioinformatics analysis was carried out to discover differential TFs and their enriched pathways. **Results and Discussion:** As a result, 485 TFs were identified totally. An average of 330 TFs were detected in mouse liver in different time points from 12 hours to 7 days after PHx. TFs were grouped into 12 classes according to their regulation along with time course. Among the 12 classes, TFs from 4 classes were up regulated at time point 0.5d, 1d, or

2d, while TFs from 4 classes were down regulated at time point 0.5d, 1d, or 2d. Analysis of target genes controlled by at least 2 TFs in each class showed that immune response was enriched in all 4 up-regulated classes and metabolism was enriched in down-regulated class. **Conclusion:** Together, we surveyed TFs regulations with proteome-scale during liver regeneration, providing a resource to the liver physiology research community for further investigation.

Keywords: Transcription Factor, Liver regeneration

CS14: MEMBRANE PROTEOMICS

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CS14.02 Membrane Protein Interactomes in Health & Disease: Application to Lung Cancer

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Abstract: My lab is focused specifically on understanding how the interactions of membrane proteins contribute to cellular disease states at a systems level. Despite extensive proteomics research in the past decade, there is a lack of in-depth understanding of protein networks associated with integral membrane proteins because of their unique biochemical features, enormous complexity and multiplicity. This is a major obstacle to understanding the biology of deregulation of integral membrane proteins which leads to numerous human diseases, and consequently hinders our development of improved and more targeted therapies to help treat these diseases. To address this problem, we developed two unique technologies specifically suited for the study of full-length integral membrane proteins in their natural cellular context; the classic Membrane Yeast Two-Hybrid (MYTH) [1-5] and the newly created Mammalian Membrane Two-Hybrid (MaMTH)[6]. Our ultimate goal is to uncover a wealth of information about protein interactions for the majority of "druggable" human membrane proteins, which should in turn greatly facilitate the discovery of new truths about diseases like cancer, schizophrenia, cystic fibrosis, hypertension and Parkinson's disease. During my talk, I will discuss our recent findings indicating that the application of MaMTH to the human Epidermal Growth Factor Receptor (EGFR) [6,7] resulted in the identification of CRK II protein as a novel interactor of oncogenic EGFR (L858R), and showed that CRKII promotes persistent activation of aberrant signaling in non-small cell lung cancer (NSCLC) cells[6]. I will also illustrate how MaMTH is a powerful tool for drug discovery as well as for investigating dynamic interactomes of human integral membrane proteins, and why it promises significant contributions to therapeutic research.

Keywords: membrane proteins, protein-protein interactions, lung cancer, Mammalian Membrane Two-Hybrid (MaMTH) assay

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CS14.03 Membrane Proteome Profiling to Discover Therapeutic Targets for HTLV-1 Associated Myelopathy

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Introduction and Objectives: Human T-cell leukemia virus type1 (HTLV-1) is a retrovirus which infects CD4⁺ T-cells. 20~30 million individuals are infected by HTLV-1 in the world and 0.3% of those develop HTLV-1 associated myelopathy (HAM/TSP). HAM/TSP is a progressive neurological disease characterized by severe motor impairment. Since no curative therapy is available, development of novel therapies for HAM/TSP is urgently required. Here we conducted quantitative proteome analysis on CD4⁺ T-cells to identify novel therapeutic targets for HAM/TSP. **Methods:** CD4⁺ T-cells from peripheral blood mononuclear cells of normal donors (ND, n = 14), asymptomatic carriers (AC, n = 21) and HAM/TSP patients (n = 21) were subjected to tryptic digestion. To focus on membrane proteome, glycopeptides were enriched by hydrophilic chromatography followed by Con-A lectin affinity chromatography in 96 well plate format. Glycopeptides desorbed by PNGase were analyzed by LTQ-Orbitrap Velos and label-free quantification was performed. To verify that expression of candidate protein was specific for infected cells, HTLV-I proviral loads were quantified by qPCR. **Results and Discussion:** 56,705 peptides were detected in LC-MS/MS analysis. Welch test (ND + AC vs HAM/TSP, $p < 0.05$) extracted 171 peptides up-regulated in HAM/TSP. MASCOT search identified 6,791 glycopeptides derived from 946 glycoproteins (FDR < 0.01). Among identified glycoproteins, 69.8% of those were annotated as surface proteins. Analysis of 171 up-regulated peptides allowed identification of 12 peptides, including Adhesion molecule A (Adm-A) as a therapeutic target candidate. The qPCR on leucocytes from 6 HAM/TSP patients revealed that HTLV-1 infected cells were specifically enriched in Adm-A⁺ subpopulation among CD4⁺ T-cells. **Conclusion:** Membrane proteome analysis on CD4⁺ T-cells identified Adm-A as a promising therapeutic target for HAM/TSP. These results show that our approach is ideally suited for discovery of pathogenic cell-specific surface antigens.

Keywords: Leukemia, Label-free quantification, membrane proteomics, therapeutic target

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CS14.04 Aβ-Induced Protein Phosphorylation Changes in the Mouse Synaptosome

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Introduction and Objectives: Alzheimer's disease (AD) is a neurodegenerative disorder characterized by the aggregation of Aβ and tau. The strongest pathological correlate of clinical dementia is not protein aggregation or neuronal death, but loss of synapses, implying that AD is a synaptic disorder. Not only is Aβ secreted at synapses, its primary toxicity also appears to be targeted against synapses. Tau, an intracellular protein, is also secreted at synapses and toxic forms of tau appear to spread along synaptic networks. The goal of this study is to understand Aβ-induced changes in protein phosphorylation patterns of brain synaptosomes. **Methods:** We prepared synaptosomes of APP/PS1 mice and conducted phosphoproteomic surveys based on quantitative mass spectrometry. Since synaptosomes are membrane-rich pellets, we used gel-assisted digestion to facilitate the detection of membrane proteins. Phosphopeptides enriched by immobilized metal ion affinity chromatography were analyzed by label-free quantitative mass spectrometry. **Results and Discussion:** We detected and quantified hundreds of

protein phosphorylation sites in the synaptosome fraction, covering both membrane and non-membrane proteins. We detected proteins of synaptic, mitochondrial, and myelin origins, consistent with the reported composition of such preparations. Many sites showed altered phosphorylation states in APP/PS1 over control mice, including many proline-directed kinase sites. Hence, proline-directed kinases may be downstream mediators of amyloid toxicity. **Conclusion:** Using gel-assisted digestion, it is possible to carry out comprehensive phosphoproteomic analysis of membrane proteins in synaptosomes, which contain biological membranes of very diverse origins. The synaptosomal proteome may be closely related to the secreted proteome of brain tissues, and therefore phosphosites showing high intensity and significant changes may have the potential to be useful biomarkers detectable in cerebrospinal fluid or blood plasma. The link between synaptosomal proteins and body fluid biomarkers should be further investigated.

Keywords: phosphorylation, amyloid, Alzheimer's disease, membrane protein

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CS14.05 Accurate Determination of Cellular Membrane Protein Copy Numbers

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Introduction and Objectives: Systems biology interpretation requires accurate quantification of cellular components. Achieving this goal for integral membrane proteins poses additional challenges, such as selecting of an adequate standard for protein quantification. Here we describe for sperm from the sea urchin A. punctulata, from cell lysis to MRM-based peptide quantification with a focus on a PSAQ membrane protein standard, how recovery can be quantified and high accuracy can be achieved in each sample preparation step. **Methods:** Determination of sperm cell number and lysis efficiency were determined with a Neubauer chamber and a CASY cell counter. Sample recovery upon SDS-PAGE and tryptic protein digestion was assessed by means of introducing a stable isotope-labeled guanylate cyclase (GC) and external standard proteins. GC, standard proteins (e.g. ovalbumin), and the cyclic-nucleotide-gated channel (CNGK) were quantified with AQUA peptides, using RP- LC separation and MRM analysis on a TSQ vantage, and Skyline software for quantification. **Results and Discussion:** It was found that cell counting with both methods did not result in significant differences. Quantification of GC with PSAQ in comparison to AQUA peptides, resulted in higher accuracy, since copy numbers were underestimated by a factor of about four with AQUA. Comparing quantification results for each peptide, usage of PSAQ delivered higher precision, reflected in a smaller standard deviation, too. It was found that the GC receptor covers about 15% of the sperm flagellar surface. An important finding for interpreting the signal transduction mechanism was the about 20fold excess of GC over CNGK, which excludes the tight association of both proteins in a signalosome complex. **Conclusion:** Reliable cell-counting methods and addition of a PSAQ standard early in the experimental workflow are mandatory for accurate quantification of cellular (membrane) protein copy numbers

Keywords: membrane, signal transduction, Absolute quantification

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TUESDAY, SEPTEMBER 29, 2015 – 11:10 – 13:00

CS14.06 Membrane Proteins of Leukocyte-Inspired Nanoparticles Improve Their Therapeutic Efficacy

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Introduction and Objectives: The use of bio-inspired nanocarriers aims to improve drug efficiency by enhancing targeting and biocompatibility, and reducing side effects. We showed that nanoporous silicon (NPS) particles coated with cell membranes isolated from leukocytes -Leukolike Vectors (LLVs) – possess cell-like properties. **Methods:** LLVs were fully characterized for their shape, size, surface charge and coating through DLS and SEM. In addition, we characterized the content and function of the leukocyte's proteins transferred onto the LLVs through LC/MS^E. The presence of leukocyte markers was validated by western blotting and FACS. For the protein corona experiment, LLVs were incubated in serum, then isolated by centrifugation, the proteins of the corona were resolved by 1D gel electrophoresis and then identified by LC/MSMS. **Results and Discussion:** LLVs escape macrophage uptake, delay sequestration by the reticulo-endothelial system and target tumor inflamed vasculature. Moreover, the proteomic analysis of the coating revealed the presence and the correct orientation of several important markers of leukocytes: CD45, CD47 and MHC-I were identified as key players in determining LLVs biocompatibility, while Leukocyte Associated Function-1 (LFA-1) and Mac-1 contributed to the LLVs targeting ability and bioactivity towards inflamed endothelium. In addition, our data showed that the membrane coating induced the formation of a singular protein corona (i.e. the protein adsorption layer) on the surface of the nanoparticles either qualitatively and quantitatively, if compared to non-coated nanoparticles. **Conclusion:** Our results show that is possible to transfer biologically active leukocyte membrane proteins onto synthetic nanoparticles, thus creating biomimetic carriers retaining cell-like functions. The targeting of the inflamed endothelium can be applied to a broad range of diseases and this approach could open new avenues for the development of next generation personalized treatments by using as cell membrane source the immune cells of patients

Keyword: Nanomedicine, membrane proteins, leukocytes, drug delivery

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CS14.07 Comparison of Membrane Enrichment Coupled Proteomics Methods for Immune Target Discovery

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Introduction and Objectives: Human monocytes play crucial role in innate immune system towards the pathogenic, bacterial and inflammatory agents. A major interest of the pharmaceutical industry is to

suppress or treat immunological disorders. It is a challenging task to find novel membrane targets since membrane proteins are low in abundance and relatively insoluble. To develop a membrane enrichment coupled proteomics strategy for human monocytes, we combined and compared multiple MS -based proteomics techniques. **Methods:** Monocytes from 20 donors were pooled and subjected to a variety of different membrane enrichment coupled proteomics and whole cell proteomics methods. Urea denaturation based and FASP based whole cell proteomics as well as cell surface biotynylation, hydrazide chemistry based glycoproteomics and two step ultracentrifugation based membrane enrichment methods were coupled with LC/MS/MS. At least three runs were completed for each method and two dimensional liquid chromatography technologies were applied due to the complexity of the sample prior to Q-Exactive MS/MS runs. **Results and Discussion:** Up to 1,800 proteins were identified by each method in single injections and together with two dimensional liquid chromatography experiments, we were able to analyze 10,400 proteins. A variety of bioinformatics analyses including transmembrane domain and gene ontology were applied to each set of experiments as well as the combined data set. The glycoproteomics method provided the highest percentages of membrane protein enrichment. However, two step based ultracentrifiguation membrane enrichment technique (47 % enrichment) was found to be most efficient in terms of protein coverage and required less cells. IPA showed antigen presentation pathway and activation of macrophages as the most enriched functions and pathways. **Conclusion:** By coupling a variety of different membrane enrichment methods with mass spectrometry based proteomics techniques and the bioinformatics analysis tools, we were able to devise and efficient strategy that can be used to scan membrane proteome of immune cells for novel target discovery.

Keywords: membrane proteomics, immune cell target discovery, Glycoproteomics, cell surface biotinylation

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CS14.08 Analysis of Membrane Proteins – Opening the Treasure Chest Protected by the Phospholipid Bilayer

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Introduction and Objectives: About one-fourth of eukaryotic genes codes for transmembrane proteins (TMPs), yet because of their low expression levels and their amphipathic nature, integral membrane protein are underrepresented in conventional large-scale proteomic analyses. Numerous methods aimed specifically at TMPs have been developed, however, low enrichment and substantial contamination by non-membrane proteins remains an issue. **Methods:** As demonstrated by Blackler et al. [1], the transmembrane alpha-helical domains protected by the phospholipid bilayer can be selectively isolated from cellular material after the unprotected hydrophilic (extramembrane) domains and all contaminating non-membrane proteins had been proteolytically degraded. With our modified protocol we performed the enrichment of the crude membrane fraction followed by opening membrane vesicles at high pH and tryptic digestion of all accessible protein material. The "shaved" membranes were then solubilized, and the transmembrane domains were re-digested with CNBr to generate shorter peptides amenable to mass spectrometric analysis. **Results and Discussion:** We applied this method to human mantle cell lymphoma (MCL) cells, and identified over 800 TMPs containing 1 to 16 transmembrane domains and originating from various membrane compartments. The majority of those proteins were identified by peptides mapping onto the predicted transmembrane domains. **Conclusion:** The method, being compatible with SILAC experiments, can complement conventional large-scale proteomic analyses by providing the semi-quantitative information on the expression of hundreds of hydrophobic integral membrane proteins. References [1] Blackler AR, Speers AE, Ladinsky MS, Wu CC. A shotgun proteomic method for the identification of membrane-embedded proteins and peptides. J Proteome Res. 2008 7(7):3028-34.

Keywords: CNBr, integral membrane proteins, transmembrane proteins, lymphoma

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CS14.09 TGFβ1 Induces Membrane Proteomce Changes in Colorectal Cancer Cells

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Introduction and Objectives: It is well understood that Transforming Growth Factor-β (TGFβ) is a potent regulator of cell growth, differentiation, migration and tumour suppression. However, during colorectal cancer (CRC) and other cancers TGFβ mRNA and protein show marked increases are associated with tumour growth. The involvement of TGFβ as tumour promoter is poorly understood. We hypothesise that the increased expression of TGFβ can be achieved by high expression levels of integrin αvβ6, which can activate latent-TGFβ and MMP-9 both of which are activators of latent-TGFβ. High expression of αvβ6 has been observed in various cancers including CRC. Cell proliferation and would healing assays showed significant differences when treated with active TGFβ. To gain more in-depth understanding of role of TGFβ in CRC we compared the membrane proteomes of TGFβ1 (10ng/mL) treated SW480, HT29 and HCT116 CRC cells with varying expresison of αvβ6 and uPAR. **Methods:** Triton X-114 phase partitioning method was used to isolate the membrane proteins from the cells. Proteomics was performed using iTRAQ on an AB Sciex TripleTOF5600. **Results and Discussion:** Proteomics identified varying numbers of significant proteins form each cell line. The IPA analysis these significantly altered proteins revealed cellular assembly and organization, cell-to-cell signalling and interaction as the top-ranked altered biological function. Ingenuity pathway analysis also showed RAN and EIF2 signalling were amongst top 5 canonical pathways identified. We identified various proteins including integrins (α3β1, α4β1, α6β1, α5β1, αv), ERK1/2, TGFβ and MAPK1/2 through IPA network analysis. All these molecules have been previously implicated in various cancers including CRC. **Conclusion:** These results show that TGFβ is enhancing the ability of the cancer cells towards progression and metastasis. It is very important to study any TGFβ associated protein-protein interactions during CRC which can assist in identifying novel key players for metastasis, and serve as biomarkers and/or drug targets to improve CRC therapy.

Keywords: membrane proteomics, TGFβ1, colorectal cancer, crosstalk

CS15: CARDIOVASCULAR AND HAEMATOLOGICAL PROTEOMICS

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CS15.02 Dynamics of Life

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Abstract: Data Science of Mitochondrial Discoveries and Clinical Translation: Recent investigations have achieved monumental milestones in omics sciences. Nevertheless, we face great challenges in translating these molecular discoveries into reusable biological knowledge. Success in this endeavor is critical and will require the effective integration, organization, and dissemination of diverse multi-scale data. Accordingly, several data science initiatives have been launched by funding agencies to address critical tasks in data analysis and clinical translation. Our work has focused on developing specific software packages for the analysis and integration of omics data to understand mitochondrial biology and medicine. An example is to delineate protein dynamics data in the mitochondria via ProTurn. The ProTurn project aims to provide bioinformatics tools for calculating biomolecule dynamics in different stable isotope labeling mass spectrometry experimental designs. ProTurn combines mass spectrum integration, kinetic curve-fitting, and statistical tools in a user-friendly package. We first demonstrated its utility on mitochondrial protein dynamics in cardiac diseases, and have now accrued turnover dynamics data on over 500 mitochondrial proteins in multiple species, genetic backgrounds, and disease states. The bulk of these data are being made available on Synapse, a public collaborative data analysis platform by our BD2K Center investigators at Sage Bionetworks. A second example is the initiation of the Cardiac Gene Wiki project, which aims to address a persistent challenge in biomedical research, namely that the broad community (scientists, clinicians, and laypersons alike) cannot readily access and benefit from published scientific knowledge. Since its birth in 2008, the Gene Wiki project has begun to document information on ~10,000 human genes, however with limited success in completion. To address this challenge, we are developing new tools and resources to engage users in gene annotation via crowdsourcing. We have demonstrated the utility of the preliminary tools by targeting mitochondrial protein pages and annotating them with cardiovascular domain knowledge. We have completed over 200 Gene Wiki pages on mitochondrial proteins (>10% of the mitochondrial proteome), with over 800 sections improved, and over 1,600 references added. The tools discussed above are open-source via the Cardiac Organellar Protein Atlas Knowledgebase (COPaKB), which provides an integrated framework that combines high-quality cardiac mitochondrial proteomics datasets, analysis tools, and information on cardiovascular phenotypes. COPaKB aggregates data from prominent community resources as well as its own relational database, enabling users to retrieve and analyze organellar protein properties of interest, including protein expression profiles and cardiovascular disease relevance. The intuitive interface, multi-resource integration capabilities, and powerful bioinformatics tools of COPaKB are designed to allow researchers within or outside of the proteomics field to dissect the molecular signatures of cardiovascular phenotypes and uncover hidden relationships. These tools now form part of an emerging data science ecosystem being cultivated at the UCLA BD2K Center and its collaborators globally. I will summarize by discussing further opportunities and challenges in the era of proteomics Big Data.

Keywords: Mitochondria, Data science, dynamics, annotation

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CS15.03 Antibody Proteomics in Plasma for Diseases Affecting the Cardiovascular System

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Introduction and Objectives: Diseases of the cardiovascular system are the leading cause of death in the Western world and because patients are most often diagnosed with advanced disease, a right classification is essential to enable tailored treatment. For a systematic exploration of proteins related to these phenotypes and across larger sets of plasma samples, we developed an affinity proteomics strategy based on suspension bead arrays assays and antibodies from the Human Protein Atlas (www.proteinatlas.org). **Methods:** Multiplexed and hypothesis-free disease studies were conducted on antibody bead arrays using directly labeled samples. We screened 384 patient samples from four distinct clinical study sets using more than 10,000 antibodies. For subsequent target validation, we employ additional study sets, several antibodies towards a common targets of interest, and mass spectrometry for the identification of proteins captured from plasma to build sandwich assays. **Results and Discussion:** An affinity-based strategy was applied to discover and validate proteins in plasma related to diseases of the cardiovascular system. We selected 720 candidates from 10,000 anitbodies for subsequent targeted analysis in > 2,000 retrospective plasma samples. Several interesting candidates were found in the context of myocardial infarction and unstable angina. Their dependency on established risk factors used for clinical management, such as BMI, smoking, and age was subsequenlty assessed. Candidate targets were then validated by immune-capture mass spectrometry with plasma, and combinations of antibodies were evaluated to build sandwich assays. Presently these sandwich tests are being implemented into a clinical routine laboratory to evaluate assay and marker performance in prospective samples and in relation to troponin and other clinical risk factors. **Conclusion:** This work describes a translational study on affinity-based plasma proteomics that provided promising candidates for cardiovascular disease and upon further clinical evaluation, these tests may support and improve current tools in clinical routine.

Keywords: Antibody bead arrays, Biomarker discovery, Assay translation, plasma

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CS15.04 Cardiac Recovery: Targeting Novel Cell Type and Chamber Specific Markers

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Introduction and Objectives: Advanced heart failure (AHF) describes the subset of heart failure patients refractory to conventional medical therapy. For some AHF patients, mechanical circulatory support (MCS) provides an intermediary "bridge" step for transplant eligible patients. Clinical observations have revealed ~1% of patients with MCS undergo reverse remodeling such that the device can be explanted. As this is clinically infrequent, targeted approaches to identify who would most benefit from MCS and predicting clinical response would be invaluable. One promising source of mark-

ers for meeting these clinical needs resides in the pool of proteins localized to the cell surface. Cell surface proteins can be valuable as live imaging markers, may be shed into the circulation and detectable as non-invasive markers, and may be informative of the molecular mechanisms involved in disease, specifically those that offer cell type and chamber specificity. **Methods:** We have used an innovative MS strategy to identify extracellular epitopes of cell surface proteins on human cardiac fibroblasts and myocytes. A pluripotent stem cell model of cardiomyogenesis allows us to identify proteins that emerge during early development which may be exploited for identifying targets that re-emerge during AHF. **Results and Discussion:** We have identified >600 cell surface proteins on cardiac fibroblasts and cardiomyocytes, including proteins unique to each cell type. Using the extracellular domain defined by the MS approach, we developed monoclonal antibodies specific to a novel cardiomyocyte marker. Also, protein abundance and subcellular localization changes are compared in failing and non-failing heart tissue. Finally, top-down MS is used to define the extracellular domain of proteins shed from the cell surface to inform targeted MS assays of serum to probe for cell-type specific markers shed during disease. **Conclusion:** We describe a promising novel approach to identifying cell type and chamber specific proteins whose tissue expression and circulating levels may allow for targeted stratification of myocardial recovery potential.

Keywords: heart failure, cell surface proteins, cardiomyocyte, stem cells

CS15.05 Label-Free Quantification of Hypoxia-Induced Changes of the Cardiac Fibroblast Secreted Proteome

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Introduction and Objectives: Cardiac fibroblasts (CF) play central roles in the outcomes of heart failure after ischemia and fibrosis. Cardiomyocyte and CF intercellular communication can occur through paracrine and ECM interactions. In addition to soluble factors, cardiac cells secrete exosomes (EXO), with evidence suggesting CF EXO modulate pathophysiology in vitro. Detailed proteomic analysis of the fibroblast secretome in normal and stressed conditions will offer insights into the role of CF in heart disease. **Methods:** Primary mouse CF were cultured for 24h in 21% (normoxic) or 2% (hypoxic) O2 for 24h in serum-free media. Conditioned media was differentially centrifuged and ultracentrifuged to obtain EXO and EXO-depleted secretome (SEC) fractions. Successful EXO isolation was indicated by CD81 enrichment via immunoblot, density measurements of 1.17-1.24 g/mL via sucrose gradient, and stereotypical morphology and size via electron microscopy. 6-step MuDPIT was performed with a LTQ-Orbitrap Discovery. Data was searched using XTandem!, OMSSA, MyriMatch, and Comet. Protein relative abundance was calculated using QSpec. **Results and Discussion:** Proteomic analysis identified 1760 unique proteins in total, with 1366 and 647 in normoxic EXO and SEC, respectively, and 1314 and 895 in hypoxic EXO and SEC, respectively. QSpec analysis identified 501 proteins differentially expressed between normoxic fractions, 152 proteins between hypoxic fractions, 150 proteins between normoxic and hypoxic SEC, and 439 proteins between normoxic and hypoxic EXO. Gene Ontology revealed hypoxic conditions increase expression of secreted proteins associated with ECM and signalling, suggesting an activated secretory phenotype. Proteins enriched in EXO and in SEC were associated with cytoskeleton and glycoprotein annotations, respectively. For functional assessment, we subjected cardiomyocytes pretreated with either CF EXO or SEC for 24h, to 60 μM H2O2 for 24h to mimic oxidative stress. MTT assays suggest a reduced viability due to CF-derived secreted factors. **Conclusion:** CF secretome proteomics reveal differential expression based on mode of secretion and oxygen-levels in vitro.

Keyword: Exosome, Secretome, MuDPIT, Label-Free

CS15.06 Source Specific cGMP Activates Distinct PKG Signaling Pathways in Cardiac Myocytes

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Introduction and Objectives: The cyclic-GMP (cGMP)/Protein kinase G (PKG) signaling cascade is a key regulator of contraction/relaxation in the myocardium and a well-recognized modulator of cardiac function. cGMP levels are regulated by guanylate cyclases (GC), which synthesizes cGMP from guanosine triphosphate, and phosphodiesterasaes (PDEs), which hydrolyzes the phosphodiester bond of cGMP. Enhancement of cGMP levels and PKG activity, in particular through cGMP specific phosphodiesterase 5 (PDE5) inhibition, has been shown to reverse the pathophysiology associated with mouse models of pressure overload induced cardiac hypertrophy; however, clinical trials testing the efficacy of PDE5 inhibitors in human heart failure have shown poor outcomes. In the current study we set out to 1) determine the PKG phosphoproteome of adult cardiac myocytes using the cGMP analogue 8-Br-cGM and 2) determine the phosphoproteome of neonatal rat myocytes resulting from inhibition of two cGMP specific PDEs (PDE5 and PDE9) and to determine if these PDEs hydrolyze cGMP generated specifically from NO or NP signaling. **Methods:** Adult cardiac myocytes were cultured for 15 minutes in the presence and absence of 8-Br-cGMP. Neonatal myocytes were cultured in the presence and absence of PDE5 or PDE9 inhibitors with and without L-NAME. Samples were digested and phosphopeptides were enriched by TiO2 and analyzed by LC-MS/MS. **Results and Discussion:** We have identified 201 phosphosites on 145 proteins that were differentially regulated in adult cardiac myocytes with 8-Br-cGMP treatment. Additionally, we showed that inhibition of PDE5 and PDE9 results in both unique and overlapping phosphoproteome changes and that the PDE5 inhibited phosphoproteome was reversed to a greater extent through the inhibition of NOS by L-NAME. **Conclusion:** These results suggest that PDE5 primarily hydrolyzes cGMP generated from the NO-soluble GC pathway while PDE9 operates in an NO-independent manner. These results could have an impact on the use of PDE9 inhibitors as potential therapeutics for cardiovascular diseases.

Keywords: proteomics, cardiology, Protein Kinase G, cyclic-GMP

CS15.07 Studying the Functional Role of Reactive Oxygen Species in Atherosclerosis by Redox Proteomics

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Introduction and Objectives: Ruptured atherosclerotic plaques activate platelets and trigger formation of thrombi that cause heart attacks and strokes, claiming millions of lives every year. Recruitment of activated monocytes to inflamed blood vessels is a critical contributor to plaque formation. Consequently, strategies that prevent platelet or monocyte activation hold enormous therapeutic potential. We have recently identified a novel mechanism by which platelet activation activates monocytes: platelet releasate (PR), secreted by activated platelets, up-regulates very late antigen 5 (VLA-5) and increases monocyte adhesiveness. PR also induces production of reactive oxygen species (ROS), leading us to investigate its role in monocyte activation. **Methods:** SILAC-labeled THP-1 cells were exposed to PR following platelet activation by physiological and pathological agonists (thrombin and lysophosphatidic acid (LPA)). To quantify all forms of cysteine oxidation by mass spectrometry, a modified biotin-switch redox proteomics approach was applied. ROS levels and expression of protein markers were quantified by FACS. For inhibition studies, cells were pre-treated with commercially available NOX inhibitors. **Results and Discussion:** We identified 104 or 120 oxidative modifications from 239 or 224 cysteine-containing peptides for thrombin- or LPA-induced PR, respectively. Although several proteins involved in glycolysis showed reduced enzymatic activity, a distinct signaling pathway did not emerge. Further experimentation revealed DPI, a non-specific NADPH oxidase (NOX) inhibitor, to greatly reduce PR-induced ROS production and reverse the increase in monocyte adhesion. Proteomics confirmed DPI to block cysteine oxidation, establishing a functional link between these events. Additional screening with isoform-specific inhibitors verified NOX involvement in monocyte activation. **Conclusion:** Our work highlights NOX as a novel therapeutic target in atherosclerosis. We currently apply this approach to evaluate the effects of NOX inhibitors also in platelet activation, which we have observed to also involve NOX activation and ROS production. We anticipate this to provide additional proof of the utility of redox proteomics in identifying and validating potential therapeutic targets.

Keywords: reactive oxygen species, atherosclerosis, platelet/monocyte interactions, redox proteomics

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CS15.08 iTRAQ Comparison of Human Plasma Proteins in HCM and Selected Cardiovascular Disorders

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Introduction and Objectives: Hypertrophic cardiomyopathy (HCM) is an inherited cardiovascular disease affecting myocardium (otherwise unexplained hypertrophy of the left ventricle). It is frequent disorder associated with high risk of sudden cardiac death, mainly in young age. Although the etiology of HCM has been extensively studied, mechanism of its pathogenesis remains largely hidden. Especially, there is a need to find suitable combination of protein biomarkers to identify this disease from patient blood and to stratify risks for patients. **Methods:** In our study, we applied a frequently used exploratory proteomic approach - iTRAQ quantitative analysis of pooled and immunodepleted plasma proteins. Individual plasma samples were collected from HCM patients and several control groups (healthy donors, aortal stenosis, dilated cardiomyopathy, arterial hypertension and ischemic heart disease). The iTRAQ analysis was based on the comparison of all identified proteins contained in pooled human plasma samples between the designed groups. **Results and Discussion:** We identified group of differentially abundant plasma proteins when we compared and statistically treated data from iTRAQ analysis. We should mention that many of these proteins could be affected with used medication or some concomitant diseases, but during designing of this study we tried to explicitly choose the most suitable objects and all volunteers in this study have complete clinical characteristics. Therefore majority of the proteins which we identified should be further studied as potential biomarkers of cardiovascular disease. **Conclusion:** In this work we identified a group of proteins which could serve as potential blood indicators of HCM. These proteins will be further analyzed using bioinformatics tools and selected proteins dividing patients and control groups could be used for validation using targeted mass spectrometry methods on individual samples. The grant no. NT/13721 is gratefully acknowledged.

Keywords: cardiomyopathy, iTRAQ, protein biomarker

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CS15.09 Discovery of Cardiovascular Disease Biomarkers in Human Plasma Using MRM-MS

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Introduction and Objectives: Cardiovascular disease, ranging from atherosclerosis to myocardial, is the leading cause of morbidity and mortality in adults worldwide. The development of a minimally-invasive test based on plasma protein biomarkers can improve patient care options, and reduce a significant burden on the healthcare system. Cardiovascular disease, ranging from atherosclerosis to myocardial, is the leading cause of morbidity and mortality in adults worldwide. The development of a minimally-invasive test based on plasma protein biomarkers can improve patient care options, and reduce a significant burden on the healthcare system. **Methods:** A total of 223 peptides from 99 protein candidates were measured by MRM-MS on 73 plasma samples from patients with angiographic coronary artery disease and subsequent cardiovascular mortality (case), and 73 from patients with coronary artery disease and no subsequent mortality (control). The maximum peptide relative ratio was used for proteins with more than 1 peptide available. Moderated t-tests (robustLIMMA) were used to rank proteins according to their differential concentrations. The top proteins (p value<0.05) were used to generate a protein classifier score using Elastic Nets. The performance of the classifier score was evaluated by cross-validation. **Results and Discussion:** A total of 14 proteins showed a significant differential concentration between patients with and without subsequent cardiovascular mortality. The most significant proteins were aggregated into a protein classifier, which can be used to classify new samples. The estimated area under the receiving operating curve (AUC) of the resulting classifier was 0.80, demonstrating the power of a plasma proteomic panel to identify patients with coronary artery disease that are at “high risk” of dying. **Conclusion:** This study provides evidence that protein concentrations in plasma may provide a relevant measure for monitoring and diagnosing cardiovascular diseases.

Keywords: cardiovascular disease, MRM-MS, Discovery, human plasma biomarkers

CS16: NEW ADVANCES IN BIOMARKER DISCOVERY

CS 16: NEW ADVANCES IN BIOMARKER DISCOVERY
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CS16.01 Sieving through the Secretome of Colorectal Cancer Cells

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Abstract: The cancer secretome, which encompasses all the proteins secreted by cancer cells, is a promising source of biomarkers as the secreted proteins are most likely to enter the blood circulation. Moreover, since secreted proteins are responsible for signaling and communication with the tumor microenvironment, studying the cancer secretome would further the understanding of cancer biology. However, secretome studies using cell line conditioned media (CM) have been traditionally hampered by inefficient methods for enrichment of secreted proteins due to the large volumes of CM collected, as well as the contamination by intracellular proteins released during cell lysis. In order to overcome these limitations, we used a commercially available hollow fibre culture (HFC) system to collect the CM samples from a pair of isogenic colorectal cancer cell lines, viz., HCT-116 and E1, and profiled their CM with the aim to discover novel biomarkers for colorectal cancer. The HFC system was able to enrich for secreted proteins in the CM samples by retaining the secreted proteins in a small media volume. Moreover, due to the large surface area in the HFC cartridge available for cell growth coupled with the dynamic removal of waste, cell lysis rates could be reduced significantly, thus lessening the extent of intracellular protein contamination in the CM. We have also taken an additional step to enrich for glycoproteins from the secretome by employing multi-lectin affinity chromatography (MLAC) since it is well established that secreted proteins are often glycosylated. The HCT-116 and E1 glyco-secretomes were subsequently analysed and compared using the label-free quantitative SWATH-MS technology, from which 149 differentially secreted glycoproteins were reported. From this analysis, Laminin β-1 (LAMB1), a glycoprotein not previously shown to be secreted in colorectal cancer cells, was found to be over-secreted in the E1 metastatic cells. We also showed that the levels of LAMB1 were significantly higher in colorectal cancer patient serum samples as compared to healthy controls by ELISA. ROC analyses indicated that LAMB1 performed better than CEA at discriminating colorectal cancer patients from controls. Moreover, the diagnostic performance was further improved when LAMB1 was used in combination with CEA. Our results thus showed LAMB1 as a potential serological biomarker that could be used in conjunction with CEA for diagnosis of colorectal cancer.

Keywords: glyco-secretomes, hollow fibre culture, multi-lectin affinity chromatography, Laminin β-1

CS 16: NEW ADVANCES IN BIOMARKER DISCOVERY
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CS16.03 The Potential Clinical Impact of the Human Protein Atlas

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Introduction and Objectives: The recently released tissue-based map of the human proteome by the Human Protein Atlas project, based on the combination of transcriptomics and antibody-based proteomics, provides an excellent resource for exploration and investigation of future drug targets and disease biomarkers. **Methods:** In the Human Protein Atlas project, thoroughly validated antibodies were used for immunohistochemistry on tissue microarrays containing samples from 44 different normal organs, combined with RNA-seq of 32 different tissues. All human protein-coding genes were characterized based on RNA expression level and tissue distribution, which allows for identification and exploration of proteins selectively expressed in a certain tissue, such as the placenta. **Results and Discussion:** 6,942 genes were found to be elevated in one tissue type as compared with all other analyzed tissues, out of which 294 genes were elevated in placenta. By combining the transcriptomic analysis with immunohistochemistry, proteins exclusively expressed in various cell types within placenta were identified, such as proteins expressed in trophoblasts, decidual cells and endothelial cells. Both well characterized proteins and proteins with an unknown function hitherto not analyzed in the context of placenta biology were identified. Given the specificity for placenta, these proteins provide interesting targets for further studies analyzing potential biomarkers for e.g. placental function and preeclampsia. The immunohistochemistry data and images available online at www.proteinatlas.org offer a unique possibility to analyze the protein expression at a single cell resolution, with intact cell structure and tissue morphology. This gives further understanding of the underlying biology and function of the protein, and the method itself is easily transferable to the clinical utility. **Conclusion:** The Human Protein Atlas represents an invaluable resource in order to gain biological insight on human proteins, and the protein expression data are likely to become useful for numerous spin-off projects in both basic and clinical research.

Keywords: proteomics, transcriptomics, tissue microarrays, immunohistochemistry

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CS16.04 Equalizer- and TMT-Based Detection of Cellular Proteins in Clinical Peritoneal Dialysis Effluents

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Introduction and Objectives: Peritoneal dialysis (PD) is a renal replacement therapy offering clear advantages to patients suffering from kidney failure. Hyperosmolar PD fluid (PDF) is instilled into the peritoneum and removes excess water, uremic toxins and salts. The effluent (PDE), drained from the patient represents a rich source of biomarkers for monitoring disease and therapy. Although this information could help guiding renal replacement therapy, little is known about the potential of PDE as a source of biomarkers, due to high abundance plasma proteins. **Methods:** Stable PD patients (n=20) received either standard PDF (Dianeal®, Baxter-Healthcare) or PD-protec™ (Dianeal® with added Alanylglutamine (AlaGln)) in an open-label, randomized, two-period, cross-over clinical trial (Eudract-2010-022804-29). PDE samples were depleted from high abundance plasma proteins using a bead-coupled combinatorial hexapeptide library and enriched low abundance proteins were subjected to TMT-labeling and FASP-LC-MS. **Results and Discussion:** Using the presented workflow, we could significantly increase the coverage of the PD effluent proteome. Whereas all recent publications together were able to identify only 144 unique proteins, our study identified more than 1100 unique proteins ranging from high abundance plasma proteins to low abundance cellular proteins and cover-

ing biological processes from all major categories. Interestingly our preliminary results show that two recently proposed markers for peritoneal membrane integrity which increase or decrease, respectively, over the time of the therapy are affected by AlaGln addition. Our results indicate that treatment with AlaGln added to the PD fluid may alter the abundance of these marker proteins in a way, resembling shorter time on chronic dialysis. **Conclusion:** Our study is the first randomized control trial investigating as well potential biomarkers for pathomechanisms as a therapeutic intervention in PD by proteomics techniques. The results of this trial should not only help to establish ideal biomarkers for disease staging and monitoring of the therapy but also for evaluation of cytoprotective interventions in PD.

Keywords: tandem mass tag, Biomarker discovery, renal disease

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CS16.05 Proteomic Alterations in Human Renal Epithelial Cells Exposed to Nephrotoxins

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Introduction and Objectives: Protein detection, as opposed to transcriptomic approaches, is more widely applicable for developing downstream assays. Also such assays can be readily applied to quantification in biological fluids, such as urine, and thus may transpire to be useful biomarkers in clinical settings. **Methods:** Differentiated transporting monolayers of the human renal proximal tubule cell line RPTEC/TERT1 were exposed to a single bolus of a sub-cytotoxic concentration of the immunosuppressant cyclosporine A (CsA, 15 µM), the cosmetic ingredient and oxidant potassium bromate (KBrO3, 0.8 mM) and the mycotoxin ochratoxin A (OTA, 0.13 µM) for 24h. Cell lysates were prepared for proteomic studies (4 replicates) at the end of the 24 h exposure. Samples were processed with FASP protocol and the resulting tryptic peptides were subjected to label free quantitation, iTRAQ labeling and subsequent MOAC-based phosphopeptide enrichment. **Results and Discussion:** From the quantitative data, nephrotoxins exposure caused significant alterations in the abundances of hundreds of proteins in RPTEC/TERT1 cell line. Major similarities were between CsA and KBrO3, both which have been previously shown to cause a strong Nrf2 oxidative response in proximal tubular cells. Interestingly, while OTA had a very large impact on the transcriptome after 24 h, it was the compound that induced the least alterations in the proteome. Ingenuity pathway analysis of the proteomic changes indeed identified the Nrf2 response as a major effect of CsA and KBrO3. Additionally, CsA impacted on the unfolded protein response and KBrO3 on cell cycle regulation. Of the differentially regulated proteins, ~60% were altered in the same direction by CsA and KBrO3. Interestingly, the Nrf2 regulated hemeoxygenase-1 was the highest increased protein. Urinary HO-1 has been previously associated with acute and chronic kidney disease. **Conclusion:** The data show that the approach used is an excellent strategy to uncover mechanistic biomarker of compound induced renal injury.

Keywords: Biomarker discovery, LC-MS, Proteome and phospho-proteome

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CS16.06 Detection of Biomarkers of Sepsis Using Affimer Microarrays

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Introduction and Objectives: Affimers, developed by Avacta Life Sciences, are combinatorial engineered proteins which mimic the specificity and binding affinities of antibodies. Using a library of Affimers, we have developed a protein array for high throughput biomarker screening. We describe the application of the technology to the search for biomarkers of sepsis in children. **Methods:** A library of 25,000 random Affimers, expressed in E. coli, was printed on glass microscope slides. Plasma from children (n=104) with sepsis and from healthy children (n=24) were fluorescently labelled using iFluor555 and were then used to challenge arrays (n=3). A technical control corresponding to a pool of all the samples was used to normalise signals between slides. Quality control and signal analysis were performed using Bioconductor. The fluorescence intensity of each Affimer feature from patients was compared to controls and hierarchical clustering and ROC curves were used to identify Affimers binding proteins linked to disease. **Results and Discussion:** Inter-slide variability was < 15% CV and fewer than 10% of the slides failed QC. Unsupervised hierarchical clustering based on the 25,000 Affimers allowed differentiation between the control and patients samples. 200 Affimers were identified as being differentially expressed between the 2 groups with a > 2 fold change. The Affimer arrays identified a strong signature of sepsis and ROC curve analysis allowed confident prediction of disease (AUROC of 0.9). Preliminary results of the identification of the proteins bound to the Affimer by affinity purification and mass spectrometry are promising and should lead to the identification of biomarkers of sepsis. **Conclusion:** This work demonstrates the scope of Affimer affinity reagents to develop a new protein array-based biomarker-discovery and validation workflow. We predict that array-based validation of signatures identified using Discovery Arrays prior to affinity purification and mass spectrometry will offer a cost- and time-effective methodology compared to purely mass spec-driven workflows.

Keywords: biomarkers, Affimer microarray, affinity reagent, proteomics

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CS16.07 Proteomic Biomarker Discovery in 1'000 Plasma Samples for Personalized Nutritional Intervention

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Introduction and Objectives: The multicenter DiOGenes project is a European project focusing on weight management in obese subjects [1]. It aims at advancing the understanding of the individual trajectory of weight loss

during a low caloric diet (800 kcal•day−1 over 8 weeks) and the influence of food macronutrient content on maintaining the reduced weight over 6 months. In the present work, more than 1'000 human plasma samples from the DiOGenes study at baseline and at the end of the study were analyzed using a highly automated MS-based proteomic discovery workflow [2]. **Methods:** 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 with a robotic liquid handler. Two SPE steps for sample purification completed the sample preparation. Samples were analyzed with RP-LC MS/MS. After protein identification and quantification, data analysis was performed using several statistical methods. **Results and Discussion:** The proteomic dataset encompassed 2'006 measurements within 1'005 human plasma samples corresponding to 518 unique subjects. On average 190 proteins were identified per subject for a total of 365 individual proteins identified throughout the study. About 110 proteins were consistently quantified, without missing value, in all samples. We first looked at those human plasma proteins with the lowest variability and explored the effect of clinical variables that typically impact clinical results. After such analytical validation and quality checks of our workflow, differential protein expression was analyzed and predictive models developed with regards to both weight loss and maintenance. **Conclusion:** Our MS-based proteomic methodology enabled the analysis of 1'000 samples and provided innovative and robust candidate protein biomarkers for personalized nutritional intervention. [1] Larsen et al., N. Engl. J. Med. 2010;363(22):2102-13 [2] Dayon et al., J. Proteome Res. 2014;13(8):3837-3845

Keywords: plasma, Clinical Research, Predictive Model, Obesity

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CS16.08 Identifying Exosome Biomarkers for Radiation Exposure Using a DDA and DIA Combined Workflow

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Introduction and Objectives: In the event of deliberate or accidental exposure to radiation, early and accurate assessment of tissue injury by radiation-responsive biomarkers is critical for triage and early intervention. Biofluids such as urine and serum are ideal sample sources because they are minimally invasive, easy to collect, and can be used for monitoring individuals over time. Secreted extracellular vesicles in the biofluids, such as exosomes, have been shown to be valuable resource for disease biomarkers. In this study, we analyzed and compared urine and serum exosomes for identifying potential biomarkers of acute and persistent radiation injury in mice exposed to total body irradiation (TBI). **Methods:** Urine and serum samples were collected from mice at 24 hours and 74 hours post 10.4 Gy TBI, and exosomes were isolated and analyzed by LC-MS/MS based workflow for radiation exposure signatures. A DDA and SWATH-MS combined workflow approach was used to identify significantly altered exosomal biomarkers that are indicative of acute or persistent radiation-induced responses at 1% false discovery rate. **Results and Discussion:** Urinary exosomes showed a different molecular composition from serum exosomes in mice exposed to TBI. Proteomic analysis identified 23 potential radiation exposure biomarkers from urinary exosomes and 24 potential radiation exposure biomarkers from serum exosomes. Urinary exosome signature also indicated different physiological changes from serum exosome signature. Urinary exosome signature showed dysfunctions of liver, kidney, GI tract, and prostate whereas serum exosome signature showed vascular injuries and acute inflammation in TBI mice compared to non-irradiated control mice. **Conclusion:** Comparisons of urinary and serum exosomes from TBI mice revealed time-dependent protein signatures in response to high dose ionizing radiation exposure. We identified 47 differentially secreted proteins in urinary and serum exosomes in TBI mice and together these data showed the feasibility for defining biomarkers that better understanding of tissue damages caused by high dose ionizing radiation.

Keywords: Exosome biomarker discovery, Mass spectrometry, Radiation exposure, Quantitative mass spectrometry

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CS16.09 Large Scale Metabolic Exploration of Human CSF Proteins Using SILAV

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Introduction and Objectives: It has been shown that certain disease states can be characterized by disturbances in protein production, accumulation, or clearance. In the central nervous system (CNS), alterations in metabolism of proteins such as amyloid-beta (Aβ), alpha-synuclein, or Tau may be the cause of neurodegenerative diseases such as Alzheimer's disease (AD) (Bateman, 2006). The SILAV approach based on the administration of a stable isotope labeled amino acid (^{13}C6-leucine) in patients, kinetics sampling and high-resolution tandem mass spectrometry analysis allow to quantify the rates of synthesis and clearance of a large scale of proteins in humans. Using these unique capabilities, it can be used to get new insights of the physiopathology of neurodegenerative disease. **Methods:** In vivo labeling was performed following the protocol of Bateman et al. CSF was collected every 3 hour during 24 hours. 40uL of CSF sample were denaturated (8M urea), reduced (DTT), alkylated (IAA), digested overnight (37°C) with LysC/trypsin mix (promega), and desalted with C18 tips. Tryptic digest was fractionated using Strong Cation eXchange chromatography (SCX) and eluted in 5 fractions. Denaturation, digestion, SCX prefractionation and clean up steps were automatized on a BRAVO assayMap system (Agilent). The samples have been analyzed using a Nano-RSLC (Dionex) coupled to an Impact II (Bruker Daltonics) UHR-Q-TOF. ^{13}C6-leucine incorporation rates have been calculated using skyline software. **Results and Discussion:** 2300 leucine-containing peptides, corresponding to 500 proteins were monitored. Thanks to the SCX prefractionation, MS spectra obtained were easier to analyze, which allowed an automated integration of MS peptides peaks despite the weak ^{13}C6-leucine incorporation (<10%). Protein incorporation rate was validated using technical triplicates and inter-individual variability was evaluated on 3 patients. Among the 500 proteins that could be monitored, 100 exhibited an incorporation of ^{13}C6-leucine during the 24 hours of injection. **Conclusion:** Two distinct groups have been identified (rapid or low synthesis rate) and the proteins pathways have been established.

Keywords: SILAV, In Vivo Human Labelling, UHRQ-TOF

CS16.10 A SWATH-MS Approach for a Comprehensive Characterization of the Secretome under Oxidative Stress

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Introduction and Objectives: The proteins secreted by cells play important roles in cellular communication and in the regulation of many physiological processes, being therefore good predictors of the cellular physiological state. As a result, the secretome is an important source of potential biomarkers and a good target for therapeutics. Oxidative stress is perhaps the most common factor involved in a large variety of disorders, however oxidative stress (de)regulation may occurs through different mechanisms, and thus leading to different cellular responses, which can be reflected in the secretome. These alterations can be associated with: the secretion of new factors; differential secretion of particular factors; and different oxidative states of the secreted proteins. Therefore, in this work it is presented a comprehensive method to evaluate the secretome changes and to integrate protein levels with their oxidative state changes. **Methods:** A cell model was treated with hydrogen peroxide, and the newly generated secretome was spiked with the appropriate internal standards, and analyzed by SWATH-MS. The entire secretome was used to count for differences in the total amount of secreted protein. Finally, differential alkylation was used to evaluate the oxidative state of the proteins. **Results and Discussion:** More than 750 proteins were quantified and 320 present a statistically meaningful difference for the controls. More importantly, the use of exogenously added internal standards, generated from our group, allowed better data normalization. Considering the toxic conditions used, it is important to determine if the protein content change is due to membrane disruption. Therefore, a careful analysis on endogenous proteins revealed a panel which can be used as cellular integrity standards. Finally, a SWATH-MS approach was developed to quantify the cysteine redox dynamics in the secretome allowing now to monitor hundreds of proteins in a single analysis. **Conclusion:** An integrative approach was introduced, and successfully applied in the identification of oxidative stress biomarkers in secretome.

Keywords: Secrotome, oxidative stress, Redoxomics, SWATH-MS

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CS17.02 Computational Advancements and Challenges in MS-Based Proteomics

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Abstract: Over the past decade there has been significant progress with developing computational tools and methods for mass spectrometry based proteomics. Nevertheless, new challenges emerged and need to be carefully addressed. These include the ever increasing size of proteomics datasets requiring revised approaches for estimation of peptide and protein identification error rates, proteogenomics applications that seek to identify novel peptides currently not represented in reference protein sequence databases, and new data acquisition strategies such as data dependent acquisition mass spectrometry. We provide an overview of these challenges, and highlight computational strategies developed in our lab and by others to address them. We will discuss and contrast several computational strategies for estimating the error rates in very large proteomics datasets. We will also discuss the computational strategies and challenges of proteogenomics (A. Nesvizhskii, Nature Methods 2014), from building custom protein sequence databases to methods for assessing the confidence in novel peptides. We will particularly stress the importance of applying more stringent filtering criteria to novel peptides, especially to peptides that are highly homologous to known peptides present in the reference protein sequence databases. Finally, we will present an update on our work on the analysis of data dependent acquisition (DIA) mass spectrometry data such as SWATH-MS, extending our published DIA-Umpire computational workflow (C.C. Tsou et al, Nature Methods 2015). In particular, we will discuss the analysis of DIA data generated using Orbitrap Fusion and Q-exactive instruments, and present a strategy that combines untargeted (i.e. without relying on a spectral library) peptide identification with targeted re-extraction, and also with external spectral library-based searching. We will conclude by summarizing the current challenges and available computational solutions. In particular, we will propose revised data analysis guidelines for the analysis of large-scale datasets and for proteogenomics studies.

Keyword: bioinformatics, false discovies in proteomics, proteogenomics, DIA mass spectrometry

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CS17.03 Using SRM-MS to Uncover Signaling Networks Regulating Mammalian Cell Differentiation

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Introduction and Objectives: Slow, ongoing terminal cell differentiation is essential for replacing aging or damaged cells and for maintaining tissue size in all adult mammals. For example, adipocytes, or fat cells, the key regulators of glucose and lipid metabolism, make up 10-40% of human body mass and are renewed at a rate of approximately 10% per year [1]. Similarily cardiomyocytes renew in humans at a rate of 1% per year, respectively. **Methods:** Here we use computational modeling, quantitative mass spectrometry [2,3] and single-cell microscopy [4] to uncover a signaling network architecture controlling the rate of adipogenesis. **Results and Discussion:** We identified a highly-feedback connected system architecture that can control low rates of differentiation in fat cells [3,4]. We have also observed that the unique architecture of this system allows it to filter physiological inputs. We have now also applied our SRM assays to mouse models of insulin resistance and uncovered relationships between differentiation and disease states of different adipose tissue types [5]. **Conclusion:** These results may have broad relevance for all terminal cell differentiation processes including those in cardiac, neuronal, hematopoietic, and other tissues. In particular, understanding how fat cells regulate such very slow, homeostatic differentiation rates will likely enable better treatments of insulin resistance, obesity, and related metabolic diseases. We are continuing to explore the kinetics and molecular mechanisms of how this system filters physiological inputs with the goal to open up new venues for therapeutically regulating adipose tissue renewal and size. [1] Spalding et al (2008). Nature. 453:783-7. [2] Abell et al, (2011). PNAS 108(35):14485-90. [3] Park et al. (2012). Cell Reports Oct 25. [4] Ahrends et al. (2014). Science 344:1384-9. [5] Ota et al. (2015). J. Lipid Research Apr 3.

Keywords: adipocytes, Signaling Networks, cell differentiation, SRM, targeted proteomics

CS17.04 Sensitive Peptide Identification in Data-Independent Acquisition by Spectral Library Search

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Introduction and Objectives: Data independent acquisition (DIA) strategies have emerged as practical methods to reproducibly quantify peptides but current targeted approaches typically require knowledge of peptide retention times (RT) and are not designed to identify peptides with no spectra in the assay library, such as peptides with post-translation modifications. MSPLIT-DIA is a new untargeted tool that outperforms current approaches and does not have the above constraints. **Methods:** MSPLIT-DIA peptide identification matches library spectra to DIA runs across the whole RT range; if the library contains RT information then these are aligned using matched spectra and a second-pass search is run requiring peptides to be found at their expected RTs. To identify peptides with PTMs, library spectra for modified peptides are predicted based on library spectra of unmodified peptides. **Results and Discussion:** MSPLIT-DIA can scale up to very large search spaces by searching complex human lysate samples against a proteome-scale spectral library (>200,000 peptides) without any prior RT information. When RT information is available in spectral libraries, MSPLIT-DIA's two-pass search accurately aligns RTs between library and DIA data without requiring spike-in synthetic reference peptides, saving cost and efforts. Benchmarking against DDA (identification state-of-the-art), we show that MSPLIT-DIA identified up to 30% more unique peptides in a complex human lysate and detected up to 28% more modified peptides. MSPLIT-DIA can also enhance targeted tools for peptide quantification by generating sample-specific assay libraries that improve sensitivity by up to 180%. Finally, combining MSPLIT-DIA's increased sensitivity and reproducibility with affinity-purification MS (AP-MS), we further show that MSPLIT-DIA improves the detection of protein-protein interactions by 40%. **Conclusion:** MSPLIT-DIA is a robust tool for untargeted peptide identification in DIA data that can significantly improve detection of protein-protein interactions and the performance of targeted tools for peptide quantification.

Keywords: data independent acquisition, Spectral library search, Peptide identification

CS17.05 The Interaction Proteome of the Human Kinome: Biochemical and Biomedical Implications

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Introduction and Objectives: Protein phosphorylation changes the physicochemical properties at more than 100000 different sites in the human proteome and thus represents a fundamental mechanism to control basic protein properties including half-life, enzyme activity, complex formation or subcellular localization. More than 500 different protein kinases catalyze protein phosphorylation in human cells. Localization, activity and substrate specificity of protein kinases result largely from interactions with other cellular proteins. Good coverage of protein interaction information is however available only for a well-studied subset of human kinases. Besides biases towards intensively studied kinases, public interaction data originate from heterogeneous experimental approaches and lack information on false discovery rates. This limits their successful integration with other data types including genome data from patients for the discovery of new disease linked kinase pathways and subsequent development of personalized pharmacological intervention. **Methods:** 314 HEK293 cell lines have been generated for inducible expression of epitope-tagged kinases and analysis by affinity purification and mass spectrometry (AP-MS). Filtering of interaction raw data using WD-scores resulted in a high confidence interaction proteome for the human kinome. **Results and Discussion:** Here applied a systematic AP-MS approach covering 314 human kinases, which resulted in over 8000 high-confidence interactions between more than 2800 proteins. Besides confirming over a thousand interactions from the literature the data represents a comprehensive resource for novel kinase-protein interactions, delineate kinase participation in specific complexes and allow biochemical classification of poorly studied kinases. The data revealed extensive kinase-kinase interaction networks whereby interactions among related kinases were overrepresented supporting existing hypothesis on the evolution of protein interactions. When we combined AP-MS data with kinase substrate predictions, we found established kinase substrate pathways and uncovered novel candidate substrate interactions. Finally, integration of AP-MS data with patient genome information identified new disease associated kinase modules and revealed an over-representation of cancer driving proteins in human kinase complexes. **Conclusion:** 'not applicable'

Keywords: protein complex, Kinase, Interaction networks, cancer

CS17.06 Construction of the Methylproteome Network Reveals a Novel Regulatory System in the Cell

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Introduction and Objectives: Methylation of proteins occurs predominantly on arginine and lysine residues in the eukaryotic cell. Until recently, its predominance was unknown and its role obscure. This presentation outlines our efforts to construct the first 'methylproteome network' for a eukaryotic cell and presents evidence that methylation modulates protein-protein interactions in this network. **Methods:** We analysed the yeast methylproteome to identify methylated proteins and precise modification sites. Targeted data acquisition - electron transfer dissociation LC-MS/MS was used, as were yeast proteome arrays (containing 4,400 chips spotted on to microscope slides). To build the intracellular methylation network, all known and putative methyltransferases in yeast were knocked out and the methylproteome re-analysed to determine which enzyme was responsible for which methylation event. Enzyme-substrate links were further investigated by the analysis of recombinant substrate proteins methylated by recombinant enzymes, by in vivo methylation assays and/or the incubation of proteome arrays with recombinant enzymes. Validated enzyme-substrate links were integrated with yeast protein-protein interactions to generate the first 'methylproteome network'. A new 'conditional two-hybrid' (C2H) system was then constructed to test whether methylation can modulate protein-protein interactions. **Results and Discussion:** Our analyses, together, showed that protein meth-

ylation is widespread in the eukaryotic cell. We discovered two new eukaryotic lysine methyltransferases, elongation factor methyltransferases 2 and 3, both of which have mammalian orthologs. Our integrated methyltransferase-substrate protein and protein-protein interaction network suggested that methylation might modulate protein-protein interactions. This was proven by or new 'conditional two hybrid' system, in that half of the protein-protein pairs involving arginine methylated proteins show a significant increase in interaction on methylation. In some cases, phosphorylation was found adjacent to methylation and we have shown that this can interfere with methylation and thus decrease certain protein-protein interactions. **Conclusion:** The 'methylproteome network', built here, reveals a novel means of regulating protein-protein interactions and thus biological function in the eukaryotic cell.

Keywords: proteome arrays, methylation, protein interaction network, conditional two hybrid system

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CS17.07 Mapping Dynamic Protein Interaction Landscapes Using a Novel Whole Network Enrichment Approach

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Introduction and Objectives: Cellular physiology is dynamic in nature, especially in response to perturbation. Despite significant advances in genetics, biochemistry and cell biology, signal transduction networks and protein effectors that mediate specific cellular responses have mostly been annotated under static (i.e. basal) cellular conditions, both at the genetic and physical interaction level. These approaches provide little information about the dynamics of a given pathway or its constituents under varying patho-physiological contexts. Proteins in these cellular networks are largely organized into protein complexes that work in concert to enable desired cellular responses. Therefore, defining the architecture and response of these complexes to perturbation is vital to our mechanistic understanding of fundamental cellular biology, and may provide critical insight into disease pathology. **Methods:** We have developed an approach to annotate the architecture and dynamics of protein complex networks by utilizing a novel whole-network affinity pull-down method coupled to novel computational analysis. This method involves generation of TAP-tagged node proteins, which are expressed, pooled and affinity-purified in parallel. Our approach allows for efficient system-wide identification and quantification of protein complex members and novel interactors in response to perturbation. We developed and utilize novel biochemical and proteomic methodologies to study network response to known perturbations in established protein networks. Specifically, as a proof-of-principle, the platform has been employed to study changes in nutrient sensing protein complexes in response to rapamycin treatment. **Results and Discussion:** The concept has been extended to the DNA damage response as well as energy homeostasis networks in yeast, and the latter has been correlated to a homologous mammalian network for investigation of metabolic dysregulation in disease. By measuring the dynamics of protein networks, we have identified novel associations within and between components otherwise missed in traditional protein-by-protein Affinity Purification-Mass Spectrometry (AP-MS) methodologies. **Conclusion:** This is the first proteomic platform to enable dynamic interaction measurements at a network-wide scale.

Keyword: Protein Network Dynamics

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CS17.08 Identifying Novel Sequences in the PRIDE Archive through Spectrum Clustering

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Introduction and Objectives: The 'PRIDE Cluster' algorithm (PMID: 23361086) was originally developed to identify reliable peptide spectrum matches (PSMs) in the PRIDE database (http://www.ebi.ac.uk/pride), using a spectrum clustering approach. Due to the success of the ProteomeXchange Consortium, the amount of data deposited to PRIDE is growing exponentially. This vast increase required an updated, more efficient version of the original algorithm. **Methods:** The new 'PRIDE-Cluster-H' algorithm (http://www.ebi.ac.uk/pride/cluster/) was designed to run in the Hadoop open-source software framework, taking advantage of its parallelisation capabilities. It can process all publicly available identified spectra in PRIDE (>75 million as of 4/2015) in less than 4 days, compared to 2 weeks (for 25% of the data available today) using the original algorithm. This significant improvement now enables to perform additional, more in-depth analysis of the data deposited in PRIDE. **Results and Discussion:** We are able to identify not only correctly but also incorrectly identified spectra through PRIDE Cluster. These clustering results enable us to specifically select clusters that represent peptides whose spectra were not correctly identified originally. This pre-selection enables a further analysis with computationally more expensive methods that can then lead to the correct identifications. **Conclusion:** We are able to identify not only correctly but also incorrectly identified spectra through PRIDE Cluster. These clustering results enable us to specifically select clusters that represent peptides but whose spectra were not correctly identified originally. This pre-selection enables a further analysis with computationally more expensive methods that can then lead to the correct identifications.

Keywords: data reliability, data repositories, Hadoop, spectrum clustering

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CS17.09 Predicting Mutations Impact in Protein Interaction Networks in Cancer

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Introduction and Objectives: Cancer is a complex disease involving wide spectrum of genes, proteins, and molecular interactions that are dynamically regulated. It is strongly associated with mutations that affect the protein's structure and function. With ~13M cancer mutations identified to date, identification of important mutations becomes a major challenge. The available mathods are not able to correctly identify many known disease linked mutations and don't consider the effect of mutations on many aspects of protein function, e.g. protein interactions. We developed an integrated approach to construct high-resolution protein interaction networks in cancer predicts the effects of mutations on the protein interactions network dynamics. **Methods:** The protein interaction predicted based on peptides binding to peptide recognition domains (PRDs) that play crucial role in protein function. The binding peptides were determined in large-scale using phage-display screening (PDS) and the results were used to build

position weight matrixes (PWMs), statistical models used to represent the binding motifs. The PWMs will be used to predict the interactions with the PRD-containing proteins. Then, the predictions are supported Bayesian integration system that uses ten different genomic and context information to filter the prediction. Using the mutation information of COSMIC database, we constructed the mutants in silico and repeated the predictions for each PRD-containing protein with the same predictions and filtration steps. This resulted in high-resolution interaction networks based on cancer mutations. By overlaying the two networks, we pinpointed rewiring events (gain or loss of interactions/functions). **Results and Discussion:** Applying the described method on ~800,000 mutations from COSMIC and the information of 117 SRC Homology (SH3) domains, we constructed high-resolution interaction networks based on wildtype proteins and cancer variants. We identified >6,000 network rewiring events that affect the interaction network dynamics. **Conclusion:** Our method provides better understanding for cancer biology by help elucidating disease mechanisms, discovering new cancer driver genes in unprecedented resolution and large-scale.

Keywords: Cancer genomics, Cancer mutations, Peptide recognition domains, Protein interaction networks

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CS17.10 Small and Big Data in Proteomics: Reprocess Public Data to Design Better Experiments

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Introduction and Objectives: In a global effort for scientific transparency, an increasing number of researchers share the experimental data supporting their findings. Vast amounts of proteomic data are thus available to the community, allowing the mining of literature knowledge to an unprecedented level of detail. However, due to their heterogeneity and complexity, utilizing public data poses numerous challenges. Available datasets therefore remain largely underexploited. Here, we present solutions to easily extract new knowledge from these, and show how this can be applied to everyday experiments. **Methods:** We developed PeptideShaker (Vaudel et al., Nature Biotechnology, 2015) an intuitive interface for the reprocessing of datasets from the PRIDE repository (www.ebi.ac.uk/pride) using multiple search engines via SearchGUI (Vaudel and Barsnes et al., Proteomics, 2011). The software has been tested worldwide for several years, and detailed tutorials were made freely available (Vaudel et al., Proteomics, 2014). **Results and Discussion:** PeptideShaker features an intuitive interface, allowing the straightforward interpretation and mining of complex datasets. It takes advantage of online resources, including gene annotation and protein structure, to provide users with new insights on the results. It can be integrated into composite workflows, thanks to the use of the mzIdentML standard format, offering various additional outputs like customizable reports, recalibrated spectra, and inclusion lists. Finally, strong efforts were put into integrating PeptideShaker in automated processing environments, as illustrated with the recent integration in GalaxyP (usegalaxyp.org). **Conclusion:** Using PeptideShaker, it is now possible to reprocess shared proteomics data with just a few clicks without advanced bioinformatic skills. This technique enables researchers to mine projects of interest, and analyze them in new contexts, e.g. search for new modifications, use different databases or different bioinformatic tools. On a larger scale, the reprocessing of multiple projects can provide an overview for specific organs, diseases of interest, and proteomics in general.

Keywords: Bioinformatics, Public Data, Reanalysis, Open Source

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CS18.01 Tumor Liquid Biopsy by Circulating Extracellular Vesicles

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Abstract: Introduction and Objectives: Recently biological significance and clinical utility of extracellular vesicles (exosomes) have been extensively investigated. Particularly exosomes are considered as ideal targets of biomarker discovery due to their molecular characteristics reflecting those of original cells. However, difficulties in exosome isolation from biological fluids have significantly hindered effective discovery of specific biomarkers. Recently we developed 3 key technologies (CD9-MSIA tips, ExoTrap columns, and EV-Second columns) allowing rapid isolation of high quality exosomes from serum/plasma or cell culture medium by simple procedures. Using these new tools, we performed proteome-wide exosomal biomarker screening for scirrhous gastric cancer. We further conducted biomarker discovery for renal adenocarcinoma using exosomes effused from resected tissues. Methods: We employed EV-Second (Extracellular Vesicle isolation by Size Exclusion Chromatography ON Drip column) to perform quantitative proteome profiling of serum exosomes from 58 individuals (10 normal controls and 48 gastric cancer patients). The EV-Second column (GL Sciences Inc.) allows surface antigen-independent isolation of exosomes with simple gravity-flow procedures. Label-free quantification and statistical analysis were performed on Expressionist proteomics server (Genedata AG). In effused exosome analysis, pairs of tumor region and non-tumor region were collected from 10 renal cell carcinoma patients immediately after surgical resection. Following incubation of tissues in medium, effused exosomes were collected and analyzed by LC/MS. Results and Discussions: Among 822 identified serum exosomal proteins, 13 proteins were significantly up-regulated in gastric cancer-derived exosomes (t-test, $p < 0.05$, fold change > 2.0, and valid value > 80%). Further investigation suggested that three of them might have enzymatic activities regulating pH and oxidative stress response in tumor microenvironment. These experiments indicated the existence of an exosome-driven positive feedback loop regulating tumor microenvironments. For mass spectrometric analysis of cultured resected tissue supernatant, we successfully detected tetraspanin molecules (CD9, CD63, CD81, CD82, TSN9, TSN14, etc.), indicating that exosomes directly secreted from renal carcinoma tissues were effectively collected and detectable. Here we found that a growth regulatory kinase was specifically involved in exosomes from tumor tissues. Conclusions: Our exosome purification technologies can provide simple, high-throughput, and in-depth proteome profiling of exosomes derived from multiple clinical specimens. The panel of cancer-associated exosomal proteins would be able to explain new mechanisms about tumor progression or metastasis.

Keywords: exosome, Extracellular vesicles, cancer, biomarker

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CS18.02 The Mitochondrial Interactome and Its Response to Apoptosis

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Abstract: The majority of proteins engage in coordinated protein interactions with an estimated 150,000 to 650,000 discrete protein interactions predicted. The propensity of proteins to form high order associations is driven by the benefits to the cell of protein association which leads to enhancements in the efficacy of processes through spatial and temporal confinement; enhanced robustness due to the formation of scale-free networks containing hubs of different functionality; the flexibility of allowing alterations of function or specificity through the exchange of subunits; and enabling control of protein abundances through by stabilization of subunit. The recognition of the human proteome as a highly interconnected network of protein-protein interaction (PPI), also known as the interactome, has changed biology's view of protein functionality as increasingly it is clear it not only the present of an disease related allele within a system but its effect on the network which leads to disease states. Although the last decade has resulted in significant progress in the elucidation of ever-larger PPI networks, our understanding of most interactomes is still incomplete. It has been estimated that only 20% of the human interactome has been mapped to date, with the majority of mapping efforts currently performed in select few cell types and typically examined using only one protein isoform. This approach ignores the diversity inherent in the proteome and the interactome, making current networks of limited use to biological research. Here we demonstrate the application of protein correlation profiling (PCP)-SILAC to monitor changes during the initiation of apoptosis in both organelle membrane and cytoplasmic complexes in response to the Fas-mediated apoptotic cascade in Jurkat T-cells and explore how the PPI landscape changes as cells becomes committed to cellular destruction. We observe the majority of interactions are unaffected by the initiation of apoptosis yet discrete alterations in both organelle membrane and cytoplasm are evident. As previous studies have suggested, the activation of proteolysis, predominately but not exclusively by caspases, during the initiation of apoptosis leads to inactive members of complexes via the removal of functional domains leading to the total loss of proteins from the proteome. Within this work we show although proteolysis mediated changes are clearly evident and support the formation of stable protein fragments, we also demonstrate dramatic changes in known caspase targets precede cleavage and are independent of changes in the protein level. This finding supports that dramatic protein interaction changes may be the harbinger to caspase-mediated proteolysis, rather than being caused by proteolysis. N-termini analysis supports that these changes in the interactome are largely due to changes in the abundance of members, due to cellular localization changes, not the formation neo-N-termini. Taken together these finding demonstrate apoptosis involves multiple alterations that are invisible without the examination of the system on the interactome level.

Keyword: PCP-SILAC, protein-protein interactions, Jurkat

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CS18.03 High Throughput Structure-Function Analysis of the Centriole-Cilia Interface

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Introduction and Objectives: The centrosome is a complex molecular assembly that functions as the major microtubule-organizing centre in human cells. The centriole plays critical roles during mitosis, and when the cell exits the cell cycle and enters G0 the centrosome undergoes a series of dramatic morphological and functional alterations culminating in the formation of a primary cilium. A combination of previous proteomics, comparative genomics and functional studies suggest that over 1000 proteins are required for the function, assembly and maintenance of cilia. Defects in cilia lead to a number of different human diseases characterized by, for example, obesity, infertility, respiratory dysfunction and renal disease. **Methods:** We have developed a pipeline to systematically identify protein-protein interactions that occur at the centriole-cilia interface. Using automated imaging and analysis tools, we have conducted siRNA-mediated knockdown to test the involvment of detected interactors in centrosome/cilia related processes. **Results and Discussion:** Applying BioID to 36 centrosome-associated and ciliary transition zone proteins under both ciliated and unciliated conditions, we have identified >4000 high confidence protein-protein interactions, and 1450 interactors. We have conducted siRNA-mediated knockdown on 500 of these genes not previously implicated in centriole or cilia function, and monitored ciliogenesis, centriole duplication and centriolar satellite biogenesis. The loss of >200 of these new centriole-associated proteins impacted one or more of these processes. Further characterization of selected hits has revealed new centriole satellite components and novel regulators of ciliogenesis. **Conclusion:** The mechanisms underpinning cilia biogenesis and function have puzzled scientists for many decades. Our work opens new perspectives in understanding centriole/cilia biology, and provides important new insights into understanding ciliopathies.

Keywords: BioID, Protein-protein interaction, Centrosome, Primary Cilium

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CS18.04 A Protein Marker-Based Physical Map of a Human Cell

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Introduction and Objectives: Compartmentalization, a basic and essential characteristic of eukaryotic life, ensures that cellular processes are performed at defined subcellular locations. While both biochemical fractionation and microscopic approaches help to better define subcellular organization, many compartments are difficult to probe with classical methods. BioID uses an abortive biotin ligase (BirA*), fused to a bait protein, to biotinylate proximal proteins in vivo. Our objective was to fuse classical subcellular compartment markers and peptide target sequences to BirA* to generate protein profiles for various compartments that include both membrane-bound (e.g. plasma membrane, endosomes, endoplas-

mic reticulum and mitochondria) and non-membrane bound structures (e.g. nucleolus, P-bodies, stress granules, cytoskeleton and chromatin). **Methods:** Each selected compartment was profiled with at least three independent protein markers selected from the literature or suggested by cell biology experts. BirA*-tagged markers were stably expressed in Flp-In T-REx HEK293, and biotinylated proteins were affinity purified in biological duplicates (on streptavidin) and identified by mass spectrometry. High confidence interactors were scored against a set of negative controls by SAINT, and subjected to correlation analysis. **Results and Discussion:** Our initial profiling of 16 compartments clearly revealed that BioID recapitulates immunofluorescence results and is capable of defining the composition of membrane and non membrane-bound organelles. BioID distinguishes between subcompartments, adjacent compartments and different membrane structures. We further sought to explore the relationship between the number of baits profiled and the spatial resolution using stress granules and P-bodies, structures which cannot be biochemically purified. In this example, as the number of baits profiled increased (to 75), clear protein complexes and functional modules could be recovered. **Conclusion:** We demonstrated that both low and high-resolution profiling of various subcellular structures is achievable through BioID. This technique complements other approaches for subcellular analysis and notably permits the analysis of membrane interactions in a large-scale format under near physiological conditions.

Keywords: subcellular, affinity purification, BioID, Protein-protein interaction

CS18.05 Plasma Membrane Proteomic Study of HIV Latent Infection

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Introduction and Objectives: The persistence of latent HIV-infected cellular reservoirs represents the major hurdle to virus eradication on patients treated with HAART. It is very important to pickup or reactive HIV latent cells. However, the biomarkers related to latent HIV-infection are very limited. **Methods:** In this work, we performed subcellular proteomic technology to identify the proteins related to HIV infection. An established human immunodeficiency virus-1 (HIV-1) latent cell models (A7) and parental cell lines (Jurket cell) were used. The plasma membrane (PM) was enriched through aqueous two-phase partition. Proteins were analyzed through 2DE-MS and iTRAQ based quantitative proteomic technology. **Results and Discussion:** 66 differently expressed proteins with 1.5 fold change between HIV-1 latent infection model and its control were detected, including 13 from 2DE technology and 53 from iTRAQ. Of which, 28 proteins were up-regulated in HIV-1 latent infection model compared with the controls. Further analysis of gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG)-Pathway enrichment demonstrated that these differentially expressed proteins are primarily related to the biological processes of binding and enzyme, et al. Of these proteins, 40% were plasma membrane or membrane-associated proteins. We confirmed further the differential expression of stomatin-like protein 2, CAPG and APR3 through western blotting, confocal technologies. **Conclusion:** These proteins such as stomatin-like protein 2 might be potential anti-HIV drugs or drug targets.

Keywords: proteomics, Plasma membrane, HIV latent, iTRAQ

CS18.06 Novel Components of Rods and Rings - A Subcellular Structure with Unknown Function

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Introduction and Objectives: Several reports have described rod- or ring-shaped structures of 2-10 μm in cultured cells. The function of the structure, called Rods and Rings, is unknown and only two components have been found, Inosine monophosphate dehydrogenase 2 (IMPDH2) and Cytidine triphosphate synthetase 1 (CTPS1), both rate-limiting enzymes in biosynthesis of GTP/CTP. The structures are present at low frequency in human cell lines but assembly can be induced by inhibition of one of the two enzymes or by glutamine deprivation. We have searched for additional components with the Human Protein Atlas and immunofluorescence. **Methods:** In the Human Protein Atlas (www.proteinatlas.org) 19000 antibodies have been analyzed with immunofluorescence in human cell lines with the aim to provide subcellular locations for all human proteins. Stainings with fibrous or rod-like appearance were chosen and the corresponding antibodies were analyzed in cells treated with the IMPDH2 inhibitor ribavirin that increases the number of cells displaying Rods and Rings. **Results and Discussion:** We found 20 proteins that are possible novel components of Rods and Rings and three of these were validated with more than one antibody. Costaining showed that all 20 proteins localize to the exact same structure. The proteins have diverse functional annotations and it is not yet clear how they relate to the formation or function of Rods and Rings. **Conclusion:** Compartmentalization to generate local high concentration of proteins can be related to function enhancement, regulation, quickly accessed storage or disposal of dysfunctional proteins. Filament formation seems to be a conserved mechanism since CTPS can also form filamentous structures in bacteria, yeast and drosophila. Rods and Rings are approximately 10 times larger than mitochondria and could potentially consist of several hundred components. Knowledge of these components is essential for further characterization and understanding of this structure.

Keywords: Human Protein Atlas, Subcellular Proteomics, Rods and Rings

CS18.07 Pathogenic E. Coli Infection Alters the Mitochondrial Proteome and Mitochondrial Proteolysis

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Introduction and Objectives: Mitochondrial proteases play crucial roles in essential mitochondrial processes, such as apoptosis and mitochondrial proteome import. Our understanding of mitochondrial dynamics and proteases could be greatly improved with a proteomics technique that identifies proteolytic events. **Methods:** We have developed ‘mito-TAILS’ to quantitatively profile the mitochondrial proteome and proteolysis, combining SILAC, mitochondrial enrichment, and amino (N)-terminal proteomics (terminal amine isotopic labeling of substrates, TAILS). In this study, mito-TAILS was used to

characterize changes in the mitochondrial N-proteome during infection with enteropathogenic Escherichia coli (EPEC). EPEC virulence factors target mitochondria; they are imported, cleaved, and promote apoptosis. Human epithelial cells were grown in light or heavy isotope-coded SILAC medium and either infected with wild-type EPEC or mock infected. Mitochondria were isolated; 5% of the pooled cell homogenate was used for a complementary whole cell analysis. N-proteomes were quantitatively compared using TAILS and tandem mass spectrometry. **Results and Discussion:** Using mito-TAILS, we identified 1273 unique proteins and 2104 unique N-terminal peptides from three biological replicates (1%FDR, Mascot). We identified altered abundance or import of mitochondrial proteins as well as novel proteolytic events in 35 mitochondrial proteins. Finally, we identified known apoptotic events, including proteolysis of the mitochondrial protease HtrA2, releasing it to the cytoplasm, where we observed proteolysis of its cytoplasmic substrates. **Conclusion:** EPEC infection alters mitochondrial proteolysis and protein import. Mito-TAILS is a valuable new approach to study mitochondrial proteome dynamics and proteolysis. This is the first application of terminal proteomics to study human mitochondria and the first study of the mitochondrial proteome during infection. By profiling both the mitochondrial and cellular proteomes, mito-TAILS can be applied to study cell-wide consequences of mitochondrial events and can be applied to study other mitochondrial pathways and pathologies.

Keywords: Bacterial infection, mitochondrial proteome, Mitochondrial proteolysis, Mitochondrial dynamics

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CS18.08 Cancer-Associated Lipid Raft Function Revealed by Subcellular Proteomics and Computational Analysis

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Introduction and Objectives: Lipid rafts are dynamic cholesterol and sphingolipid-enriched membrane nano/micro-domains that regulate cell function. Many oncogenes and tumor suppressor genes/pathways are associated with lipid rafts, for example, caveolin-1 and HER2. By proteomic profiling of lipid raft subcellular fractions, we have previously identified PTRF/cavin-1 as an essential cofactor for formation of caveolae (1) then further characterised the role of PTRF/cavin-1 as a tumor suppressor for caveolin-1-positive prostate cancer (2). Here, we employed a computational approach using existing and new proteomics data, to test the hypothesis that lipid raft dysfunction is a common mechanism for tumor progression. **Methods:** Cancer cell models with modulated lipid raft proteins were established and characterised. Lipid rafts were prepared from SILAC-labelled cells using detergent-resistant membrane method, and analysed by LC-MS/MS. Statistical analysis was performed using a permutation method (3). Lipid raft proteins correlating with cancer cell phenotypes were compared with published lipid raft proteomics data, using RaftProt database(4). Protein-protein interaction network analysis was performed on the modulated lipid raft proteins. **Results and Discussion:** In defining a set of high confidence lipid raft proteins in RaftProt, we have allowed identification of a protein by more than one lipid raft preparation method, and/or sensitivity to the cholesterol-disrupting agent methyl-b-cyclodextrin as the criteria, leading to ~28% of all reported lipid raft proteins being classified as high confidence. **Conclusion:** Meta-analysis of lipid raft proteomics datasets reveal altered cytoskeletal-lipid raft membrane linkage as a common feature of aggressive progression in tumors. References (1) Hill et al. Cell. 2008;132:113-24. (2) Moon et al. Oncogene. 2014; 33(27):3561-70. Nassar et al. Oncotarget. 2013;4:1844-55. Inder et al. Mol Cell Proteomics. 2012; 11:M111.012245. (3) Nguyen et al., Journal of Integrated Omics. 2: DOI: 10.5584/jiomics.v2i2.109. Chen et al., J Proteome Res. 2014;13:4184-91. (4) Shah et al., Nucleic Acids Res. 2015;43(Database issue):D335-8.

Keywords: lipid raft, cancer, caveolin, cholesterol

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CS18.09 Abundance and Turnover of Synaptic Proteins by Mass Spectrometry and Super-Resolution Microscopy

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Introduction and Objectives: Chemical synapses are a defining structure of the nervous system that allows the transfer of information from a presynaptic neuron to a postsynaptic cell. This is achieved through the release of neurotransmitters that are stored in synaptic-vesicles (SVs) - small organelles that release their content upon electrical stimulation in the synaptic-cleft. The release of SVs is a tightly regulated process, requiring the orchestrated interaction of a number of proteins necessary for exocytosis, endocytosis and vesicular recycling. Despite substantial current knowledge of the molecular processes occurring at the synapse, the abundances and temporal turnovers of synaptic proteins are not extensively studied. Here, we combine biochemical and biophysical techniques, including a modified version of the SILAC mice approach, to describe the quantitative and dynamic functioning of synapses. **Methods:** The physical characteristics of synapses and the distribution of proteins were investigated by electron and "stimulated-emission-depletion" (STED) microscopy. The absolute amount of the synaptosomal proteins were determined by quantitative-western-blot and label-free "intensity-based-absolute-quantitation" (iBAQ) using mass-spectrometry. For temporal protein-dynamic studies, mice were fed with a lysine6-diet for defined time frames. Protein turnover rates were determined by mass spectrometry according to their heavy-to-light ratios. **Results and Discussion:** The various techniques used for determination of copy-numbers of the proteins involved in the specific steps of SV-recycling yielded results that correlate remarkably well. On the basis of the copy-numbers – together with other physical, biochemical and biophysical data – we modeled the 3D architecture of the synaptic terminal. We further observed a correlation between protein-turnover rates and copy-numbers of synaptic proteins. In general, protein lifetimes in-vivo (i.e. in mice) follow tendencies that have been previously reported in cell cultures of neurons; however, protein-turnover rates, and thus their lifetime's in-vivo, are substantially prolonged as compared with the values observed in in-vitro cell culture. **Conclusion:** Abundances and lifetimes of proteins within synapses are correlated.

Keywords: synaptic vesicle, protein quantification, Protein turnover, synapse

CS18.10 A Dynamic Picture of the Ubiquitinome upon Proteasome Inactivation

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Introduction and Objectives: The 26S proteasome is a 2.5 MDa protein complex, which degrades unneeded and damaged proteins in the cell. As such, it is critical in regulating proteostasis and controls key regulator abundance levels. Malfunctioning of the ubiquitin–proteasome system has been implicated in diseases such as cancer and neurodegenerative disorders. On the other hand, in cancer therapeutics the induction of apoptosis by proteasome inhibition using drugs is widely used. Current strategies are directed towards the development of more selective inhibitors that target the proteasome regulatory subcomplex and have less side-effects. We take a proteomics approach to dissect the molecular mechanisms of the proteasome regulatory subcomplex, which is essential for the development of better proteasome inhibitors. **Methods:** Targeted proteasome inactivation by selective RNAi knockdown or drugs is monitored at the proteome and ubiquitinome levels using a SILAC approach in Drosophila. **Results and Discussion:** Over 5,000 proteins and 10,000 diGly peptides were identified and quantified. After brief inactivation by drugs, proteins involved in stress response, cell cycle regulation, apoptosis and the UPS were upregulated (e.g., Hsp proteins) and accumulated. After prolonged inactivation, the abundances of several 100s of proteins were altered. Similar effects were observed after inactivation of the proteasome with RNAi knockdown of different subunits. Protein ubiquitination dramatically increased upon proteasome inactivation. Interestingly, many proteins showed dynamic ubiquitination changes in opposite directions on different target lysine residues within the same protein. Proteomic analysis of individual RNAi knockdown of three proteasome bound deubiquitinating enzymes indicated that each of them has a different and specific function. Finally, proteasome interactome profiling under different experimental conditions using LFQ based quantitation suggested that the proteasome itself is a dynamic complex that recruits different partners and/or (sub)complexes under specific conditions. **Conclusion:** Global analysis of the dynamic proteome and ubiquitinome after proteasome inactivation gives detailed insight into regulatory mechanisms of the proteasome.

Keywords: proteasome, SILAC, ubiquitin, diGly peptide enrichment

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CS19.01 The Progress and Challenges of the HUPO Human Proteome Project

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Abstract: Introduction: The HUPO Human Proteome Project (HPP) has two overall goals: (1) stepwise completion of the Protein Parts List, the draft human proteome, identifying and characterizing at least one protein product plus [EWD1] PTM, SAP, and splice variant isoforms from each human protein-coding gene; (2) making proteomics an integrated counterpart to genomics throughout the biomedical and life sciences community through advances in instruments, assays, reagents, and proteomics knowledgebases. **Methods:** 50 HPP research teams are organized in the Chromosome-centric C-HPP, the Biology and Disease-driven B/D-HPP, and the Antibody, Mass Spectrometry, and Knowledgebase resource pillars. PeptideAtlas and GPMDB reanalyze all major mass spectrometry datasets available through ProteomeXchange with standardized protocols and stringent quality filters; neXtProt curates and integrates MS and other findings. **Results & Discussion:** Enhanced with several major new datasets published in 2014 and reanalyzed by PeptideAtlas, the Human Proteome presented as neXtProt version 2014-09-19 has 16,491 unique confident proteins (PE level 1), up from 13,664 at 2012-12 and 15,646 at 2013-09. Thus, we began 2015 with 2948 missing proteins with protein existence PE 2-3-4, plus 616 uncertain proteins (PE 5). >40 manuscripts are under review for the 3rd annual J Proteome Research C-HPP special issue. Many identify missing proteins in specific tissues, such as testis, or by chromosome, or explain why many proteins may not be detectable. We recognize that claims of detecting missing (PE2-3-4), uncertain (PE5), or novel (from lncRNAs) proteins require scrutiny of the PSMs and of alternative protein matches for the presumed proteotypic peptides that represent single amino acid substitutions or isobaric PTMs. The B/D-HPP has created comprehensive SRM resources, generated priority-protein/ popular-protein lists to guide targeted proteomics assays for specific diseases, and launched an Early Career Researchers initiative.

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CS19.02 Strategic Points for Dealing with Missing Protein Mapping in the C-HPP

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Abstract: One of the major goals of the C-HPP is to identify and characterize missing proteins that lack mass spectrometric (MS) evidences. Despite some difficulties, a substantial progress on mapping missing proteins has been made during the past years as evidenced by publications of three consecutive JPR special issues since 2013. Recently there emerged some issues about accuracy in missing protein identification that accounts for 15% of 20000 proteins. Therefore, we wish to discuss some strategic points to deal with this issue which seems important for the C-HPP. The human placental tissues were obtained with informed consent in accordance with institutional review board guidelines from the Yonsei University College of Medicine (Seoul, Korea). Tissue samples were usually reduced, alkylated, and subjected to tryptic digestion for mass spectrometric analysis (e.g., Orbitrap). Among those mapping steps of missing proteins-profiling, identification, in vitro verification, functional validation, mechanism-we focused on the identification and validation of missing proteins because they are important components for accurate annotation. First, to facilitate efficient protein identification, we constructed and utilized an expanded hybrid spectral library with the MS/MS spectrum simulation of each sequence. From this work, total 11 additional alternatively spliced proteins were identified (e.g., SNX3). Second, to make stringent verification of missing proteins (e.g., SLC9A3R1), cross analysis was made between the western blot and MS analysis followed by the transcriptional analysis (e.g., mRNA quantification, siRNA treatment, RNA Seq). Third, for the biological validation of the missing proteins, the phenotype change of the C. elegans mutants of the human

orthologues encoding missing proteins (e.g., SLC9A3R1) was assessed for identifying their possible roles in germ cell development. From our efforts to improve mapping process of missing proteins, it was concluded that there remain a lot more challenge to overcome in the areas of intact sample selection techniques, integrated omics databases and analytical platforms (M/SRM, SWATH).

Keywords: C-HPP, Missing proteins, Spectral library, functional validation

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CS19.03 Controlling False Discovery Rates (FDRs) in Genome-Wide Proteomics Datasets

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Introduction and Objectives: With the growing size of proteomics datasets there is an urgent need for rigorous control of false protein identifications. Recently, two studies reported nearly complete human proteome coverage. Strikingly they strongly deviate from each other in the identification criteria they apply. In one of the studies, false discoveries are controlled only on the level of PSMs, while in the other a peptide level FDR is applied additionally. We introduce a computational workflow suitable for genome-wide proteomics data and apply it to several large-scale studies. **Methods:** We apply a two-tier target decoy-based filtering with FDR control of PSMs and protein groups. In order to identify novel protein-coding genomic regions we use a hierarchy of protein sequence databases, ranging from validated SwissProt entries to translated pseudo-genes and non-coding RNAs. A prioritization scheme prefers simple explanations. **Results and Discussion:** We analyzed several large datasets including the proteome draft from the Pandey lab. We confirm applicability of conventional target-decoy approaches by comparing protein score distributions for forward and reverse hits which we find to be identical at low-scoring protein identifications. At protein FDR of 1% the number of identified protein groups for the draft human proteome is 13,242. Surprisingly we find with 13,533 protein groups for data by Neuhauser et al. (JPR 2013, p2858) a higher number. In the original analysis of the draft proteome many olfactory receptors were unexpectedly reported as identified. In our analysis of the draft proteome we do not find a single olfactory receptor. The amount of seemingly translated pseudo-genes and non-coding RNAs increases strongly and will be severely over-predicted if they are not undergoing strict filtering as we provide with the protein FDR. All reported algorithms will be freely available in MaxQuant. **Conclusion:** We present a computational workflow for the reliable analysis of genome-wide proteomics data correctly applying protein-level FDR to human draft proteome.

Keywords: Human Proteome, False discovery rates, Protein FDR, MaxQuant

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CS19.04 How Does an Extra Chromosome 21 Modulate the Quantitative Human Proteome?

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Introduction and Objectives: The chromosome is the high level biological unit of genetic information for gene expression in eukaryotic organisms. Aneuploidy, the condition defined as the presence of an abnormal number of chromosomes in a cell, is found in most solid tumors and a major cause of various human diseases. For example, Down's syndrome results from trisomy of Chromosome 21 (T21). It is therefore crucial to investigate the multilayered genetic and -omic consequences of the extra chromosome 21 in human cells, for studying both basic biology and translational medicine. **Methods:** We used primary fetal skin fibroblasts derived from a pair of monozygotic twins discordant for T21, which uniquely allowed us to characterize the proteome changes due to the supernumerary T21, without the noise of genomic variability. To compare and generalize our finding, we also analyzed the fibroblasts from 11 unrelated T21 individuals bearing Down's and 11 controls. To achieve high data reproducibility and quantitative accuracy, we applied SWATH-MS to profile the proteomes. Data were analyzed by OpenSWATH using the human assay library for ~10,000 proteins. **Results and Discussion:** We reproducibly quantified 3455 unique proteins by 1% protein FDR (controlled by Mayu) across all the samples (R=~0.98 between bio-replicates). The quantitative correlation between protein-mRNA abundances in each sample was ~0.45, whereas the T21/normal fold-change correlation was much lower, indicating substantial post-transcriptional regulation buffering effects of the extra chromosome. The detectable 37 proteins encoded in chromosome 21 on average displayed a much higher expression in all T21 cases compared to controls, which is more significant than the global proteome changes. Subsequent computational analysis suggested that members of heteromeric protein complexes in particular seem to be exempt from responding to the copy number alteration. **Conclusion:** Our data revealed the prevalent proteomic consequences of an extra chromosome 21, which involved significant post-transcriptional regulation and a possible buffering mechanism on protein level.

Keywords: Down's syndrome, SWATH mass spectrometry, Chromosome 21, Monozygotic twins

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CS19.05 Mining Missing Proteins Base on the Transcriptomics and Proteomics to the Individual Testis Tissues

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Introduction and Objectives: On the basis of latest neXtProt database, a total of 2936 human genes have remained yet to be verified as the corresponding protein products. Herein, we proposed that a careful proteomics to human testis tissues could provide additional evidence in exploration of more missing proteins. **Methods:** The transcriptomes in the testis tissues were separately acquired by RNA-Seq using IonProton, while the testis proteomes were achieved from SDS-PAGE coupled with LC MS/MS using OrbiTrap Velos MS. The proteins were finally identified through IPeak search engine upon MS/MS data. **Results and Discussion:** A total of 16370 transcripts and 10444 proteins were identified in the three testes tissues, in which 94 transcripts that were never reported previously were detected by RNA-Seq, and 403 proteins that were defined as missing proteins were identified by LC MS/MS. Moreover, over 67% of the mRNAs that enabled encoding missing

proteins were detected in all three individual testes, whereas only 26% of such proteins was commonly shared in the samples. Comparison analysis towards the mRNA abundance revealed that the mRNAs encoded from the missing protein genes displayed the similar abundance distribution as the total mRNAs identified from the testis tissues, whereas the proteins elicited from missing proteins clearly exhibited lower abundance as compared with the average abundance of the overall proteins identified, suggesting that the poor abundance was indeed a characteristics of the proteins belonging to missing proteins in human testis. Further analysis upon the mRNA abundance obtained from our study and other reports divulged that except the missing proteins co-detected by RNA-Seq and LC MS/MS, the transcripts derived from the genes encoding missing proteins had relatively higher abundance in testis tissues, implicating that the expressing of missing protein genes was enriched in testis. **Conclusion:** Comprehensive and integrated proteomics was necessary to detect the missing proteins.

Keywords: Missing proteins, testis tissue, proteomics, transcriptomics

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CS19.06 Call for a Testis-Epididymis Proteome Project

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Introduction and Objectives: About 15% of couples encounter fertility problems and, in about half cases, the cause is of male origin. Due to its structural complexity, the testis is difficult to study in terms of organization, function, regulation and disorders. In particular, the mechanisms leading to the coordinated expression and relocation of a myriad of functional protein products during spermatogenesis and gamete maturation need to be unravelled. This project, currently developed in the framework of C-HPP initiatives on chromosomes 14 and 2, combines next-generation sequencing, transcriptomic and proteomic datasets to: 1. decipher the proteomes of testicular and epididymal cells, seminal plasma and spermatozoa, with a focus on missing proteins, novel protein-coding genes and testis-specific proteoforms; 2. prioritize proteins potentially involved in male reproduction for functional studies; 3. study the dynamics of proteins' post-translational modifications during the epididymal maturation of spermatozoa and correlate them with fertilization properties. **Methods:** For proteomics studies, state-of-the-art shotgun mass spectrometry using stringent validation criteria were applied. Immunohistochemistry on testis was performed with antibodies from the Human Protein Atlas. **Results and Discussion:** The high potential of the testis to discover missing proteins was demonstrated by combining transcriptomics and proteomics data. Over 100 missing proteins were evidenced in either total testis or enriched mobile spermatozoa extracts, and the expression pattern of a dozen of them was monitored. In parallel, some proteins from the seminal plasma were identified as valuable biomarkers for infertility. **Conclusion:** This project contributes to new knowledge on the pathophysiology of testicular function, with promising clinical applications. It is now time to build, with the Human Proteome Organization, a "testis/epididymis proteome project", to facilitate comparisons between Omic datasets for researchers active in the field of male reproduction and bridge the gap between C-HPP and B/D-HPP. There is no doubt that this effort will yield significant discoveries in the field of male reproductive pathologies.

Keywords: Infertility, Epididymis, integrative omics, Testis

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CS19.07 Refining the Human Proteome: Analyzing Human Tissues by RNA-Seq, Proteomics and Antibodies

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Introduction and Objectives: Having established a baseline proteome profile of the human body, we are now in the process of refining this draft map. The comprehensive and deep proteome analyses of 32 healthy human tissues profiled in the Human Protein Atlas project (www.proteinatlas.org) are now integrated with histological information, antibody-based staining of >18,000 human proteins and sample-matched RNA Seq information. **Methods:** Fresh frozen tissues (n=32) were lysed and tryptically digested in solution using a urea-based procedure. Peptides were analyzed by LC-MS/MS followed by identification and label-free quantification using Maxquant. **Results and Discussion:** The molecular profiling of 32 human tissues by RNA seq, proteomics and post-translational modifications (PTMs) covers the three most important layers of information flow in a biological system. Each tissue has been profiled to a depth of 15,000+/-2,000 mRNAs and 9,000+/-1,000 proteins (at 1% protein FDR). Preliminary results indicate that individualized tissue-specific RNA Seq databases enable the reliable identification of variant peptides and considerably alleviated the protein inference problem. Comparing transcriptome and proteome abundance levels corroborates previous observations that mRNA abundance distributions are considerably tighter than the proteome abundance range (2-3 vs 5-6 orders of magnitude) leading to a poor correlation between transcriptome and proteome. However, comparing individual transcript and protein levels across all tissues reveals extremely stable protein-mRNA ratios. Deviations from this rule point to post-transcriptional, (co-)translational and post-translational regulation. Comparing proteome and PTM levels uncovered tissue-specific, specialized PTMs and networks tightly linked to tissue function. Additional analyses include the comparison of this integrated multi-omics map to previously published draft maps of the human proteome, the integration of histological information, antibody-based staining information of >18,000 human proteins and further explorations of the utility of such datasets. **Conclusion:** The refinement of the human proteome reveals unprecedented insights into tissue- and cell specific biology covering e.g. proteome complexity, tissue-specific isoform expression and transcriptional and translational regulatory mechanisms.

Keywords: Refined human proteome, www.proteomicsdb.org, Human Protein Atlas

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CS19.08 Urinary Proteins Originating Uniquely from Each Nephron Segment

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Introduction and Objectives: This study aims to find urinary proteins, which are uniquely derived from four nephron seg-

ments (glomeruli, proximal and distal tubules, and collecting ducts) by shotgun proteomic analysis of urine and those tissues. **Methods:** We construct proteomic databases for human urine and kidney nephron segments. The urine and nephron databases are constructed from 10 datasets and 21 datasets (5 for glomeruli, 7 for proximal tubules, 4 for distal tubules and 5 for collecting ducts). We analyze nephron datasets to extract unique proteins for each segment, and then compare them with urine database to find those also identified in the urine proteome. Subsequently, plasma proteins are filtered out of these potential proteins by using plasma proteomic dataset described by Human Plasma Proteome Project (HPPP). Finally the urinary proteins derived from the nephron segments are screened by immunohistochemical images provided by Human Protein Atlas (HPA). **Results and Discussion:** Our data show 2831 proteins in the urine proteome database and 1808 proteins in the nephron database. Proteomic analysis extracts urinary proteins, which are uniquely derived either from glomeruli, proximal tubules, distal tubules or collecting ducts. Compared with the HPA immunohistochemical labelling in kidney, the urinary proteins are selected as high-confident ones originated from glomerulus (6), proximal tubule (24), distal tubule (3) and collecting duct (3). These proteins could be used to reflect the biologic or pathologic states of each nephron segment. **Conclusion:** In our study, the in-depth proteomic profiles of human urine, nephron segments and plasma are analyzed and integrated. In combination with immunohistochemical study, we extract potential urinary proteins uniquely derived from each nephron segment, which may reflect the biological or pathological states there.

Keywords: urinary protein, nephron segment, shotgun proteomics

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CS19.09 Detection of Chromosome 16 Missing Proteins - Spanish HPP

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Introduction and Objectives: According to neXtProt database annotation, there are still proteins without conclusive experimental evidence, which are termed "missing proteins". We have developed a specific protocol based on the generation of recombinant forms, to develop MRM methods for the identification of chromosome 16 missing proteins in biological matrices. **Methods:** 48 missing proteins from chromosome 16 were expressed using IVTT cell free expression system. Protein expression was assessed by targeted methods that were developed using Skyline. Peptides for the MRM were proteotypic and were chosen considering the following restrictions: no trypsin misscleavages, 7-25 aminoacids length, no Met, Trp and Cys residues. Upon method optimization proteins were searched on different biological samples, including biofluids and cell lines. **Results and Discussion:** According to the last release of neXtProt database, 17 of the 48 expressed proteins of chromosome 16 are still annotated as missing proteins. All of them were expressed and detected in the expression mixture with at least one peptide by MS/MS . Based on the information found in public repositories (PRIDE) and on the expression probability of these 17 missing proteins in cell lines, normal tissues and cancer tissues from the analysis of transcriptomics experiments (J Proteome Res. 2015 Mar 6; 14(3):1350-60) the biological matrices with the highest probability of detecting theses proteins were selected. Analyses are ongoing and the results will be presented. **Conclusion:** Expression of recombinant forms of missing proteins emerges as a useful method to optimize MRM methods and enhance the probabilities of detection.

Keywords: Missing proteins, Spanish HPP, Chromosome 16

CS20: PROTEIN MODIFICATIONS (OTHER THAN PHOSPHOPROTEINS)

CS 20: PROTEIN MODIFICATIONS (OTHER THAN PHOSPHOPROTEINS)
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CS20.03 Using the Ubiquitin-Modified Proteome to Monitor Protein Homeostasis Function

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Introduction and Objectives: Insults to endoplasmic reticulum (ER) homeostasis activate the unfolded protein response (UPR), which elevates protein folding and degradation capacity and attenuates protein synthesis. While a role for ubiquitin in regulating the degradation of misfolded ER-resident proteins is well described, ubiquitin-dependent regulation of translational reprogramming during the UPR remains uncharacterized. **Methods:** Our previous development and utilization of quantitative proteomic approaches to interrogate endogenous ubiquitylation events in a site-specific manner demonstrated that regulatory, non-degradative ubiquitylation is a pervasive modification across the proteome. Application of this quantitative proteomic approach to characterize alterations in protein ubiquitylation upon acute protein homeostasis dysfunction led to the identification of individual 40S ribosomal proteins that are ubiquitylated in a regulatory manner upon protein homeostasis stress. **Results and Discussion:** We demonstrate that regulatory 40S ribosomal ubiquitylation (RRub) occurs on assembled cytoplasmic ribosomes and are stimulated by both UPR activation and translation elongation inhibition. We further show that ER stress-stimulated regulatory 40S ribosomal ubiquitylation occurs on a timescale similar to eIF2α phosphorylation, is dependent upon PERK signaling, and is required for optimal cell survival during chronic UPR activation. **Conclusion:** These results demonstrate the power of using quantitative proteomics to interrogate site-specific alterations in the ubiquitin modified proteome upon acute protein homeostasis stress. In total, these results reveal regulatory 40S ribosomal ubiquitylation as a previously uncharacterized and important facet of the eukaryotic translational control.

Keywords: ubiquitin, ribosome, protein homeostasis stress, protein quality control

CS20.04 An Effective Method to Site-Specifically Analyze N-Sialoglycosylated Proteome on the Cell Surface

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Introduction and Objectives: Glycoproteins on the cell surface are very common and essential for cells to interact with other cells and the extracellular matrix. Surface sialoglycoproteins can markedly impact cell adhesion, mobility and solubility, which are correlated with cancer cell invasiveness and metastasis. Considering that cell surface sialoglycoproteins are critically involved in many cellular activities, comprehensive and site-specific analysis of N-sialoglycosylated proteins on the cell surface will help us better understand glycoprotein function and cellular activities, which will have profound biomedical implications. **Methods:** Here we have integrated metabolic labeling, copper-free click chemistry, and MS-based proteomics to perform the global identification and quantification of surface N-linked sialoglycoproteins site-specifically. Sialoglycoproteins located on the cell surface were selectively tagged, and after cell lysis and protein digestion, sialoglycopeptides tagged with biotin were enriched with NeutrAvidin beads. The newly developed method was employed to quantify the surface N-sialoglycoproteome in cancer cells with distinctive invasiveness. **Results and Discussion:** For HEK293, we identified 395 unique sites on 213 surface proteins. The molecular function analysis demonstrated that functions of these surface sialoglycoproteins are in very good agreement with the well-known ones of surface proteins. Combining with quantitative proteomics, we systemically investigated surface N-sialoglycoproteins for cancer cells with distinctive invasiveness. In striking contrast, the number of up-regulated sialoglycopeptides was nearly three times more than the number of down-regulated peptides. Among up-regulated N-sialoglycoproteins, clustering analysis showed that the most highly enriched function was cell adhesion. Additionally, proteins corresponding to cell motion and cell-matrix adhesion were also enriched. These results demonstrated that surface sialoglycoproteins are related to cell adhesion and mobility. **Conclusion:** The current strategy integrating metabolic labeling, click chemistry, and MS-based proteomics is effective to analyze surface N-sialoglycoproteins. For the first time, we site-specifically quantified the surface N-sialoglycoproteome between breast cancer cells with distinctive invasiveness. This effective method can be extensively applied to biochemical and biomedical research.

Keywords: N-sialoglycoproteome, MS-based proteomics, Targeted enrichment method, Cell Surface

CS20.05 Development of Methods for Site-Specific Analysis of N-Linked Protein Glycosylation

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Introduction and Objectives: As one of widespread post-translational modifications (PTMs), glycosylation plays essential roles in biological functions of proteins, as well as processes of protein folding, signal transduction, cell communication and cell apoptosis. And the aberrant of glycoforms is associated with many diseases[1-3]. Herein, a series of integrated technologies have been developed for mapping of N-glycosylation sites, quantification of glycoproteins, identification and quantification of aberrant N-glycosites as well as characterization of intact glycopeptides. **Methods:** In order to improve the efficiency of glycopeptide enrichment, mesoporous nanoparticles were prepared and electively modified with two different functional groups step by step (NP-MCM-S-NH2). Taking the advantages of superior hydrophilicity in the internal surface and size-confine of the mesopores, the prepared NP-MCM-S-NH2 particles were adapted as adsorbent for extraction of glycopeptides in complex biological samples. A platform for determination of N-linked site-specific glycoforms was fabricated, which combined the enrichment of glycopeptides by using functional mesoporous nanoparticles, optimization of HCD fragmentation and bioinformatics interpretation. A novel quantification system was further developed for large-scale quantification of site-specific N-glycoforms by the combination of the above strategy and stable isotope dimethyl labeling method. **Results and Discussion:** And about 800 N-linked glycosites were identified from only 20 μg mouse liver digest, indicating high specificity and sensitivity of enrichment strategy by using functional mesoporous nanoparticles. The glyco-sylation of human serum was analyzed, and about 942 site-specific glycopeptides representing 90 N-linked glyco-sites with both glycan composition and peptide sequence were determined with high throughput and efficiency. About 7100 proteins and 4000 site-specific N-linked glycoforms corresponding to 1800 glycosites were quantified from epithelial-mesenchymal transition (EMT) induced by TGF-β. **Conclusion:** In summary, the above methods could map N-glycosylation sites, quantification of glycoproteins, identification and quantification of aberrant N-glycosites as well as characterization of intact glycopeptides, which might be in favor of potential clinical biomarkers screening during the processes of tumor metastasis.

Keyword: Glycosylation of proteins; HILIC enrichment; Mesoporous materials; Site-specific glycoforms;

CS20.06 A Chemical Proteomics Approach for Lysine Monomethylome Profiling

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Introduction and Objectives: Lysine methylation plays critical roles in chromatin function and in diverse disease development. Nevertheless, non-histone methylation substrates remain largely unknown. Thus, proteome-wide analysis of Kme substrates will reveal chromatin-independent protein targets and pathways. Because of the limited physiochemical difference between mono-methyllysine and unmodified lysine residues, it is difficult to efficiently enrich mono-methylated peptides. An effective approach for system-wide analysis of lysine mono-methylation is lacking. We describe a chemical proteomics approach for global screening for mono-methyllysine substrates. **Methods:** RPMI1640 or DMEM medium was reconstituted with 12CH3-methionine or 13CD3-methionine. Chemical derivatization of the protein lysate with propionic anhydride was performed, followed by tryptic digestion. Affinity enrichment of the propionyl-methyl peptides was carried out using an anti-propionyl-methyllysine antibody (PTM BioLabs, Chicago, IL, USA). The enriched propionyl-methyl peptides were subjected to nano-HPLC-MS/MS analysis on an LTQ-Orbitrap Elite mass spectrometer.

Results and Discussion: We developed a novel strategy to enrich mono-methyllysine peptides, involving three steps: 1. Chemical derivatization of the mono-methylated ε-amine group of the lysine residue with propionyl anhydride to form propionyl-methyl ε-amine. 2. Affinity enrichment of the propionyl-methyllysine-containing peptides using a newl pan antibody against propionyl-methyllysine with high specificity. 3. HPLC/MS/MS analysis of the resulting peptides to map the modification sites. We cultured the cells in 13CD3-methionine media to distinguish the isotopic labeled monomethyllyine from other possible modifications or amino acid mutations with identical mass shift. Using this approach, we identified, with high confidence, 446 lysine monomethylation sites in 398 proteins, representing the largest data set of protein lysine monomethylation described to date. Our data not only confirms previously discovered lysine methylation substrates, but also reveals new substrates associated with diverse biological processes. **Conclusion:** This study provides a robust methodology for dynamic analysis of lysine monomethylation substrates, and dramatically extends the inventory of monomethylated proteins for further functional study.

Keywords: Lysine mono-methylation, Affinity enrichment, Global analaysis, Chemical propionylation

CS 20: PROTEIN MODIFICATIONS (OTHER THAN PHOSPHOPROTEINS)
TUESDAY, SEPTEMBER 29, 2015 – 14:30 – 16:20

CS20.07 Analysis of Arginine Methylation in Primary T Cells Reveals Roles in Cell Signalling and Fate

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Introduction and Objectives: Arginine methylation is the most prevalent form of protein methylation and appears to be particularly important for the function of human T cells of the immune system. However the repertoire and regulation of methylated proteins in these cells remains largely unknown. We aimed to carry out a comprehensive analysis of arginine methylation in primary human T cells using recently developed antibodies and a novel methyl SILAC labelling (iMethyl SILAC). We further sought to follow changes in arginine methylation occupancy during primary human T cell stimulation and differentiation. **Methods:** Primary human T cells were isolated from blood and labelled by iMethyl SILAC to provide high identification confidence of methylated peptides. Proteins were digested and methylated peptides were enriched with anti-methylarginine antibodies. To follow changes in arginine methylation, both methylated and non-methylated peptides were quantified to derive fold changes in arginine methylation occupancy. **Results and Discussion:** In the iMethyl SILAC labelling strategy, methylated peptides occur as 1:1 methyl SILAC pairs, allowing unambiguous corroboration. Using this labelling in T cells, 3 different proteases and 2 different anti-methylarginine antibodies, we performed the most comprehensive analysis of arginine methylation to date, resulting in the identification of 2500 unique methylation sites in >1200 proteins. Numerous transcription factors critical to T cell differentiation were found to be methylated on arginine. Following changes in arginine methylation during primary T cell stimulation and differentiation revealed a small group of splicing factors displaying altered methylation occupancy. **Conclusion:** Labelling by iMethyl SILAC provides high confidence corroboration of methylated peptides and is applicable to any form of protein methylation. Primary human T cells contain a large number of arginine methylated proteins, among these, a small group of splicing factors change in methylation occupancy during differentiation and are likely to be functionally important.

Keywords: heavy methyl SILAC, arginine methylation, T cells, Antibodies

CS 20: PROTEIN MODIFICATIONS (OTHER THAN PHOSPHOPROTEINS)
TUESDAY, SEPTEMBER 29, 2015 – 14:30 – 16:20

CS20.08 Protein Citrullination - Novel Insight into Triggers of Autoimmune Diseases

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Introduction and Objectives: Protein citrullination is the enzymatic catalyzed deimination of arginine resulting in an altered protein fold and the exposure of neoepitopes which subsequently may trigger an autoimmune response. Dysfunctional events of citrullination is emerging as a central clue in the disease etiology of multiple inflammatory and neurodegenerative diseases, e.g. Multiple sclerosis, Alzheimer's disease, and rheumatic diseases including rheumatoid arthritis. Citrullinated proteins remain technically difficult to discover. We have developed new and improved acquisition and bioinformatics approaches for identifying citrullinated proteins from tissue biopsies, cerebrospinal fluid and synovial fluid using mass spectrometry-based strategies, enabling the detection of hundreds of unreported sites including novel neo-autoantigens for clinical diagnostics as well as new insight in the biological role of this modification. **Methods:** Tissue colon biopsies, cerebrospinal fluid and synovial fluid were collected from patient cohorts diagnosed with RA or MS followed by optimized PTM specific sample preparation and label free quantitative mass spectrometry-based strategies. The acquisition strategies include data dependent, neutral loss triggered, and data independent analysis of the complex mixtures followed by an optimized data analysis and datamining. **Results and Discussion:** We have optimized data acquisition and data mining strategies based on a wide range of synthetic stable-isotope labeled citrullinated peptides and in-vivo citrullinated proteins. Our analysis have enabled an 60% reduction of false-positively annotated citrullination sites and identifyed more than 220 novel sites of citrullination in human body fluids and tissues allowing us to improve the insight of PAD enzymes, their biological targets and clues to role in inflammatory and neurodegenerative diseases. **Conclusion:** The development and implementation of an optimized citrullination identification workflow has enabled the analysis of citrullination in a number of tissues and body-fluids with a complex protein composition, with a higher sensitivity than ever before. Utilizing the workflow we have investigated the association between citrullination and a number of inflammatory and neurodegenerative diseases.

Keywords: PTM, proteomics, Citrullination

CS20.09 Lysine Succinylome Analysis of the Model Cyanobacterium Synechococcus sp. PCC 7002

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Introduction and Objectives: Lysine succinylation is a newly identified protein post-translational modification present in both prokaryotic and eukaryotic cells; however, its extent and function in cyanobacteria remain unexplored. Synechococcus sp. PCC 7002 is a model cyanobacterium and has been used extensively for studies concerned with photosynthesis and environmental adaptation. **Methods:** Here, we performed a global succinylome analysis of the Synechococcus by using nano-LC-MS/MS in combination with the enrichment of succinylated peptides from digested cell lysates and subsequent peptide identification. **Results and Discussion:** In total, 265 lysine succinylation sites on 129 proteins were identified in Synechococcus. The identified succinylated proteins are implicated in diverse biological processes, such as photosynthesis. Among all identified succinylated proteins involved in photosynthesis, the photosystem II (PSII) manganese-stabilizing protein (MSP, also known as PsbO) was found to be succinylated on lysine 89, 99, 219, and 234. Both non-succinylated and mimic-succinylated mutants were constructed by using site-directed mutagenesis. The in vivo characterization of the PsbO mutants showed that succinylation is a negative regulatory modification on the function of the photosystem II (PSII). Molecular dynamics simulations revealed that succinylation affects the conformational stability of PsbO, which is critical for the activity of PSII oxygen-evolving complex (OEC). Further functional studies demonstrated that succinylation of PsbO protein can lead to PSII photodamage and reduce the electron transport rate from PSII to PSI. **Conclusion:** This study reveals novel insights into the molecular mechanisms of protein succinylation in the regulation of photosynthesis and provides a rich source for functional studies of lysine succinylation in cyanobacteria.

Keywords: manganese-stabilizing protein, Lysine succinylation, cyanobacteria, photosynthesis

CS21: PROTEOMICS OF MICROBES AND INFECTIOUS DISEASES

CS 21: PROTEOMICS OF MICROBES AND INFECTIOUS DISEASES
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CS21.03 Pathogenic E. Coli Manipulate Global Proteolysis within Human Intestinal Cells during Infection

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Introduction and Objectives: Proteases regulate cellular processes relevant to infection, including innate immunity and cell death. Accordingly, this study aims to characterize T3SS-mediated alterations in the human proteome, including proteolysis, by using an amino (N)-proteomics approach to enrich protease-generated peptides. Furthermore, this work aims to identify substrates of the T3SS protease NleC. **Methods:** Polarized human intestinal cells were infected for 1.5 hours with wild-type EPEC or a mutant unable to secrete T3SS effectors. In parallel, intestinal cell lysates were incubated with either active or inactive NleC protease to identify novel substrates. For both experiments, N-proteomes from three replicates were quantitatively compared using terminal amine isotopic labeling of substrates (TAILS), tandem mass spectrometry (Orbitrap Velos), and MaxQuant . **Results and Discussion:** The abundance of 452 N-terminal peptides was altered >1.5-fold during EPEC infection (146 altered >2-fold). We identified 298 N-terminal peptides corresponding to proteolytic events occurring in a T3SS-dependent manner during EPEC infection; many also occurred in an NleC activity-dependent manner. Preliminary data indicate proteolysis of proteins involved in known T3SS-mediated pathways, including innate immunity and cytoskeletal regulation, as well as novel proteolytic events in proteins known to interact with EPEC T3SS effectors. Candidate NleC substrates were identified in transcriptional, ion channel, and innate immune pathways. Finally, novel, NleC-mediated proteolytic events in a cytoskeletal GTPase may provide further mechanistic details on how the T3SS mediates host cytoskeletal rearrangements during infection, a hallmark of EPEC infection. **Conclusion:** Examining how T3SS effectors alter the human cellular N-proteome has shed light upon mechanisms behind T3SS-mediated processes during infection. Ongoing studies aim to identify the impact of NleC substrates on human cellular pathways and EPEC disease phenotypes.

Keywords: Terminal proteomics, Virulence factors, proteases, Bacterial infection

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CS21.04 A Proteomics Approach for Characterization of the Human Adaptive Immune Response to S. Aureus

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Introduction and Objectives: Staphylococcus aureus is a pathogen that plays a major role in community-acquired infections. Treatment becomes more complicated due to the spread of antibiotic resistances. Highly sensitive proteomics approaches provide an unprecedented view of the interactions of this pathogen with its host. Immunoproteomics approaches in particular offer the option of a personalized approach to characterize the humoral immune response in detail. **Methods:** S. aureus was cultivated in infection mimicking conditions such as iron limitation or isolated after internalization by human host cells and proteins were analyzed on a Q Exactive™. In addition, nasal polyps from patients carrying S. aureus were also analyzed. Selected, heterologously expressed antigens were coupled to beads and the FlexMap 3D-bead technology was used to study the adaptive immune response of plasmas of human subjects either colonized with S. aureus in the nose or not (each N=16). Additionally, the composition of corresponding plasma samples was analyses by LC-MS/MS. **Results and Discussion:** The combined analysis of S. aureus grown in iron-restricted conditions and nasal polyps identified 1,710 proteins. Proteins of the Isd-family, SirA and FhuC were present at higher level in iron-restricted conditions. Furthermore, virulence factors (splF, sspB, clfB, SCIN , fib) were detected in nasal polyps and about 80 of these proteins were finally used for immunoproteomics screening. In an attempt to elucidate if nasal carriers display different immune responses to S. au-

reus, 16 plasmas of each group were profiled and significantly higher serum IgG levels for HlgB, HlgC, Geh, Ecm, Map, PrsA and SACOL0480 were observed in carriers. The study was complemented by plasma proteomes of the same subjects who also revealed significant differences. **Conclusion:** The established S. aureus specific FlexMap 3D multiplex assay allows now a broad profiling of the specific antibody response and can be used to identify S. aureus antigens that might serve as vaccine candidates or diagnostic markers.

Keywords: S. aureus, in vivo proteomics, nasal polyp, immunoproteomics

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WEDNESDAY, SEPTEMBER 30, 2015 – 11:10 – 13:00

CS21.05 A Novel Proline Specific Protease from C. Difficile Involved in Adhesion

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Introduction and Objectives: The regulation of adhesion and motility of pathogenic bacteria is important for bacterial virulence and cell surface and secreted proteins are important mediators of these processes. The aim of the current study was to functionally characterize novel secreted proteins of the human pathogen Clostridium difficile. **Methods:** We used a proteomic approach to analyze the proteins secreted by Clostridium difficile and identified a novel secreted protease. Applying a variety of biochemical, mass spectrometric (MS) and in vitro proteolytic assays enabled us to identify the cleavage site specificity and determine the parameters of the enzyme kinetics. Cell surface shaving experiments, collagen binding assays and gene knockouts allowed us to reveal the endogenous protease substrates and enabled us to decipher the function of the protease. **Results and Discussion:** First of all, we have identified CD2830 as one of the major secreted proteins from C. difficile strain 630. We show that CD2830 is a metalloprotease with a striking preference for cleaving between two proline residues in an overall proline rich motif. Subsequently, we identified two C. difficile cell surface proteins, CD2831 and CD3246, as genuine CD2830 substrates. Using mass spectrometry, we demonstrated that CD2830 efficiently cleaves the CD2831 collagen binding protein at multiple locations, both in vitro as well as from live cells. The CD2830-mediated release of CD2831 from the cell surface coincides with lower affinity of these cells for a collagen matrix, compared to CD2830 knockout cells. **Conclusion:** Our data demonstrate CD2830-protease mediated release of cell surface adhesion proteins and as such support a role for CD2830 in switching from a sessile to a motile phenotype in C. difficile.

Keywords: Clostridium difficile, metalloprotease, adhesion, secretome

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CS21.06 Quantitative Mass Spectrometry Meets Molecular Epidemiology and Vaccinology: Factor H Binding Protein (fHbp) from Neisseria meningitidis

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Introduction and Objectives: fHbp is a surface-exposed lipoprotein of Neisseria meningitidis and a protective antigen composing the recently licensed Bexsero® vaccine. fHbp is a highly variable meningococcal protein, with remarkable sequence variability, and classified in three variants with limited cross-protection. Furthermore the level of fHbp expression varies significantly among strains. Different methods have been used to assess fHbp expression on meningococcal strains, however all these methods use anti-fHbp antibodies, and for this reason the results are affected by the different affinity that antibodies can have to different antigenic variants. **Methods:** To overcome the limitations of an antibody-based quantification, we developed a Selected Reaction Monitoring (SRM) approach, emerged as a powerful MS tool for quantifying proteins in complex mixtures. We identified nine ProteoTypicPeptides (PTPs), which are uniquely associated to fHbp and allowing to cover hundreds of different MenB strains. This approach, highly sensitive, quantitatively accurate and highly reproducible, was used to quantify the absolute amount of fHbp antigen in total extracts of 105 serogroup B strains, evenly distributed among the three main variant groups and selected to be representative of the fHbp circulating subvariants around the world. **Results and Discussion:** We found that the amount of fHbp can vary among different strains and a significantly higher level of expression was measured for variant 1 strains compared to strains carrying fHbp variant 2 and variant 3. **Conclusion:** To date this is the first comprehensive fHbp expression profiling in a large panel of strains driven by an antibody-independent MS-based methodology to fully characterize the Neisseria meningitidis "landscape" to fight with vaccination. We extended the study at the genetic level investigating the correlation between differential level of expression and promoter sequences. The implications of fHbp expression on the susceptibility of the strain to killing by anti-fHbp antisera are also investigated.

Keywords: Molecular Epidemiology, SRM, Vaccine, Neisseria meningitidis

CS 21: PROTEOMICS OF MICROBES AND INFECTIOUS DISEASES
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CS21.07 The Listeria Monocytogenes Proteotype

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Introduction and Objectives: Listeria monocytogenes (Lm) is a facultative intracellular bacterium responsible for a severe foodborne infection known as listeriosis. Here, we aim to establish an experimental workflow for studying the combinatorial function of bacterial and host cellular factors that contribute to the Lm cell attachment, invasion and propagation. **Methods:** The colon epithelial cells (Caco2) and the Lm reference strain EGDe were chosen as model system. The surfaceome of Caco2 cells was analyzed using the Cell Surface Capture (CSC) protocol coupled to mass spectrometric analysis. Ligand-based Receptor-Capture (LRC) technology was adapted to allow for the receptor identification of intact Lm on host cells. An optimized experimental workflow for shotgun proteomic analysis of Lm cells was undertaken for the generation of an Lm spectral library. **Results and Discussion:** In preparation for a combined analysis of Lm host interactions, we developed a Data Independent Acquisition (DIA)-MS workflow for the relative quantitative interrogation of the Lm proteotype upon

perturbation. The spectral Lm library covers more than 80% of the predicted Lm proteome and enables repetitive identification and quantification of the Lm proteotype upon perturbation. Given that host cell surface proteins could be monitored as extracellular markers of intracellular network changes upon Lm invasion, we established a surfaceome map for Caco2 cells. CSC analysis enabled the detection of 550 N-glycosylated Caco2 cell surface proteins including the E-cadherin; a known Lm host cell interaction partner. In order to investigate host cell proteins for Lm host cell docking and internalization, we adapted the TRICEPS-based LRC technology towards the detection of host cell receptors for orphan bacterial ligands. **Conclusion:** We generated a DIA-MS platform that enables the sensitive, repetitive and quantitative interrogation of the Lm proteotype upon perturbation. The DIA-based strategy in conjunction to the CSC and LRC workflows provide us with the opportunity to investigate bacterial mediators that enable Lm invasion in mammalian cells.

Keywords: host-pathogen interactions, ligand-receptor capturing, Listeria spectral library

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CS21.08 A DIA-MS Approach for the Investigation of S. Aureus Specific In Vivo Host-Pathogen Interactions

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Introduction and Objectives: S. aureus related diseases range from mild to severe infections. Proteome analyses are able to identify key components important for disease pathophysiology. However, general proteome analysis approaches using data-dependent acquisition (DDA) are known to provide lower reproducibility and comprehensiveness when compared to data-independent acquisition (DIA). A spectral library, which is suitable for the analysis of DIA data, was generated, benchmarked with a well-characterized biological standard data set, and used for an in-depth analysis of in vitro S9 cell and in vivo murine infection experiments. **Methods:** Protein samples from S. aureus grown under different conditions were extensively fractionated to obtain a comprehensive proteome map for routine proteomics approaches. The DDA data (N=152) were collected on a Q Exactive and integrated to provide a spectral library suitable for DIA analysis. The comprehensive S. aureus DIA library was then used to perform DIA-based proteome studies in vitro and in vivo. **Results and Discussion:** Benchmarking of the S. aureus DIA library, which covers approx. 72% of the S. aureus proteome, reveals that this library provides very high reproducibility (majority of CVs < 10%) and cross-MS compatibility. When analyzing 47 Staphylococcus strains, peptides of each strain covered > 80% of our library. Thus the library can be used as a global staphylococcal DIA library. DIA analysis of murine infection samples revealed up-regulation of proteins involved in oxidative stress and down-regulation of proteins involved in dNTP synthesis and protein biosynthetic activity, which was confirmed by the S9 cell infection experiments. DIA data allowed a deeper insight in the pathogen adaption to the host during infection. **Conclusion:** A newly generated S. aureus DIA spectral library represents a valuable resource for the scientific Staphylococcus community. The comprehensive and quantitative DIA data revealed similar adaption patterns of the pathogen after internalization by non-professional phagocytes and in a murine in vivo infection model.

Keywords: in vivo proteomics, DIA, mouse model, S. aureus

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CS21.09 Phosphorylation and Thiol-Redox Modifications as Molecular Switches in Host-Microbe Interactions

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Introduction and Objectives: The respiratory airways, including bronchial epithelial cells and specialized immune cells, constitute a frontline barrier against airborne bacterial pathogens such as the pneumonia causative agent Staphylococcus aureus. Proteinogenic regulatory switches, in particular phosphorylation or cysteine thiol oxidation, are essential components of cell signaling networks ensuring rapid modulation of cellular responses. We hypothesize that interaction of bacterial pathogens with cells of the airway epithelium is accompanied by characteristic alterations in posttranslational modifications (PTM) that are either associated with defense mechanisms of the host or establishment and progression of the infection. Characterization of the regulatory switches can therefore lead to a greater understanding of the molecular pathomechanisms of infections and the establishment of novel host-centric targets for anti-infectives. **Methods:** We utilize models of S. aureus-infected human bronchial epithelial cells and a monocyte/macrophage model in combination with enrichment techniques for protein kinases, phosphopeptides and thiol-redoxmodificationstocomprehensivelycharacterizechangesinphosphorylation and thiol-redox-modifications under infection-relevant conditions. **Results and Discussion:** Functional data analysis on the investigated PTMomes not only identified affected biological processes and molecular functions, our studies also highlight key regulatory proteins. For example, in human bronchial epithelial cells the activity of the epidermal growth factor receptor was found to be critical for sensitivity towards staphylococcal alpha toxin. In addition, temporal phophosphorylation signatures indicate a key role of cycline and calcium-dependent kinases during the early phase of infection and pharmacological inhibition of relevant kinases significantly diminished the bacterial load. TAK1 kinase in THP-1 monocytic cells, on the other hand, was found to be implicated in monocyte-to-macrophage transition, cytokine/chemokine production and bacterial phagocytosis. Eventually, our studies indicate thiol-redox and phosphorylation cross-talk and emphasize the important role of hydrogen peroxide as a second messenger. **Conclusion:** Mass spectrometry-based PTMomics can reveal alterations in cellular signaling during host-microbe interactions and highlight relevant regulatory hubs that might be suitable for pharmacological intervention.

Keywords: Cell signalling, airway cells, PTMomics, host-microbe interaction

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CS21.10 Proteomic Investigation of a Potential Type I Secretion System in the Syphilis Spirochete, Treponema Pallidum

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Introduction and Objectives: Treponema pallidum, the causative agent of syphilis, is a persistent and invasive pathogen that can be acquired through sexual transmissionor vertically from a pregnant woman to her fetus. This non-cultivable, obligate human pathogen undergoes rapid dissemination from the site of infection and crosses both the placental and blood-brain barriers. Bioinformatic analysisidentified an operonencoding a potential Type I Secretion System (T1SS). As with other bacterial pathogens, we hypothesize this systemfunctions in T. pallidum to export virulence factors,including the hostcomponent-degrading proteasepallilysin, and hence contributes to the unique invasiveness of this pathogen. The objective of our studies is to characterize the potential T1SS using proteomic methodologies and determine if it secretestreponemal virulence factors to the host environment. **Methods:** Plate-based binding studies, pull-down assays, crosslinking studies and mass spectrometry analyses were used to investigate potential protein-proteininteractions between pallilysin and the periplasmic component of the T1SS that has been shown to play a role in secretion. Additionally, since the related spirochete Treponemaphagedenis also encodes a potential homolog of the T1SS, we performed an unbiased pull-down assay using recombinant pallilysin (bait) and a T. phagedenis lysate (prey) followed by mass spectrometry analyses for protein identification. **Results and Discussion:** Plate-based binding assays showed a low level ofinteraction between the periplasmic component of the T1SS and pallilysin, and this was confirmed by pull-down assays using recombinant T. pallidum proteins.In vitro crosslinking studies followed by SDS-PAGE analysis and in-gel trypsin digestion/mass spectrometry showed co-migration of both proteins at an approximately additive molecular mass. Finally, the unbiased pull-down experiments identifiedadditional potential protein interaction partners. **Conclusion:** This constitutes the first investigation of a potential T1SS within T. pallidum, and insight gained will lead to a better understanding of the mechanisms facilitatingT. pallidumhost invasion.

CS22: PHOSPHOPROTEOMICS AND CELL SIGNALING

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CS22.01 Multi-Layered Proteomics Reveals Molecular Switches Dictating Biased Ligand-Dependent EGFR Signaling

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Abstract: Quantitative phosphoproteomics is a powerful technology for unbiased analysis of cellular signaling networks. One of the most intriguing and unanswered questions in the field of cell signaling concerns how stimulation of the same receptor with distinct ligands generates specific cellular outputs. The concept of biased ligand signaling is well-established in the field of G-protein coupled receptors, where different ligands binding to the same receptor activates full or only partial downstream signaling networks. This notion of functional selectivity by ligands activating the same receptor is now emerging as an equally important concept in receptor RTK signaling, as we have recently shown for FGF receptor family. Here we present a time-resolved Integrated Multi-layered Proteomics Approach (IMPA) and performed quantitative interaction proteomics, phosphoproteomics and ubiquitome analysis to delineate EGF receptor signaling dynamics activated by the recycling ligand TGF-α or by EGF, a ligand that induces receptor degradation. Although the majority of regulated phosphoproteins, ubiquitylated proteins and interaction partners are shared between the two ligands, their dynamics are often different. We found that EGF generally induce transient phospho-signaling, whereas TGF-α activates sustained phospho-signaling, which leads to increased cell proliferation and migration. In particular, proteins that are part of the endocytic machinery were differentially regulated and from these we identified and functionally validated 'cellular switches' that control the endocytic trafficking of the EGF receptor and ultimately decide the cellular outcome. Notably, by manipulating the expression level of or phosphorylation of the differentially regulated endocytic proteins in cancer cells of different origin we showed that the TGF-α-dependent cellular response switched to an EGF-like response and vice versa. These results, based on a multidisciplinary approach, which combines multi-layered proteomics and functional assays, identify ligand-dependent mechanisms for the control of EGFR intracellular fate and for the specification of long-term responses.

Keywords: quantitative interaction proteomics, EGF receptor signaling, global ubiquitin analysis, phosphoproteomics

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CS22.02 The Chronoproteome of the Brain

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Abstract: The circadian system is an important biological clock that coordinates our physiological processes by aligning internal biological processes with the day/night cycle. Increasing evidence links disruption of circadian cycles with aging- and neurodegenerative(ND)- disorders, and other diseases. A core set of genes involved in the control of the clock have been identified and studied in different organisms. However, very little is known about the effects of the clock system on proteins, protein networks, and pathways. Moreover, although the primary clock is located in a specific region of the brain called the suprachiasmatic nucleus, secondary clocks are also present in other regions of the brain and their understanding is even more rudimentary. I will present results that demonstrated that the rhythmic nature of the brain circadian cycle extends far beyond the core clock to many rhythmic proteins, and cellular processes, and different regions of the brain, responsible for different functions, have their own unique oscillating proteomic profile.

Keyword: proteomics, circadian, phosphorylation

CS 22: PHOSPHOPROTEOMICS AND CELL SIGNALING
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CS22.03 Kinome, Total Proteome and Phosphoproteome Analysis of the CRC64 Cell Line Panel

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Introduction and Objectives: The CRC64 panel is a collection of colorectal cancer (CRC) cell lines, the majority of which were recently shown to be representative models of the main molecular subtypes of primary cancer (Mouradov et al., 2014). Here, we analyze the kinome, as well as both the total proteome and the phosphoproteome of the CRC64 panel, in order to find proteomic markers of drug sensitivity and resistance. **Methods:** Kinases were enriched from total cell lysates (TCLs) using Kinobeads gamma (Médard et al., 2015). Phosphopeptides were enriched from in-solution digests of TCLs using Fe-IMAC columns (Ruprecht et al., 2015). For total proteome analysis, in-solution digests of TCLs were fractionated into 48 fractions using hSAX (Ruprecht et al., 2015), followed by desalting (Rappsilber et al., 2007) and combination into 24 fractions. Kinobead enrichments, total proteomes and phosphoproteomes were measured on an Orbitrap Elite, an Orbitrap Velos and a QExactive Plus, respectively. We performed label-free quantification using MaxLFQ (Cox et al., 2014) and used elastic net regression (Zou and Hastie, 2005) to identify proteins/peptides, which are associated with drug sensitivity or resistance. **Results and Discussion:** More than 200 kinases were quantified using Kinobeads, with a mean of 142 kinases per cell line. The MaxLFQ quantification for selected kinases correlates well with densitometry measurements of western blots (Pearson's r=0.916). Hierarchical clustering using kinome data revealed five subgroups in the CRC64 panel. Total proteome measurements resulted in the quantification of more than 8,000 protein groups per cell line. On average, phosphoproteomics identified more than 12,000 unique phosphopeptides and more than 8,000 unique class 1 sites per cell line. Elastic net regression resulted in the identification of known and novel markers of drug sensitivity and resistance. **Conclusion:** A holistic view on the proteome of the CRC64 panel exemplifies how proteomics enables the discovery of novel markers of sensitivity and resistance towards common drugs.

Keywords: Drug Sensitivity, colorectal cancer, Cell Lines, phosphoproteomics

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CS22.04 Integrated Proteomic and Phosphoproteomic Analysis of TCGA Ovarian Tumors

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Introduction and Objectives: Ovarian cancer remains the most lethal gynecological malignancy in the developed world, despite recent advances in genomic information and treatment. To better understand this disease, define an integrated proteogenomic landscape, and identify factors associated with overall survival, we gathered global proteomic and phosphoproteomic data from a subset of high grade serous ovarian cancers previously characterized at the genome level (sequencing, methylation, miRNA and mRNA expression) by The Cancer Genome Atlas (TCGA). **Methods:** Isobaric peptide labeling (iTRAQ) for quantitation in conjunction with extensive fractionation and high performance reversed phase liquid chromatography and high-resolution tandem mass spectrometry was used for proteomics measurements. **Results and Discussion:** Correlation of CNAs with protein abundances identified loci with significant trans regulatory effects mapping to pathways associated with proliferation, cell motility/invasion, and immune regulation, three known hallmarks of cancer. Using the trans regulated proteins we also created models significantly correlated with patient survival by multivariate analysis. Analysis of proteomics and phosphoproteomics data highlighted a set of distinct and overlapping pathways compared to more traditional gene expression data. Further we use novel analysis approaches to integrate proteomic and genomic data to identify pathway activation and show that data integration improves resolution of activated pathways in short- and long- term survivors and in different clinical and molecularly-defined subtypes. We employ data integration and network analysis approaches to identify novel potential drivers of cancer progression. **Conclusion:** This work illustrates the ability of proteomics to build on insights from genomics experiments to elucidate protein networks that drive cancer biology and how these pathways alter in correspondence with clinical phenotypes. This enhanced molecular view may ultimately accelerate our understanding of the molecular basis of cancer, thereby linking genotype to proteotype, and ultimately to phenotype.

Keywords: CPTAC, Proteogenomics, phosphoproteomics, Ovarian cancer

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CS22.05 Selective Enrichment of Phosphotyrosine Peptides Using Plastic Antibodies

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Introduction and Objectives: Tyrosine phosphorylation is a fundamentally important mechanism of signal transduction and regulation in all eukaryotic cells, but it is challenging to characterize. This is because of low abundance of many of the proteins targeted for phosphorylation, phosphorylation is typically substoichiometric, and, only ~0.05% appears on tyrosine residues. Highly efficient and preferably site-selective enrichment procedures are therefore required. A Marie Curie project termed PEPMIP have with this objective applied novel use of molecular imprinted polymers (MIPs) for phosphotyrosine (pY) enrichment. **Methods:** The affinities of plastic antibodies are tunable by tweaking the chemistry of their production, and our collaboration partners synthesized an array of different pY MIPs. Their formats were either SPE-MIPs made from grinded bulk MIPs or monolith-MIPs capillaries prepared by grafting polymerized capillaries with pY templates. We tested and demonstrated their use on either simple protein digests followed by MALDI with manually evaluated data, or on digested rat liver lysates with LC-MS analysis and data processed by Proteome Discoverer. **Results and Discussion:** The most promising pY selective MIPs were firstly screened for and complementary selectivity to state-of-the-art sample preparations; titanium dioxide (TiO2) and immunoprecipitation (IP) was demonstrated. These MIPs were secondly used to develop a sample preparation workflow for tissue samples which comprised both TiO2 and SPE-MIPs. The optimized workflow was finally evaluated against a reference sample preparation (TiO2) were the MIPs workflow unveiled other phosphotyrosine and phosphorylated peptides than the reference: It gave ~140% additional peptides (n=62) with tyrosine sites whereas 15 are never before reported, and ~80% additional peptides (n=1274) with other phosphorylated sites. **Conclusion:** Plastic antibodies unveiled novel phosphorylated tyrosine sites and demonstrated complementary selectivity to current "gold standard" phosphoenrichment tools. The tunable affinity of MIPs also allows for specificity adjustment and use for other post translation modifications which should and will be investigated in the future.

Keywords: tyrosine phosphorylation, targeted phosphorylation enrichment, molecular imprinted polymers

CS22.06 Profiling Protein Expression, Modifications and Interactions with Antibody Microarrays

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Introduction and Objectives: Antibody microarrays permit the sensitive and semi-quantitative analysis of the expression, covalent modification and interactions of proteins recovered from lysates of cells and tissues. At Kinexus, we have developed antibody microarrays that feature over 875 pan- and phosphosite-specific antibodies for tracking protein kinases, phosphatases and other low abundance cell signalling proteins. **Methods:** Several variations of the use of our Kinex™ KAM high content antibody microarrays were examined to monitor protein levels, phosphorylation and interaction. One method involved capture of in vitro dye-labeled proteins from lysates from cells subjected to diverse treatments. Another method involved the detection of general changes in protein phosphorylation with the pIMAGO stain or more specifically with our generic phosphotyrosine-specific PYK rabbit polyclonal antibody. To explore the interactions of adapter, scaffolding and chaperone proteins with hundreds of potential target signal transduction proteins, we have also developed sandwich antibody microarrays that utilize dye-labeled reporter antibodies for these highly interactive proteins. **Results and Discussion:** We used several cancer cell lines (e.g. A431, HeLa, Jurkat, MCF7) subjected to diverse treatments to identify biomarkers of the actions of these agents. Reproducible results were obtained with as little as 25 µg of lysate protein, with a dynamic range of detection exceeding 6000-fold, and a median error range for duplicates measurements of ±12%. Typically 10-15% of the proteins tracked with these arrays demonstrate perturbations exceeding 50%. More than a quarter of the leads from our antibody microarrays were confirmed by immunoblotting studies. The major limitation associated with validation by Western blotting was the much lower sensitivity of this method. **Conclusion:** The goal of our proteomics and bioinformatics studies is to use the experimental results from the application of these microarrays to map the architecture of signalling networks in a cell- or tissue-specific manner. Antibody microarrays are a cost-effective, sensitive and quantitative method available for such purposes.

Keywords: Antibody microarray, Protein phosphorylation, Cell signalling, Biomarker identification

CS22.07 Mapping Signalling Network Intersection Downstream of Major Cell Surface Receptors

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Introduction and Objectives: Reversible protein phosphorylation forms vast signalling networks that rapidly respond to intra- and extra-cellular cues to orchestrate molecular responses, linking the cell's genome with its environment. The misregulation of such signals cause or exacerbate the onset of complex diseases including cancer and ageing, and their rectification is therefore a major area of biomedical research. This is however complicated by the fact that major signaling nodes are functionally pleiotropic, being involved in several biological processes. Indeed, different cellular stimuli often signal through shared network branches. Designing effective therapies against dysregulated signalling therefore requires a more complete picture of the signalling landscapes downstream of cell surface receptors and the extent of network overlap. **Methods:** Using a new scalable, single-run phosphoproteomics platform, we quantified the response of signalling networks of diverse cell lines to a large panel of ligands, targeting pro-survival (e.g. EGF, IGF, VEGF, PDGF) and pro-inflammatory pathways (e.g. TNFα, TRAIL, IL1β, IL-6). **Results and Discussion:** Here, we quantified over 20,000 phosphorylation sites, from which comparisons of ligand induced cellular signaling networks have been visualized. This has revealed highly specific signalling network branches, as well as large tracts of shared components. **Conclusion:** These systems-wide data provide an extensive view of ligand-induced signalling networks in different cell lines, greatly extending the current knowledge of the signalling occurring downstream of major cell surface receptors.

Keywords: phosphoproteomics, signal transduction

CS22.08 Phosphoproteome Analysis Identifies Oncogenic Kinases in Hepatocellular Carcinoma

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Introduction and Objectives: The somatic mutation of kinases /phosphorylation drived singnaling abberant is very common in many cancers. To detect the phosphorylation phenomenon is critical to find some aberrant activated kinases, especially some important signaling or target proteins in HCC. **Methods:** The paired tumor and no tumor HCC tissue's phosphoproteome were analyzed to gain a large landscape of the pathways. With the sustained and rapid technique development of mass spectrometry, label-free has emerged as an alternative approach in PTMs quantification. In this study we present an excellent robust and fast method for the phosphopeptides enrichment and separation combined with high-resolution mass spectrometry identifications. **Results and Discussion:** We identified 6,555proteins, 36,955 phosphopeptides, 33,421 sites from 42 paired HCC samples. The pathway enrichment revealed that most pathway were highly activated, like as tight junction pathway and VEGF pathway, which are critical in tumor metastasis and formation of new blood vessels, respectively. The certain sites of proteins are generally phosphoylated by certain kinases families, and the motifs of the phosphosites also reveal the activities of the kinases. 262 kinds of kinases are found in our dataset, the majority are involving CDK,MAPK,PKA signaling pathways, suggesting the two kinases families AMGC and AGC active highly responsible for the detected phosphosites. **Conclusion:** Analysis of the phosphoproteome in HCC and the paired no tumor tissue showed kinases drived pathways specific phospho-active in HCC, like EGFR and VEGFR, which may indicate the deregulation of pathways in HCC.

Keywords: Phosphoproteome, kinases, Hepatocellular carcinoma

CS22.09 In-Depth Phosphoproteomic Analysis of Immune Signaling Pathways in Response to Salmonella Infection

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Introduction and Objectives: Innate immune detection of intracellular Gram-negative bacteria (e.g. Salmonella) is largely based on the recognition of cytosolic bacterial LPS (Lipopolysaccharide). In murine macrophages, cytosolic LPS can activate an inflammasome including caspase-11. Since phosphorylation is a main regulator of many signaling cascades, we analyzed the global cellular phosphorylation changes within the first 8 hours upon Salmonella infection to characterize which innate immune signaling cascade are important for caspase-11 activation and establish their temporal dynamics. **Methods:** We extracted and digested proteins of primary wt/B6 bone marrow derived macrophages (BMDM) infected with ΔorgA Salmonella Typhimurium for 0.5, 1, 2, 4 and 8 hours and subjected them to phosphopeptide enrichment using TiO2. Phosphopeptides were either directly analyzed by 1D-LC-MS using a Q Exactive-HF hybrid quadrupole-Orbitrap or after phosphatase treatment. Subsequently, peptides were subjected to label-free quantification using the Progenesis QI software followed by statistical analysis using SafeQuant. **Results and Discussion:** Around 5000 phosphopeptides could be quantified across all 6 time points with more than 700 showing a significant change in at least one time point. The appropriate phosphoproteins cover many known immune signaling pathways, like TLR, IFN, NF-κB, NOD1/2 or MAPK signaling, however, the coverage of several pathways was incomplete and known phosphoproteins (e.g. Jak/Stat) were not included in this first dataset. Therefore, we tried to extend phosphorylation site coverage by analyzing our efficiently enrichment phosphopeptide samples before and after phosphatase treatment. Interestingly, the removal of phosphorylation groups significantly increased the MS-response of all treated peptides by 5-10-fold, allowing us to quantify a total of 13000 phosphopeptide candidates from the same samples. **Conclusion:** The newly detected proteins from the phosphatase treatment extended the picture of activated signaling pathways acquired previously and for a couple of reasons, which will be discussed, we can assume that these peptides were actual phosphopeptides.

Keywords: Inflammasome, Phosphopeptides, Label-free quantification

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CS22.10 The Molecular Mechanism of PI3K Mutations Implicated in Immunodeficiencies

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Introduction and Objectives: Phosphoinositide 3-kinases (PI3Ks) are responsible for the generation of the key lipid signalling molecule phosphatidylinositol (3,4,5) tris-phosphate (PIP3), which is vital for transduction of extracellular signals at the plasma membrane. The production of PIP3 is essential in regulating downstream pathways involved in cell growth, death and proliferation. PI3K is composed of one of four catalytic subunits (p110α, p110β, p110Δ, and p110γ) and the activity of the p110 catalytic subunit is regulated by its tightly controlled interaction with the regulatory protein p85α. Binding of p85α to the p110 subunit inhibits catalytic activity and activating mutations affecting this interaction result in a variety of human diseases. Recently identified patient mutations in PIK3CD and PIK3R1, the genes encoding p110Δ and p85α, have been implicated in primary immunodeficiencies. Analysis of patients with these mutations showed an increase in phosphorylation of downstream signaling molecules. Further investigation has shown that these are activating mutations that result in a hyperactive kinase, indicating that the interaction between p110Δ and p85α has been affected. Our objective is to identify how patient mutations are affecting the interaction between p110Δ and p85α. **Methods:** This membrane associated protein complex has required a combination of biochemical assays and hydrogen-deuterium exchange mass spectrometry (HDX-MS) to effectively answer our key research questions. Using these techniques, we have been able to investigate the molecular mechanisms of how these clinically relevant mutations alter the structural dynamics of the p110Δ/p85α protein complex. We also are able to relate these dynamic structural changes to the mechanism of how PI3Ks both interact with membranes and catalyze substrate turnover. **Results and Discussion:** not applicable
Conclusion: not applicable

Keywords: Signalling, Immunodeficiency, HDX-MS, PI3K

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CS23.01 Integrated Omics to Study the Human Proteome

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Abstract: Integrated omics to study the human proteome Mathias Uhlen, Science for Life Laboratory and Albanova University Center, Royal Institute of Technology (KTH), Stockholm, Sweden The human proteins constitute the major building blocks for the function of the various processes necessary for human life. The mapping of the human genome has allowed us to predict that each human has approximately 20,000 genes encoding for proteins. In the field of proteomics, these proteins are studied using various tools such as mass spectrometry, antibody-based profiling, chromatography, bioimaging, crystallography and spectroscopy. In addition, the new tools in genomics based on next generation sequencing have open up the possibility to study human variation and expression levels in a quantitative manner not possible only a few years ago. We have classified all the protein coding genes in humans using a combination of genomics, transcriptomics, proteomics and antibody-based profiling. We have used this data to study the global protein expression patterns in human cells, tissues and organs as well as a discovery tool to find potential biomarkers for disease, such as cancer. Selected own references: 1. Uhlen et al (2015) Science 347: 394 2. Martinoglu et al (2014) Nature Communication, 5:3038. 3. Wein et al (2014) Nature Medicine 20: 992-1000 4. Agren et al (2014) Molecular Systems Biology, 10:3. 5. Kampf et al (2014), FASEB J 28(7): 2901-2914. 6. Forsström et al (2014) Mol Cell Proteomics 13: 1585- 1597 7. Edfors et al (2014) Mol Cell Proteomics 13: 1611-1624. 8. Zang et al (2014) Proc Natl Acad Sci U S A. 111: 1149-58 9. Stadler et al (2013) Nature Methods 10(4):315-23 10. Danielsson et al (2013) Proc Natl Acad Sci U S A. 110(17): 6853-8

Keywords: transcriptomics, Tissue-based map, Human Proteome, Antibody profiling

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CS23.03Integrative Analysis of RNA, Translation and Protein Level Variation across Humans

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Introduction and Objectives: Elucidating the consequences of genetic differences between humans is essential for understanding phenotypic diversity and personalized medicine. Although variation in RNA levels, transcription factor binding and chromatin have been explored, little is known about global variation in translation and its genetic determinants. **Methods:** We used ribosome profiling, RNA sequencing, and mass spectrometry to perform an integrated analysis in lymphoblastoid cell lines from a diverse group of individuals. **Results and Discussion:** We find significant differences in RNA, translation, and protein levels suggesting diverse mechanisms of personalized gene expression control. Combined analysis of RNA expression and ribosome occupancy improves the identification of individual protein level differences. Finally, we identify genetic differences that specifically modulate ribosome occupancy - many of these differences lie close to start codons and upstream ORFs. **Conclusion:** Our results reveal a new level of gene expression variation among humans and indicate that genetic variants can cause changes in protein levels through effects on translation.

Keywords: translation, human genetic variation, gene expression, integrative analysis

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CS23.04 A Multi-Omics Perspective of a Population-Based Cohort - A Molecular Epidemiology Approach

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Introduction and Objectives: Omics approaches are widely applied in the screening for diagnostic and prognostic biomarkers and population-based cohorts provide an extremely valuable resource particularly for the study of prevalent diseases. One such study, the Study of Health in Pomerania (SHIP) which is acknowledged for its comprehensive high-quality phenotyping has been established since 1997 at the University of Greifswald (1). It is our goal to enrich these epidemiological data with molecular phenotypes and to assess to which degree such multi-omics data will provide new insights into the pathophysiology of common diseases. **Methods:** Comprehensive phenotyping of the SHIP cohort was performed as previously described (1). Omics data were generated by genotyping with Illumina 2.5 bead chips, whole blood transcriptional profiling with Illumina bead-chips, plasma miRNA profiling with multiplexed qRT-PCR, plasma, urine and saliva proteome profiling using LC-MS/MS and metabolic profiling of saliva, plasma and urine using NMR and LC-MS/MS. **Results and Discussion:** We recorded multi-omics data sets (genomics, transcriptomics, circulating miRNA profiles, proteomics and metabolomics) of several hundred participants of SHIP-TREND and analyzed the correlation of these data among each other (2,3) and with common phenotypes. In the presentation we will present the outcome of this complex data analysis using common phenotypes such as BMI, age and blood pressure as examples and integrate data from three biofluids (saliva, plasma and urine). **Conclusion:** Conclusion Combination of molecular phenotyping with epidemiological research can provide new insights into pathophysiology of common diseases. References 1) Völzke et al. 2011 Int J Epidemiol 40, 294-307, 2) Suhre et al. 2011 Nature Genet 43, 565-569, 3) Homuth et al. 2012 J Endocrinol 215, 17-28

Keywords: multi-omics approach, common diseases, biomarker screening, population-based values

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CS23.05 Multi-Omics to Study the Effects of Diclofenac on Wild Type and Hepatic Reductase Null (HRN) Mice

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Introduction and Objectives: Systems biology can provide detailed insights into drug metabolism and mechanisms of toxic action. Hepatic CPY reductase null (HRN) mice are compared with the phenotypically normal strain (C57BL6J) to study the effects of diclofenac on liver structure and function. Integrated metabonomic, lipidomic and proteomic profiles of liver extracts were assessed to explore the downstream metabolic and proteomic consequences of an absence of functional CYP450s in the HRN vs normal mice and the effects of diclofenac administration. **Methods:** Wild type (C57BL6J) and hepatic CYP reductase null (HRN) mice were administered a daily dose diclofenac for 7 days. Liver tissue (20 mg) was prepared for metabonomics, lipidomics and proteomics. Metabonomics and lipidomics were performed on aqueous and organic extracts, respectively, by reversed-phase UPLC-MS analysis in both positive and negative ESI mode. Protein extracts were proteolysed with trypsin and the resulting peptides separated by a reversed-phase nanoscale LC gradient. Proteomic acquisitions utilized ion mobility and the data were processed and searched using Progenesis QI against a protein sequence database, providing normalized label-free quantitation results. **Results and Discussion:** Metabonomic and lipidomics highlighted differences in bile acid and lipid profiles between wild type and HRN mice pre- and post-administration of diclofenac. Perturbed lipid metabolism was particularly evident in HRN liver extracts compared with controls, and in both phenotypes lipid expression was elevated post-administration of diclofenac. Proteomic analysis resulted in 1400 highly curated protein identifications based on mass accuracy (< 10ppm) and peptide score, with additional filtering yielding 200 proteins of interest. Pathway analysis on the complimentary datasets provides an opportunity for a better understanding of the underlying biology of differentially expressed proteins, metabolites and lipids. **Conclusion:** An integrated "omics" approach was used to study the effects of genetic modification in mice resulting in non-functional hepatic CYP450s compared to wild type animals, and the effects of diclofenac exposure on both phenotypes.

Keywords: Drug metabolism, Hepatotoxicity, proteomics, Metabolomics

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CS23.06 Omic Analysis of Lung Cancer Reveals Proteome Signatures with Prognostic Impact

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Introduction and Objectives: The cancer phenotype is a product of a set of processes, each subject to selective pressure and prone to dysregulation in

cancer, acting at each stage of the sequence continuum DNA→RNA→Protein. Non-small cell lung carcinoma (NSCLC) has the highest cancer mortality worldwide, and with outcomes that have not improved in decades. Our objective was to characterize lung cancer-associated proteome remodeling, and to integrate these data with genomics and clinical information in order to identify polygenic cancer genes and prognostic molecular signatures. **Methods:** We constructed a genetic map in the form of an integrated omic array comprising measures of DNA gene copy number, mRNA expression, and MS-based protein abundance from a series of patient-matched NSCLC primary tumours, patient-derived xenograft (PDXs), and normal lung. **Results and Discussion:** Dysregulated proteins were encoded throughout the genome including, but not limited to, examples that map into cancer-associated regions of recurrent DNA amplification/ deletion, but which have not previously been implicated as important to the cancer phenotype. The ability of a primary tumour to engraft is the best known prognostic indicator of recurrence in early stage NSCLC (John et al. Clin Cancer Res, 2011). Clustering revealed metabolism proteome signatures especially highly recapitulated between matched primary and PDX tumours. Genes encoding signature metabolism proteins were not highly mutated in cancers, but when amassed as signatures identified cohorts of patients (from a non-overlapping set of patients; TCGA/cBioportal) with differential overall survival. Proteome clusters and patient subgroups with differential outcome were different between adenocarcinoma and squamous cell carcinoma subtypes. Metabolism proteome expression was also identified as a determinant of primary tumor engraftment. **Conclusion:** Integrated omic analysis indicates that selective pressure for proteome remodeling is a key component of tumorigenesis and/or maintenance of the cancer phenotype, and that metabolism proteome signatures may provide a basis for tumour classification and treatment.

Keywords: omic integration, metabolism, cancer, prognostic signature

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CS23.07 Serum Proteomes Distinguish Children Developing Type-1 Diabetes

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Introduction and Objectives: The Finnish Type 1 Diabetes (T1D) Prediction and Prevention Study (DIPP) began in 1994 with collection of follow-up samples from children with increased genetic risk for T1D. Here we report the results of comparing serum proteomes during the early stages of life in children who progressed to develop T1D to identify potential changes that could be associated with disease onset or activity and detected before the appearance of T1D associated autoantibodies. **Methods:** Profiles were generated by a combination of iTRAQ and label free analyses on 26 subjects from approximately six time points per patient representing periods from early infancy, to seroconversion and diagnosis. Comparisons were made between children who seroconverted and progressed to type 1 diabetes and those who remained persistently autoantibody negative, matched by age, gender, sample periodicity and risk group. The samples represented the pre-diabetic period and ranged from the age of three months to twelve years. **Results and Discussion:** We determined longitudinal serum proteomics profiles from children with HLA-conferred type 1 diabetes susceptibility to identify changes that could be associated with the β-cell damaging process and detected before seroconversion and the appearance of type 1 diabetes associated autoantibodies. Functional annotation enrichment analysis highlighted proteins related to lipid transport and inflammatory response. Prior studies on transcriptomics, metabolomic and genetic network analysis will be compared to the proteomic data. **Conclusion:** On the basis of top scoring pair analysis, classification of the T1D developing subjects was observed with a success rate of 91% indicating that we have identified new candidates whose levels change in children during the early development of T1D. Importantly, differences in abundance of a set of proteins were consistently detected already before development of autoantibodies in children who developed type 1 diabetes.

Keywords: Type 1 Diabetes developing children, Longitudinal sampling from birth to onset, label-free proteomics, proteomic correlation to multi-omics data

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CS23.08 Multilayered Genetic and Omics Dissection - A New Age for Biomedical Researches

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Introduction and Objectives: The manner by which genotype and environment coordinately effect phenotype is a fundamental question in biology and medicine. On the one hand, the proteome is more than a mere translation of a genome. On the other hand, genome-wide association analysis (GWAS) and quantitative trait locus (QTL) analysis have been successfully applied to identify genes driving phenotypic variations and have provided valuable evidence on disease-driven SNPs on gene expression. Large genetic reference populations (GRPs), such as the BXD mice strains, are frequently used to determine to which extent phenotypic variation is driven by genetic variants. To dissect the genotype-proteotype-phenotype relation, we integrated transcriptomics, proteomics and metabolomics strategies to dissect metabolic disease in the BXD mice genetic reference population. Combined with the measurement of phenotypes, this integrated omics dataset provides novel evidence of how genotypic variation are translated into proteome and eventually affect phenotypes. **Methods:** We have applied SWATH proteomics in 40 genetically diverse BXD mice, and the proteomic layer is complimented with full genome coverage , complete transcriptome measurements and extensive coverage of metabolites. Furthermore, all mouse cohorts were profiled for dozens of clinical metabolic phenotypes, and fully analyzed in response to different environmental conditions, such as chronic feeding of chowor high fat diets. Together, these layers provide detailed information along the entire spectrum from genotype to phenotype. **Results and Discussion:** We obtained full coverage of 2,622 proteins in the livers across 80 cohorts, and quantified 25,136 transcrpts using Affymatrix Mouse Gene microarray. Meanwhile, 979 unique mass-charge ratios were identified for metabolomics. Together, these layers provide detailed information along the entire spectrum from genotype to phenotype, providing an unprecedentedly comprehensive view of cellular mechanisms in a mammalian population study. **Conclusion:** Integrated omics enables the identification and explanation of novel genetic, environmental and gene-by-environment factors underlying metabolic disease in a mammalian population study.

Keywords: Integrated omics, metabolic disease, genotype-proteotype-phenotype relation, mouse genetic reference population

CS 23: INTEGRATED OMICS
WEDNESDAY, SEPTEMBER 30, 2015 – 11:10 – 13:00

CS23.09 Impact of THS 2.2 and Conventional Cigarette Smoke on the Lung Proteome of ApoE-/- Mice

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Introduction and Objectives: Cigarette smoking causes many diseases, including cardiovascular disease and lung diseases such as COPD and emphysema. The aim of this study is to compare the effects of the aerosol from a candidate modified risk tobacco product called THS 2.2 with those caused by smoke from a reference combustible cigarette (3R4F) on the lung proteome of ApoE$^{-/-}$ mice. **Methods:** ApoE$^{-/-}$ mice were exposed for up to 8 months to either fresh air (Sham), smoke from 3R4F or an aerosol from THS 2.2 for 3 hours/day, 5 days/week to a target nicotine concentration of 30 µg/L. After 2 months exposure to 3R4F, cessation and switching groups were further exposed for up to 6 months to fresh air, or THS 2.2 aerosol, respectively. Lung tissue was collected at months 1, 2, 3, 6 and 8, proteins were extracted, and labelled using TMT 6-plex. Tryptic peptides were separated on an Easy nanoLC 1000 instrument connected online to a Q-Exactive mass spectrometer (Thermo Scientific) and analyzed with a custom workflow. **Results and Discussion:** Quantitative proteomics demonstrated an increasing effect of 3R4F exposure on the lung proteome over time. Proteomics captured the broad biological impact of 3R4F exposure including effects on the immune-system, xenobiotic and oxidative stress, metabolism, and ER stress protein clusters. In contrast, THS 2.2 exposure showed only a limited effect on the lung proteome. The cessation and switching groups showed a comparable decline of the observable effects relative to Sham group levels over time. **Conclusion:** This work is part of our larger systems toxicology assessment framework, including transcriptome and lipidome measurements and classical toxicological endpoints. Our work demonstrates the advantage and contribution of proteomics analyses to study the biological impact of cigarette smoking and to investigate the molecular mechanisms of smoking cessation as well as switching to aerosol exposure of candidate modified risk tobacco product.

Keywords: quantitative proteomics, systems toxicology, tobacco-heating

CS 23: INTEGRATED OMICS
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CS23.10 Utilizing Proteomic and Genetic Methodologies to Discover Pathways Affecting Cytokinesis

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Introduction and Objectives: Cytokinesis, the final step in the cell cycle, is the physical separation of a cell into two daughter cells. Although cytokinetic failure is usually lethal, mounting evidence suggests that it contributes to tumorigenesis via tetraploid intermediates. Here, we use the fission yeast Schizosaccharomyces pombe as a model organism for studying cytokinesis, primarily because S. pombe cells divide symmetrically, like a conventional animal cell, through formation and constriction of an actomyosin contractile ring (CR). Screens in fly, worm, and mammalian cells have revealed overlapping sets of cytokinetic proteins, indicating that the principle features of S. pombe cytokinesis are shared across species; thus discoveries about CR regulation in S. pombe will inform us about this process in many organisms. The goal of these studies is to identify and connect regulatory mechanisms of CR formation by identifying functional modules emanating from the essential F-BAR scaffolding protein Cdc15. **Methods:** Pull-down and tandem affinity purifications (TAP) coupled with 2D-LC-MS (LTQ-Velos, Thermo) were used to identify interacting partners of Cdc15 and other CR components and map phosphorylation sites. Deletion strains and a synthetic genetic array were used to identify genetic interactions between strains lacking genes whose products localize to the cell division site. Microscopy, biochemistry, radio-labeling and other cell biological techniques were also utilized. **Results and Discussion:** Proteomics revealed that Cdc15 binds C2 domain Fic1, scaffold protein Spa2, paxillin-like Pxl1, and Rho-GEF Rgf3. Loss of both fic1 and spa2 makes cells sick; the SGA screen revealed a potential link between these two—an inositol phosphatase that might regulate lipids at the cell division site. We are investigating the role of CR protein phosphorylation (e.g. Fic1, Imp2) on CR regulation and integrating these networks of physically- and genetically- linked genes/proteins to continue to define and characterize CR regulatory modules. **Conclusion:** Integration of proteomic and genomic methodologies reveals pathways impinging on cytokinesis.

Keyword: Cytokinetic regulation, proteomics, genetic screen, phosphoregulation

CS24: SAMPLE PREPARATION FOR PROTEOMICS

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CS24.01 High-Throughput Protein Microarrays for Personalized Diagnostics

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Abstract: One of the most compelling steps in the post-genomic era is learning the functional roles for all proteins. To help address this question we have assembled a large collection of cDNA clones in recombinational cloning vectors that supports protein expression in a broad variety of high throughput experimental platforms. In one approach, we have adapted these clones for use in high throughput protein purification, including the use of cell free expression and automation. In another, we have developed a method to produce proteins in situ on protein microarrays. These self assembling protein microarrays can be used to study protein-protein interactions, protein-drug interactions, search for enzyme substrates, and as tools to search for disease biomarkers. In particular, recent experiments have focused on using these protein microarrays to search for autoantibody responses in cancer patients and identifying the substrates of a recently discovered form of post-translational modification, called AMPylation.

CS 24: SAMPLE PREPARATION FOR PROTEOMICS
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CS24.02 Sample Collection Method Bias Effects in Quantitative Phosphoproteomics

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Introduction and Objectives: Current advances in selective enrichment, fractionation and MS detection of phosphorylated peptides allowed identification and quantitation of tens of thousands phosphosites from minute amounts of biological material. One of the major challenges in the field is preserving in-vivo phosphorylation state of the proteins throughout sample preparation workflow. This is typically achieved by using phosphatase inhibitors and denaturing conditions during cell lysis. Here we accessing if the upstream cell collection techniques could introduce changes in protein phosphorylation. To determine the effect of sample collection protocols on global phosphorylation status of the cell, we compared different sample workflows by metabolic labeling and quantitative mass spectrometry. **Methods:** We assessed three sample collection workflows: two that used denaturating conditions and involved mixing of cell cultures with either an excess of ethanol (EtOH) at -80°C or trichloroacetic acid (TCA), and a third under non-denaturing conditions by suspension and washing cells in cold phosphate buffered saline (PBS). We used stable isotope labeling by amino acids in cell culture (SILAC) to compare changes in protein phosphorylation profiles to a reference condition in which cell cultures were directly frozen in liquid nitrogen to instantaneously halt all enzymatic activities. **Results and Discussion:** We identified highly similar phosphopeptides for cells harvested in ice cold isotonic phosphate buffer, cold ethanol, trichloroacetic acid, and liquid nitrogen. However, quantitative analyses revealed that the commonly used phosphate buffer unexpectedly activated signalling events. Such effects may introduce systematic bias in phosphoproteomics measurements and biochemical analysis. **Conclusion:** Sample collection workflows can significantly affect quantitative phosphoproteomics measurements, and the conclusions drawn. Our data suggest that either TCA or EtOH sample collection protocols introduce lower sample collection bias compared to the more commonly used ice cold PBS protocol. We believe that results obtained in this study will be useful for future experimental designs involving measurements of highly dynamic protein PTMs.

Keywords: phosphoproteomics, Sample Preparation, SILAC, osmotic shock

CS 24: SAMPLE PREPARATION FOR PROTEOMICS
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CS24.03 Targeted Quantification of 97 Proteins in Dried Blood Spots

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Introduction and Objectives: The integration of dried blood spot (DBS) microsampling with mass spectrometry (MS) has proven advantageous for quantifying small molecule metabolites in biomarker screening and drug development. To leverage these benefits for proteomic applications, we report a multiplexed LC-MRM-MS method for the precise quantification of 97 endogenous proteins in DBS samples. **Methods:** DBS samples were transferred to a 96-well plate for extraction, denaturation, and digestion with trypsin. Matching stable isotope-labeled standard (SIS) peptides were spiked in the digests to enable precise, relative quantitation of targeted proteins. All liquid handling steps in sample preparation were automated on a Tecan Freedom EVO 150 robotic system. Samples were then analyzed by UHPLC-MRM/MS on an Agilent 6490 triple quadruple instrument. **Results and Discussion:** Standard curves were generated to characterize each of the 173 target peptides, representing the 97 proteins. The linearity was excellent with an average linear dynamic range of 207-fold and an average R^2 value of 0.986. These proteins span almost 5 orders of magnitude in endogenous concentration from serum albumin at 18.0 mg/mL down to cholinesterase at 190 ng/mL. The average CV for the intra-assay precision and inter-assay precision ranges for 6 biological samples were 6.1-7.5% and 9.5-11.0%, respectively. Furthermore, the majority of target peptides were stable in DBS samples (within 20%) when stored at room temperature for at least 4 weeks. Finally, we will present our strategy to incorporate quality control into DBS measurements for ensuring optimal data quality across larger scale experiments. **Conclusion:** We have developed a MRM method for quantifying 97 proteins in DBS samples. Overall, these results demonstrate the DBS-MRM holds considerable promise for quantitative clinical proteomics applications.

Keywords: quantification, Mass spectrometry, Dried Blood Spots, multiple reaction monitoring (MRM)

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CS24.04 An Integrated Sample Preparation System for Targeted Proteomics

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Introduction and Objectives: Introduction and Objectives; The objectives of this work were to develop a system for targeted proteomics that integrates plasma extraction from a drop of blood, collection of a 2.4 uL plasma aliquot, heavy isotope labeled internal standard addition, proteolysis, affinity purification, and direct transport into an LC-MS/MS using disposable membrane cartridges. **Methods:** Methods; A membrane stack that absorbs 30 uL of blood was fabricated and the requisite buffers, anticoagulants, antibodies, and enzymes preloaded at specific sites before drying. Applied samples were pulled through the membrane stack serially by capillary action, dissolving and mixing reagents in plasma by convection and intra-membrane diffusion along with removing cells by filtration; all without an external energy source. Samples thus prepared were eluted into an LC-MS/MS using a clamp-type eluter. **Results and Discussion:** Results and Discussion; Sample loading occurred in 3 min, at which time four thing had happened; i) the system was loaded and convective flow had stopped, ii) 2.4 uL of plasma had been collected, iii) reagents had mixed with the sample, and iv) sample preparation reactions were proceeding. The top two membrane layers were then removed and incubation completed in the collection membrane. Prepared samples were either dried and sent to an MS lab or eluted directly into an LC-MS/MS or onto a MALDI plate. Samples acquired and prepared in this fashion were comparable in top-down and bottom-up analyses to those prepared by conventional methods. **Conclusion:** Conclusions. Targeted proteomics can be greatly simplified and accelerated by integrating preparation sample steps in a single, disposable paper device. This will be a substantial asset in enabling large scale, high throughput proteomics along with clinical diagnostics.

Keyword: sample preparation, plasma extraction, membrane filtration, targeted proteomics

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CS24.05 Comparison of Acetone Precipitation and FASP II for Protein Identification through Bottom up MS

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Introduction and Objectives: Sodium dodecyl sulfate (SDS) is commonly used in proteomic workflows to aid in cell lysis, protein solubilization, enzyme digestion, and to impart mass-based separations. However, high concentrations of SDS (> 0.01 %) can impede chromatographic separation and can cause MS signal suppression. It is therefore necessary to employ a method to deplete SDS from the protein samples ahead of LC/MS analysis. The efficiency of available methods is typically described through the number of identified proteins / peptides. Here we present a comparison of two techniques for SDS depletion, FASP II and acetone precipitation, in terms of identified proteins / peptides. We also investigate differences that may exist between the peptides identified caused by the characteristics of the sample (cellular component, hydrophobicity, mass, and isoelectric point). **Methods:** Using GELFrEE fractionated E coli proteins, a low mass and a high mass fraction containing 1 % SDS were subject to both SDS depletion methods prior to analysis on an Orbitrap Velos Pro. The identified peptides were further assessed based on the characteristics previously listed. **Results and Discussion:** Although FASP II depletes more SDS (99.99 % vs 99.9 % for acetone), the precipitation approach provides higher protein recovery (80-100 %) than FASP (< 50 %). Acetone precipitation yielded a 17% average increase in the number of identified proteins (30% average increase in identified peptides) and there appears to be no discernable difference in the type of peptides identified by each method that would account for this increase. **Conclusion:** These results were the same for both high and low mass fractions, indicating that acetone precipitation is a favorable strategy for SDS depletion in a proteomics workflow.

Keywords: FASP II, Acetone Precipitation, proteomics, protein identification

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CS24.06 Solvent-Based Protein Precipitation as a Tool for Detergent Removal Ahead of LC/MS

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Introduction and Objectives: SDS is ubiquitous for proteome processing, However, given incompatibility with LC/MS the surfactant must be removed from the sample. Numerous approaches are available to do so (column approaches, membrane filtration, electrophoresis…) The most favorable approach considers analyte purity as well as protein recovery. Protein precipitation in organic solvent is often overlooked, with concerns relates to poor recovery, both during precipitation and through resolubization. Here we demonstrate solvent precipitation affords exceptional recovery, even at nanogram levels of protein. Moreover, cold formic acid is demonstrated to quantitatively resolubilize the protein. We observe that these reagents can chemically modificy protein, and present steps to avoid these artifacts. **Methods:** Standards are precipitated in acetone or chloroform as per published methods (Botelho, J. Proteome Res. 2010, 9, 2863-70). Proteins are resolubilized in 80% formic acid, prechilled to -20°C. Residual SDS is quantified by LC/MS^2 while protein recovery is assessed through LC/UV. Intact proteins are observed through SDS PAGE or by LC/MS on an orthogonal microTOF instrument (Bruker), while bottom-up MS was on an Orbitrap Velos Pro. **Results and Discussion:** Solvent precipitation depletes 99.9% of SDS, while FASP & in-gel digestion deplete 99.99%. Considering recovery, solvent precipitation demonstrated the highest yields of all methods assesses (between 80-100% recovery, vs <50% for FASP). High recovery (>50%) is possible with as little as 50 ng. We continue to assess protein recovery at lower analyte concentration. Cold formic acid (80%) will quantitatively resolubilize the protein pellet. At room temperature, protein formylation (m/z +28) is a significant occurance. The modification is essentially eliminated at low temperature. We also observe an abundant +98 modification of proteins, and trace this back to the acetone. The modification is not observed with chloroform/methanol. **Conclusion:** Solvent based protein precipitation affords exceptional purity and protein recovery and is a favoured approach for detergent removal ahead of LC/MS.

Keywords: Formic acid, Protein modification, SDS removal, Protein precipitation

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CS24.07 A Simple Affinity Proteomics System for Fast, Sensitive, Quantitative Analysis of Proteins in Plasma

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Introduction and Objectives: The improving efficacy of many biological therapeutics and identification of low level biomarkers are driving the analytical community to deal with extremely high levels of sample complexity. Many protein quantitation and biomarker validation procedures utilize immunoaffinity enrichment to purify the sample and maximize sensitivity. Afterwards, to generate surrogate peptides with better mass spectrometric properties, protein enrichment is coupled with proteolytic cleavage. This is a time-consuming, multistep, serial process. This workflow enables rapid protein enrichment and proteolytic cleavage to be performed in a single, easy to use reactor in under 4 hours. **Methods:** Samples containing the analyte of interest, a biotinylated antibody targeting the analyte, and co-immobilized avidin and heat-activated temperate-stable trypsin were incubated together in a 1.5mL tube at room temperature for 2 hours. Following incubation, the resin was washed repeatedly with a wash buffer at room temperature. Afterward, digestion buffer was added and the entire system was incubated at 70°C and 1300rpm for 1 hour. Samples were analyzed using LCMS. **Results and Discussion:** Initial screening for the detection of human IgG from murine plasma demonstrated the ability to effectively capture the protein out of plasma using a biotinylated antibody and the resin with co-immobilized avidin and trypsin without loss of signal due to digestion during capture or washing. Complete digestion of IgG took 1 hour. Capture time was independent of order of addition of the components and took 2 hours. Prewashing with plasma did not result in a decreased loading capacity as might have been expected due to biotin or biotin-like compounds in plasma. Accurate and reproducible (CV<%10) detection of IgG was performed on concentrations as low as 5 ng/mL in 400 µL of plasma in less than 4 hours. **Conclusion:** We have developed a new method for accurate quantitation of single ng/mL protein levels and below from complex matrices in under 4 hours.

Keywords: immunoaffinity, proteomics, Bottom-up, plasma

CS24.08 Isolation and Identification of Human Cell Line Proteins Using Acid-Labile Detergents on LC-ESI-TOF

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Introduction and Objectives: Isolating and identifying plasma membrane proteins from human cells are terrific analytical challenges. Classical methods use antibodies and rely on their specificities to detect proteins indirectly. Alternatively, proteomics can directly identify proteins representing an unbiased approach to unscramble the proteome of homogenous mixtures. In this study, we developed a simple, gel-free nano-LC-ESI-TOF method to isolate and identify proteins of NALM-16. Since many detergents are detrimental to mass spectrometers, we explored a new class of acid-labile detergents. **Methods:** We used $2x10^7$ cells, isolated proteins using the detergent AALS II (sodium 2,2-diheptoxypropyl sulfate) and digested with Lys-C/Trypsin. Clean-up was with C18/SCX. Peptides were measured on a nano-LC-ESI-TOF system. **Results and Discussion:** To our knowledge, no group has yet combined these methods in order to identify human cell lines. We identified five membrane proteins (CD10, CD19, CD20, CD138 and HLA-DR) which clearly identified the cell line according to DSMZ (German cell bank). Crucial steps for this method were solubilisation of membrane proteins, complete digestion and separation by LC. NALM-16 is a well-characterized human cell line with a B cell precursor leukemia cell type. We verified the most abundant peptides and identified CD44E, CD46, CD53 and CD99 which have not yet been correlated with this cell line. Furthermore, we will undertake quantifying key proteins on the peptide level by classical stable isotope dilution experiments. This method is suitable for cell culture quality control, as an alternative to DNA-typing, or to identify new phenotypical proteins. We will transfer this method to human primary cells, e.g. isolated T/B/NK cells or tissues. This workflow could assist in developing an individualized treatment of patients with an aberration in distinct proteins causing malignancies such as cancer. **Conclusion:** Our results show that the current workflow is suitable for isolating and identifying human cell membrane proteins in NALM-16 and is fully compatible with our LC-TOF system.

Keywords: human cell line, membrane proteins, acid-labile surfactants, NALM-16

CS24.09 Automated Sample Preparation Solutions for MS-Based Proteomics

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Introduction and Objectives: Mass spectrometry (MS) has become a vital tool for proteomics as well as biopharmaceutical fields due to its high sensitivity, precision and accuracy. However, a prerequisite for high quality data is the quality and reproducibility of sample preparation. Salts, detergents, as well as a host of other contaminants need to be removed efficiently with minimum loss of target protein(s). In addition, automation is required to deal with high-throughput workflows. **Methods:** Contaminants were removed by a combination of hydrophilic interaction chromatography (HILIC) and electrostatic repulsion hydrophilic interaction chromatography (ERLIC) from protein and peptide samples using carboxylate functionalized magnetic micro-particles, as previously described by Hughes et al. (2014). Trypsin immobilized on magnetic particles was used for digestion of protein samples prior to MS-based analysis. **Results and Discussion:** The common contaminant SDS was removed from protein samples following binding of proteins to carboxylate functionalized particles in the presence of 50% acetonitrile (organic component) and under acidic conditions. SDS was removed from peptide samples following binding of peptides to carboxylate functionalized particles in the presence of > 95% acetonitrile at neutral pH conditions. These findings are in agreement with studies previously performed by Hughes et al. (2014). Immobilized trypsin was tested against conventional in-solution sequencing grade trypsin. Reproducible and efficient cleavage was achieved within 1 hour for immobilized trypsin in comparison to 16 hours for sequencing grade trypsin, showing potential for high-throughput applications. Immobilized trypsin showed a greater tolerance for denaturants and detergents, maintaining activity in the presence of 5M Urea and 0.5% SDS. Autolysis of in-solution trypsin, which is significantly reduced for immobilized trypsin, may further decrease the sensitivity of MS-based analyses by masking low abundance proteins. **Conclusion:** Both types of micro-particles used in the current study have magnetic properties; therefore, are being tested for use in a single automated high-throughput workflow using a magnetic particle processor.

Keywords: proteomics, Sample Preparation, Carboxyl microparticles, Trypsin microparticles

CS24.10 Optimized Clinical Use of RNALater and FFPE Samples for Quantitative Proteomics

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Introduction and Objectives: The availability of patient samples is essential for clinical proteomic research. Biobanks worldwide store mainly samples stabilized in formalin-fixed and paraffin embedded (FFPE) biopsies or in RNAlater typically for NGS. Biobank material is a potential source for clinical proteomics to provide retrospective information concerning biomarkers for diagnosis, prognosis and novel drug discovery. In this study, we have assessed the influence of sample stabilization using RNAlater (Qiagen) on human derived samples for quantitative proteome analysis and pathway mapping, which we compare to FFPE and snap-frozen samples as godl standard. **Methods:** From the sigmoideum of two healthy participants' twenty-four biopsies were extracted using endoscopy. The biopsies was stabilized either by being directly snap-frozen, RNAlater treated, FFPE imbedded or incubated for 30 min at room temperature prior to FFPE. Furthermore, human mastoid bone and human peripheral blood mononuclear cells were stabilized in a similar manner (freezing or RNALater treatment). The characterization of the protein content was analysed by high throughput gel free quantitative proteomics, followed by an in-depth analysis of post-translational modifications and label free quantification, using ProteinPilot and MaxQuant respectively. **Results and Discussion:** Our optimized sample preparation methodology enabled identification of a similar high number of proteins regardless of sample stabilization method, as well the abundance in RNAlater and frozen was close to identical. The most abundant post-translational modifications were assigned and recommendations for the database search and the impact for accurate label free quantification.

Our data demonstrated the feasibility in performing proteome analysis on samples stored in RNAlater or by FFPE. Furthermore, we show that pathway mapping information within 30 min incubation prior to FFPE did not interfere with the quantitative information information. **Conclusion:** We have demonstrated that quantitative proteome analysis and pathway mapping of samples stabilized in RNAlater or by FFPE is feasible with minimal impact on the quality of protein quantification and post-translational modifications.

Keywords: RNALater, PTM, FFPE

CS25: TOP DOWN PROTEOMICS AND MACROMOLECULAR COMPLEXES

CS 25: TOP DOWN PROTEOMICS AND MACROMOLECULAR COMPLEXES
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CS25.01 Top-Down Mass Spectrometry: What Does the Future Hold?

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Abstract: Characterization and quantitation of intact proteins, so-called top-down mass spectrometry (MS), has the potential to transform our understanding of biological systems and enable physiologically relevant studies of microbes and higher eukaryotes. Critical information including stoichiometry of modifications on a single proteoform, [Smith LM et al. Nat Methods. 2013, 10(3):186-7] or presence of a combination of multiple modifications on a single proteoform, can only be inferred from top-down (i.e. intact protein) MS; hence this approach is rapidly becoming an indispensable avenue for proteomic studies.

There are, however, significant technical challenges associated with nearly all aspects of the top-down approach. Recent advances in MS instrumentation, separation, and bioinformatics significantly increased the throughput of top-down proteomics, allowing the identification of hundreds of intact proteoforms. Yet many challenges persist. For instance, effective separation of proteoforms remains an unsolved problem. Most of current efforts involve several sample pre-fractionation steps, which are often labor intensive, require large sample sizes, and are inadequate in terms of quantitation. Herein, we describe practical one-dimensional LCMS workflows developed to tackle proteoform separation challenge. However, perhaps an even bigger challenge relates to MS performance. Fourier transform ion cyclotron resonance (FTICR) offers the highest mass resolving power and accuracy of any mass analyzer. And, because all key measures of FTICR MS performance improve with increased magnetic field strength, a high-field FTICR spectrometer will arguably provide that next level of performance needed to bring the top-down MS to the mainstream. Herein, we describe initial results acquired using a 21T FTICR spectrometer, recently brought online at EMSL, a DOE national scientific user facility located at PNNL, suggesting this capability has a potential to push the current limits of top-down MS and facilitatehigh-throughputcomprehensivecharacterizationofthefunctionalproteomes.

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CS25.03 Top-Down, High-Throughput Proteomics of Allergens Using Complementary MS/MS Fragmentation Strategies

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Introduction and Objectives: Over 15 million Americans including 8 percent of children in the US suffer from food allergies and the amount of people affected has been steadily increasing for the past decade. LC/MS/MS provides an accurate and sensitive way to test for allergens in food products. Here, we present a protein-based method that identifies the most common commercially available fish species in minutes using a simple protein extraction protocol couple to multiplex top-down proteomics in an Orbitrap Fusion Tribrid mass spectrometer modified with a UVPD source. **Methods:** Reference samples from commercial fish species were included in the work. Protein extraction was carried out by mechanically homogenizing 1 g of muscle. Water soluble proteins were centrifuged, the supernatant heated at 90 ºC for 5 min and centrifuged again. Soluble proteins were diluted 1/100 and 5 uL were used for analysis using an Orbitrap Fusion Tribrid mass spectrometer coupled to an Ultimate 3000 RSLCnano LC system. MS/MS acquisition was performed using ETD, EThcD, HCD or UVPD fragmentation. Data analysis was performed using Thermo Scientific Deconvolution 4.0 and Prosight PD node in Proteome Discoverer 2.0 software. **Results and Discussion:** Parvalbumins considered as the major fish allergen were successfully purified from the sarcoplasmic protein fraction by heat treatment. The high mass accuracy and resolution, and the different fragmentation modes allow the classification of proteins with high protein sequence homology, but species dependent amino acid substitutions. UVPD was the most efficient fragmentation technique providing a dramatic increase in protein sequence coverage compared to the other fragmentation techniques. **Conclusion:** Overall, this strategy offers a very reliable and widely used top down proteomics method to detect allergen and to authenticate food in the same inspection test. Comparing with the current available DNA methods this strategy represents a major breakthrough in terms of time, specificity and effectiveness.

Keywords: Top-down, UVPD, Allergens, Fusion

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CS25.04 Applying a Proteoform Profiling Method for Neurological Disorder Biomarker Discovery

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Introduction and Objectives: Measuring the intact mass of proteins in tissue samples or biofluids has the advantage over bottom-up approaches to be able to track directly the results of major biological processes like alternative splicing, proteolytic processing or modification of PTM pattern distribution, as this information is encoded in the intact mass of proteins. In this study we have used a last-generation UHRQ-Tof to perform a protein profiling approach with the objective of detecting and afterwards identifying proteoforms which are discriminating for neurological disorders.

Methods: Pooled CSF samples were obtained from control patients with ethical consent. 1,5 µl CSF samples have been separated on a 75µmX15cm µm pepmap C4 column (TheroFischer Scientific), coupled to an impact II benchtop UHR-Q-Tof (Bruker Daltonics) via a CaptiveSpray nanoBooster ion source (Bruker Daltonics). All data have been automatically processed in Data Analysis 4.2 (Bruker Daltonics). Statistical analyses have been performed on a slightly modified version of the Profile Analysis 2.1 Software. Identifications have been performed using the Top-Down Sequencing search functionality of BioTools 3.2 (Bruker Daltonics) and Mascot 2.4 (Matrix Science). **Results and Discussion:** We could easily detect over1500 proteoforms in each sample with high reproductibility. The analysis of the first technical replicates from a small pool of biological replicates enabled to detect several discriminating proteins. Using a Scheduled Precursor list, an auto-LC-MS/MS run enabled the identification of a glycoform of a a human Chromogranin-A fragment. The maximum mass shift observed for this fragment over 4 injections was 0,19 ppm, and the resolution was exceeding 45000.The study is now continuing with samples issued from larger cohorts of patients in order to validate the early biomarker candidates which have already been detected . **Conclusion:** We have established the applicability of a protein profiling approach by UHR-Q-TOF for the analysis of our daily clinical samples.

Keywords: Protein Profiling, UHR-Q-TOF, Biomarker discovery, CSF

CS 25: TOP DOWN PROTEOMICS AND MACROMOLECULAR COMPLEXES
WEDNESDAY, SEPTEMBER 30, 2015 – 11:10 – 13:00

CS25.05 Top-Down Structural Analysis of Intact Antibodies Using H/D Exchange and ETD on an Orbitrap-MS

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Introduction and Objectives: Although digestion-based hydrogen/deuterium exchange (HDX)-MS and the intact-protein based top-down approach each have advantages for antibody structural elucidation, the top-down approach is preferable for biopharmaceutical characterization as it requires less manipulation of the protein sample, and has the potential to reach amino-acid resolution. Nonetheless, most HDX-MS studies on antibodies have used the digestion-based approach. Here we present a new top-down approach for structural characterization of intact antibodies by combining HDX, subzero temperature chromatography, and electron transfer dissociation (ETD). **Methods:** HDX of the antibody was performed with 80% D2O. Subzero temperature HPLC was controlled by a deep freezer. MS data were acquired on a Thermo Scientific Orbitrap Fusion mass spectrometer equipped with ETD. **Results and Discussion:** Top-down ETD spectrum of the antibody, Herceptin (HER), is characterized by a number of distinct fragment ions. The most intense ions were found to be fragments containing about 100 residues from the terminus of both the heavy chain and light chain. Combining top-down ETD with HDX, individual IgG domain-level deuteration information was obtained for 6 out of the 12 IgG domains of HER, which included the antigen binding sites. This is the first time that top-down HDX has been applied to an intact protein as large as 150 kDa. Structural differences (~10 Da), induced by protein ligand binding to HER, were determined to be located only on the variable region of the light chain. Although the presence of disulfide bonds prevents this approach from obtaining amino acid level information within disulfide-linked regions, the advantages such as minimal sample manipulation, fast workflow, very low level of back exchange, and simple data analysis, make this technique well suited for fast comparative structural evaluation of intact antibodies. Determination of structural differences in the protein binding partner is ongoing. **Conclusion:** A top-down HDX-MS approach has been developed to characterize higher-order structure of intact antibodies.

Keywords: Top-down proteomics, H/D exchange, electron transfer dissociation (ETD), Orbitrap-MS

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CS25.06 MS3ID: A Novel MS3-Based Method Coupled to a Supervised Learning Algorithm for Top-Down Proteomics

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Introduction and Objectives: Post-translational modifications (PTMs) play an important role in the biology of the cell and of disease processes but traditional bottom-up mass spectrometry (MS)-based proteomics approaches are typically unable to distinguish subset proteins or to accurately identify PTMs that co-occur at remote positions within a protein sequence. By contrast, top-down MS allows the identification of these PTMs, but the techniques necessary for this approach require expertise in the field. Herein, we present a novel top-down MS pipeline (MS3ID), including key innovations in separation, instrumentation, and computational analysis that makes top-down proteomics more accessible to non-experts. **Methods:** MS3ID was designed using a hybrid quadrupole mass filter/linear ion trap/Orbitrap mass spectrometer. The architecture of this instrument and its improved scan speed and parallelizability allowed us to develop an MS3-based technique that simplifies data analysis and protein isoform differentiation. MS3ID also includes a novel algorithm based on a logistic regression classifier that takes advantage of the popular SEQUEST database search algorithm to statistically assess the confidence of protein identifications and differentiate protein isoforms. **Results and Discussion:** We present the results of the analysis on the Thermo Fusion Tribrid Orbitrap Mass Spectrometer of simple protein mixtures as well as complex protein lysates from human HEK293 cells. A variety of fragmentation techniques, including CID, HCD, and ETD, are also compared in the context of MS3ID. We demonstrate that our computational analysis allows for confident protein identifications and differentiation of protein isoforms. In addition, we show that MS3ID provides a better sequence coverage for identified proteins than traditional bottom-up approaches. **Conclusion:** MS3ID allows the application of a top-down proteomics protocol without the need of expert knowledge. Our approach allows non-experts to more easily identify and differentiate protein isoforms in a given sample and therefore facilitates the elucidation of the underlying mechanisms of the biology of the cell and of disease processes.

Keywords: Top-down proteomics, Bioinformatics, post-translational modification, MS3

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CS25.07 Intact Protein Signatures in Substantia Nigra Associated with Lewy Bodies and Neuronal Loss

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Introduction and Objectives: Lewy body protein aggregates, along with loss of dopaminergic neurons in the substantia nigra, have been associated with onset and progression of Parkinson's disease. We applied a novel intact protein profiling platform to analyze post-mortem substantia nigra tissue from a 51 sample cohort of human subjects with confirmed Lewy body pathology and matched controls. By analyzing undigested protein extracts we are able to characterize the endogenous protein species contributing to pathology. **Methods:** Brain tissue samples were homogenized by bead mill in denaturing buffer to extract proteins. Extracts we then filter using 100K MWCO filters to remove contaminants and large proteins not amenable to RPLC-MS/MS analysis. Samples are then desalted by buffer exchange using 10K MWCO filter. The proteins are separated by C5 reverse phase chromatography and analyzed by an Orbitrap Elite mass spectrometer. Spectral identification was performed using MSAlign and MSPathfinder software. To quantify the proteoform abundances we applied three different approaches: spectral counting and quantification using chromatographic peak area using in-house developed IQ and ProMex software. The ProMex algorithm does not depend on MS/MS identifications but instead tracks LC-MS features allowing for the quantification of unidentified proteoforms, greatly increasing the information content of the analysis. **Results and Discussion:** Overall, our platform identified over 950 unique proteoforms. On average, 550 proteoforms from 230 proteins were identified per sample run. The mass range of observed protein species extends from approximately 3K-30K Da. This allows us to profile many small proteins as well as truncation and degradation products from larger genes that are obscured in bottom-up proteomic investigations of the same sample set. Preliminary statistical analysis indicated proteoforms with abundance correlated with Lewy bodies and neuronal loss. **Conclusion:** Intact protein profiling shows the potential to identify novel proteomic markers of Parkinson's disease pathology as well as potential mechanistic insights obscured in traditional bottom-up proteomic measurements.

Keywords: Top-down, Biomarker discovery, Mass spectrometry, Parkinsons disease

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CS25.08 Comprehensive Glycosylation Characterization of Therapeutic mAbs by Top- and Middle-Down MS

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Introduction and Objectives: Antibodies represent a class of proteins that are key to mammalian immunological defense systems. They bind to an antigen (protein, glycoprotein, DNA, etc.) with a high degree of specificity thus neutralizing foreign biological material and xenobiotics. This process makes them extremely valuable for use in diagnostics, general research, and as therapeutics. Since their introduction in the late 1980s, therapeutic monoclonal antibodies (mAbs) have become very popular drug candidates due to this high specificity and low toxicity. **Methods:** We sought to study protein sequencing and the posttranslational modification (PTM) status of an IgG1 mAb and a glycosylated IgG1-fusion protein by top- and middle-down MS with multiple fragmentation techniques including electron transfer dissociation (ETD), electron capture dissociation (ECD), and matrix-assisted laser desorption ionization in-source decay (MALDI-ISD). Fourier transform ion cyclotron resonance (FT-ICR) or high performance liquid chromatography electrospray ionization (HPLC-ESI) on an Orbitrap was employed. **Results and Discussion:** These experiments provided a comprehensive view on the glycosylation level in these two proteins determined by heterogeneous combinations of glycosylation on intact form of the proteins. Pyroglutamate modification and lysine truncation were also shown to occur respectively on N-terminus and C-terminus of the proteins. In particular, we demonstrated the advantage of MALDI-ISD over ESI for analysis of a protein with high sialic acid modification, and for improved sequence coverage by a single MALDI-ISD analysis of IdeS digest without sample pre-fractionation. **Conclusion:** In conclusion, top- and middle-down methods enabled: 1) detection of heterogeneous glycosylated proteoforms and sites in intact IgG1 and fusion proteins with high mass accuracy, 2) estimation of relative abundance levels of proteoforms in the sample, 3) confirmation of the proteins termini structural information, and 4) improved sequence coverage by MALDI-ISD analysis for the internal regions of the proteins without sample pre-fractionation.

Keyword: Therapeutic, mAb, Top-down, Middle-down

CS26: STRUCTURAL PROTEOMICS

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CS26.01 The Advancement of Cross-Linking/Mass Spectrometry in Structural Proteomics

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Abstract: not applicable not applicable During the last 15 years, chemical cross-linking combined with mass spectrometry (MS) and computational modeling has advanced from investigating 3D-structures of isolated proteins to deciphering protein interaction networks [1-3]. Chemical cross-linking relies on the introduction of a covalent bond between functional groups of amino acids within one protein, to gain insight into the conformation of a protein, or between interaction partners to elucidate interfaces in protein complexes. Based on the distance restraints derived from the chemical cross-linking data, three-dimensional structural models of proteins and protein complexes can be constructed. Most commonly, homobifunctional amine-reactive cross-linkers, such as N-hydroxysuccinimide esters, are used for studying protein-protein interactions. One of our goals is to extend the arsenal of existing cross-linkers to obtain complementary structural information. To facilitate the identification of cross-linked products, we have designed novel MS/MS cleavable cross-linkers creating characteristic marker ions [4]. A direct way to probe in vivo protein-protein interactions is by site-specific incorporation of genetically encoded photo-reactive amino acids or by non-directed incorporation of photo-reactive amino acids. In my talk, I will give an overview of different cross-linking strategies and illustrate them for two examples: (i) Mapping the interaction sites between the basement membrane proteins laminin and nidogen [5] and (ii) elucidating the topology of the tetrameric tumor suppressor protein p53 [6]. Literature References: [1] Sinz, A., Expert Rev. Proteomics 2014, 11, 733. [2] Herzog et al., Science 2012, 337, 1348. [3] Rappsilber, J., J. Struct. Biol. 2011, 173, 663.

[4] Müller, M.Q. et al., Anal. Chem. 2010, 82, 6958. [5] Lössl et al., PLoSOne 2014, 9:e112886. [6] Artl et al., Proteomics 2015 online available. not applicable

Keyword: cross-linking, mass spectrometry, protein 3D-structure, protein-protein interactions

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CS26.02 Towards Solving Protein Structures by Structural Proteomics

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Abstract: The combination of protein chemistry methods with modern mass spectrometry (MS) has crystallized into the distinct field of structural proteomics. A variety of protein structural questions, ranging from defining protein interaction networks to the study of conformational changes of single proteins, can be addressed by using multiple MS-based structural proteomics approaches. Each technique provides specific structural information which can be used as experimental constraints in protein structural modeling; combining numerous experimental constraints from different approaches enables unequivocal determination of protein structures. Here, we present our recent developments in limited proteolysis, surface modification, hydrogen-deuterium exchange (HDX) and crosslinking -- all combined with modern MS techniques -- for the solving unknown protein structures. Limited-proteolysis sites were determined by SDS-PAGE combined with in-gel digestion and MS characterization of the cleavage products, and by direct-infusion ESI-FTMS of the reaction mixtures. Top-down HDX was performed using ECD-ESI-FTMS. Differential surface modification of native and unfolded with 8M urea protein states was performed using light and heavy isotopic forms of isotopically-coded reagents. Crosslinking was performed with a panel of isotopically-coded crosslinking reagents, followed by digestion and analysis of the crosslinked peptides by LC/ESI-Orbitrap MS. For de novo protein structural modeling, the importance of short-distance constraints for the arrangement of the secondary structure elements was reiterated. To provide such constraints,a line of non-selective isotopically-coded hetero- and homo-bifunctional short-range photo-reactive reagents is being developed. Reagents carrying aryl-azido and diazirine photo-activatable groups were shown to be able to modify a wide range of amino acid residues and thus provide short-distance crosslinks between adjacent protein structural elements. The constraints obtained were used to select threading protein model candidates and for experimental constraint-driven ab initio protein-structure modeling for several proteins with unknown structures, including Tau, α-synculein, and aggregated prion. Recent advancements in structural proteomics techniques make constraint-driven solution of protein structure attainable.

Keywords: hydrogen/deuterium exchange (HDX), limited proteolysis, surface modification, Crosslinking

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CS26.03 Investigating the Basis of Nav1.5 Fast Inactivation Using a Crosslinking Unnatural Amino Acid and MS

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Introduction and Objectives: Fast inactivation of voltage gated sodium currents is critical for the function of excitable tissues. Halting the flow of ions is achieved by efficient and rapidly reversible conformational changes in the cytoplasmic linker between domains III and IV (DIII-IV) in the channel complex and its putative receptor site. A variety of inherited mutations in this complex impair channel inactivation, which results in prolonged sodium conductance that can lead to a myriad of pathophysiological disorders. Although the amino acid residues that comprise the inactivation gate are well characterized (IFMT within the DIII-IV linker), the location and nature of its binding site remains uncharacterized. Here, we utilized the incorporation of a photo-crosslinking unnatural amino acid and mass spectrometry to identify the binding site of the fast inactivation gate. **Methods:** The photo-crosslinking unnatural amino acid, p-benzoyl-L-phenyl alanine (Bpa), was genetically incorporated into Nav1.5 channels using the amber stop codon suppression system. Mutagenesis was preformed at F1486, a critical residue in the fast inactivation gate. **Results and Discussion:** Simultaneous patch clamp recording and UV irradiation of channels specifically during inactivation demonstrated an accumulating and permanent loss of conductance indicating covalent crosslinking of the fast inactivation gate to its receptor. To determine the site of crosslinking, control and UV treated channels were purified, digested and analyzed by LC-MS/MS. MS search results confirmed the incorporation of Bpa, demonstrating a +104 Da mass shift at F1486. Candidate receptor sites were identified by analysis with Crossfinder, a custom software application for determining UV induced Bpa crosslinks. Manual validation of high scoring crosslinked MS/MS spectra indicated sites of interaction between the IBMT peptide and the sodium channel. **Conclusion:** Using these techniques we have demonstrated the incorporation of the unnatural amino acid, induced UV crosslinking and determined a candidate binding site for the Nav1.5 fast inactivation gate.

Keywords: Unnatural amino acid mutagenesis, Photo-crosslinking, Mass spectrometry, Voltage gated sodium channel

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CS26.04 Large-Scale Detection of Unstructured Protein Regions by Pulsed Proteolysis

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Introduction and Objectives: Protein folding is essential to maintain functional proteomes and can be impaired by various cellular stress conditions, neurodegenerative diseases, and aging. Chaperones support protein folding and thus protect from cytotoxic protein aggregation. We are interested in the detection of the in-vivo folding state of proteins on a proteome-wide scale. Further, we would like to better understand how chaperones and protein degradation pathways affect protein folding under stress conditions. **Methods:** Pulse proteolysis by thermolysine was used to degrade unstructured proteins in E.Coli under normal growth and heat stress conditions during cell lysis. Quantitative changes of degraded proteins were detected by metabolic SILAC labeling based mass spectrometry **Results and Discussion:** Out of the detected 2500 E.Coli proteins, 530 proteins were significantly degraded under normal growth conditions. In addition, 220 proteins were found to be only degraded under heat stress conditions, thus defining the thermosensitive proteome. These proteins were larger in size and more acidic compared to the whole detected E.Coli proteome. We found that the degraded proteins, which tend to be dis-

ordered, also tend to be more resistant to aggregation, in particular for the proteins that are degraded under normal conditions. Bioinformatic analysis further revealed that proteins degraded under normal growth conditions were the most unstructured proteins, the non-degraded proteins were the least unstructured proteins, and thermosensitive proteins were in an intermediate range. Experimentally detected cleaved protein regions correlated well with the predicted unstructured protein regions. **Conclusion:** Pulse proteolysis is an efficient method for the proteome-wide detection of unstructured protein regions under various conditions. The detected thermosensitive E.Coli proteome correlates well with bioinformatic predictions. The developed method will be further applied to investigate the role of chaperones and cellular protein degradation mechanisms for the folding states of the proteome.

Keywords: pulse proteolysis, protein folding, structural proteomics, proteostasis

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CS26.05 Global Analysis of Protein Structural Changes in Complex Proteomes

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Introduction and Objectives: Protein conformational changes often result in an alteration of function and can range from small local motions, such as allosteric regulation, to drastic structural rearrangements, such as amyloid formation. Despite their important role in the functioning of a protein, not much is known about intracellular conformational switches and the consequences thereof, mostly due to the lack of suitable techniques to study protein structural transitions in their native cellular environment. Structural techniques such as X-ray crystallography or NMR are incapable of dealing with complex matrices, while FRET-based techniques or in-cell NMR require protein tagging/labeling and are thus not applicable at high-throughput.To overcome these limitations, we developed a novel mass spectrometry (MS)-based strategy. **Methods:** In order to identify and quantify protein conformational switches within a complex biological matrix, the presented approach combines a biochemical tool – limited proteolysis (LiP) – with latest generation targeted proteomics techniques. The essence of the sample preparation is a double protease digestion step applied to a whole proteome extract (Feng et al. Nat Biotech 2014). **Results and Discussion:** We benchmarked the reproducibility and performance of our approach using complex cell extracts spiked with model proteins that undergo well-characterized conformational changes of different magnitude. As a next step, we demonstrated the feasibility of a proteome-wide survey on structural changes by comparing the proteomes from yeast grown under two different nutritional conditions where structural features of over 1000 proteins were probed simultaneously and ~300 proteins were found to be structurally altered during the given metabolic shift. Our data revealed that while some branches in carbon metabolism are transcriptionally regulated, others are governed by conformational changes of various enzymes. **Conclusion:** In conclusion, this method enables multiplexed extraction of conformational markers for different protein conformations, allowing to quantify the extent of structural transitions within a ~10 amino acid resolution, directly in the cellular matrix.

Keywords: Proteome-wide structural probing, Small molecule-protein interactions, Protein structural change, LiP-SRM

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CS26.06 Investigation of Protein-RNA Interactions by UV Induced Cross-Linking and Mass Spectrometry

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Introduction and Objectives: Protein-RNA complexes play an important role in fundamental cellular processes such as gene expression and regulation. The investigation of protein-RNA interactions in such ribonucleoprotein complexes is of absolute importance for the functional understanding of these cellular processes. UV induced protein-RNA cross-linking and mass spectrometry have emerged as a straightforward approach not only for the identification of proteins that interact with RNA but also for the unambiguous identification of the exact amino acids and nucleotides mediating these interactions. This approach was utilized to investigate RNA binding regions in single (recombinant) proteins such as the human spliceosomal helicase Aquarius as well as in multi-subunit protein assemblies such as the S. cerevisiae Lsm1-7 heptameric ring. **Methods:** Pre-assembled protein-RNA complexes were subjected to UV irradiation at 254 nm, followed by RNase and endoproteinase digestion. Cross-linked peptide-RNA heteroconjugates were enriched using C18 desalting and TiO2 enrichment. The purified crosslinks were analyzed using HCD fragmentation on Orbitrap Velos and QExactive HF. The MS data was analyzed with a novel automated data analysis tool RNP^{XL}, implemented in OpenMS that allows for efficient pre-filtering of spectra and generation of peptides precursor variants putatively cross-linked to RNA with varying length and nucleotide composition. **Results and Discussion:** In the Aquarius-polyU-ADP ribonucleoprotein complex, the cross-linked amino acids were mapped to the RecA-like domains known for their RNA binding properties. Additional cross-links were observed in the β-barrel and the stalk region which are the putative binding sites for the RNA in other helicases. The heptameric ring of the S. cerevisiae Lsm proteins 1-7 was cross-linked with polyU, the cross-linked amino acid residues were observed in the central channel of the ring indicating the RNA binding pockets for the passage of the RNA. **Conclusion:** Our cross-linking results validated protein-RNA interactions reported in X-ray studies, in addition to identifying new RNA binding regions in these proteins.

Keyword: UV cross-linking, protein-RNA interactions, structural proteomics

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CS26.07 Protein-RNA and Protein-DNA Cross-Link Identification Pipeline Integrated in Proteome Discoverer 2.0

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Introduction and Objectives: UV cross-linking of proteins with DNA and RNA and identification of the resulting products has been used to assign novel DNA/RNA binding regions and exact binding sites in proteins. The large number of potential cross-linked amino acids and oligonucleotides poses a data analysis challenge and has to be accounted for in MS database searching. To address this issue, we recently introduced a processing pipeline, RNPxl, implemented in the OpenMS framework. Here we report on its further development by integration of a dedicated search engine for peptide-oligonucleotide cross-links. It allows for automated assignment of the cross-linked amino acid based on characteristic product ions of the cross-linked species. In addition, we have integrated RNPxl into Proteome Discoverer 2.0. **Methods:** To account for the complex fragmentation behavior of cross-linked moieties we implemented a novel search engine designed for peptide-RNA/DNA cross-link identification. Manually annotated MS/MS spectra derived from several studies were used to define characteristic marker ions, cross-linked immonium ions, and characteristic production ions derived from the cross-linked peptide moiety. Using this information a localization score allows the search engine to automatically determine cross-linking sites or regions. **Results and Discussion:** The improved RNPxl performs database searches more than ten times faster than the first version of RNPxl (Kramer et al., Nat. Methods, 2014, 11, pages 1064-1070). It allows for automated localization of the cross-linked amino acids in the cross-linked peptide sequence. The novel software pipeline is available free of charge as open-source software and has additionally been integrated into Proteome Discoverer. **Conclusion:** We introduce a software pipeline in Proteome Discoverer for identification and localization of peptide–RNA/DNA cross-links which is easy to handle. It thus meets the needs of structural biologists, researchers working in the field of DNA/RNA binding proteins as well as proteomic researchers that investigate DNA/RNA binding in various cellular states contexts.

Keywords: computational MS/MS workflow, site localization, protein-RNA/DNA cross-linking

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CS26.08 Role of Drosophila Memory Related Fatty Acid Binding Protein on Transporting Fatty Acids

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Introduction and Objectives: Drosophila fatty acid binding protein (dFABP) found in brain tissue is a member of fatty acid-binding protein superfamily. This protein shares approximately 50% amino acid sequence homology with mammalian brain-type fatty acid binding protein 7 (FABP-7), and it has recently been reported to be involved in the enhancement of long-term memory consolidation. However, the conformation relationship between ligand binding and interaction with membrane remains unclear. **Methods:** In this study, we utilized various biophysical methods including crystallography, fluorescence, circular dichroism spectrometry (CD), isothermal titration calorimetry (ITC), nuclear magnetic resonance (NMR) and molecular dynamic simulateion (MD) in attempt to elucidate not only the atomic correlation between stability and binding of dFABP in the presence or absence of binding ligands, but also how dFABP transports ligands. **Results and Discussion:** Much to our surprise, we found that even though the overall secondary conformation of dFABP remains mostly the same, the tertiary structure seems to be rather dynamic, hence its thermal stability decreased at lower pHs or in the absence of ligand. Besides, the presence of ligands protects dFABP from deuterium exchange via NMR H/D exchange experiments, while bound state of mutation R30A has apparent less protection to that of wild-type dFABP. **Conclusion:** Based on the sequence alignment results and the surface electronic analysis, we are at present studying the key positively charged residues of dFABP that has been found through our ITC analysis to impede ligand binding or to gate toward membrane by applying membrane mimetics, which together may fulfill the explanation of how this dynamic protein works between membranes.

Keywords: Drosophila fatty acid-binding protein (FABP), Thermol stability, Helical region, Basic residues

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CS26.09 Enrichment of Cross-Links from Complex Samples by Charge-Based Fractional Diagonal Chromatography

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Introduction and Objectives: Understanding complex protein interactions on the molecular level is an essential step to obtain a comprehensive insight into cellular mechanisms, as protein complexes are the basis for cellular signaling, energy transfer, motion, stability and cellular metabolism. Protein cross-linking – the covalent linking of amino acid residues – in combination with mass spectrometry is a powerful tool to study protein structures and complexes. Due to the low efficiency of Cross-Linking, which goes along with low abundance of Cross-Linked peptides, enrichment techniques may enhance the MS-based identification of Cross-Links in complex samples. As Cross-Linked peptides on average have higher charge states than unmodified peptides, Strong Cation Exchange (SCX) chromatography can be used for enrichment. We recently developed a 'charge-based fractional diagonal chromatography' (ChaFRADIC) approach, that enables us to effectively enrich Cross-Linked peptides from complex samples – including the identification of so far non-identified Cross-Linking sites. Here we evaluated the applicability of our new approach to highly complex samples. **Methods:** The approach is based on a two dimensional separation of peptides by SCX at pH 2.7. After LysC digestion peptides are separated into fractions representing distinct peptide charge states in the first dimension. Afterwards, the high charge state fractions are additionally digested with trypsin, thus inducing a charge state shift that can be used to enrich cross-linked peptides in a second dimension SCX. **Results and Discussion:** Our ChaFRADIC approach for Cross-Linked peptides resulted in an effective enrichment for model systems of low complexity, even when spiked into a complex background. Furthermore, we applied our method to enrich Cross-Linked peptides from highly complex samples and to study protein complexes in order to demonstrate the applicability beyond model systems. **Conclusion:** Our novel ChaFRADIC approach allows effective enrichment of Cross-Linked peptides from samples of low, medium and high complexity and provides new opportunities for analysis of Cross-Links out of complex samples.

Keywords: Enrichment, Strong Cation Exchange Chromatography, Protein Cross-Linking

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CS26.10 Ion Mobility and Surface Topology Mapping Reveals the Cause of the Protein G-IgG Affinity Switch

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Introduction and Objectives: Efficient affinity chromatography / enrichment systems require strong and specific binding of ligands to capture molecules as well as fast and nearly complete releases of ligands upon triggering the "affinity switch". The protein G - IgG system is widely used for purification and enrichment of biomolecules from all kinds of biological sources and has been applied in the clinics in apheresis therapies, e.g. with Rheumatoid Arthritis patients. Structure dynamics of interacting partners under binding and elution conditions was studied at the molecular level using IM-MS, ESI-MS, and surface topology mapping to develop a model of conformational changes in protein G. **Methods:** Hydroxy-radical labeling by Fast Photochemical Oxidation of Proteins (FPOP) was used to map surface topology alterations of protein G under neutral and acidic conditions. IM-MS, ESI-MS, and CD-spectroscopy were applied to monitor pH-related tertiary and secondary structure changes in protein G, a protein that consists of three IgG-binding domains and an N-terminal His-tag, all linked together by short spacer peptides. **Results and Discussion:** FPOP under acidic conditions revealed that oxidations were spread all along the surface of protein G, encompassing all domains and spacer sequences. Interestingly, under neutral conditions less oxidation was observed in the first spacer, but more in the IgG-binding domains. CD spectroscopy showed that secondary structure features nearly remained unchanged despite varying pH. By contrast, substantial tertiary structure changes, affecting domain assemblies, were found by ESI-MS charge structure analysis and by collisional cross section determination using IM-MS. Hence, we conclude that dropping pH caused (i) opening-up of the over-all globular assembly of the IgG-binding domains with respect to each other and (ii) simultaneously resulted in tightening the individual domain folds by which otherwise exposed hydrophobic areas were kept covered. Consequently, the associated affinity switch is responsible for release of antibodies. **Conclusion:** Dynamic protein G domain reorganization causes affinity switch towards IgG.

Keywords: Fast Photochemical Oxidation of Proteins, Ion Mobility-Mass Spectrometry, Affinity switch, Protein Structure Changes

CS27: CNPN

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CS27.02 Prognostic Significance of Head and Neck Cancer Biomarkers: Translation into Oral Surgery Clinics

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Introduction and Objectives: There has been much criticism about discovered biomarkers not making it into clinics. A reason for this paucity is that the skill sets for discovery and translation are different, and success requires building collaboration and having the right collaborators at the appropriate time. Here we present our experience in head and neck cancer (H&NC). **Methods:** H&NC biomarkers were identified by MS-based differential proteomics using iTRAQ and verified using immunohistochemistry. Their prognostic values were assessed for oral premalignant lesions' (OPLs') potential for transformation into cancers. A commercial test has been developed for use in oral maxillofacial surgery clinics. **Results and Discussion:** iTRAQ analyses enabled the identification of a suite of biomarkers, including 14-3-3 zeta, 14-3-3 sigma, S100A7, hnRNPK and prothymosin alpha, for H&NCs and OPLs, and verified on >300 patient samples. We tested their effectiveness on an additional set of 110 patients with biopsy-proven OPLs with known clinical outcomes. The best-performing biomarker in correlating expression with high-risk malignant transformation is S100A7 (p-value = 0.014, odds ratio = 4.706). Patients who overexpressed S100A7 had a mean oral-cancer-free survival period of ~70 months versus ~120 months for those who did not. S100A7's expression impacts upon beta-catenin, and promotes tumor differentiation and secretion of cytokines. The commercial version of the test, Straticyte, has been developed by Proteocyte Diagnostics Inc. Straticyte is an immunohistochemistry-based test that assesses the expression and distribution of biomarkers, compares these to a reference database, and generates a score that expresses the progression risk of a given OPL. The score is intended to aid the surgeon in deciding the best personal therapy: high-risk lesions may require immediate attention, probably surgery plus radio- and/or chemotherapy; low and moderate risk lesions could be monitored closely. **Conclusion:** Not applicable

Keywords: Head and Neck Cancer Biomarkers, Prognosis of Malignant Transformation, Commerical Test Developed, Aid to Oral Maxillofacial Oral Suregons in Developing Best Personal Therapy

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CS27.03Diagnosis of Urological Disorders with Protein Biomarkers Measured in Seminal Plasma

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Introduction and Objectives: Non-invasive diagnostics of urological disorders is a recognized unmet need in the urology clinics. In our search for biomarkers, we focus on the proteome of seminal plasma (SP), a proximal fluid suitable for discovery of novel markers and development of non-invasive diagnostics. Using mass spectrometry, we previously identified over 3,000 proteins in SP of men with various urological disorders. We designed biomarker development pipelines for male infertility, prostate cancer and prostate inflammation. In this presentation, we will introduce our biomarker discovery platform and discuss in detail development of biomarkers in SP. **Methods:** Our platform integrates proteomic profiling of SP by shotgun mass spectrometry followed by verification and validation of candidates by SRM, immuno-SRM and in-house developed ELISAs (Korbakis et al. Mol. Cell. Proteomics, 2015). **Results and Discussion:** Previously, we discovered a testis-specific protein TEX101, which differentiated between normal spermatogenesis and azoospermia forms with absolute specificity and sensitivity. Here, we produced monoclonal antibodies and ELISA, and validated clinical per-

formance of TEX101 in 832 SP samples from fertile men and men with subfertility and infertility. A cut-off of 170 ng/mL in SP distinguished between normal spermatogenesis and obstructive azoospermia with 100% sensitivity, and between normal spermatogenesis and non-obstructive azoospermia with 93% sensitivity, at 100% specificity. Furthermore, we identified and verified by SRM additional 12 testis- and germ cell-specific proteins, which are novel markers of spermatogenesis and male infertility. Our prostate cancer pipeline resulted in 83 proteins which were verified by SRM in 51 SP samples, and 22 proteins which were validated by SRM in 258 SP samples. We proposed a panel of SP markers for differentiation between prostate cancer and benign disease. **Conclusion:** SP is a promising fluid to discover novel markers of male infertility and prostate cancer. Our work may result in an SP-based biomarker panel for non-invasive and differential diagnosis of numerous male urological disorders.

Keyword: seminal plasma, male infertility, prostate cancer, SRM

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CS27.04Proteomic Analyses of Macrophage Response to Mycobacterium Tuberculosis Infection

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Introduction and Objectives: Mycobacterium tuberculosis, the etiological agent of Tuberculosis (TB), is one of the most prevalent infectious agents worldwide. An estimated 8.6 million new cases and approximately 1.3 million deaths occur annually giving TB the highest mortality rate of any infectious bacteria. Alveolar macrophages, paradoxically, serve both as first line of defence against M. tuberculosis and the bacterium's natural habitat. To better understand the evasive nature of the tubercle bacilli and its molecular manifest on the macrophage response to infection, we conducted a global quantitative proteomic profiling of macrophages infected with various strains of M. tuberculosis. **Methods:** The wild-type H37Rv strain (WT), the gene knockout strain ΔptpA (KO), and the corresponding complemented strain (CO) were used in the study. Four independent sets of cells were infected. Samples were trypsin digested and iTRAQ labeled peptides were separated HPLC followed by LC-MS/MS. All data was analyzed using Proteome Discoverer 1.4 (Thermo Fisher Scientific) and the search engine MASCOT v2.4 (Matrix Science). Raw data files were searched against the Uniprot-SwissProt database with allspecies filter and mammalian species only. Statistical Analysis of data files was performed using R (gnu). **Results and Discussion:** 42,007 peptides and 4,868 distinct proteins were detected. Of these, 845 macrophage proteins showed significant modulation in expression upon infection. 27 proteins were significantly regulated outside of a 90% confidence interval about the mean. Modulation of expression was validated individually for selected proteins. These host proteins represent the macrophage transcriptional, translational, and innate immune response to infection as well as its signaling capacity. **Conclusion:** Our studies provide new information about the macrophage response to M. tuberculosis infection, creating a better understanding of Tuberculosis and its associated pathophysiological complexity, and give insights into novel therapeutic approaches.

Keywords: Tuberculosis, iTRAQ, macrophages

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CS27.05 Using TAILS N-Terminomics to Identify Missing Proteins and Study Inflammatory Gingival Diseases

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Introduction and Objectives: We aimed to harness the power of TAILS N-terminomics to decipher the roles of extracellular proteases and their substrates, allowing a system-wide understanding of the dynamic protease network and its interaction with the proteome in inflammatory diseases. To access acute inflamed human tissue we sought an accessible tissue and therefore we used peri coronal gingiva above it around impacted wisdom teeth. **Methods:** Terminal Amine Isotopic Labelling of Substrates (TAILS) was performed on proteome extracts for identification of proteins and their N-termini. Simultaneous labelling and blocking of primary amines was achieved via dimethylation. Labeled proteomes were then digested with trypsin producing internal peptides possessing free primary N-terminal amines, which are subsequently targeted to covalently bind for removal to a commercially available polyaldehyde polymer (www.flintbox.com). Contrary, unbound peptides —representing original and processed protein N-termini, were recovered using ultra filtration and analysed by tandem mass spectrometry. **Results and Discussion:** In our preliminary analysis of six paired specimens and four paired mouth rinse samples, we identified >15,000 unique peptides and >4,000 proteins by our combined shotgun/N-terminomics approach (<0.01 FDR). We identified several inhibitors and mediators of inflammation differentially cleaved in health and disease, highlighting the importance of proteolytic processing in inflammation and its resolution and pinpointing new control points. Furthermore, due to the combination of rare proteome sources with a non-shotgun proteomics technique, we were able to identify a myriad of proteins which were invisible for proteomics so far, and thus classified as "missing" by neXtProt. **Conclusion:** Our ongoing studies on human inflamed tissues highlight the power of N-terminomics and TAILS to study inflammation and proteolytic processing, and represent highly valuable data for the Canadian Chromosome 6 initiative within the global Human Proteome Project.

Keywords: proteolytic processing, TAILS N-terminomics, inflammatory diseases, Missing proteins

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CS27.06 Antibody Colocalization Microarray Quantifies 108 Proteins in 35 μL of Serum at fM Concentration

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Introduction and Objectives: Biomarkers useful for early diagnosis of diseases are ideally detectable in bodily fluids, but will likely only be found at very low concentration. For complex diseases, biomarker panels will also be needed for diagnosis. Hence, there is a need for highly sensitive, multiplexed protein analysis technologies. The antibody colocalization microarray (ACM) is a cross-reactivity free antibody array using a sandwich im-

munoassay format and co-localizing each capture and detection antibody pairs, avoiding mixing of reagents while facilitating the addition of new targets. Here we report our recent progress in the development of the ACM. **Methods:** 40 slides were printed at once with 16 arrays of 108 antibodies against cancer proteins, cytokines, transcription factors etc. printed in triplicate with a customized Nanoplotter (GeSIM) using homemade high liquid capacity silicon pins for analyzing 640 samples in a single experiment. Capture antibodies were printed using an optimized mixture of hygroscopic additives preventing evaporation, followed by incubation with samples and overlay spotting of detection antibodies. **Results and Discussion:** The printing buffers which avoid evaporation of solutions during microarray fabrication lead to an intra-slide printing reproducibility of 5.9%. Limiting the variation of humidity within the spotter chamber was found to be critical for good reproducibility. The use of a fluorescent calibrant co-spotted with capture antibodies allows to achieve a coefficient of variation of < 11% in assay results. The limit of detection of the ACM currently is 0.6 pg/mL for IL-1b (35 fM) and the median for 108 proteins is < 100 pg/mL. **Conclusion:** The ACM with 108 targets is currently the largest high sensitivity, multiplex sandwich assay platform. The good reproducibility and the ease of adding new targets further contribute in making the ACM a versatile and powerful platform for the targeted discovery of protein biomarkers in large number of samples thus achieving good statistical power.

Keyword: Antibody microarray

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CS27.07 Quantifying the Active Kinases in Ovarian Cancer Cell Lines to Explore Cisplatin Resistance

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Introduction and Objectives: Kinases are intimately involved in regulating cell growth and have been implicated in the etiology and pathophysiology of cancer. Cisplatin is a widely used anti-cancer therapy for ovarian cancer, yet a subset of most patients that initially respond to treatment become resistant over time. To determine the role played by kinases in cisplatin resistance, we used multiple kinase inhibitors to enrich the kinome from ovarian cancer cell lines that are sensitive or resistant to cisplatin and quantified their abundance by Data Independent Acquisition (DIA; SWATH) analysis. **Methods:** Cell cultures from the cisplatin-sensitive ovarian cancer cell line A2780 and its resistant derivative A2780cis were treated with cisplatin and kinases were enriched from protein lysate with a layered kinase inhibitor bead column. Enriched kinases were trypsin digested on column, and the resulting peptides were analysed by DDA and SWATH (DIA) on a AB SCIEX 5600 mass spectrometer. Data was analyzed using ProteinPilot, the SWATH microapplication within PeakView and MarkerView software. **Results and Discussion:** In applying the established methodology of multiple inhibitor bead kinase enrichment, we assessed the impact of different protein amounts, kinase elution methods and different kinase binding methods. More kinases were identified from capture with a layered inhibitor bead column as compared to batch incubation binding. Similarly, more kinases were identified through trypsinization of kinases directly on inhibitor beads than from protein elution and digestion. Over 200 kinases are detected in each sample type, with reproducible quantitative differences limited to a small subset of kinases. As expected, differences between cell lines were noted and may provide clues to the origin of cisplatin-resistance in these paired cell lines. **Conclusion:** By using multiple kinase inhibitors to purify the kinome we are able to identify a subset of kinase proteins that are up-regulated in cisplatin-resistant ovarian cancer cells.

Keywords: DIA, SWATH, Kinase, cancer

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CS27.08 Investigation of the CK2-Dependent Phosphoproteome Using Mass Spectrometry

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Introduction and Objectives: Phosphorylation of proteins by kinases is critical for cellular regulation. CK2 is a constitutively active serine/threonine kinase that is overexpressed in several cancers. Global proteomics studies of phosphoproteomes perturbed by radiomimetic drugs or ionizing reveal dynamic regulation of CK2 motifs, implicating CK2 in the DNA Damage Response (DDR). Using quantitative phosphoproteomics by mass spectrometry the main objectives are to identify dynamic indicators of CK2 inhibition using CK2 inhibitors followed by investigation of CK2-dependent phosphorylation sites required for DDR execution. **Methods:** SILAC heavy labeled HeLa cells were treated with 20 μM CX-4945 inhibitor for 1 hour with light label HeLa cells treated with vehicle. Following this both populations of cells were treated with 20 ng/mL neocarzinostatin (NCS) for 75 minutes to induce DNA double-stranded breaks. Additional experiments using only CX-4945 inhibitor were performed at time points 1, 12, and 24 hours. Lysates were processed, digested by trypsin and prepared using SPE cartridges followed by TiO2 enrichment. Phosphopeptides were fractionated by SCX chromatography and analyzed using a Q-Exactive in DDA mode. Raw data was searched and quantified using MaxQuant and analyzed in Perseus. Phosphorylation sites were analyzed using KinomeXplorer to identify associated kinases. **Results and Discussion:** Analysis of the phosphoproteome across different treatments revealed over 3000 quantified sites. Phosphorylation sites that decreased in response to CK2 inhibition mapped to kinases such as CK2, PAK1, GSK-3, and PKC. Sites increasing contained serine-proline motifs, and many of these sites were predicted to be CDK1-dependent. It is anticipated that the phosphorylation sites identified will serve as a panel of biomarkers that will enable the monitoring of the kinome response during CK2 pharmacological inhibition. **Conclusion:** Inhibition of CK2 using CX-4945, an inhibitor currently undergoing clinical trials, reveals a dynamic cellular phosphoproteome. Identification of phosphorylation sites increasing and decreasing in response to CK2 inhibition suggests an adaptive response of the kinome.

Keywords: CK2, kinase inhibitor, DNA Damage

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CS27.09 Negative Phosphoregulation of Protein Interaction Domains by Tyrosine Kinase Receptors

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Introduction and Objectives: Cells respond to extracellular stimuli in order to survive and to adapt to their environment. Signals from cell surface receptors are often relayed via adaptor proteins, such as NCK1 and NCK2. Their main function is to couple pTyr docking sites on activated tyrosine kinase receptors (RTKs) via their single SH2 domain, to cytoplasmic effectors containing Pro/Arg-rich motifs via one of their three SH3 domains. We sought to determine whether NCK proteins are phosphorylated and how this regulates their function. **Methods:** We used mass spectrometry to map pTyr residues on NCK1/2 and to analyse the effect of this modification on protein interactions in vitro and signaling networks in vivo. **Results and Discussion:** We identified 15 non-redundant pTyr on NCK1/2. We showed that a few of these sites are phosphorylated by the RTK EphA4, a direct binder for these adaptor proteins. We determined that one of the Tyr is located within the binding pocket of NCK1/2 SH3 domains and is conserved during evolution. We further demonstrated that phosphorylation of this Tyr abrogates NCK1/2 SH3 domains interactions with their substrates, both in vitro and in vivo. **Conclusion:** Our data suggests that RTKs are able to terminate signal transmission directly by phosphorylating their substrates, including adaptors proteins such as NCK1/2.

Keywords: adaptors proteins, Tyrosine kinase receptor, phosphorylation, cell signaling

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CS27.10 Assessment of SUMO/Ubi Kinetic in Human Cells Using an Optimized Peptide Immunopurification

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Introduction and Objectives: The small ubiquitin like modifier (SUMO) is a protein member of the family of Ubiquitin like modifier that are conjugated on lysine residues of acceptor substrates. SUMO and Ubiquitin are known to interact with each other in a synergistic and competitive manner. In this study, we optimized a SUMO remnant immunoaffinity enrichment method to profile temporal changes of protein SUMOylation and ubiquitylation upon MG132 treatment. **Methods:** HEK293 SUMO3 mutant cells were grown in DMEM and treated for various times with MG132. Protein extracts were enriched on Nickel NTA beads prior to tryptic digestion. SUMOylated and Ubiquitinated peptides were purified using custom monoclonal antibodies directed against their respective remnants. Peptides were analyzed by LC-MS/MS on an Orbitrap Fusion. MS/MS spectra were acquired in HCD mode and LC-MS/MS data were processed using MaxQuant. **Results and Discussion:** The dual affinity enrichment method developed here enabled the simultaneous profiling of protein SUMOylation and Ubiquitination from the same cell lysate. Under proteasome inhibition, 60% of both SUMOylation and Ubiquitination sites were upregulated 16 hours post-treatment. Moreover, 25% of all identified proteins were both SUMOylated and Ubiquitinated. Interestingly, several proteins including MCM7 showed a unique behavior, where we observed an overall increase of SUMOylation and ubiquitylation, except for specific residues. For example MCM7 K648 showed an over Ubiquitylation correlated with a decreased in SUMOylation, suggesting an interplay between these modifications. **Conclusion:** This method allows for the simultaneous identification of more than 1000 SUMOylated and ubiquitylated sites from low amounts of starting material and is suitable for routine experiments using multiple treatments and time points.

Keywords: SUMOylation, Mass spectrometry, Peptide IP

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CS28.01 Integrative Personal Omics Profiling During Periods of Disease, Weight Gain and Loss

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Abstract: While significant genetic and environmental risk factors are known that contribute to the development of Type 2 Diabetes (T2D), overall our ability to predict which individuals will eventually develop T2D and when this will occur is woefully inadequate. To better understand these factors, we present a longitudinal multi-omic personalized medicine pipeline for the comprehensive molecular profiling of blood- and microbiome-based analytes that we apply to track the progression to T2D in a cohort of 75 individuals over periods of health, illness and weight gain and loss. Multi-omic profiling (transcriptome, DNA methylome, proteome, metabolome etc.) revealed significant differences in multiple ‘omes between prediabetics and healthy controls at steady state, implicating pathways related to chronic inflammation and insulin regulation as well as novel connections to T2D. A subset of participants was then placed on a short-term high caloric diet, followed by additional multi-omic profiling. The dietary perturbation was associated with a wealth of biomolecular expression changes concomitant with weight gain and spanning multiple ‘omes including the microbiome, and the omic response to weight gain differed between prediabetics and healthy controls. To better understand interaction of the multi-omes during the diet perturbation, we applied integrative analysis on those large-scale multi-omes dataset, also incorporating other phenotypical data of participants, including standard clinical lab tests and physiological self-assessments. We therefore identified groups of co-variable analytes corresponding to different stages of the diet perturbation, with their biological functions and significance subject to further investigation. In total, these large-scale longitudinal data offer a novel and comprehensive view of the dysfunction in cellular networks associated with the progression to T2D and may offer new strategies for predicting and preventing the disease.

Keywords: Type 2 diabetes, Insulin sensitivity, Integrated omics, Diet perturbation

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CS28.02 Cancer Proteomics in the Era of Precision Medicine

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Abstract: Introduction and objectives: Cancer is a genetically and clinically variable disease. Cancer patients often exhibit different response to treatments even when they are diagnosed at the same clinical stage. To improve the clinical outcome, the precious diagnosis and treatments have been required. Recently, a numerous number of novel molecular targeting drugs have been developed and applied to various types of malignancies. Those drugs exhibit remarkable effects on tumors, but at the same time, they also results in unexpected side effects, and often extremely expensive. To establish therapeutic strategies using novel drugs, we need to make more precious diagnosis using novel molecular biomarkers. We found that tissue proteomics also have great potentials for biomarker development. **Methods:** We developed several experimental tools for tissue proteomics, such as the application of ultra-high sensitive fluorescent dyes for laser microdissected tissues, a large format 2D-PAGE device, and the cancer proteome database. Using biobanking resource in our institute, we developed many biomarkers to predict effects of treatments, recurrence and metastasis, and validated the results by a nation-wide collaboration. **Results and Discussion:** We developed predictive and prognostic biomarkers in sarcomas. In gastrointestinal stromal tumor (GIST), we identified pfetin as a novel prognostic biomarker, and validated its prognostic utilities in more than 500 GIT cases in 7 hospitals by immunohistochemistry. The prospective study is undergoing in “Study on treatment after complete resection of high risk gastrointestinal stromal tumor (STAR ReGISTry)” in Japan. The molecular backgrounds of prognostic utilities of pfetin were examined, supporting the clinical applications in near future. Biomarkers should be developed in the way to reply to the urgent clinical demands. Thus, the researchers and clinicians should share the information of current problems in the cancer treatments, and the potentials of cancer proteomics.

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CS28.03 Identification of Tumor Antigens for Personalized Immunotherapy by Analysis of the HLA Peptidome

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Introduction and Objectives: Treatment of many cancers calls for development of more specific and effective treatments, such as immunotherapy. The HLA peptidome provide a rich source of tumor antigens for development of a personalized cancer immunotherapy. This research aimed to identify large repertoires of cancer cells' HLA peptides, in order to enable selection of tumor vaccine candidates. **Methods:** Since the expression of HLA class I molecules is significantly lower on many cancer cells, we treated the cancer cells with interferon gamma, which increases the expression levels of the membranal HLA. In addition, we induced recombinant expression of specific soluble HLA alleles to obtain larger number of peptides. After immunoaffinity purification of the HLA molecules, the bound peptides were extracted and identified by capillary chromatography and tandem mass-spectrometry using a HCD fragmentation. **Results and Discussion:** In total, we identified above close to a hundred thousand different HLA peptides from different cells lines and tumor tissues. Of these, the vast majority were derived from the membrane HLA class I molecules, while the peptides isolated from the soluble HLA class I peptides could be easily associated with the studied HLA allele. Tumor antigens potentially useful for vaccination are selected according to a number of criteria: 1) Antigens not expressed in normal essential tissues, such as tumor testis antigens and embryonal antigens; 2) Antigens that can elicit immune response; 3) Antigens encoded by genes of the two above groups that are known to be sufficiently expressed in the studied tumor cells. Using such databases and bioinformatics analyses we could select a few hundreds of such putative candidate HLA peptides for further testing as cancer immunotherapeutic. Identification of large numbers of HLA peptides enables selection of the few potentially useful peptides for subsequent human treatment by personalized immunotherapy. **Conclusion:** HLA peptidome analysis is approaching maturation for clinical use in personalized cancer immunotherapy.

Keywords: personalized cancer vaccine, HLA peptidome

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CS28.04 Immune Response Proteins Predict HCV Treatment Outcome

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Introduction and Objectives: The goal of this study was to better understand the biology of hepatitis C virus treatment response as well as to predict at baseline who is more likely to respond to therapy. **Methods:** An unbiased label-free mass spectrometry approach was taken to analyze the serum proteome in patients infected with HCV. Samples were collected prior to treatment in one of three clinical trials for the investigational drug Telaprevir. Responders and non-responders to therapy were characterized based on reduction in viral load at week 4. A multiplexed MRM assay was developed, based on 71 proteins identified in the discovery study and was used to confirm protein identities and relative expression differences between responders and non-responders at baseline. **Results and Discussion:** In the initial discovery study, protein abundance was compared between 25 responders and 25 non-responders to standard of care (SoC) treatment (pegylated interferon and ribavirin). A response signature was identified and verified on an independent cohort of 87 patients treated with SoC plus Telaprevir. In a subsequent analysis, using the MRM assay, 15 of the original 71 differentially expressed proteins were found to be the best classifiers of response, All 15 are liver-derived proteins, the site of the infection, and 7/15 are part of the focal adhesion complex. These focal adhesion proteins make the connection between actin filaments and the cell surface integrins and are involved in a variety of key processes. These 7 proteins were shown to be coordinately regulated with the greatest expression differences observed in patients with no or low levels of fibrosis **Conclusion:** In conclusion, focal adhesion proteins are upregulated at early stages of HCV infection. These proteins and others were able to classify patients at baseline into responders and non-responders to therapy. The classifier was based on subjects treated with SoC and verified in those treated with SoC plus Telaprevir.

Keywords: HCV, host response, serum proteome, predicting outcome

CS28.05 Substrate and Chaperone Binding Sites in α-Galactosidase Identified by Proteolytic Affinity MS

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Introduction and Objectives: Fabry's Disease (FD) is a rare metabolic disorder caused by deficiency of the lysosomal enzyme α-galactosidase A, leading to blocked substrate breakdown and detrimental effects on organ functions. Although FD can be successfully treated by enzyme replacement therapy (ERT), a new promising therapy avoiding potential immunological and toxicity complications of ERT employs pharmacological chaperones that can reconstitute enzyme activity. The chaperone 1-deoxygalactonojirimycin (DGJ), a structural analogue of galactose, is currently evaluated in clinical trials, and is thought to bind at the identical site as galactose. We report here the identification of the galactose and DGJ binding sites using a new affinity-mass spectrometry approach by selective proteolytic digestion of the enzyme- galactose and –inhibitor complexes. **Methods:** Proteolytic-extraction mass spectrometry was used for identification of chaperone binding structures. The protein was first digested in solution and the resulting fragments bound on a column with divinylsulfone- immobilized carbohydrate. Non-binding peptides were removed by washing and binding fragments recovered. Affinity-bound peptides were eluted with ACN: 0.1%TFA 2:1, for 15 min at 37 °C. Supernatant, washing and elution fractions were collected and analyzed by MALDI-MS. To evaluate unspecific binding of peptides, a control experiment was performed using unmodified matrix; **Results and Discussion:** Proteolytic extraction affinity- MS of human α-galactosidase A identified two galactose-binding peptides, hαGAL[83-100] and hαGAL[141-168], which contain the essential residues involved in galactose binding. The binding of hαGAL[83-100] and hαGAL[141-168] to galactose was inhibited by addition of DGJ to the digest mixture, indicating high affinity for DGJ. The binding site of DGJ was with the same protocol as for galactose. DGJ is a structural analogue of galactose and was found to bind to the original substrate binding site. Several hydrogen bonds established with the galactose binding were also formed when DGJ was bound. **Conclusion:** Affinity- MS is a powerful tool for obtaining molecular information on carbohydrate-binding peptides.

Keyword: alpha-galactosidase; chaperone-enzyme complexes; proteolytic extraction-mass spectrometry;

CS28.06 Risk Assessment of Development Impairment in Preterm Babies by Cord Blood Proteome Profiling

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Introduction and Objectives: "Intrauterine-growth-restriction (IUGR)" is a pathological pregnancy condition in which the fetus does not reach its genetically given growth potential. IUGR is a risk factor for cardiovascular and metabolic diseases later in life. By contrast, other preterm babies, categorized as "small for gestational age (SGA)", are not associated with such risks, yet at birth they are difficult to distinguish by clinical means. Our affinity mass spectrometry-based assay reliably identifies IUGR babies and differentiates them from other preterm (SGA) newborns upon molecular profiling of cord blood serum proteins with high confidence. **Methods:** Cord blood was collected and serum was prepared and deposited onto "plasma collection discs" (Noviplex; Shimadzu Europe, Darmstadt, Germany). The discs were soaked with 0.1% RapiGest solution. MagSi-proteomics C8 beads (MagnaMedics Diagnostics, Geleen, NL) were added and intact serum proteins were eluted and spotted onto a MALDI target. After adding ferulic acid matrix protein ions were recorded in a mass range from 4 to 20 kDa. **Results and Discussion:** Serum samples were stored and shipped by mail without loss of quality when deposited on a "plasma collection disc" (Noviplex ™). Serum proteins were eluted intact via bead surfaces onto stainless steel targets from which protein profiles were analyzed by MALDI-ToF MS. All 60 mass spectra showed ca. 50 ion signals, each, used for profiling and scoring of an individual spectrum. Multiparametric and biostatistical analyses sorted nearly all spectra from the IUGR samples into the IUGR group (true positive) and almost all spectra from the control samples into the control group (true negative). From these results a sensitivity of 0.87 (true positive rate) and a specificity of 0.93 (true negative rate) was calculated. The robustness of all involved steps, beginning in the case room, makes this assay attractive to maternity clinics world-wide. **Conclusion:** Proteome profiling of cord blood serum proteins enables differentiation between IUGR and controls.

Keywords: proteome profiling, personalized risk assessment, preterm baby screen, cord blood serum analysis

CS28.07 Urine Proteome Analysis for Differential Diagnostics of Respiratory Tract Pathologies in Newborns

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Introduction and Objectives: Infectious and noninfectious respiratory disorders are the leading cause of death in neonatal intensive care. Manifestations of infectious diseases in newborns with severe morphological and functional immaturity, especially in children with very low and extremely low birth weight (VLBW and ELBW), are often nonspecific. Incorrect diagnosis and late start of targeted therapy are common failures in the treatment of neonatal patients. A serious problem during intensive care and nursing of premature infants is the invasiveness of many examination methods. The aim of this research was to develop an approach for differential diagnosis of respiratory disorders for newborns being treated in the Neonatal Intensive Care Unit (NICU) based on urine proteome analysis. **Methods:** Urine samples from newborns were collected at the V. I. Kulakov Research Center of Obstetrics, Gynecology and Perinatology. All newborns had respiratory disorders of different diagnosis and required specific therapy in the Neonatal Intensive Care Unit. Urine samples of newborns were prepared and LC-MSMS analyzed according to the protocol described previously (Pastushkova LK, et al. (2013). Plos One;8(8):e71652) with minor modifications caused by low sample volume.invasiveness of many examination methods. The aim of this research was to develop an approach for differential diagnosis of respiratory disorders for newborns being treated in the Neonatal Intensive Care Unit (NICU) based on urine proteome analysis. **Results and Discussion:** Proteins reliably distinguishing newborns with respiratory disorders of infectious and non-infectious genesis

were determined. Different clinical parameters of the patients such as length of the gestation period, age and body weight were evaluated. **Conclusion:** A number of proteins with potential use for the differential diagnostics of congenital pneumonia and respiratory diseases of non-infectious origin (transient tachypnea, respiratory distress syndrome, apnea of prematurity) were identified.

Keywords: newborn, Mass spectrometry, respiratory tract disorders, urine proteome

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CS28.08 Prediction of Mortality in Acute Respiratory Distress Syndrome

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Introduction and Objectives: Acute Respiratory Distress Syndrome (ARDS) continues to have high mortality. We have identified early differences in the protein expression profile in pooled bronchoalveolar lavage fluid (BALF) from ARDS survivors and non-survivors. The objective of this study is to determine the differences in these two groups by characterizing BALF from individual ARDS subject. **Methods:** BALF was depleted of the high abundance proteins, trypsin digested and labeled with eight-plex iTRAQ® reagent. The labeled peptides was analyzed by 2D LC-MS/MS on an Orbitrap Velos system in HCD mode for DDA tandem MS. Protein identification and relative quantification were performed with ProteinPilot within Galaxy P platform. Protein identification ambiguity across different MS experiments was resolved using Protein Alignment Tool (AB Sciex). Pooled BALF from 27 patients was used as a global internal standard for protein quantification. Variance weighted student t-test was used to identify the differentially expressed proteins between survivors compared to non-survivors. To identify proteins that predict outcomes, we used a logistic regression model for the survivorship status with leave-one-out-cross-validation. **Results and Discussion:** BALF from 20 ARDS survivors and 16 non-survivors was studied. There was no difference in the ARDS day when BALF was obtained, PaO2: FiO2 ratio BALF cell counts between survivors and non-survivors. We performed six iTRAQ LC-MS/MS experiments and identified 850, 606,1055, 865, 976 and 879 proteins respectively resulting in a total of 1122 unique BALF proteins. Variance weighted t-test identified 249 proteins to have differential expression between ARDS survivors and non-survivors. Similar to our prior studies these proteins participate in acute inflammatory response and collagen metabolic processes. We also identified several proteins that predict survival in ARDS using the linear regression model. **Conclusion:** We identify key differences in biological processes between ARDS survivors and non-survivors. These differences will result in development of predictive biomarkers and also provide therapeutic targets in ARDS.

Keywords: biomarker, ARDS, Prognosis

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CS28.09 Serological Epithelial Component Proteins Identify Intestinal Complications in Crohn's Disease

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Introduction and Objectives: Crohn's disease (CD) is a chronic relapsing inflammation of the gastrointestinal tract that affects a young, working age population and is on the rise in developing countries. Half of all CD sufferers will experience stricturing or fistulizing intestinal complications that require extensive surgeries and prolonged hospitalizations, and neither genes nor clinical risk factors can predict this debilitating natural history. We applied discovery and qualification phase proteomic studies along the NCI-FDA biomarker development pipeline to interrogate differences in the low-mass (<25kDa) blood serum fraction between CD behavioral phenotypes. **Methods:** Quantitative proteomic profiling was performed on Inflammatory (ICD) and Complicated (stricturing and fistulizing) CD (CCD) serums using label-free Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS). DAVID 6.7 (NIH) was used for functional annotation analysis of detected proteins and immunoblotting and Multiple Reaction Monitoring (MRM) to verify a priori findings in an independent cohort of active (untreated/balloon dilated strictures & non-healed abscesses/fistulas) CCD, ICD, and intestinal, Th1/17 systematic inflammation and non-disease controls. **Results and Discussion:** 172 serum proteins were modulated in CCD versus ICD by LC-MS/MS (P<0.05, q<0.01), annotating to pathways of epithelial barrier homeostasis (P<0.01). A 3-protein assay developed from discovery proteomics data distinguished CCD from all other groups (P=0.041), correlated with Erythrocyte Sedimentation Rate (ESR) (r=0.492, P=0.032) and discriminated complication in CD (70% sensitivity and 72.5% specificity at score ≥1.907, AUC=0.777, P=0.007). An MRM assay secondarily confirmed increased biomarker candidate levels in CCD (P<0.001). In a longitudinal subanalysis-cohort, biomakrer candidate levels were stable over a two-month period with no behavioural changes (P=0.099). **Conclusion:** Genomic, endomicroscopic and ex-vivo studies have established dysregulated epithelial barrier functions in CD. Increased circulation of epithelial barrier component proteins are associated with intestinal CD complications, confirming epithelial defects at the in-vivo proteome-level and suggesting viability of monitoring intestinal injury by serological tests.

Keywords: Inflammatory Bowel Disease, Translational Medicine, biomarkers, multiple reaction monitoring

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CS28.10 Tear Proteome Correlates with Better Clinical Signs and Symptoms in Glaucoma Patients

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Introduction and Objectives: The goal of the study was to determine if changes in the tear proteome could be shown to underlie the benefits of changing glaucoma medi-

cation from a preserved type to a non-preserved type. **Methods:** Thirty glaucoma patients were entered in the study approved by Tampere Hospital Ethics Committee. Inclusion criteria: >3 years of glaucoma, >2 years of Xalatan® usage, clinical symptoms of dry eye using the OSDI. Patients were changed on to preservative free Taflotan® glaucoma medication, Schirmer type I samples were taken at 0, 1, 3, 6 and 12 months after the change. Patients clinical symptoms and signs were recorded at each visit. Tear proteins from Schirmer strips were analysed with NanoLC-TripleTOF mass spectrometer SCIEX 5600+ using iTRAQ for relative quantification. **Results and Discussion:** Most patients completed the study (28/30). Using iTRAQ 150-500 proteins were quantified in each sample. Clinical signs and symptoms including redness, tear breakup time, Schirmer's value, blepharitis and corneal staining began to improve by 1.5 months after changing medication. Pain and discomfort decreased p=0.00289 at 12 months. Underlying inflammation as shown by the S100 proteins A6 and A4 both decreased by 1.5 months as did alpha enolase. The lacrimal gland protein, lacritin, which acts to maintain the corneal epithelium increased after 3months. At 3 months patient confort improved, continuing to the study conclusion. **Conclusion:** This is the first study to demonstrate the value of tear proteomics in a clinically important situation. Individual differences were noted and these could be used to monitor medication. The significant tear proteins represented inflammation and biological processes associated with a dry eye state, but which tended to return to a more normal condition. MRM could be used in a future study for the 6 proteins needed to monitor the condition of the eye and augment the clinical exams.

Keyword: tears, dry eye, glaucoma, cornea

CS29: PROTEOGENOMICS

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CS29.03 Towards Improving the Genome Annotation of the Honey Bee (Apis mellifera)

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Introduction and Objectives: Honey bees (Apis mellifera) are important pollinators in managed agriculture as well as natural ecosystems. Recently, the sequencing and annotation of the bee genome has allowed proteomics to become a powerful technique to probe aspects of bee biology; however, one troubling trend that emerged from these studies is that honey bee samples consistently result in lower peptide identification rates compared to other organisms. This suggests that either the genome annotation can be substantially improved, or some atypical biological process is interfering with the mass spectrometry (MS) workflow. **Methods:** We used a publically available MS dataset (Peptide Atlas; 1,472 raw files) in a proteogenomic approach to search for missing genes, new exons, and to revive discarded annotations. To do this, we searched the data against a six-frame genome translation, a three-frame refSeq RNA translation and a database that included sequences lost from previous annotations. We also considered unexpected post-translational modifications (PTMs), high genetic diversity and endogenous proteolysis as alternative explanations for low peptide identification rates. **Results and Discussion:** While we found no significant effects of PTMs, sequence diversity or proteolysis in tissues other than the gut, we did discover 1,454 new continuous coding regions matched by two or more peptides (1% FDR), including twelve sequences that were previously annotated as non-coding RNAs. In a separate search, we matched 748 previously annotated proteins that had not been retained in the current official gene set (OGS). Importantly, when the sequences were added to the OGS protein database (increasing the database size by 13.5%), this improved MS identification rates across tissues. **Conclusion:** Using this proteogenomic strategy, we have improved the completeness of the honey bee genome annotation. The information we present here can facilitate further research on this important insect and this template can be used to aid the genome annotation of other under-studied species.

Keywords: honey bees, genome annotation, Proteogenomics

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CS29.04 Deep Proteogenomic Profiling of 55 Breast Cancer Cell Lines Reveals Novel Insights in Cancer Biology

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Introduction and Objectives: Genomic alterations in human cancers have been extensively characterized, but it remains challenging to prioritize for cancer driver genes. Integration with proteomics data allows for identification of altered and novel protein coding genes associated with cancer. We therefore performed deep proteome profiling and proteogenomic data integration of 55 breast cancer cell lines. **Methods:** Tryptic digests were 10-plex TMT labeled, fractionated by HILIC and on-line separated using nanoLC-MS. Mass spectra were generated by Fusion Tribrid Orbitrap in MS^3 mode in duplicate prioritizing the most and least abundant precursor ions for fragmentation, respectively. Raw MS data were processed in the trans-proteomic pipeline and searched against the Uniprot human reference proteome, including all isoforms. For all cell lines previously generated genomics data were available, RNAseq data of breast tumors were downloaded from TCGA. **Results and Discussion:** In total 3,320,471 MS/MS spectra were generated, resulting in 1,523,367 PSMs and 160,852 identified peptides. These peptides inferred to 9,234 proteins at <0.6% FDR, of which 7,599 were >20% sequence independent. Cell lines readily clustered according to known molecular subtypes, and proteins corresponding to oncogenes of the PI3K, p53 and pRB pathways were observed. Median correlation between gene and protein abundance was R=0.47. Negative correlation (R<-0.139) was observed for 109 proteins, which were enriched in nuclear and luminal proteins, such as polymerases and transcriptional regulators. These nuclear lumen proteins were more highly abundant in the estrogen receptor negative breast cancer subtype. To dig deeper into the proteome, we will extract high quality non-matched spectra peptides and re-search against a custom sequence database based on matching oncogenic sequence data complemented with TCGA RNAseq data. **Conclusion:** We have generated a large and high quality quantitative proteomic dataset of 55 breast cancer cell lines, and integrated it with previously generated genomics data. This proteogenomic approach provides novel insights into breast cancer biology.

Keywords: Breast cancer, proteome profiling, TMT labels, Proteogenomics

CS29.05 Peppy 2.0: New Software Addressing the Sensitivity Problem in Proteogenomics

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Introduction and Objectives: Proteogenomics is severely impacted by high false positives and false negatives. Searching a full, 6-frame translation of a eukaryote genome creates a theoretical protein set that is ~600x larger than a reference protein database. Larger search spaces increase the possibility of false positive identifications and thus require increasing confidence thresholds to maintain an appropriate FDR. Unfortunately, higher confidence thresholds result in discarding many true identifications that would normally be accepted with typical peptide searches. To address these issues, we have created major improvements and additions to the proteogenomic search software Peppy. **Methods:** Peppy 2.0 incorporates mass error tolerance optimization and three extra-pass search techniques. Before the peptide identification begins, an initial analysis is performed to find optimal precursor and fragment tolerance values. Then, three search methods are applied: 1) mass spectrum sequential subtraction (MSSS). 2) Targeted 6-frame regions surrounding non-canonical matches identified with MSSS. 3) Blind modification searches preformed with the remaining spectra using the set of identified peptides as the search database. For each of the search passes performed, all spectra identified at the FDR cutoff are removed from all subsequent passes. **Results and Discussion:** Mass error tolerance optimization and multiple database searches improve sensitivity by appropriately limiting the peptide sets being considered when assigning peptide-spectrum matches. MSSS involves searching spectra first on protein-only databases before 6-frame. Positives are increased due to the lower FDR confidence threshold required of the protein databases. False positives are reduced in non-canonical matches as MSSS effectively applies separate false-discovery analysis. Targeted searches use the theorized novel coding locations found in the preceding searches to intelligently limit the 6-frame search space. Blind modification searches allow extra PSMs identified without the space multiplication of variable modification searches. **Conclusion:** In addition to addressing sensitivity, Peppy 2.0 adds GUI, HTML reports, error reports. This software is freely and immediately available. http://geneffects.com/peppy

Keyword: FDR, FP, FN, MSSS

CS29.06 Impact of Individual Single Nucleotide Variants on Signal Transduction Networks in Cancer Cell Lines

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Introduction and Objectives: Single nucleotide variants (SNVs) are the most commonly occurring variants in the human genome. Many of the SNVs are non-synonymous (nsSNVs), resulting in altered protein sequences, such as amino acid substitutions, that in turn can change the modification status of a protein. Several types of cancer are known to be caused or enhanced by the nsSNVs in the key regulatory proteins underscoring the importance to study the impact of nsSNVS on signal transduction networks. **Methods:** We used publicly available RNA-Seq data of melanoma and leukemia cancer cell lines to assess the extent of nsSNVs and to assemble cell line-specific protein databases. We analyzed phosphoproteomes, acetylomes and ubiquitylomes of the individual cancer cell lines using LTQ Orbitrap Elite or QExactive HF mass spectrometers and processed MS data using MaxQuant software. **Results and Discussion:** The extent of nsSNV detected by RNA-Seq data ranged between 12,583 and 13,196 in melanoma and leukemia cell lines, respectively. About 30% of all nsSNVs affected modifiable amino acids (Ser, Thr, Tyr, Lys) and about 20% fell into kinase target motifs, underscoring the potential of nsSNVs to rewire signal transduction networks. Database search of acquired high-accuracy MS/MS spectra against cell type-specific databases resulted in the identification of up to 14,500 phosphorylation, 4,100 ubiquitylation, and 1,600 lysine actelytion events in each cell line. Preliminary analysis revealed the presence of 872 variant peptides, of which 150 were found to be modified. Corresponding proteins mapped to various physiological processes and signaling pathways, such as cell cycle regulation and ubiquitin/proteasome system. We are currently assessing the impact of individual variant modification sites on major signal transduction networks. **Conclusion:** This approach has potential to discover novel protein modification sites that depend on individual genetic variants and will serve as a foundation for further application to clinical samples from cancer patients.

Keywords: Proteogenomics, personalized medicine, Bioinformatics

CS29.07 Proteome-Scale Discovery of Protein Isoforms, including Those Predicted from RNA-Seq Analysis

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Introduction and Objectives: The majority of human genes are expressed as alternatively spliced transcripts. When translated, these result in protein isoforms that diversify the function of proteins within and between cells and tissues. With the advent of RNA-seq, it has become increasingly possible to define the mRNA splice variants in a cell type or tissue. Here we comprehensively explore how RNA-seq transcriptomics data, and proteomic analysis of the same sample, can be used to identify protein isoforms. **Methods:** RNA-seq data from undifferentiated human mesenchymal (hMSC) stem cells were analysed with our new TranscriptCoder tool, to generate a database of protein isoform sequences. MS/MS data from matching hMSC samples were then matched against the TranscriptCoder-derived database, and also Ensembl. The results were finally analysed with our PG Nexus Pipeline (Pang et al 2014 J Proteome Res 13: 84-98) to determine the peptides which were exonic and those which spanned exon-exon splice junctions. This also permitted the co-visualisation of mRNA splice isoforms and peptides in the context of the genome (in the Integrated Genome Viewer). **Results and Discussion:** The unambiguous identification of protein isoforms is difficult, as it requires the presence of peptides that are found in isoform-specific exons or in isoform-specific exon-exon junctions. By querying the RNA-seq-derived isoform database or Ensembl a total of ~450 isoforms could be identified, as they contained unique isoform-specific proteotypic peptides. This included candidate hMSC-specific isoforms for the genes DPYSL2 and FXR1. Where isoform-specific peptides did not exist, we found that sets of non-isoform-specific proteotypic peptides could specifically identify 65 further isoforms. However, our analysis also revealed that some isoforms will be impossible to identify unambiguously as they do not have peptides that are sufficiently distinguishing from other isoforms of the same protein. **Conclusion:** We have shown that isoform identification, largely ignored by proteomics to date, can be achieved. This paves the way for isoform-based

analyses of normal and diseased human proteomes.

Keywords: Proteogenomics, RNA-seq, isoform identification

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CS29.08 Dynamic Linking of Public Proteomics Data in Ensembl Using TrackHubs

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Introduction and Objectives: Mass spectrometry proteomics is an important platform for post-genomic investigations, but an efficient and up-to-date integration between proteomics and genomics information in public resources has only occurred to a limited extent so far. We aim to provide dynamic integration between world-leading resources at EMBL-EBI connecting the public-domain proteomics data available in the PRIDE database (http://www.ebi.ac.uk/pride) and the genome information available through the Ensembl genome browser (http://www.ensembl.org). **Methods:** Submitted peak list files and/or raw data from selected projects submitted to PRIDE (which is part of the ProteomeXchange Consortium) are reprocessed using the 'ProteoAnnotator' pipeline (PMID: 25297486, http://www.proteoannotator.org). Data reprocessing is focused on human and the main model organisms represented in PRIDE, which are part of Ensembl or Ensembl Genomes: human, mouse, rat and Arabidopsis. All the resulting peptide related information will be shown in Ensembl, by extending the TrackHub technology to represent proteomics data. **Results and Discussion:** TrackHubs for representing proteomics data have been standardized using the Bed and BigBed formats. A registry for TrackHubs has already been implemented and other data types in addition to proteomics will be supported as well. The first human datasets have now been reanalyzed using ProteoAnnotator and will be included as soon as possible in Ensembl, once the data export pipeline from PRIDE is finalized. **Conclusion:** This project aims to provide a higher exposure for public proteomics data, and to take advantage of it to help improving genome annotation efforts.

Keywords: databases, public repositories, Proteogenomics, data integration

CS30: STANDARDIZATION IN PROTEOMICS

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CS30.01 A Multi-Centric Study to Evaluate the Use of Relative Retention Times in Targeted Proteomics

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Introduction and Objectives: The use of relative retention times (RT), defined on the basis of RT peptide standards, has emerged as a useful tool in targeted proteomic analysis. In order to evaluate the robustness and reproducibility of this strategy, we set up a multi-centric study (PME10) carried out at 25 laboratories of the spanish ProteoRed network, and others within EuPA. **Methods:** Each participant received a sample consisting of a mixture of: i) a tryptic digest of a human cancer cell line (MCF7); ii) the Sigma-Aldrich set of retention time standards MSRT1 (14 isotopically labeled peptides covering a wide range of hydrophobicity) and iii) 16 labeled peptide standards corresponding to 16 different proteins known to present in the MCF7 proteome. Samples of the pure peptide mixtures ii) and iii) were also supplied. The sample was analyzed at each laboratory by SRM or other targeted LC-MS methods. Analysis were performed in triplicate runs of three different gradient lengths (60, 90 and 120 min), using diverse columns and chromatograpy equipment. Additionally, the MSRT1 standard mixture alone was also run on each of the three gradients. MS acquisition methods were set to monitor the 14 RT standard peptides, plus the 16 labeled/endogenous peptide pairs of the selected MCF7 proteins, and 9 additional endogenous peptides from the same proteins, with no labeled standard pair. **Results and Discussion:** Each laboratory reported results on the relative RT of the 25 MCF7 peptides, as determined by a) external calibration, based on the RT of the MSRT1 peptides from a separate run of pure MSRT1 in the same conditions, and b) internal calibration, based on the RT of MSRT1 present in the mixture from the same run **Conclusion:** The global results demonstrate an excellent reproducibility of the measured relative retention times, across different gradients and laboratories, supporting the usefulness of relative RT in targeted proteomic analysis.

Keywords: Relative Retention Times, Targeted analysis, Muticentric study

CS 30: STANDARDIZATION IN PROTEOMICS
WEDNESDAY, SEPTEMBER 30, 2015 – 14:30 – 16:20

CS30.03 Multi-Site Assessment of Quantitative and Qualitative Performance of SWATH Mass Spectrometry

Yansheng Liu[1], Ben Collins[1], Christie Hunter[2], Stefani Thomas[3], Dan Chan[3], Hui Zhang[3], Samuel Bader[4], Robert L. Moritz[4], Birgit Schilling[5], Bradford Gibson[5], Christoph Krisp[6], Mark Molloy[7], Guixue Hou[8], Siqi Liu[9], Mio Hirayama[10], Sumio Ohtsuki[10], Nathalie Selevsek[11], Ralph Schlapbach[11], Shin-Cheng Tzeng[12], Jason Held[13], Brett Larsen[14], Anne-Claude Gingras[15], Ruedi Aebersold[1]
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Introduction and Objectives: Reproducibility is a key requirement for scientific research. According to recent studies, SWATH-MS which combines data independent acquisition (DIA) and targeted data analysis, was able to quantify thousands of proteins across large sample sets with high data completeness and quantitative accuracy. However, whether SWATH-MS is sufficiently robust to support the generation of reproducible proteomic datasets across laboratories has not been demonstrated. Here we performed a multi-laboratory comparative study in which eleven research teams in different locations and countries performed SWATH-MS measurements on standardized samples.

Methods: All labs performed variable window SWATH acquisition on the TripleTOF 5600. A complex HEK293 matrix with 30 synthetic peptides dosed in across a broad dynamic range was analyzed. These samples were run repeatedly during the course of a week in each lab to investigate the intra- and inter-laboratory reproducibility and detectability of peptides. The data were analyzed centrally by OpenSWATH and MultiQuant™. **Results and Discussion:** We analyzed the quantitative performance of the 30 synthetic peptides spiked in to the HEK293 matrix. Good linearity was consistently observed across 4.5 orders of magnitude. The number of peptides confidently quantified at each concentration level showed excellent reproducibility between labs. The intra-day reproducibility was ≤10% CV for all labs, and the inter-day CV was typically similar in the majority of labs. We also compared the set of proteins that could be detected from the HEK293 matrix across all samples from all labs by targeted analysis of the SWATH data using a combined human assay library containing peptide coordinates for ~10,000 proteins. Essentially the same set of proteins (~3700) was detected across all sites. The median Pearson correlation coefficient for protein abundances (log2) across all files from all labs was 0.985. **Conclusion:** Our data suggest that the reproducibility of SWATH acquisition across labs is sufficiently high to support consistent and large-scale protein quantification across labs.

Keywords: SWATH mass spectrometry, Cross-lab study, Reproducibility

CS 30: STANDARDIZATION IN PROTEOMICS
WEDNESDAY, SEPTEMBER 30, 2015 – 14:30 – 16:20

CS30.04 Can Certified Reference Materials Support the Validation of Biomarkers?

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Introduction and Objectives: The validation of the clinical relevance of potential biomarkers requires a considerable number of reliable measurement data. They have to be of sufficient accuracy and comparability and appropriate quality assurance measures have to be implemented. The experience from various measurement areas shows that reference materials play a fundamental role. They are needed already in the course of method developments, but in particular for the establishment of sustainable and globally accepted reference measurement systems and for performance controls within and among medical laboratories. The necessary characteristics for materials and the available information on them depend on their intended role in the measurement process and the validation study. Reference materials for HbA1c (biomarker for diabetes) will be discusses as an example. **Methods:** A certified reference material (CRM) containing different mixtures of highly purified HbA1c and HbA0 has been developed, produced and certified for establishing a global reference measurement system for this biomarker. An internationally validated and agreed on reference method has been used together with routine laboratory methods to investigate the homogeneity, stability and commutability of the material, and to characterize its properties. **Results and Discussion:** The measurements confirmed the purity of the starting materials and the proper preparation of the final mixtures. The CRM units were homogeneous and stable under the chosen storage and shipping conditions. The characterization measurements allowed assigning amount-of-substance values. Moreover, a feasibility study for the preparation of an HbA1c matrix material from human haemolysate of diabetic patients was successfully performed. It could be demonstrated that this material is commutable to patient samples. **Conclusion:** The examples demonstrate two application levels of CRMs. The pure material mixtures are providing the metrological anchor for HbA1c measurement results on patient samples to the international system of units. The matrix material under investigation should ensure a proper quality control of the measurements of this biomarker.

Keyword: Reference Materials, commutability, traceability, validation

CS 30: STANDARDIZATION IN PROTEOMICS
WEDNESDAY, SEPTEMBER 30, 2015 – 14:30 – 16:20

CS30.05 Affinity Binder Knock-Down Initiative

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Introduction and Objectives: There are an enormous amount of publicly available affinity binders. Currently the open-access database Antibodypedia (www.antibodypedia.com) list antibodies from about 20% of antibody suppliers worldwide, and the database holds data on 1.8 million antibodies. Several studies suggest that many affinity binders are not working as expected, somewhere between 25-50% of all antibodies are successful in a particular application. The global spending on affinity reagents is estimated to be around $2 billion in 2014 and around $3 billion 5 years from now. A lot of material, time, and money can be saved with a quality assurance program in proteomics research. **Methods:** The Affinity Binder Knock-Down Initiative's mission is to engage the scientific community to contribute to a systematic validation of the quality of affinity reagents using knock-down experiments to verify the correct binding. **Results and Discussion:** The crowdsourced data is publicly available through Antibodypedia. There is a need to gather the knowledge in a systematic and standardized system to assure the quality of the affinity binders. Reproducibility of results depends on identification of good affinity reagents. **Conclusion:** Most research is in one way or another built on or connected to other scientific findings; identifying good affinity reagent is key to reproducibility and advancement of science. There are existing validation and refund programs for affinity reagents, many run by or supported by private companies, but there is no systematic collection of data and no standardized system for validation of affinity binders to our knowledge. A systematic exploration of affinity reagents to verify the quality will improve research outcome in all areas of proteomics.

Keywords: Knockdown, Validation, Quality assurance

CS 30: STANDARDIZATION IN PROTEOMICS
WEDNESDAY, SEPTEMBER 30, 2015 – 14:30 – 16:20

CS30.06 DIGESTIF: A Universal Quality Standard for the Control of Bottom-Up Proteomics Experiments

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Introduction and Objectives: In bottom-up mass spectrometry-based proteomics analyses, variability at any step of the process, particularly during sample proteolysis, directly affects the sensitivity, accuracy, and precision of peptide detection and quantification. Currently, no ge-

neric internal standards are available to control the quality of sample processing steps. This makes it difficult to compare MS proteomic data obtained under different experimental conditions. To meet this need, we have generated and validated a universal protein standard, called DIGESTIF, that has been specifically designed to release indicator peptides directly reflecting how well a generic protein sample is digested. **Methods:** The DIGESTIF standard was designed as a recombinant protein which can be spiked into biological samples and digested with trypsin alongside endogenous proteins. The DIGESTIF standard includes in its sequence the 11 peptides of the iRT peptide set (Escher et al, 2012). To simulate the differing digestion properties encountered in a complex protein mixture, iRT peptide cleavage sites were flanked with amino acids to either favor or hamper trypsin digestion. After sample processing, the retention time and relative intensity pattern of the released iRT peptides can be used to assess the extent of digestion and the performance of the LC-MS system. **Results and Discussion:** We assessed how DIGESTIF performed in different proteolysis conditions applied to various matrices (liver lysate, urine, plasma). We demonstrated that DIGESTIF can be used to evaluate digestion efficiency in complex matrices and that it is useful in identifying conditions where proteolysis is incomplete. It also allows to select the optimal digestion protocol. Finally, we examined how the DIGESTIF standard could be used to assess digestion efficiency and reproducibility across different laboratories. DIGESTIF clearly identified the most efficient protocol when comparing inter-laboratory results. It also unequivocally highlighted inter-laboratory variations in digestion efficiency. **Conclusion:** In conclusion, DIGESTIF has great potential to enhance the quality and reproducibility of bottom-up proteomics experiments.

Keyword: Standard, Proteolysis, Trypsin, Biomarkers

CS 30: STANDARDIZATION IN PROTEOMICS
WEDNESDAY, SEPTEMBER 30, 2015 – 14:30 – 16:20

CS30.07 Comparison of iTRAQ Data Processing Approaches with Respect to Analytical and Biological Variability

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Introduction and Objectives: The iTRAQ multiplex labelling method have been widely used in quantitative proteomic analysis of biological samples. Typically, the relative abundance of a protein is estimated by aggregating signals of reporter ions of MS/MS spectra belonging to the same protein using weighted or unweighted mean or median with or without the usage of a common reference. **Methods:** In this study, in order to better understand the impact of data processing on data analytical precision and preservation of biological signal, we compared seven different iTRAQ data processing approaches using data from a large scale proteomics study of 122 biological samples and 10 co-randomized repeats of a single quality control (QC) sample. Specific metrics used for evaluation include the coefficient of variation (CV) of proteins in the QC repeats, the signal to noise ratio (SNR) of proteins defined as the ratio of variance in biological samples over that in QC repeats, and the Spearman's correlation between protein abundance and its mRNA gene expression across the biological samples. Comparison was performed at various levels of allowed missing values. **Results and Discussion:** Our results indicated that overall approaches with the use of a common reference provided a more precise estimate of relative abundance of proteins ($p<0.01$). Among them, the weighted mean approach statistically outperformed the others in both analytical precision and SNR, especially when the allowed proportion of missing value increased ($p<0.05$, K-S). For example, allowing at most 30% samples having missing values, the weighted mean approach resulted in mean and median CVs of 8.55% and 7.02%, respectively, and mean and median SNRs of 10.22db and 9.99db, respectively, over a total of 7,012 proteins. **Conclusion:** In conclusion, this study used a uniquely available large dataset to systematically compare approaches in iTRAQ data processing using metrics relevant to proteomic data analysis and interpretation.

Keywords: iTRAQ data processing, analytical precision, comparison study, preservation of biological signals

CS 30: STANDARDIZATION IN PROTEOMICS
WEDNESDAY, SEPTEMBER 30, 2015 – 14:30 – 16:20

CS30.08 Harmonization of Proteomics Analyses: A Simple Method to Assess System Suitability

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Introduction and Objectives: Quantitative mass spectrometry-based studies are widely adopted in biology and biomedical research. Initially used by few laboratories, the technology has matured and gained broad acceptance by the community, which has a variety of platforms and protocols. The comparison and integration of data across laboratories requires a standardization of the 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. **Methods:** 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. **Results and Discussion:** The addition of two distinct isotopically labeled internal standards for each peptide analyzed allowed, in a single LC-MS analysis, the monitoring of the different stages of the analytical process. The measurement of the signals triplets of the different isotopic variants provides a straightforward read-out, enabling the assessment of the digestion efficiency, the recovery of the full sample preparation, and in turn the reproducibility of the experiment. The chromatographic and MS characteristics of the reference peptides were systematically 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. **Conclusion:** A protocol has enabled the assessment of both sample preparation and LC-MS performance in one simple analysis; it can easily be adapted and implemented in workflows routinely employed in quantitative proteomics.

Keywords: standardization, isotope dilution, quantification, digestion

CS 30: STANDARDIZATION IN PROTEOMICS
WEDNESDAY, SEPTEMBER 30, 2015 – 14:30 – 16:20

CS30.09 Glycome Profiling of Mouse Tissues Using a Standardized Method Combined LMD and Lectin Microarray

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Introduction and Objectives: In our previous work, we have constructed a reproducible method combined laser microdissection (LMD) and lectin microarray for mouse tissue glycome mapping. In this study, we used the established method and performed glycome profiling of different organs from two C57BL/6J mice. **Methods:** Formalin-fixed paraffin-embedded tissue sections (5 mm thickness) of five organs including brain, liver, kidney, spleen and testis from each mouse were adopted for this study. An area of 0.91 mm^2 was collected from each tissue section by LMD. 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:** The glycome profiling of different organs showed that the level of fucosylation was much higher than that of sialylation in the brain and renal cortex, while it was opposite in the liver, spleen and renal medulla. These results were highly consistent with previous reports analyzing the N-glycome by mass spectrometer (Medzihradszky et al. Mol Cell Proteomic 2015; Ji et al. Anal. Chem. 2015; Gizaw et al. Biochim. Biophys. Acta. 2015; Powers et al. PLOS ONE. 2014) and confirmed the reliability of our technology. In addition, we also found that the intense signals of O-glycan binders in testis were much stronger than those in the other organs, which was consistent with the previous report using lectin histochemistry (Manning et al. HistolHistopathol. 2004). **Conclusion:** The standardized method has been proved as a powerful tool to perform in-depth and precise tissue glycome profiling from a tiny amount of biological specimens. These approaches would be applied to variousdisease model mice and facilitate further studies on disease-related glyco-biomarker discovery.

Keywords: lectin microarray, LMD, mouse tissue, glycome profiling

HPP SESSIONS

HPP 01: CANCER HPP
MONDAY, SEPTEMBER 28, 2015 – 07:30 – 09:00

HPP 01 Cancer HPP

Understanding the proteomic differences in multiple human tumor types is the current requisite and central theme of the cancer human proteome project (Cancer-HPP) with the ultimate goal of defining expression and interactions of these proteins. This will greatly increase our knowledge of human cancer biology and disease progression. Overall, the Cancer-HPP attempts to characterize different cancer proteomes, determine the correlation of transcriptome and proteome, identify the high priority proteins for each tumor type and generate and disseminate assays and resources to support the analysis of complex biological networks or clinical specimens underlying different disease processes. The use of immunoassays and protein assays as well as the emerging mass spectrometry (MS)-based platforms such as selected reaction monitoring (SRM), Parallel reaction monitoring (PRM), and targeted data extraction for candidate proteins from SWATH-MS data have become reliable popular methods for quantitative analysis of high priority target proteins. Data from multiple laboratories studying different cancer types has confirmed the supremacy of these technologies over conventional assays. Therefore, we propose an international cancer proteomic effort similar to The Cancer Genome Atlas (TCGA) project to identify and validate cancer proteins for different cancer types. Data deposition, quality control, and public availability are always a priority for the key proteomics journals and some funded programs such as CPTAC and EDRN, and they are the key components of the Cancer-HPP. We will further support and propose an open discussion on procedures how to accrue/share data for a list of target proteins from each cancer type, the strategy for assay development, quality control, and procedure and materials needed for disseminate the established assays to cancer biology or clinical laboratories. Toward this goal, it will be essential to involve the whole cancer community including not only proteomics but also genomics and cancer biologists and clinical oncologists in Cancer-HPP.

HPP 02: HPPP (PLASMA)
MONDAY, SEPTEMBER 28, 2015 – 07:30 – 09:00

HPP 02 HPPP (Plasma)

Due to its circulation throughout the human body and ease of acquisition, blood provides a unique window into human health and disease. However, analyzing blood plasma or serum still poses one of the major challenges for proteomics in terms of sensitivity and analytical depth. With growing numbers of plasma samples being systematically collected, stored, and made available through biobanks, proteomic methods and technologies advancing, and the remaining need for novel clinical markers to determine and monitor health and disease, the effort to understand the plasma proteome is becoming an increasingly active area of research. This HPPP session will host a selection of short talks on recent advances in plasma protein analysis that cover different technologies, diseases, and concepts. We bring together different contributions to the field in mass spectrometry and affinity-based assays. The presentations will demonstrate success stories, technological possibilities, viewpoints and perspectives on how plasma proteomics has and will continue to advance. The invited speakers will cover topics from the field of biomarker research to their translation into clinical use.

HPP 03: CARDIOVASCULAR INITIATIVE WORKSHOP
MONDAY, SEPTEMBER 28, 2015 – 07:30 – 09:00

HPP 03 Cardiovascular Initiative Workshop

The HUPO Cardiovascular Initiative Workshop updates cardiovascular researchers and general biological scientists with progresses in the Cardiovascular Human Proteome Project (HPP), whose goal is to understand proteome regulations in cardiovascular diseases and promote the translation of proteome technologies. The Cardiovascular Workshop this year will take place on Monday, September 28th 7:30 am to 9:00 am in Vancouver, Canada (http://hupo2015.com/). The Workshop will feature the work of multiple young investigators from around the world. Selected topics will include advances in redox modifications, their regulations, and their particular importance in the diagnosis and prognosis of cardiovascular diseases; proteomics toolsets to elucidate context-dependent disease pathways in aortic aneurysm; novel quantification workflow using TMT10-TAIL; data science methods to expedite the translation of targeted quantification assays for cardiovascular-centric proteins; and others. Dr. Pothur Srinivas from NHLBI will begin the Workshop session with an overview on the current achievements and future directions of cardiovascular proteomics. A panel discussion period will be dedicated for open discussion to promote interactions with our panelists on the growth of cardiovascular proteomics.

HPP 03: CARDIOVASCULAR INITIATIVE WORKSHOP
MONDAY, SEPTEMBER 28, 2015 – 07:30 – 09:00

HPP03.02 Optimizing a Proteomic Toolset to Elucidate Context-Dependent Disease Pathways in Aortic Aneurysm

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Introduction and Objectives: The overarching goal of this project is to delineate the molecular mechanisms underlying aneurysm of the thoracic aorta. The specific objectives of the current work were to (1) construct a library of aortic vascular smooth muscle cell (VSMC) proteins using data dependent acquisition MS and (2) query this library against data independent acquisition MS samples from VSMCs cultured in conditions relevant to tissue context in aortic aneurysm. **Methods:** For library generation, 144 separate MS runs were conducted on fractionated lysate from both primary and immortalized lines of mouse-derived VSMCs. Raw data were processed using the TransProteomicPipeline (version 4.8.0), ultimately using Spectrast to construct a master VSMC spectral library, with raw retention times converted to indexed retention time using a set of internal reference peptides. DIA samples were acquired on a TripleTOF 5600 from unfractionated VSMC lysates of differing embryonic lineage of origin and/or subsequent to treatment with the cytokine TGFB (10ng/mL), vehicle, or AT1R-receptor blockade with telmisartan (10 uM). **Results and Discussion:** We compared expression of > 2,300 unique proteins between primary lines of VSMCs from different embryonic lineages of origin (N=3/lineage). Expression data pointed to a handful of transcription factors predicted to be differentially active between disparate VSMC lineages. Using the same reference library, we quantitatively screened the response of over 2,900 proteins to pharmacological treatments, indicating that treatment with TGFB induced significant changes in expression among 227 VSMC proteins. AT1R blockade attenuated TGFB-induced responses in more than half (n=162) of these proteins. We are integrating this downstream expression analysis with the rapid upstream phosphopeptide response to TGFB to pinpoint the precise molecular intersections between these two signaling systems. **Conclusion:** Our ultimate goal is to apply these molecular discoveries toward the identification of novel candidates for therapeutic interventions that will allay the progression of aneurysm in aortic disease.

Keywords: Aortic Aneurysm, Vascular Smooth Muscle Signaling, DIA-MS, TGFB

HPP 03: CARDIOVASCULAR INITIATIVE WORKSHOP
MONDAY, SEPTEMBER 28, 2015 – 07:30 – 09:00

HPP03.03 Novel CaMKII Phosphorylation Sites Identified on NaV1.5 by Label-Free Mass Spectrometry

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Introduction and Objectives: The cardiac voltage-gated sodium channel, NaV1.5, drives the upstroke of the cardiac action potential and is a critical determinant of myocyte excitability. Recently, Calcium (Ca)/Calmodulin(CaM) dependent protein kinase II (CaMKII) has emerged as a critical regulator of NaV1.5 function through phosphorylation of multiple residues including S516, T594, and S571 and these phosphorylation events may be important for the genesis of acquired arrhythmias, as occur in heart failure. However, phosphorylation of full-length human NaV1.5 has not been systematically analyzed and NaV1.5 phosphorylation in human heart failure is incompletely understood. **Methods:** Human NaV1.5 was expressed and purified in pairs from HEK293 cells with one sample subsequently used for in vitro phosphorylation by recombinant CaMKIIdC and the other sample serving as a baseline control. NaV1.5 was digested in-gel with chymotrypsin and trypsin. Peptide spectra were collected by LC-MS/MS with an Orbitrap Q Exactive Plus, searched and scored with SEQUEST and X! Tandem, and filtered with Scaffold. The relative abundance of phosphorylation at identified sites was determined using spectral counts and MS1 parent precursor ion peak areas (Skyline). Human failing ventricular heart tissue was probed by Western blot with a novel phospho-S516 antibody. **Results and Discussion:** In the present study, we used a label-free mass spectrometry based approach to assess phosphorylation of human NaV1.5 with full coverage of non-transmembrane sites and identified 23 sites that were phosphorylated by CaMKII in vitro (n=5). We also identified 12 sites that were basally phosphorylated in unstimulated HEK293 cells (n=5). We confirmed phosphorylation of S516 and S571 by LC-MS/MS and found a decrease in S516 phosphorylation in human heart failure vs non-failing donor controls (n=10). **Conclusion:** This work furthers our understanding of the phosphorylation of NaV1.5 by CaMKII under normal and disease conditions, provides novel CaMKII target sites for functional validation, and provides the first phospho-proteomic map of full-length human NaV1.5.

Keywords: phosphorylation, arrhythmia, heart failure, sodium channel

HPP 03: CARDIOVASCULAR INITIATIVE WORKSHOP
MONDAY, SEPTEMBER 28, 2015 – 07:30 – 09:00

HPP03.04 TMT10-TAILS Analysis of Lymphocytes to Unravel the Role of Proteolysis in B Cell Activation

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Introduction and Objectives: Proteases are integral in initiation and fine-tuning of signaling pathways in lymphocytes. Mutations in these proteins, or lack of regulation leads to severe immunodeficiencies or autoimmunity via not well elucidated mechanisms. To investigate the role of proteolytic processing in lymphocytes, we used B-cells from a patient suffering from combined immunodeficiency. We probed changes in the intracellular proteome and N-terminome upon activation of the B-cell receptor pathway using 10-plexed TMT-TAILS (Terminal Amine Isotopic Labeling of Substrates) to enrich for N-terminal peptides allowing us to identify proteolytic cleavage events with high confidence. **Methods:** Proteomes (200 µg each) were collected from EBV-immortalized B-lymphocytes of the patient, heterozygous sibling and mother grown with or without PMA-ionomycin stimulation. Primary amines were blocked using 10-plex TMT-labels before trypsinization. N-terminal peptides were negatively enriched using an aldehyde-functionalized polymer (http://www.flintbox.com/public/project/1948) and analyzed on a nanoLC-ESI-Orbitrap Fusion platform. Spectra were searched using the Byonic search node and N-terminal peptides were quantified using Proteome Discoverer 2.0. **Results and Discussion:** In two biological replicates 7,498 unique N-termini (acetylated or TMT-labeled) were identified with high confidence in 3,173 proteins (FDR <<1%). Combining results from preTAILS and TAILS we identified 30 missing proteins for the HPP initiative. We observed 2,665 unique quantifiable N-terminally TMT-labeled peptides which contain proteolytic cleavage sites. Significant ratios were identified by boxplot-and-whiskers analysis. We identified previously unknown cleavages in proteins involved in B-cell function, the NF-κB pathway and revealed a potential cross-talk between two pathways instrumental in lymphocyte activation. **Conclusion:** By using TAILS to reduce sample complexity combined with TMT10 multiplexing and high end mass spectrometry we generated, to our knowledge, one of the largest N-terminome datasets without any fractionation prior to LC-MS analysis. The lymphocytes obtained from the immunodeficient patient provided us with a unique opportunity to investigate B cell biology and has led to new insights into the role of proteolysis therein

Keywords: Lymphocytes, N-terminal proteomics, proteases

HPP04: HPP – DIABETES
MONDAY, SEPTEMBER 28, 2015 – 07:30 – 09:00

HPP04: HPP – Diabetes

The aim of the HUPO Human Diabetes Proteome Project (HDPP) initiative is to enhance the understanding of mechanisms in diabetes development by performing and analyzing large-scale network biology-based experiments.

Diabetes is a disease characterized by inability of normalizing circulating levels of glucose resulting in hyperglycemia. In addition, persons with diabetes often have increased concentrations of fatty acids especially if they are overweight or obese. Therefore, elevated circulating concentrations of glucose and lipid, separated or combined have been implicated in the impaired function observed in different cell types and tissues in individuals with diabetes. Within the HUPO community special expertise is available to generate and analyze complex data sets generated from cells and tissues obtained from healthy and diabetic subjects, animal models of the disease or cell lines.

At previous HDPP workshops, which are held twice a year with one at the HUPO meeting, persons with interest in pursuing diabetes research related to the aim of HDPP have been invited to join the initiative. The resulting group of researchers has worked on different topics. One topic has been to define sets of proteins of interest for understanding diabetes and identify how these sets could be measured and analyzed in an efficient way. Information from these workshops has been disseminated to the general public by the HDPP homepage (http://www.hdpp.info).

The 8th HDPP workshop, to be held at the HUPO 2015 meeting in Vancouver, will focus on how sets of proteins of interest discovered by classical omics strategies can be translated in robust assay development to be applied on large cohort of patients. Among the speakers are Dave Goodlett and Ann-Catrin Andersson, who together with the chair and co-chair of the session will address the topic.

HPP 05: HUMAN ANTIBODY INITIATIVE
MONDAY, SEPTEMBER 28, 2015 – 07:30 – 09:00

HPP 05 Human Antibody Initiative

The recent discussions within the scientific community and in journals about reproducibility and the quality of antibodies throw light on topics that impact the daily work of most life science researchers. Antibodies and other affinity reagents are commonly used tools across all disciplines of life science, including proteomics, and they are well known to be cross-reactive and have variable success depending on the application. There is a strong need to improve reproducibility in antibody-based proteomic research. Undertaking a number of steps will help reach this goal; first of all affinity reagents must be properly identified and secondly proper validated for the particular application is needed. This session will focus on research programs and initiatives working on identification and quality improvement for affinity reagents, and how we all can contribute to higher quality in proteomic research.

HPP 06: EYEOME - PROTEOMICS: TOWARDS UNDERSTANDING BIOLOGICAL PATHWAYS IN THE EYE
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HPP 06 EyeOME - Proteomics: Towards Understanding Biological Pathways In The Eye

The EyeOME session has eight speakers who place an emphasis upon biological pathways in the eye that have been revealed through proteomic approaches. The topics include identification of pathways and biomarkers associated with age-related macular degeneration through study of aqueous humor (Hyewon Chung) and tears (Lei Zhou) using SWATH-MS, use of laser capture microdissection and pressure cycle technology to study specific retinal substructures (Sascha Dammeier), and discovery of molecular networks in diabetic retinopathy using a novel transgenic pig model (Stefanie Hauck). New mechanisms of glaucoma have been found through proteomic investigation of the trabecular meshwork (Sanjoy Bhattacharya) and retina (Franz Grus). SWATH-MS has been used to identify dynamic changes in the proteome of human induced pluripotent stem cells in their differentiation to retinal pigment epithelial cells (Richard Semba). A screening approach was used to identify retinal substrates for a disease-associated variant in the protease CAPN5 (Vinit Mahajan).

HPP 06: EYEOME - PROTEOMICS: TOWARDS UNDERSTANDING BIOLOGICAL PATHWAYS IN THE EYE
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HPP06.01 SWATH-based Comparative Proteomic Analysis of the Aqueous Humor in Patients with Dry Age-related Macular Degeneration

Hyewon Chung[1], Suk Ho Byeon[2], Daehan Lim[1], Jin Kim[1], Yun Jeong Kim[1], Je-Hyun Baek[3]
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Abstract: The molecular mechanisms underlying dry age-related macular degeneration (AMD) remain largely unknown; there has been no proven treatment for dry AMD and its progression to geographic atrophy or neovascular AMD as of yet. To uncover novel biomarkers related to the pathogenesis of dry AMD, we first investigated the aqueous humor (AH) proteome using a data-independent acquisition method (SWATH-MS) for dry AMD patients according to their phenotypes, including: soft drusen, reticular pseudodrusen and geographic atrophy. These were compared to controls. The SWATH-MS experiments were evaluated by quality controls with a beta-Galactosidase digest, confirming the repeatability of a proteomics workflow. In this study, we identified 160 proteins (FDR 1%) from dry AMD patients and control subjects and extracted quantitative information from 104 proteins using SWATH-MS, which enables fast and reproducible conversion of a limited amount of clinical samples such as AH into a permanent digital file representing a proteome of the samples. Extracellular superoxide dismutase, apolipoprotein A-I, actin (alpha 1), and 6 other proteins increased while pigment epithelium-derived factor and cathepsin D, plus 8 other proteins decreased in the AH of dry AMD patients compared to controls. Identifying such proteins could enhance our understanding of the protein-based etiology of this multifactorial disease and enable the matching of specific markers to specific target-based therapies in AMD patients with various phenotypes.

HPP 06: EYEOME - PROTEOMICS: TOWARDS UNDERSTANDING BIOLOGICAL PATHWAYS IN THE EYE
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HPP06.02 Tear Protein Biomarkers for Detection of Age-Related Macular Degeneration

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Abstract: Age-related macular degeneration (AMD) is the leading cause of irreversible blindness in elderly people in Western countries and increasingly in Asia. Early detection has the potential of significantly improving the visual outcome of patients. The aim of this study was to determine whether tear protein biomarkers can be detected that would differentiate patients with AMD as compared to healthy controls. Tear samples were collected from 22 wet AMD and 10 age-matched normal controls using a Schirmer's type I procedure. Tear proteins were analyzed with 1D nanoLC-MS/MS using SWATH technology. Human tear samples from 1000 healthy subjects with no eye complaints (411 male, 589 female, average age 55.5 years, SD 14.5 years) were pooled into a single global control sample for building human tear protein spectra library. A human tear protein spectra library was established which included 1508 proteins generated from combining of 30 LC-MS/MS runs using information dependent acquisition (IDA) mode. Close to 700 tear proteins were quantified and at least 70 tear proteins showed significant changes (ratio of AMD/Control: > 1.5, or < 0.67; $p < 0.05$) between AMD group and control group. Pathway analysis implicates the angiotensin system (p-value: 2.61E-09, FDR: 4.22E-07) and kallikrein system (p-value: 8.16E-09, FDR: 6.61E-07) as the top two pathways which may be involved in the pathogenesis of wet AMD after enrichment using MetaCore. Tear protein biomarker candidates identified in this study may be useful as a tool for screening AMD.

HPP 06: EYEOME - PROTEOMICS: TOWARDS UNDERSTANDING BIOLOGICAL PATHWAYS IN THE EYE
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HPP06.03 Use of Laser Capture Microdissection with Pressure Cycle Sample Preparation to Analyze the Proteome of Retinal Sub-Structures

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Abstract: The combination of laser capture microdissection (LCM) and sample preparation based on pressure cycle technology (PCT) provides a

means to study proteins that are specific for cell subpopulations or anatomic regions. This proof-of-concept study examined whether this approach can achieve separation of retinal sub-structures, e.g. outer segments of photoreceptors, from the surrounding tissue and detect proteins characteristic to these specialized structures. Eyes of wild-type mice were removed and fixed with cross-linking (10% buffered formalin) or denaturing (methanol or acetone) fixatives. We examined the ability of these commonly used fixative regimens to preserve both cell morphology and protein quality. LCM was used to isolate minute amounts of outer segments (~200,000 mm^3). Catapulted tissue patches were either processed directly or subjected to PCT, in order to facilitate bottom-up proteomic analysis. Methanol was the best fixative for tissue preservation, while acetone fixation increased the number of identified proteins substantially (PFA: 771 vs. MeOH: 681 vs. acetone: 1154). Nevertheless, tissue morphology was inadequate for proper isolation of outer segments in this latter case. Formalin fixation gave best overall results. More than 300 proteins of the outer segments were identified without PCT, whereas PCT not only abridged processing, but vastly improved protein identification to greater than 700 identifications, while elevating the average sequence coverage 1.8-fold. The results presented here suggest that the combined use of LCM and PCT during proteomic sample preparation represents a promising tool to be effective in detecting protein profiles within the eye specific to a cell type or to certain pathologies.

HPP 06: EYEOME - PROTEOMICS: TOWARDS UNDERSTANDING BIOLOGICAL PATHWAYS IN THE EYE
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HPP06.04 Ocular Pathology in the INSC94Y Transgenic Pig, a Novel Model for Diabetic Retinopathy

Cornelia A. Deeg[1], Patrizia B. Uhl[1], Barbara Amann[2], Andreas Blutke[3], Simone Renner[4], Sieglinde Hirmer[1], Marius Ueffing[5], Juliane Merl-Pham[5], Ruediger Wanke[3], Eckhard Wolf[6], Stefanie M. Hauck[5]
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Abstract: Diabetes mellitus is associated with sight threatening pathophysiology at different sites of the eye. Diabetic retinopathy (DR) is a major cause of blindness, because of two vision threating complications, proliferative retinopathy and significant macular edema. Good animal models that enable investigation of underlying pathomechanisms are currently lacking. Therefore, we analyzed and characterized the ocular phenotype of the novel model INS^{C94Y} transgenic pigs with differential proteome analyses and immunohistochemistry. Interestingly, in all INS^{C94Y} transgenic pigs, besides cataract formation, there was significant macular edema detectable. Further, inflammation, microglia activation and a change in retinal vessels was evident. These changes are very similar to pathophysiology in DR patients; therefore this novel animal model is well suited to investigate molecular mechanisms. Differential proteome analyses (label-free LC-MSMS) revealed several clusters of significantly altered proteins in eyes of INS^{C94Y} pigs, indicating a significant role of these molecular networks in DR. Upregulated proteins clustered to interesting canonical pathways in INS^{C94Y} pigs, for example to “role of pi3k subunit p85”, “regulation of actin organization and cell migration”, “RAC1 signaling pathway”, “C-MYC transcriptional activation”, “CDC42 signaling events”, “basic mechanisms of sumoylation” and “calcium Wnt signaling pathway”. In contrast, proteins from molecular pathways “visual signal transduction: rods”, “visual signal transduction: cones”, “endogenous TLR signaling”, “posttranslational regulation of adherens junction stability and disassembly” and “amb2 integrin signaling” were significantly lower abundant in INS^{C94Y} pigs. This comprehensive molecular phenotyping of a relevant model for human DR offer the unique opportunity to discover novel molecular events driving pathology and potentially novel treatable targets.

HPP 06: EYEOME - PROTEOMICS: TOWARDS UNDERSTANDING BIOLOGICAL PATHWAYS IN THE EYE
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HPP06.05 Lack of Basement Membrane Protein Degradation in Glaucomatous Trabecular Meshwork

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Abstract: Glaucoma is a group of progressive blinding disease affecting over 65 million people worldwide. The disease is frequently characterized by elevated intraocular pressure (IOP). Elevated IOP in glaucoma is due to impeded aqueous humor (AH) outflow at the level of trabecular meshwork (TM). The AH flows from the TM into Schlemm’s canal (SC). Developmentally, SC cells elicit a blend of endothelial and lymphatic cell characteristics. For example, they show expression of the lymphatic marker Prox1 but lack expression of Lyve1. Basement membrane (BM) protein mutations, such as that of LTBP2, have been found in glaucoma. Changes in BM proteins in the TM remains poorly investigated in glaucoma. We aim to determine if BM proteins undergo a significant change in glaucomatous TM. Basement membranes from normal and glaucomatous cadaver eyes were prepared with a detergent solution followed by deglycosylation and additional solubilization steps. The sample was then separated on a 4-15% 1D SDS PAGE gel under reducing conditions. The gel bands were trypsin digested and subjected to iTRAQ labeling following established procedures. The selected low abundance BM proteins (LTBP2, Gasdermin, alpha-tectorin, WolframinS1) were elevated in glaucomatous TM. Western analyses show that increased level of these proteins in glaucomatous TM are contributed by full-length molecular weight proteins. Taken together with other independent experimental investigations, our results are consistent with decreased degradation of BM proteins in glaucoma. BM protein degradation is significantly reduced in glaucomatous TM for selected proteins suggesting possible implications of this component in the development of increased intraocular pressure.

HPP 06: EYEOME - PROTEOMICS: TOWARDS UNDERSTANDING BIOLOGICAL PATHWAYS IN THE EYE
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HPP06.06 Dynamic Changes in the Proteome During the Differentiation of Human Induced Pluripotent Stem Cells into Retinal Pigment Epithelium

Richard Semba[1], Pingbo Zhang[2], Alexey Lyashkov[3], Michelle Shardell[3], Ceereena Ubaida-Mohien[3], Srinivasa Rao Sripathi[1], James T. Handa[1], Karl Wahlin[1], Donald J. Zack[1], Luigi Ferrucci[4]
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Abstract: Induced pluripotent stem cell (iPS)-based approaches show great promise in the treatment of age-related macular degeneration (AMD). Safe, simple, efficient, and affordable protocols are needed to generate retinal pigment epithelium (RPE) from a patient’s own stem cells. A major goal in stem cell research is a molecular understanding of pluripotency and differentiation. The intracellular signaling events and key regulators during the differentiation of iPS to RPE are not well characterized. We compared the

proteome of iPS cells, line IMR90-4, in their differentiation into iPS-derived RPE at days 0, 30, and 58. Whole cell lysates were prepared for LC-MS/MS and analyzed using SWATH on a Sciex 5600+ TripleTOF mass spectrometer. We identified 2689 non-redundant, quantifiable proteins (1670, 1927, and 2293 proteins at days 0, 30, and 58, respectively). Pathway overrepresentation analysis revealed many regulated pathways involved in cell differentiation and pluripotency. There was enrichment of pathways involving cell cycle regulation and DNA synthesis at the iPS stage and extracellular matrix regulation and several microRNA regulated pathways at the differentiated stage. Further studies are in progress to examine more detailed time-dependent changes in the proteome during differentiation of iPS to RPE.

HPP 06: EYEOME - PROTEOMICS: TOWARDS UNDERSTANDING BIOLOGICAL PATHWAYS IN THE EYE
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HPP06.07 Glaucoma-Associated Proteomic Changes Provide New Insights in Neurodegenerative Pathways

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Abstract: Glaucoma as a neurodegenerative disease is one of the leading causes of blindness worldwide. In previous studies we could demonstrate the involvement of an autoimmune component in the disease and that immunoproteomic approaches could detect the disease by a sensitivity and specificity of more than 90%. In this study, we were interested to explore if proteomics of retina and tear fluid of glaucoma patients could provide further hints of involved pathways in glaucoma. By use of a state-of-the-art mass spectrometric platform we could achieve a deep inside view to the human retinal proteome under glaucomatous neurodegenerative condition mapping more than 600 retinal proteins. Importantly, we found distinct changes in the abundance of retinal proteins related to glaucoma disease. Many of the altered retinal proteins are associated to apoptotic pathways, thus reflecting cellular stress condition, e.g. increased crystalline, cytochrome c oxidase or glutathione S-transferase Mu 3 levels. Especially, mitochondrial proteins indicating metabolic changes of stressed cells like ADP/ATP translocase 3 were affected in the course of glaucomatous progression. Importantly, diminished nuclear proteins like methyl-CpG-protein 2, known to modulate gene expression, open a new perspective to regulation of neurodegenerative pathways. Beside intracellular retinal changes, proteomic changes could be observed in the tear fluid of glaucoma patients shifting diagnostic potential to a non-invasive available patient sample material. Furthermore inflammatory and stress related secretory tear proteins, intracellular proteins observed in the tear film of glaucoma patients could give new insights in neurodegenerative processes underlying glaucoma disease. Especially, the role of intracellular enzymes detected in the human tear film, e.g. protein glutamine gamma glutamyl transferases 2 as well as retinal dehydrogenase, provide important hints in understanding metabolic pathways in glaucoma patients.

HPP 06: EYEOME - PROTEOMICS: TOWARDS UNDERSTANDING BIOLOGICAL PATHWAYS IN THE EYE
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HPP06.08 Protease Proteomics: Global Identification of Biological Pathways that Activate Uveitis, Retinal Angiogenesis and Degeneration, and Intraocular Fibrosis

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Abstract: Proteases activate key intracellular disease signaling pathways, but identification of substrates is a limiting factor in mechanism-based therapies. Mutation in the calcium-activated, regulatory protease CAPN5 (calpain-5) causes a complex ocular phenotype that mimics posterior uveitis, proliferative diabetic retinopathy, retinitis pigmentosa, and proliferative vitreoretinopathy. The mutant differs at a single amino acid and is proteolytically overactive, even at low calcium levels. Natural retinal targets of CAPN5 were determined in an unbiased screen for protein cleavage events as detected by mass spectrometry analysis of SDS-PAGE mobility of proteins and proteolytic fragments in the presence and absence of active CAPN5. This global proteomics analysis suggested several hundred possible candidates, so cleavage by CAPN5 was verified in in vitro activity assays and with a highly selective calpain inhibitor and in vivo. The screen identified known calpain substrates, substrate networks, and over 30 novel substrates that implicate several signaling pathways in pathogenesis (i.e., synaptic plasticity, phototransduction, angiogenesis, fibrosis, autoimmune inflammation, and necrosis). Two of the substrates were also validated in CAPN5 patients. Since elevated calcium and calpain activation is a common event in a various retinal diseases, these findings provide critical insight into protease-dependent biochemical events that can be therapeutically targeted.

HPP 07: HPP BIOINFORMATIC SESSION
MONDAY, SEPTEMBER 28, 2015 – 13:15 – 14:15

HPP 07 HPP Bioinformatic Session

Abstract: In this workshop, the current and future HPP data guidelines will be discussed. The goal of the workshop is to understand where the existing guidelines have served the HPP well and poorly, and discuss a proposed set of new guidelines, specifically in the context of advancing confident detections of missing proteins. Each subtopic will be introduced separately, followed by discussion by the workshop participants. Full audience participation is encouraged. The major subtopics include: current HPP data guidelines, data deposition in ProteomeXchange, the 1% protein-level FDR requirement, manual inspection of extraordinary claims, consideration of alternate explanations of the data, use of synthetic reference peptides, and the use of SRM to confirm shotgun results.

HPP 08: CPTAC HPP - CPTAC DATA, TOOLS, AND ASSAYS FOR CANCER BIOLOGY
TUESDAY, SEPTEMBER 29, 2015 – 07:30 – 09:00

HPP 08 CPTAC HPP - CPTAC Data, Tools, And Assays For Cancer Biology

The Clinical Proteomics Tumor Analysis Consortium represents a network of proteomic scientists at multiple locations who coordinate research approaches and data sharing to comprehensively interrogate genomically characterized specimens, initially obtained from The Cancer Genome Atlas collection of highly annotated tumor samples. CPTAC is dedicated to combining state-of-the-art standardized proteomic technologies with genomic analysis and a technological tour de force approach in order to deeply interrogate biospecimen cohorts sized for adequate statistical power. The desired outcome is not only new insights into cancer but also the development of publicly available tools (data, assays, reagents) in proteomics for future studies by the cancer research community. In this workshop CPTAC investigators will describe the tools under development as part of the initial analysis of TCGA tumor specimens, leading to a discussion of the best strategies for moving forward to develop the most informative targeted assays of disease insight.

HPP 09: PROTEOMICS STANDARDS INITIATIVE AND PROTEOMEXCHANGE CONSORTIUM
TUESDAY, SEPTEMBER 29, 2015 – 07:30 – 09:00

HPP 09 Proteomics Standards Initiative And Proteomexchange Consortium

The HUPO Proteomics Standards Initiative (PSI) defines community standards for data representation in proteomics to facilitate data comparison, exchange and verification. We contribute to data management and integration in all other HUPO initiatives, and have published a modular set of standards for proteomics data representation. For details of the HUPO PSI please see http://www.psidev.info. Based on the HUPO PSI standards, we have developed ProteomeXchange, an international consortium to standardize collection and dissemination of public proteomics data worldwide (http://www.proteomexchange.org). In this session, we will provide an overview of current PSI activities, with an emphasis on the formats currently in development that need further input from the community. We will also present the current status and future plans for aspects of data deposition in ProteomeXchange. In addition to presentation, there will be time for open discussion and feedback from the community on these activities.

HPP 10: PROTEOMICS STANDARDS INITIATIVE AND PROTEOMEXCHANGE CONSORTIUM
TUESDAY, SEPTEMBER 29, 2015 – 07:30 – 09:00

HPP 10 Proteomics Standards Initiative And Proteomexchange Consortium

Session theme

Coordination of proteomics and glycomics for establishment of Glycoproteome Atlas

Most of proteins in the living body are glycosylated and present as glycoproteins. In the previous glycomics initiatives, many technologies were focusing on the analysis of glycans released from glycoproteins. Since two years ago, the Biology/Disease-driven Glycoproteome Project has been aiming at development of new glycoproteomics technologies that are applicable to the biological and medical studies. Glycoproteomics is an Omics-technology for analysis of (1) glycosylation sites, (2) glycan structures, and (3) peptide sequences of intact glycopeptides. This workshop introduces the latest glycoproteomics technologies with six leading researchers who would provide us the recent updates on MS-based glycoproteomics approach, lectin array-based glycan profiling and its application, and perspective on rapidly advancing glycan-related databases. Although there still remain technical difficulties toward the "perfect" glycoproteomics analysis of biological samples such as serum and tissue, we will discuss the possibility of our current technologies for application in the biological and medical areas.

HPP 12: HBPP - BRAIN
TUESDAY, SEPTEMBER 29, 2015 – 07:30 – 09:00

HPP 12 HBPP - Brain

The HUPO Brain Proteome Project (HBPP) is an international interdisciplinary initiative focusing the investigation of the human brain. The brain is the most complex organ of the human system, which shows various patterns of different tissue layers and cells. In order to investigate the function of the human brain the HBPP members use a broad spectrum of methods combined with a couple of functional analyses such as: mass spectrometry coupled with label free quantification studies, immunohistochemistry, immunological techniques and laser-micro dissection. The strong cooperation between scientist from different fields (affinity proteomics, bioinformatics and biostatistics, proteomics, analytical biotechnology, clinical science, neurobiology, biochemistry, neuroanatomy, neuropathology) is triggered by the aim to understand the biogenesis of neurodegenerative diseases. Special attention is given to the biogenesis of the most common form of dementia, Alzheimer's Disease (AD), Parkinson's Disease (PD), Frontotemporal Dementia (FTD) and other neurodegenerative diseases are topics of the HBPP initiative. The search for biomarkers is also an essential task of the HBPP initiative.

The 24th HUPO Brain Proteome Project (HBPP) workshop will focus on new insights in methods of biomarker research of neurodegenerative diseases. A special tribute will be given to the most important part of the human brain, the hippocampus. But also new hypothesis of the biogenesis of neurodegenerative diseases will be discussed at the 24th HBPP workshop.

The HUPO BPP is an open project – thus, anyone interested in the project shall be welcomed cordially. The latest information will always be publicly available at http://www.hbpp.org.

HPP 13: PEDIOME – PAEDIATRICS AND PROTEOMICS: BACK TO THE BEGINNING
TUESDAY, SEPTEMBER 29, 2015 – 07:30 – 09:00

HPP 13 PEDIOME – Paediatrics And Proteomics: Back To The Beginning

In this session recent advances in pediatric proteomics will be presented and discussed. The presentations will highlight the use of a wide range of proteomics technologies in different clinically relevant settings pertaining to pediatric patient population. Examples will include the application of a wide range of technologies including iTRAQ, SILAC, label free quantification, data independent acquisition methods, SomaScan and ELISA to identify and verify biomarkers for a wide range of pediatric diseases: i) diagnostic urinary biomarkers for different causes of abdominal pain, ii) prognostic serum biomarkers to prognosticate onset and/or activity of Type 1 Diabetes (T1D) before the appearance of T1D associated autoantibodies, and iii) predictive serum biomarkers to predict disease progression and response to therapies Duchene muscular dystrophy patients. A part of the session will be devoted to a discussion led by the session chairs about the progress of the PediOme to date, as well as the specific plan for future progress of this important initiative.

HPP 15: MTHPP (MITOCHONDRIA)
WEDNESDAY, SEPTEMBER 30, 2015 – 07:30 – 09:00

HPP 15 mtHPP (Mitochondria)

In this session the speakers will focus on the update of the mitochondrial HPP project, an initiative started as an activity of the Italian Proteome Association and subsequently extended to involve collaborators from other countries. After a broad summary of previous activities, four talks will span from interactomics to metabolism, from methodology to spatial proteomics. Sufficient time is allotted for general discussion at the end of the session.

HPP 16: HUMAN LIVER PROTEOME INITIATIVE
WEDNESDAY, SEPTEMBER 30, 2015 – 07:30 – 09:00

HPP 16 Human Liver Proteome Initiative

Topics for discussion:

- Configuration of protein priority lists in regard of liver physiology and disease (NAFLD, liver fibrosis, HCC).
- Proteogenomics in the liver (interactions with TCGA and CPTAC).
- Meetings

Abstract: The liver is a central organ in human body that controls metabolic homeostasis, provide essential substances to the organism and allow detoxification of xenobiotics. In addition to its biological function, liver physiology is peculiar in different aspects, including its regeneration capacity. Despite the intense research performed during the last couple of decades,there are still many open questions in regard of the molecular mechanisms underlying liver function and, most importantly, liver disease. This constraint largely restricts the development of more efficient diagnostic and therapeutic strategies for the better management of the patients. In the postgenomic era, the Human Liver Initiative started in 2002 aiming to define a comprehensive and dynamic map of the human liver proteome. During this session latest results about key issues in liver biology will be presented and discussed, including methods to define cell specific proteome profiles within the liver, definition of factors responsible for maintaining the differentiated phenotype of hepatocytes and mechanisms by which regulation of the protein methylation pattern might contribute to liver injury.

HPP 17: IMOP (MULTI ORGANISM PROTEOMES)
WEDNESDAY, SEPTEMBER 30, 2015 – 07:30 – 09:00

HPP 17 iMOP (Multi Organism Proteomes)

iMOP, the initiative on multi organism proteomes, aims to present proteome research from non-human species. iMOP members are working with classical animal models to study human diseases but also focus on a wide range of species which greatly affect human health, including farm animals and crop species, pathogens, as well as the microbiome of humans and farm animals. This year's workshop will include presentations on animal models for studying inflammatory bowel disease (Allan Stensballe) and protegenomic approaches to improving the health state of honeybees (Leonard Foster), HipA-mediated mechanisms in E.coli (Maja Semanjscki), and studies of mucosal microbiota from rat colon, (Nico Jehmlich). As always, we will include a plenum discussion on how the iMOP community can improve proteome research in non-human species.

HPP 18A: HUMAN PROTEOMICS AT EXTREME CONDITIONS
WEDNESDAY, SEPTEMBER 30, 2015 – 07:30 – 08:15

HPP 18a Human Proteomics at Extreme Conditions

This will be the second session of the Extreme Conditions initiative. This year discussion of research in molecular bases of physiology of sport of high achievements will be included into the program.

HPP 18B: SKELETAL MUSCLE PROTEOME
WEDNESDAY, SEPTEMBER 30, 2015 – 08:15 – 09:00

HPP18b.01 Mapping the Skeletal Muscle Proteome: A Functional Approach

Luigi Ferrucci[1], Kurt Hojlund[2]
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Abstract: Maintenance of the contractile function and energy metabolism of skeletal muscle is essential for autonomy and quality of life in humans. Though, the health and function of muscle is threatened in many situations. Muscle dystrophy in its many forms is the main genetic diseases affecting children and young adults. A progressive decline in muscle mass and strength as well as changes in energy metabolism and mitochondria in muscle are a hallmarks of aging that may cause physical disability. A rapid decline in muscle mass, often referred as cachexia, accompanies debilitating diseases, such as chronic infections, cancer, kidney failure and heart failure. Common chronic diseases, such as diabetes, autoimmune diseases and osteoarthritis often accelerate the age-associated decline in muscle mass and contribute to disability. The specific mechanisms that lead to impaired muscle function in these conditions have been poorly characterized but probably include functional anomalies in energetic metabolism. Etiological diagnoses are based on medical history and physical examination complemented by genetic studies and histological examination of biopsies, which are laborious and have limited sensitivity and specificity. A comprehensive proteomic analysis of skeletal muscle biopsies may increase our understanding of the mechanisms leading to different clinical forms of muscle pathology and also improve diagnosis. Likely specific skeletal muscle proteins undergo specific changes in abundance and/or post translational modifications (PTMs) such as e.g. phosphorylation and acetylation in aging, type 2 diabetes and different other pathologies and the stochiometric relationships between different proteins and their PTMs change as well. A number of studies have reported maps of skeletal muscle proteins humans, using a number of different discovery-mode and targeted proteomic technologies, usually in small sample on unselected individuals or patients with selected diseases. An assemblage of the data from these studies suggests that as many as 7000 proteins can be detected in muscle tissues. However, an analysis of changes in muscle proteins with aging in individuals free of major diseases and drug treatments is currently not available. These data could serve as a critical reference to dissect changes in proteins that occur with disease as opposed to "normal" aging, therefore increasing their contribution in our understanding of musculoskeletal diseases and expanding their diagnostic value.

In this session, we will provide both an introductory overview of the potential values and uses of skeletal muscle proteomics in aging, type 2 diabetes and other diseases. We present the design and preliminary data from the Genetic and Epigenetic Signatures of Translational Aging Laboratory Testing (GESTALT) a study performed by the Intramural Program of the National Institute on Aging in Baltimore whose aims include the characterization of muscle proteins with aging in a group of very healthy men and women dispersed over a wide age range (20-100 years). Finally, we present studies of the proteomes and phosphoproteomes of human skeletal muscle and isolated muscle mitochondria, as well as quantitative studies of changes in the muscle proteome associated with obesity and type 2 diabetes.

Both the introductory remarks and the examples reported should stimulate a wider interest in proteomic characterization of muscle in humans and, hopefully, will encourage more collaborative research between international groups of this important research topic.

HPP 19 Infectious Diseases (HID)-BD-HPP

The HID-BD-HPP initiative was established last year at the HUPO-2014 in Madrid. The main goal of this initiative is to organize a community of scientists working in Infectious disease proteomics. Infectious diseases are caused by pathogenic microorganisms, such as bacteria, viruses, parasites or fungi. These infectious are a leading cause of illness and death throughout the world, in particular in low income countries. Lower respiratory infections, HIV/AIDS and diarrheal diseases are ranked in the top ten causes of death globally, whereas malaria and tuberculosis are two of the major ten causes of death in low income countries. In developed countries infectious diseases are also important in immunosuppressed patients and transplant recipients. New diagnostic tests, therapeutic agents and vaccines are required to control these infectious diseases. International collaboration of scientists working in Infectious diseases and proteomics is essential to promote these researches.

Topics for discussion:

- How can the scientists working in Infectious disease proteomics collaborate?
- Development of SRM methods for detection and quantification of human and microbial proteins
- Detection and quantification of these proteins by affinity or antibodies based technologies
- Building of a web page to freely-access data
- Diffusion of HID-HPP activities (reports, special issues,..)
- Organization and future actions.

HPP 20: PROTEOMICS OF PROTEIN MISFOLDING AND AGGREGATION DISEASES
WEDNESDAY, SEPTEMBER 30, 2015 – 07:30 – 09:00

HPP 20: Proteomics of Protein Misfolding and Aggregation Diseases

Protein misfolding and aggregation diseases (PMAD), exemplified by Parkinson's or Alzheimer's disease and systemic amyloidoses, are characterized by an abnormal deposition of protein aggregates of regular three-dimensional structure (amyloid). The B/D PMAD working group aims at developing proteomics assays for proteins that are relevant to the study, diagnosis and therapy of protein aggregation diseases. These assays are tested and refined on a set of relevant patient samples (for clinical applications) and on samples from model organisms and cell culture (for basic research). Besides developing assays for measuring protein abundances, a peculiarity of our initiative is that it will attempt also the development of proteomics assays for "aberrant protein conformations", those typically generated in PMADs. In this workshop of the PMAD working group we will present the current status of the project, summarize the assays for PMAD targets developed and validated so far and discuss future directions.

INTEREST GROUPS

EDRN WORKSHOP: PROTEOMIC BIOMARKERS FOR CANCER DETECTION AND DIAGNOSIS IN PRECISION MEDICINE
MONDAY, SEPTEMBER 28, 2015 – 13:15 – 14:15

EDRN WORKSHOP: Proteomic Biomarkers For Cancer Detection And Diagnosis In Precision Medicine

The development of new biomarkers for early cancer detection that can change clinical practice and ultimately have an impact on overall survival and mortality from the disease is a lengthy process that begins with the discovery of promising candidate biomarkers, rigorous validation, and implementation in the clinic. The success of this process requires a complex, dedicated infrastructure that facilitates the coordination, management and collaboration among many institutions, both from academia and industry, with the involvement of scientists and clinicians with diverse expertise. Since its inception, the main focus of the EDRN has been to bring new biomarkers to clinical validation. Early on, EDRN investigators recognized that the biomarker field was quite nascent, and consequently took on the responsibility to establishing guidelines for a phase-based biomarker development, as well as study design criteria for rigorous clinical validation. These have now been well accepted and adopted by the biomarker research community at large.

Over the past five years, EDRN investigators invested significant efforts for enriching the proteomic biomarker development pipeline to address significant unmet clinical needs in the early detection of cancer. The proposed session will highlight the proteomics in early cancer detection and its implication for precision medicine.

WORKSHOP: TRANSLATING PROTEOMICS IN DIABETES AND METABOLIC DISEASES
TUESDAY, SEPTEMBER 29, 2015 – 07:30 – 09:00

WORKSHOP: Translating Proteomics In Diabetes And Metabolic Diseases

The aim of the workshop is to present some novel aspects on diabetes and metabolic diseases and their complications thus stimulating discussions between people addressing these topics by using different approaches. The format of the workshop will be six short presentations followed by a general discussion. The presentations will cover omics, cellular and patient data giving new aspects of pathophysiology and biomarkers of diabetes and metabolic disease. The translational approach of the workshop prompted the involvement of the HUPO journal “Translational Proteomics” that will publish a special issue to be entitled “Diabetes and Metabolic Disease”.

AFFINITY - MASS SPECTROMETRY WORKSHOP
TUESDAY, SEPTEMBER 29, 2015 – 07:30 – 09:00

Affinity - Mass Spectrometry Workshop

This workshop focuses on research activities and methodology development of affinity isolation and determination strategies combined with mass spectrometry. Pre-fractionation methods are the key issue when it comes to addressing the complexity of biological samples and when analyzing low abundant analytes from biological/clinical material. Many applications of affinity-based separation methods have been used and are continuously developed to increase the selectivity and sensitivity in mass spectrometry-based proteomics; now spreading out into many scientific and medical application areas.

Lately, immuno-affinity approaches which make use of defined sets of specific capture molecules such as antibodies have enabled the rapid and unequivocal identification of binding structures and surfaces (e.g. epitopes) by mass spectrometry, particularly by new developments of online MS approaches. With novel state-of-the-art “wet-lab” as well as “dry-lab” developments quantitative and functional analyses in model studies and even in patient samples can be tackled using affinity-MS procedures, thereby opening the field of structure- /function correlations with broad application potentials in cutting-edge research areas.

WORKSHOP: PROTEOMICS IN FOOD AND NUTRITION
TUESDAY, SEPTEMBER 29, 2015 – 07:30 – 09:00

WORKSHOP: Proteomics In Food And Nutrition

The central role of food proteins in nutritional science are indisputable, the well known affirmation that ‘we are what we eat’ and that food can be our medicine is not just philosophy but a true story. In fact it is clear that the food after ingestion is transformed and also contains proteins that our body uses, in a complex direct relationship. Study of the proteome of a given food makes us aware that, once it has been ingested and transformed by the human organism, it can change the structure of the proteins in the latter. Furthermore, every nutritional process involves huge number of proteins that are expressed at different levels, from cell to whole organism. Moreover, the global composition of diet, from microbiome to nutrient, including life style, can affect every step from gene expression to protein synthesis until degradation, lead to modulation of metabolic function in a multi-factorial way. Proteomics can help personalized medicine and nutrition, play important role in solving major nutrition problem in human and animals, on the verge of one health approach, including obesity, metabolic and cardiovascular disease, cancer, ageing, allergy and fetal health and development, also in relation to gut microbiome. Profiling food, microbiome, and biomarkers of nutritional status and disease from proteomics point of view lead to a new pillar of personalized medicine. This include also a special focus to food safety issues, providing new insights relate to safety aspects, from microbioma and consortia, food authenticity, detection of animal species in the food, until identification of food allergens.

AFFINITY - MASS SPECTROMETRY WORKSHOP
TUESDAY, SEPTEMBER 29, 2015 – 07:30 – 09:00

Risk Assessment of Preterm Newborns by Affinity Mass Spectrometry

Michael O. Glocker
Proteome Center Rostock, University Medicine Rostock, Rostock, Germany

Abstract: Mass spectrometric profiling of severe forms of pregnancy complications has been successful with cord blood serum upon affinity enrichment of serum proteins using a magnetic bead system [1-3]. As of now, plasma or serum is prepared directly in the clinics immediately after blood withdrawal. Yet, one of the remaining challenges for making a mass spectrometry-based profiling assay attractive for clinical use is to bridge the distance between the delivery room and the mass spectrometry laboratory. To overcome hitherto existing limitations we have developed a robust and reliable sample delivery system making use of a novel membrane-based serum / plasma storage device. The key step for success is resolubilization of intact proteins whose relative abundances are individually profiled by MALDI-ToF mass spectrometry.

Serum proteins can be stored intact at room temperature for weeks and shipped by regular mail in a clean envelope without loss of quality when

deposited on a "plasma collection disc" (Noviplex TM). Proteins can be eluted from the "plasma collection disc" using detergent-containing solutions (Rapigest TM) at pH 8. Transfer of such resolubilized serum proteins onto bead surfaces (ClinProt TM) is possible by acidification to pH 3, by which the acid-labile detergent is destroyed and the solubilized serum proteins readily adsorb onto the magnetic bead surfaces. Intact serum proteins can be eluted from the hydrophobic bead surfaces into salt-free and volatile buffers that contain organic co-solvents. Proteins from such solutions are subjected to linear MALDI-ToF MS profiling.

We tested investigated cord blood serum samples from newborns. Two groups of premature babies, i.e. small for gestational age (SGA) and intra-uterine growth restricted (IUGR) infants, were analyzed. As babies from both groups are equally small at birth, a molecular pattern of cord blood was determined that allowed to differentiate the two. Multiparametric scoring and biostatistical analyses sort spectra from the IUGR samples into the IUGR group (true positive) and spectra from the control samples into the control group (true negative) with high confidence. The robustness of all involved steps makes this assay attractive to clinics world-wide. Clearly, mass spectrometric profiling of intact serum proteins desorbed from dried serum spots enables reliable differentiation between IUGR and control samples.

[1] M. Wölter, C. Röwer, C. Koy, T. Reimer, W. Rath, U. Pecks and M.O. Glocker (2012). A proteome signature for Intra-Uterine Growth Restriction derived from multifactorial analysis of mass spectrometry-based cord blood serum profiling. Electrophoresis, 33, 1881-1893.

2] M.O. Glocker, C. Röwer, M. Wölter, C. Koy, T. Reimer and U. Pecks (2014). Multiparametric analysis of mass spectrometry-based proteome profiling in gestation-related diseases. In: Handbook of Spectroscopy, 2nd ed., Wiley-VCH GmbH & Co. KgaA. G. Gauglitz and D.S. Moore (eds.). Part IV: Methods 3: Mass Spectrometry, Chapter 12, pages 407–428.

[3] U. Pecks, I. Kirschner, M. Wölter, D. Schlembach, C. Koy, W. Rath, and M.O. Glocker (2014). Mass Spectrometric Profiling of Cord Blood Serum Proteomes to Distinguish Intra-Uterine-Growth-Restricted Infants from Small-for-Gestational-Age and Control Individuals. Transl. Res. 164, 57-69.

AFFINITY - MASS SPECTROMETRY WORKSHOP
TUESDAY, SEPTEMBER 29, 2015 – 07:30 – 09:00

A Novel Mass Spectrometry-Based Approach in Immuno-Diagnostics

Petr Novak[1], Michael Volny[2], Petr Pompach[2]
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Abstract: Modern biochemical tests, used in medicine, veterinary, research and industry, are often based on the interaction of the protein with its biological partner. In case the protein is an antibody and the analyte an appropriate antigen, the test refers to the immunoassay. Mostly, the protein is anchored to a solid surface such as a plastic plate or magnetic beads. It enables manipulation after the antigen has been captured. The major advantage of this procedure is the test specificity and enrichment of the antigen, which significantly helps to improve the limit of detection and quantification. The antigen can be visualized by several different techniques like chemiluminescence, fluorescence and radiation. Since these techniques relays on the specificity of used antibody a novel detection procedure exploring desorption mass spectrometry has been introduced. Currently, there are several possibilities for antibody immobilization from non-covalent adhesion to covalent chemical cross-linking. Unfortunately none of them are perfect. In our pioneering study an extremely effective method has been discovered for preparation of functionalized surfaces, which can be used for both standard chemiluminescence detection and mass spectrometric analysis. The method combines native electrospray and reactive landing. Antibody is electrosprayed from solution to the gas phase in its native state and subsequently transferred using electrostatic lenses to a conductive surface. The potential for the immunodiagnostics is going to be discussed.

AFFINITY - MASS SPECTROMETRY WORKSHOP
TUESDAY, SEPTEMBER 29, 2015 – 07:30 – 09:00

iMALDI for Quantifying Akt1 and Akt2 Expression and Phosphorylation in Colorectal Cancer

Christoph H. Borchers[1], Robert Popp[1], Andrew G. Chambers[1], Adriana Aguilar-Mahecha[2], Oliver Poetz[3], Mark Basik[2]
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Abstract: Targeted treatment of colorectal cancer (CRC) only works in a minority of patients, and reliable methods to quantify signaling pathway activity are lacking. We therefore set out to develop immuno-MALDI (iMALDI) assays combined with on our phosphatase-based phosphopeptide (PPQ) quantitation approach to determine expression levels and stoichiometry of critical phosphorylation sites in Akt1 (P31749) and Akt2 (P31751) in cancer cells and tumors using a Bruker Microflex LRF benchtop MALDI-TOF instrument.

We have developed iMALDI assays to quantify tryptic non-phosphorylated Akt1 and Akt2 peptides (aa 466-480, and aa 468-481, respectively) within a linear range of 0.5 – 10 fmol from 10 µg lysate protein. We were able to quantify expression levels and phosphorylation stoichiometry of Akt1 and Akt2 from 10 µg protein of parental (Akt1: 3.4 ±0.3 fmol; Akt2: 2.3 ±0.04 fmol) and EGF-induced (Akt1: 3.9 ±0.1 fmol ; Akt2: 2.8 ±0.1 fmol) MDA-231 breast cancer cell lysates. As expected, EGF-induced cells showed increased phosphorylation stoichiometry (Akt1: 12.7 ±3.3%; Akt2: 19.8 ±5.2%) than parental cells (Akt1: 3.5 ±8.2%; Akt2: 4.9 ±7.5%). In addition, we showed the applicability of this method to measure cancer tissue lysates by determining expression levels and phosphorylation degree for so far two surgical breast cancer tissue samples.

In conclusion, we have developed iMALDI Akt1 and Akt2 assays for quantitation of expression levels and phosphorylation stoichiometry in cancer tissue samples. Next steps are optimization of precision, including automation of all liquid handling steps on an Agilent Bravo, full assay validation, and extension of biological samples to CRC tissue lysates.

AFFINITY - MASS SPECTROMETRY WORKSHOP
TUESDAY, SEPTEMBER 29, 2015 – 07:30 – 09:00

Studying Protein-Small Molecule Affinities by Quantitative Proteomics

Hans Christian Eberl
Cellzome GmbH, a GSK Company, Heidelberg, Germany

Abstract: The characterization of small molecule – protein interactions is one of the central challenges in the drug discovery process. This includes the identification of the primary target of a bioactive small molecule but also of off-targets related to adverse drug events. Chemical proteomics technologies enable to quantitatively assess compound selectivity against the proteome under (close to) physiological conditions.In this course I will present two complementary approaches, affinity enrichment-based chemoproteomics and thermal proteome profiling, which enable unbiased target identification and affinity determination of small molecules to endogenous proteins. In chemoproteomics a small molecule is immobilized on a solid support to enable affinity enrichment of target proteins from

cell extracts. When combined with quantitative mass spectrometry, dose-dependent competitive binding experiments with free compound enable the simultaneous determination of potency and selectivity for all detected targets. In thermal proteome profiling, drug-target interactions are inferred from changes in the thermal stability of a protein. These experiments are performed either in lysate or on live cells and thus enable monitoring of drug targets and downstream effectors on a proteome-wide scale. Although focused on drug discovery, the approaches and principles presented here can be transferred to many other research areas.

AFFINITY - MASS SPECTROMETRY WORKSHOP
TUESDAY, SEPTEMBER 29, 2015 – 07:30 – 09:00

Multiplexed Protein Quantification Using Peptide Group-Specific Capture Molecules and Mass Spectrometry

Oliver Poetz[1], Weiss Frederik[1], Van Den Berg Bart1, Helen Hammer[1], Hannes Planatscher[1], Thomas O. Joos[2]
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Abstract: Mass spectrometry (MS) is an emerging tool for targeted protein quantification. However, usually a biological sample is too complex to be analyzed directly in an MS. Therefore the sample has to be pre-fractionated, which limits the sample through-put.Within the last few years, various novel approaches that employ a targeted enrichment of a small subset of peptides have been integrated in the proteomics workflow to speed up the process. Sample preparation strategies that reduce the complexity of tryptic digests by using immunoaffinity based methods lead to a substantial increase in throughput and sensitivity (Zhang et al. 2003, Anderson et al. 2004).One bottleneck in immunoaffinity based approaches is the availability of the appropriate peptide specific capture antibody. Here we present a strategy that uses short terminal specific antibodies – TXP Antibodies – designed for the enrichment of groups of peptides sharing the same terminal sequence (Poetz et al., 2009, Volk et al., 2012, Eisen et al. 2013, Weiss et al., 2015). Using these antibodies as affinity capture reagents subsets of hundreds of peptides with identical termini are enriched and a sensitive mass spectrometry read out allows the detection and quantification of these peptides.We will present data for the application of such antibodies for the quantification of plasma proteins, membrane receptors and drug transforming proteins.

AFFINITY - MASS SPECTROMETRY WORKSHOP
TUESDAY, SEPTEMBER 29, 2015 – 07:30 – 09:00

Online Biosensor-Mass Spectrometry Combination: Principles and Application to Elucidation of Antibody Epitopes

Michael Przybylski[1], Loredana Lupu[1], Stefan Slamnoiu[1], Adrian Moise[2], Camelia Vlad[2], Bastian Hengerer[3], Mary Murphy[4], Jeff Bornheim[4]
[1]*Steinbeis Centre for Biopolymer Analysis and Biomedical Mass Spectrometry, Ruesselsheim, Germany,* [2]*Department Of Chemistry, University of Konstanz, Konstanz, Germany,* [3]*Zns Research Dept., Boehringer Ingelheim Pharma GmbH, Biberach, Germany,* [4]*Ametek-Reichert Technologies, Depew, NY, United States of America*

Abstract: Bioaffinity analysis using biosensors such as surface plasmon resonance (SPR) and surface acoustic wave (SAW) has become an established technique for the detection and quantification of biomolecular interactions. However, a principal limitation of biosensors is their lack of providing structure analysis of affinity-bound ligands. We have developed a continuous, automated online biosensor-MS combination with electrospray ionization mass spectrometry (ESI-MS), using both SAW and SPR biosensors. Here we report the first online SPR-MS) combination that enables the simultaneous affinity capture/isolation, chemical structure determination and affinity quantification of protein ligands, dissociated from protein-ligand complexes on a gold chip. Key tool of the SPR-MS combination is a new automated interface that provides sample concentration and in-situ desalting for the MS analysis of the ligand eluate [1]. ESI-MS systems from several MS manufacturers can be coupled, using a in house developed software connection the MS-acquisition and biosensor operation. First applications of the online SPR-MS show broad analytical potential for direct interaction studies from biological material, as diverse as antigen-antibody and lectincarbohydrate complexes, with affinity binding constants (KD) from milli- to nanomolar ranges [2, 3]. Moreover, first applications of online- SPR-MS to the direct analysis of biological samples are shown, such as the “top-down” structural characterization of proteolytic intermediates and oligomers of Parkinson’s Disease key protein, alphasynuclein (αSyn) [4] from brain homogenate. The broad application areas amenable with the online SPR-MS include affinity-based biomarker identification, identification of protein and peptide epitopes, precise antibody affinity determinations, and direct label-free antigen quantification.

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POSTERS

P01: POSTER SESSION - HUMAN PROTEOME PROJECT

P01.01 Virtual-Experimental 2-DE in Combination with ES LC-MS Gives a Clearer View of Proteomes

Stanislav Naryzhny[1], Victor Zgoda[1], Maria Maynskova[1], Swetlana Novikova[1], Natalia Ronzhina[2], Igor Vakhrushev[1], Elena Khryapova[1], Alexander I. Archakov[1]
[1]*Institute of Biomedical Chemistry, Moscow, Russian Federation,* [2]*Petersburg Nuclear Physics Institute, Gatchina, Russian Federation*

Introduction and Objectives: Human cellular and plasma proteomes are tremendously complex and composed from diverse and heterogeneous gene products (proteoforms). Recent developments in mass spectrometry and systematic approaches (technology and methodology) promise to bring new insights into this complexity. A combination of mass spectrometry with classical biochemical separation technologies is particularly attractive for this systematic investigation. Among biochemical methods, two-dimensional gel electrophoresis (2-DE) is a most powerful protein separation technique that allows not just separating proteoforms but determining their physic-chemical parameters (pI and Mw). In our study, we performed the global analysis of cellular and plasma proteins using a combination of virtual (in silico) and experimental 2-DE with high-resolution nano-liquid chromatography-mass spectrometry. This approach is moving proteomics study on the next level of the acquisition of knowledge about proteomes **Methods:** Separation of proteins by 2-DE. 2D images analysis by ImageMaster™ 2D Platinum v 7.0. The tryptic peptides separation by chromatography on Agilent HPLC system 1100 Series with following tandem mass spectrometry on Orbitrap Exactive Plus mass spectrometer. Protein identification and relative quantification using Mascot. Virtual (in silico) 2D maps generation. **Results and Discussion:** In total, up to 1000 unique proteins were identified in each gel by Mascot search. To get better impression about diversity of proteoforms in a particular proteome, the graphs were drawn where experimentally measured physico-chemical parameters of proteoforms were plotted against the theoretical (in silico) parameters of corresponding proteins. This approach shows a clear difference between cellular proteomes and plasma proteome in terms of level of PTMs. **Conclusion:** A combination of high resolution 2-DE, nano-liquid chromatography-mass spectrometry, and virtual 2-DE gel representation of proteomes based on theoretical and experimental parameters (pI/Mw) allows to getting a clearer view of the general state at the scale of complete proteomes and a single gene products (proteoforms) as well.

Keyword: 2-DE, mass spectrometry, LC/MS, proteoforms

P01.02 New Insights into the Evolutionary Characteristics of Missing-Protein-Encoding Genes

Aishi Xu[1], Guang Li[1], Dong Yang[1], Ping Xu[2], Fuchu He[2]
[1]*Beijing Proteome Research Center, Beijing, China,* [2]*State Key Laboratory Of Proteomics, Beijing Proteome Research Center, Beijing, China*

Introduction and Objectives: Although the “missing protein” is a temporary concept in C-HPP, the biological information for their “missing” could be an important clue in evolutionary studies. In this study, we focused on these important questions: how and why did these special types of genes emerge during evolution? What are the functional requirements and biological effects of these evolutionary events? **Methods:** The missing-protein-encoding genes were classified into two groups, MPNTGs (no transcript evidence, i.e., PE3, and PE4) and MPWTGs (with transcript evidence, i.e., PE2) according to neXtProt database. The gene evolutionary characteristics were retrieved from Ensembl using BioMart. **Results and Discussion:** We found missing-protein-encoding genes distribute unevenly among different chromosomes, chromosomal regions, or gene clusters. In the view of evolutionary features, MPNTGs tend to be young genes, spreading at the non-homology chromosomal regions, evolving at higher rates, and having a much higher proportion of singletons. More importantly, most of the MPNTGs belong to the newly duplicated members of the paralogous gene groups, which mainly contribute to special biological functions, such as “smell perception”. These functions are heavily restricted into specific type of cells, tissues or specific developmental stages, acting as the new functional requirements which facilitated the emergence of the missing-protein-encoding genes during evolution. In addition, the criteria for the extremely special physical-chemical proteins were firstly set up based on the properties of PE2 proteins. **Conclusion:** Overall, a model of the evolutionary features for the formation of missing-protein-encoding genes can be inferred. In brief, the requirements of some special biological function resulted in the specific OTCs/conditions for the expression of certain genes during evolution. These acted as the functional constraint facilitating the emergence of the missing-protein-encoding genes by the new origination or new duplication with specific physicochemical properties.This study is expected to be highly instructive for proteomics and functional studies in the future.

Keywords: Paralogous gene group, Missing protein, Spatial-temporal specific genes, Chromosome evolution

P01.03 Antibodypedia - The Wiki of Antibodies

Tove L. Alm, Kalle Von Feilitzen, Mathias Uhlen
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Introduction and Objectives: Antibodies are widely used as binders in many experiments, such as characterizing the components of the human proteome, or validating potential biomarkers. There are an enormous number of antibodies available on the market, and redundancy is not uncommon. Antibodypedia, www.antibodypedia.com, is a freely available and searchable knowledge database of annotated and scored antibodies. **Methods:** Antibodypedia offers a standardized system for sharing validation data and references about publicly available antibodies. Antibody and antigen information is provided by academic institutions and commercial companies. Users of the database may also submit their data to complement the existing knowledge about an antibody. **Results and Discussion:** When searching for a particular gene or protein, a list of all associated gene products is generated. The list displays a short summary of each gene/gene product and the number of providers and antibodies available in Antibodypedia. The first version of Antibodypedia was released in 2008, and at that time holding information on a little less than 4000 antibodies. Today Antibodypedia contains over 1.8 million antibodies, primary data from more than 700.000 experiments, and over 200.000 references. The database is continuously updated and currently covers 94% of the human protein-coding genes. **Conclusion:** Scientists are frequently facing the issues of getting an overview and distinguish between antibodies. The enormous number of available antibodies is not reflecting a diversified market but rather the same antibody is sold by many providers under different names. Usually there are no links to the original source and little chance to trace back the antibody. Depending on the sample and the context, the antibody may work very well in one application, but not be reliable at all in another. Displaying as much information about the antibody as possible, including clone number, antigen sequence, and binding epitope, will clearly improve the probability for a researcher to select a successful antibody.

Keywords: Antibodies, Validation, Human Proteome

P01.04 Most Genes Have a Single Highly Expressed Dominant Splice Isoform

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Introduction and Objectives: Although eukaryotic cells can express a wide range of alternatively spliced transcripts it is not clear whether genes can express a range of transcripts simultaneously across cells, or whether one of these variants might be described as dominant. Large-scale investigations into the pattern of transcript dominance across distinct tissues have produced contradictory results. **Methods:** To shed light on this complex problem, at least at the protein level, we interrogated 8 large-scale human proteomics experiments using a rigorous peptide identification strategy. We carried out an analysis of alternative splicing at the protein level to determine whether genes have a dominant splice variant. We identified peptides for 12,716 human protein coding genes, but found peptide for alternative splicing events in only 246 genes, suggesting that the vast majority of protein-coding genes may have a single dominant protein isoform. We identified a main proteomics isoform for 5,011 genes. We compared this main isoform to reference isoforms from four orthogonal sources. **Results and Discussion:** The main isoforms we identified in the proteomics analyses were overwhelmingly supported by reference isoforms from two sources, the CCDS consensus variants agreed upon by manual genome curation teams, and the APPRIS database which uses patterns of protein conservation, structure and function to select principal isoforms for each gene. For those genes where all three methods chose a reference isoform, the agreement was 99.5%. The agreement with RNAseq dominant isoforms from a large-scale experiment was just 80%. **Conclusion:** The clear agreement between three orthogonal sources significantly reinforces the probability that the main proteomics isoform is the dominant protein isoform in the cell. In particular, the agreement with APPRIS principal isoforms demonstrates that the cellular machinery tends to express the most conserved splice isoform and the one that best preserves the conserved structural and functional features of the protein.

Keywords: alternative splicing, RNAseq, Dominant isoforms

P01.05 Automated Quantitative Method for Biomarker Assessment of Salivary Proteins

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[1]*University Of Victoria – Genome British Columbia Proteomics Centre, University of Victoria, Victoria, BC, Canada,* [2]*Innovative Medicines, S-431 83, AstraZeneca R&D, Mölndal, Sweden*

Introduction and Objectives: One increasingly popular method of absolute quantitation for biomarker screening is the MRM approach with stable isotope-labeled standards (SIS) peptides. Much effort in biomedical and clinical research has been devoted to blood plasma using this approach. Considering that non-invasive biofluids present potentially attractive alternatives for protein disease biomarker evaluation, we are developing an automated quantitative method for the rapid assessment of multiplexed, candidate protein biomarker panels in human saliva. **Methods:** Human plasma and saliva were collected from healthy donors (n = 32) by Bioreclamation according to standard procedures. While Bioreclamation was collecting the saliva samples, we performed initial automation experiments on the AssayMAP Bravo platform using 96-well microplates and human plasma as the test biosample. The final automated method included protein solubilization (with 50 mM Tris), denaturation (via urea), reduction (via TCEP), alkylation (via iodoacetamide), trypsinization (TPCK-treated at a 10:1 substrate:enzyme ratio), SIS peptide addition, and solid phase extraction. All eluates from the extraction plate were processed by UHPLC/MRM-MS on a 6490 triple quadrupole instrument, with the XICs analyzed and quantified using our Qualis-SIS software. **Results and Discussion:** The preliminary results with the plasma matrix revealed excellent repeatability, with CVs approximating 5% for the endogenous protein concentrations of 35 targets. This paves the way for the translation of this method to saliva. For the saliva assay, an enlarged panel of >2000 in-house synthesized peptides (corresponding to >600 proteins, includes the glandular-derived histatins, mucins, and cystatins) will be interrogated. Key development steps will involve detectability evaluation, interference screening, quantitative reproducibility evaluation, and inter-laboratory method transferability testing for assessing the utility of a biomarker assessment kit in discovery/verification studies. **Conclusion:** We are currently translating a fully automated plasma method to human saliva for the rapid and highly reproducible quantitation of a large panel of candidate protein biomarkers for diseases of the oral cavity.

Keywords: Saliva, multiple reaction monitoring (MRM), protein, quantitation

P01.06 Gene-centric Knowledgebase as a Tool for Estimating Protein Species Number

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Introduction and Objectives: The main goal of the international Human Proteome Project is creating a comprehensive map of the proteome including different proteoforms (protein species) encoded by the same gene. These proteins are products of alternative splicing (AS), post-translation modifications (PTMs), RNA editing. However, the most of protein variability is coming from the presence of various gene allele variants: non-synonymic SNP (nsSNP), ethnic-specific or pathogenic. **Methods:** To estimate the number of protein species we suggest using Gene-centric content management system (CMS) for consolidating different types of the data on protein variability at the genomic, transcriptomic and proteomic levels. At the genome level we analyzed the gene allele variants found in two most extensive genomic surveys – 1000 Genomes and NHLBI GO Exome Sequencing Projects (ESP). Using open access to the NCBI Sequence Read Archive (SRA) data, the contribution of transcriptome diversity coming from AS was evaluated; the diversity in individual samples was accessed in the liver tissue of three individuals and HepG2 cells using both AB SOLiD and Illumina HiSeq sequencing. All data were integrated into CMS for joint analysis and visualization. **Results and Discussion:** We performed meta-analysis of the 34 studies freely available in NCBI SRA for eight human tissues. The total number of possible proteoforms arising from AS was evaluated as 3200 (Chr18) and 235,000 (complete genome). In total, 743 (Chr18) and 59,000 (genome) AS-variants and 101 (Chr18) and 7758 (genome) nsSAPs were confirmed using NGS. A set of proteotypic peptides for 64 Chr18 proteoforms was generated for future validation at the protein level using SRM approach in the same samples. **Conclusion:** Integrating different types of postgenomic data with CMS (www.kb18.org) opens up a possibility to predict the number of protein species forming the human proteome and select the most suitable items for experimental validation at the protein level.

P01.07 Platelet-Derived Extracellular Vesicles - A Key to Understand Alzheimer's Disease

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Introduction and Objectives: Platelets are the smallest of the three major types of blood cells. They are produced by megakaryocytes and contribute to hemostasis. During their lifetime of 8 to 10 days they release so called extracellular vesicles (EVs) which make up till 90 % of the circulating EVs in the bloodstream. These platelet derived extracellular vesicles (PL-EVs) are involved in various cellular processes like autoimmunity, chronic inflammation, thrombosis and neurodegeneration. Function, activity and composition of the PL-EVs are highly heterogeneous due to their subcellular origin. **Methods:** To analyze this heterogeneity we used differential centrifugation, filtration and density gradient ultracentrifugation, ending with five distinct microvesicle (PL-MV) fractions. Further analysis regarding the protein and lipid composition revealed significant differences between the PL-MV fractions. **Results and Discussion:** The detection of the amyloid precursor protein APP, the hallmark protein of Alzheimer's disease and the Parkinson's disease related protein alpha-synuclein indicates a contribution of PL-EVs in the manifestation of neurodegenerative disorders. **Conclusion:** not applicable

Keyword: Alzheimer, extracellular vesicles, platelets

P01.08 Proteomics Analysis of Human Ureter for Urine Biomarker Discovery

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Introduction and Objectives: Urine has evolved as one of the most important biofluids in clinical proteomics due to its non-invasive sampling and stability. However, it is only used in clinical diagnostics of several disorders by detecting changes in its components including urinary proteins or polypeptides. In this study, we performed a comprehensive proteomics analysis of ureter to assemble the first unbiased ureter proteome dataset for discovery of urine biomarker candidates of ureter disorders. **Methods:** Human ureter samples were obtained from kidneys removed for renal cancer therapy. The proteins were fractionated by OFFGEL electrophoresis and peptides were analyzed by Q-Exactive mass spectrometry. Proteins and peptides were identified by MASCOT and ProluCID search engines implemented in the integrated proteomics pipeline; IP2 (peptide: FDR> 0.1%, protein: FDR>1% using DTASelect19). **Results and Discussion:** The current analysis indicated 2217 non-redundant ureter proteins. Interestingly, the result showed that 24.3% of the ureter proteins were detected in previous studies in urine proteome analysis. Moreover, 21% of ureter proteins had been identified in urinary exosomes. Forty eight proteins were not found in our kidney proteome datasets and 21 proteins retained as unique in the ureter proteome by referring to urinary bladder and prostate IHC images in The Human Protein Atlas database. **Conclusion:** We compared the ureter proteome dataset with urine, urinary exosome, and kidney datasets and demonstrated that 21 proteins could be ureter disease-associated urine biomarkers. The ureter proteomic dataset may provide a valuable resource for urine biomarker discovery.

Keywords: Urine, ureter, proteome, biomarker

P01.09 Proteogenomics Analysis of Novel Transcripts and Isoforms Using the Human MiTranscriptome Assembly

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Introduction and Objectives: We have developed a bioinformatics workflow for the detection and quantification of novel peptides based on the MiTranscriptome consensus human transcriptome. This assembly, obtained from the analysis of 7256 public RNA-Seq experiments, contain 384066 different transcripts, classified as protein coding, read-throught, pseudogene, lncRNA or transcript of unknown coding potential in accordance with their coding potential. **Methods:** After the annotation of known transcripts using GENCODE as reference we have generated two databases to perform the proteogenomic study, differentiating between known and unknown aminoacid sequences. In the case of non-annotated transcripts a 6-frame translation strategy is followed in order to create the database. Public shotgun experiments stored in PRIDE database have been analyzed using the guidelines provided by the C-HPP consortium (FDR < 1 %, protein level). First, we have identified and quantified peptides corresponding to annotated transcripts, and after that we have used the set of non-assigned spectra for the detection of the translation sequences obtained from MiTranscriptome novel transcripts. **Results and Discussion:** We have compared the distributions of identified peptides, peptide scores (using Mascot, Sequest and Andromeda search engines), sequence coverage and expression levels in both cases as a function of the coding potential classification of the transcripts. Finally, all data generated in the analysis (databases, processed spectra and peptide identifications) have been included in the dasHPPboard, a bioinformatics tool for the storage of the spHPP consortium results. **Conclusion:** Proteogenomics analysis of the MiTranscriptome assembly as performed in the spHPP consortium for chromosome 16 emerges as a useful tool to increase the peptide coverage of the proteome.

Keyword: Proteogenomics, Human Proteome Project, RNA-Seq, Shotgun

P01.10 Changes on the Methyl Proteome Triggered by a Deficiency on MTAP in Liver Cells

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Introduction and Objectives: 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 processes, in part through the regulation of protein methylation. MTAP is expressed in a wide range of cell types and tissues and its deletion is common in cancer cells and iliver injury. The aim of this study was to investigate the alterations of the methyl proteome triggered by MTAP deficiency in liver cells to define novel regulatory mechanisms of central cellular processes and their pathophysiological implications. **Methods:** The protein methylation profiles of SK-HEP1 cells lacking MTAP expression and transfected SK-HEP1+ cells expressing normal levels of MTAP were compared. Protein extracts (10 mg) were digested with trypsin and the resulting peptides were immunoprecipitated with a monomethyl R specific antibody. Pulled-down peptides were then analyzed by C18 LC-ESI-MS/MS. Data analysis was performed with Mascot and R-CH3 peptide candidates were confirmed by de novo sequencing.

Results and Discussion: R-methyl proteome analysis lead to the indentification of 74 differentially methylated proteins between SK-Hep1 and SK-HEP1+ cells, including 47 new methylation sites. Restoring normal MTA levels in SK-Hep1+ cells parallels the specific methylation of 56 proteins, including KRT8, TGF and CTF8A, which provides a novel regulatory mechanism of their activity with potential implications in carcinogenesis. Inhibition of RNA binding proteins methylation is specially relevant upon accumulation of MTA. As an example, methylation of quaking protein 1 in R242 and R256 in SK-HEP1+ cells may play a pivotal role in the regulation of its activity as indicated by the upregulation of its target protein p27kip1. **Conclusion:** Our data support that MTAP deficiency leads to MTA accumulation and deregulation of central cellular pathways, increasing proliferation and decreasing the susceptibility to quimiotherapeutic drugs, in part due to differential protein methylation.

Keywords: protein methylation, methylthioadenosine, liver

P01.11 New Features of Genomewide PDB v 2.0

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Introduction and Objectives: Despite some success in identifying missing proteins since the Chromosome-centric Human Proteome Project (C-HPP) was launched in 2012, there still remain more than ~1800 missing proteins. To expedite our search for the remained missing proteins, it was suggested to develop the targeted proteomics approach (e.g., S/MRM) and the innovated bioinformatics platform which enables selection of target peptides for the distinct alternatively spliced products that are rarely expressed. To this end, we attempted to improve the functions of the current GenomewidePDB with new features, which may contribute to mapping the remained missing proteins. **Methods:** The information as to the proteins encoded by genes located on chromosomes was from neXtProt (2011-08-23 to 2015-0-01, 24 releases). NCBI UniGene (Build 236) was used for building up the gene expression data among those various tissues. Human Protein Atlas (ver 13) was referenced for tissue-specific expression of mRNA that had been obtained by RNA-Seq and protein expression by immunohistochemistry. **Results and Discussion:** We made substantial improvement on the GenomewidePDB, which was previously developed as proteomic database, by installing a few new features. This newly upgraded GenomewidePDB (v2.0) not only contains the experimentally identified proteins but also can be expandable upon the end users' needs. The other features include, but not limited to, (1) gene expression data for various tissues, (2) tryptic peptides of each protein and their isoforms with annotated information such as uniqueness, (3) advanced protein search interface that enables identifying missing proteins for targeted proteomic approaches. **Conclusion:** The updated GenomewidePDB v2.0 has many new features that enable targeted proteomics by integrating genomic dataset and search methods. This updated database will be useful for mapping the missing proteins in a genome-wide fashion.

Keywords: C-HPP, database, Expression, Chromosome 13

P01.12 SRM-Assay Database for Reproducible Protein Quantification in Different Types of Biomaterial

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Introduction and Objectives: Selected reaction monitoring (SRM) is considered to be the most accurate high throughput targeted protein quantification technology. Despite its impressive sensitivity it is not widely used in clinical and biomedical practice. There are several technical contingencies, but the key limitation of SRM is the absence of reliable automated data processing approaches. The human factor effects SRM research at the stage of sample preparation, data acquisition and data processing. At the present moment there are several quality control kits, reducing the human effect at the stages of sample preparation and data acquisition [Percy A.J. et al.2013]. But at the present moment SRM-data processing requires manual inspectation. In combination with the high interference it increases nubmer of false-positive results and prevent from accurate quantification for biomarker search. **Methods:** We analyzed huge SRM data array derived in the course of the russian part of Human Proteome Project. To estimate the reproducibility of protein SRM-measurments we used statistical analysis based on the multiple parameters (such as intencity, retention time, interference probability) distribution in individual sample, between biological or technical replicates. The most reproducible proteins were validated with data from SRMAtlas, PRIDE and Plasma Proteome Database. Overmore we estimated propriety of the usage of universal SRM-assays and methods for all types of biological samples. **Results and Discussion:** Integrating statistical analysis and bioinformatics research helped us to reveal the most reproducible proteins. Without dependence of its localization or concentration they are suitable for biomarker reseach. Unfortunately, this proteins constitute only 8% of the whole proteome, according to our estimates. SRM-detection of the other 92% requires multiple quality control because it is limited by human factor, biological sample complexity and proteome variability. Besides, our analysis showed that its quite necessary to use individual SRM-assays and methods in different types of biological materials. **Conclusion:** 'not applicable'

Keywords: Bioinformatics, mass-spectrometry, Human Proteome Project, selected reaction monitoring

P01.13 The First Master Proteome of Single Chromosome: Example of Human Chromosome 18

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Introduction and Objectives: Last year scientific world was impressed by two Nature articles by Pandey and Kuster, which declared that a draft of the human proteome is created. Deciphering the human genome has taken 20 years, since the core invention of gene amplification by PCR was made in 1986. The proteome, a much more complex system, was cracked in just five years from 2010 when the Human Proteome Project was launched in Sydney. We do not share the worldwide enthusiasm about the completion of the human proteome. Taken in a fist all of the resources Russians beat into a single target, which is human chromosome 18. **Methods:** The main approach for proteomic profiling was chosen the targeted proteomic employing selected reactions monitoring (SRM). The SRM-signals of Chr18 encoded proteins were detected and quantified by using natural proteotypic peptides and corresponding synthetic stable-isotope standards in blood plasma, liver tissue cells and HepG2 cells. **Results and Discussion:** Our results coincide with the seminal repots

of Pandey and Kuster that about 80% of the genes produce detectable amount of proteins. By taking three types of biomaterial it is possible to achieve up to 95% coverage of their proteomes. It has taken over 5000 hours of runs performed on the modern mass-spectrometers, to collect and to validate the SRM-coordinates for Chr18. After this work is now done, the measurement of 269 proteins coded by Chr18 takes one working day. **Conclusion:** In the approach exploited by Kuster/Pandey groups the deep proteome achieved by "shotgun" technology, which totally addresses the whole genome. For every next biological sample shotgun discharge would require exactly the same volume of equipment time, manpower and computer resources, as was spent by the pioneers. Oppositely, the targeted approach sufficiently reduces the resources needed to proceed to the next steps in the exploration of the protein universe.

Keywords: proteome, Mass spectrometry, Human Proteome, chromosome 18

P01.14 The Combo-Spec Search Method Improves the Current Search Methods Used to Identify Missing Proteins

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Introduction and Objectives: Spectral library searching takes all of the spectral features into accounts, such as peak intensities, the natural loss of fragments, and various unknown fragments that are specific to certain peptides. Thus, it shows greater sensitivity and better matching of results than sequence database searching. In this study, we describe a new strategy, which uses a combination of multiple spectral libraries (e.g., a reference spectral library and a simulated spectral library) for spectrum-spectrum matching to identify the proteins of interest in cell or tissues. **Methods:** We built two spectral libraries. The one of them was built by combining reference spectral libraries. We collected peptide-spectrum matches which matched to the human tryptic peptide sequences and incorporated into human spectral library to make a human spectral library with expanded proteome coverage (iRefSPL). The another was built by simulated peptide tandem mass spectra (simSPL). Two spectral library searches and FDR filtering were performed independently and all results were combined. We termed the approach "Combo-Spec Search Method". **Results and Discussion:** The Combo-Spec Search method shows higher sensitivity and low error rates than multiple sequence database search method. The iRefSPL shows more detectability than original reference spectral library. Combination of simSPL and iRefSPL shows improved peptide identification rates. When we analyzed previously published human placental tissue dataset, we found novel unique peptides of 12 missing proteins. **Conclusion:** We found that the Combo-Spec Search Method was helpful to detect more peptideswith higher speed and sensitivity. The peptide identification results which matched with reference spectra show great discrimination score. Other results which matched with simulated spectra show less score than the former, but it shows similar to or better performance than sequence database search approach. We expect that this approach would be useful for identifying missing proteins efficiently in the chromosome-centric human proteome project.

Keywords: Chromosome-centric Human Proteome Project, proteomics, Missing protein, Spectral library search

P01.15 What Can Housekeeping Gene/Protein Studies Tell Us?

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Introduction and Objectives: The searching of human housekeeping (HK) genes/proteins has been a long quest since the emergence of transcriptomics, and is instrumental for us to understand the structure of genome and the fundamentals of biological processes. The resolved proteins are frequently used in evolution studies and as normalization standards in quantitative analysis. Within the past 20 years, more than a dozen HK-gene/protein studies have been conducted, yet none of them sampled human tissues completely. We believe an integration of these results will help remove false positives owing to the inadequate sampling. **Methods:** To test this hypothesis, we merged 15 human HK-gene/protein lists obtained from the public domain to increase the tissue coverage (> 90% with more than 180 tissue and cell types included). We observed large discrepancy across all the lists, and to identify the cause of difference, we conducted a series of analyses. First we studied the number of unique HK genes followed by a relationship analysis using hierarchical clustering. Then we performed a deviation analysis using leave-out approach, and a pairwise similarity comparison. To examine the impact of detection sensitivity to the observed results, we further explored expression quantity of all the genes/ proteins. In the end, we concerned their enriched biological functions. **Results and Discussion:** For a total of more than 12,500 HK genes obtained, only one gene was shared by all the studies, and 17 genes were in 14 out of 15 datasets. Our further analysis suggests that it might not be appropriate to rigidly define HK genes as expressed in all tissue types that have diverse developmental, physiological, and pathological states. It might be beneficial to use more robustly identified HK functions for filtering criteria. **Conclusion:** The housekeeping functions are essential to every living cell, but the proteins carrying out these functions are not necessarily the same in every cell.

Keywords: housekeeping gene, housekeeping protein, Human Proteome, housekeeping function

P01.16 Chromosome-Centric Approach to Unraveling the Human Interactome

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Introduction and Objectives: The human interactome has been estimated as 650 000 interactions and visualized as 'hairballs'. This complex organization makes difficult the investigation of the structure and nature of protein interaction networks underlying in biological processes. Also the experimental results of analysis protein-protein interaction (PPI) are often contradictory and incomprehensive. Using chromosome-centric approach to analyze the data of PPI allows to reduce network keeping its structure and thus to identify new information of protein interaction. **Methods:** Data of PPIs was obtained from popular interactome resources: STRING (score>0.7), IntACT, BioGRID, MINT and DIP using the proteins coded by the chromosome 18 as baits. We used the gene-centric knowledgebase (kb18.org) to analyze interactome data and their annotation based on results of transcriptomic and proteomic experiments performed in the course of Russian part of Human Proteome Project and data from the global protein and gene databases. **Results and Discussion:** Totally the 12863 data of protein interaction for chr 18 were obtained from interactomic database. By sum of PPI's data there were 221 "bait" (out of 276 gene of chromosome 18) having at least one interaction partner. Based on the fact that 10% of all proteins are the hubs and the

distribution of protein-coding genes of the chromosomes is independent the hub proteins was determined. Comparison of the obtained list of hub proteins for each interactome resource revealed 13 hubs including NDC80, SMAD-proteins, BCL2, NEDD4L, YES1 et al. For this hub list of partners about 40% correspondence among PPI's resources. The functional annotations of these proteins showed a high expression in different tissues according to the results of transcriptomic and proteomic profiling. Also the most of hubs associated with cancer and directly or indirectly related to each other in network. **Conclusion:** Combination of chromosome-centric approach and genomic and postgenomic data can be used to productive investigate of protein interaction networks.

Keywords: hub proteins, Protein-protein interaction, chromosome-centric approach, Interactome

P01.17 Reference Molecular Map of Individual Monocyte Lineages of the Spanish Healthy Population

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Introduction and Objectives: Inflammation is a protective response mediated by endothelial and lymphoid cells that is essential for survival. However, disturbances of the inflammation process can have harmful effects. In fact, it is believed that the tissue damage associated with the progression of many diseases may be due to the development of a chronic inflammation state where monocytes and macrophages play a key role in its pathophysiology. After a short period circulating in blood, monocytes interact with endothelial cells, migrate to different tissues and differentiate into macrophages. During the inflammatory process, chemokines and cytokines released by macrophages recruit monocytes to the inflammation focus. It is now clear that there is heterogeneity among monocytes and that the outcome of inflammation may be determined by the activity of the different monocyte lineages. The Spanish Chromosome 16 Consortium is integrated in the Human Proteome Project. One of the projects of its B/D Program, together with the Spanish Biobanks Platform, is the construction of protein profiles of pure monocyte lineages of the Spanish healthy population. These data will serve as a reference molecular map to understand the inflammatory processes associated with liver, cardiovascular, rheumatic, neoplasic or infectious diseases. **Methods:** A multicentric pilot study has been carried out to construct the proteomic profile of FACS purified CD14^{+} and CD16^{+} monocytes from several healthy donors. The experimental approach includes protein fractionation by SDS/PAGE followed by shotgun LC-MS/MS analysis of the gel slices. Protein abundance has been determined by label-free methods. **Results and Discussion:** We have evaluated the variability in protein identification and quantification among different individuals, laboratories, and instrumentation used. Although the experimental strategy, simple and robust, provides good results, additional procedures are required to increase the membrane protein coverage. **Conclusion:** The present study set the foundation toward the construction of a reference dataset of individual monocyte lineages of the healthy population.

Keywords: monocytes, protein profile, reference dataset

P01.18 Comprehensive Proteome Analysis of OCT Embedded Frozen Human Renal Cortex by LC-MS/MS

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Introduction and Objectives: A novel stratagem to extract proteins from OCT embedded human frozen tissues for proteomics analysis was introduced. More hydrophilic peptides were detected. Polymeric effect of OCT on MS spectrum was not found. **Methods:** In this study OCT compound was removed from tissues by washing with PBS twice on ice roughly first. Then homogenized 10seconds three times by Precellys-24® with 7M Guanidine HCl buffer, aliquot of 100μl was performed Chloroform / Methanol precipitation procedure and protein pellet was suspended by using 50mM Ammonium Bicarbonate (ABC) directly. Trypsin digestion was done completely and its digest was filtrated by Millipore 0.45μm centrifuge unit and vacuum dried. Peptide powder was kept at -80°C until use. Same tissue without OCT compounds was in-solution digestion with Urea base buffer and peptide was purified by C18 column. Data dependent acquisitions were done by SCIEX and Thermo mass spectrometers with same gradient schedule in HPLC method. Protein identification was done with search engines MASCOT and SEQUEST as their optimal search parameters. **Results and Discussion:** Data dependent acquisition results from SCIEX 5600+ and Thermo MS show very similar results between OCT embedded and normal frozen tissues. There are 2780 proteins were identified with high stringency from Thermo Fusion MS in 135-min. acquisition with injection of 0.67ug peptides solution. The effect of OCT compounds on MS analysis was not found in our results. And there are much more hydrophilic peptides were identified from the samples get from our new method. **Conclusion:** MS base proteomics samples can be obtained easily from OCT embedded frozen tissues by using the new stratagem, which was introduced in this study. C18 column free in-solution digestion method can increase peptide discovery from same tissues. Comprehensive proteomics data can be provided by this method on any types of tissues. We hope this method can be developed as popular method for tissue proteomics.

Keywords: Renal cortex, OCT compounds, Millipore Unit, Hydrophilic peptides

P01.19 Human Proteome Project in Cancer

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Introduction and Objectives: Understanding the proteomic differences in multiple human tumor types is the current requisite and central theme of the cancer human proteome project (Cancer-HPP) with the ultimate goal of defining expression and interactions of these proteins. This will greatly increase our knowledge of human cancer biology. Overall, the Cancer-HPP attempts to characterize different cancer proteomes, determine the correlation of transcriptome and proteome, identify the high priority proteins for each tumor type and generate and disseminate assays and resources to support the analysis of complex biological networks or clinical specimens underlying different disease processes. **Methods:** The use of immunoassays and protein assays as well as the emerging mass spectrometry (MS)-based platforms such as selected reaction monitoring (SRM), Parallel reaction monitoring (PRM), and targeted data extraction for candidate proteins from SWATH-MS data

have become reliable popular methods for quantitative analysis of high priority target proteins. Data from multiple laboratories studying different cancer types has confirmed the supremacy of these technologies over conventional assays. Therefore, we propose an international cancer proteomic effort similar to The Cancer Genome Atlas (TCGA) project to identify and validate cancer proteins for different cancers. **Results and Discussion:** Data deposition, quality control, and public availability are the key components of the Cancer-HPP. By working together, we can create a synergistic effort to develop reliable validated assays to each cancer type and critical proteins involved in cancer biology pathways. We will further support and propose an open discussion on procedures how to accrue/ share data for a list of target proteins from each cancer type, the strategy for assay development, quality control, and procedure and materials needed for disseminate the established assays to cancer biology or clinical laboratories. **Conclusion:** Toward this goal, it will be essential to involve the whole cancer community including not only proteomics but also genomics and cancer biologists and clinical oncologists in Cancer-HPP.

Keywords: Human Proteome Project, cancer

P01.20 Quantitative Nuclear Proteomics of Schizophrenia

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Introduction and Objectives: Schizophrenia (SCZ) is a chronic neuropsychiatric disorder characterized by impairment of higher mental functions, exhibiting positive symptoms (delusions and hallucinations) and negative symptoms (social withdrawal, diminished affective responsiveness, speech and movement). This debilitating disorder may affect about 1% of the world population, specifically young adults (16 to 30 years old) with the same risks for both genders. Here, we follow the goal set by the Chromosome-centric Human Proteome Project Organization to characterize disease-associated proteins **Methods:** An enriched nuclear (NUC) subcellular fraction of post-mortem brain tissues was prepared from the orbitofrontal cortex region collected from 12 schizophrenia patients and 8 controls free of psychiatric disorders following Cox and Emili protocol[1]. Once isolated, nuclear proteins were digested with trypsin and labeled with iTRAQ 4-plex; three reporter groups were used for schizophrenia samples and the fourth labeled a pool of all control samples. Peptides were separated by RP-nLC and analyzed with a LTQ Velos Orbitrap **Results and Discussion:** Mass spectrometry identified and quantified 904 proteins by at least 2 unique peptides. Neurogranin (NRGN) and brain acid soluble protein 1 (BASP-1) (n =11) showed the highest fold change. NRGN is involved in the regulation of calmodulin (CaM)-mediated signalling. In the same way, BASP-1 cytoskeleton-associated protein, a calmodulin-binding protein, is widely expressed during brain development, regulating neuritic outgrowth as well as actin cytoskeleton. Former results in a synaptosomal fraction (n = 6), showed CaM as the lower abundant in half of patients; it plays important role in calcium metabolism exerting different functions in cytoskeletal and ion channel regulation. The imbalance of proteins associated with calcium metabolism, has been linked to the function of dopamine receptors and glutamatergic synapses. **Conclusion:** These quantitative proteomics results contribute to a better understanding of cellular calcium metabolism in the pathophysiology of SCZ

Keywords: Calcium metabolism, Schizophrenia, Nuclear brain proteins

P01.21 Integration of -Omics Datasets for Comprehensive Protein Expression Profiling for the C-HPP

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Introduction and Objectives: A comprehensive study of the behavior of the cells can be confronted from two different but complementary perspectives: genomics and proteomics. After the Genome era, Proteomics has emerged as a powerful tool with a great potential to simultaneously screen thousands of different proteins. In this regard, a Chromosome-centric Human Proteome Project (C-HPP) has promoted the full human proteome characterization together with the availability of in depth transcriptomics. **Methods:** We focused our research i a lymphocytic B-cell line (Ramos) performing an LTQ-Orbitrap experiment to profile its proteome. The integration of these datasets and the transcriptomics information revealed an 82% of overlapping in protein identification between both -omics approaches. **Results and Discussion:** The integration of these datasets and the transcriptomics information revealed an 82% of overlapping in protein identification between both -omics approaches. The functional enrichment analysis also showed an enrichment of several functions directly related to the functional and morphological characteristics of B-cells. In addition, up to 30% of total protein-coding genes present in the human genome were identified in this study (an average of 30% per chromosome and 85% in the case of the mitochondrial chromosome) **Conclusion:** In summary, the results provided a high coverage of the proteome and a deep insight into the biological processes of lymphocytic B-cells by joining genomics and proteomics technologies.

Keyword: C-HPP, integration omics data

P01.22 Chromosome-Based Proteomic Study for Identifying Novel Protein Variants from Human Hippocampus

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Introduction and Objectives: The goal of the Chromosome-centric Human Proteome Project (C-HPP) is to fully provide proteomic information from each human chromosome, including novel proteoforms, such as novel protein-coding variants expressed from non-coding genomic regions, Alternative Splicing Variants (ASVs), and Single Amino-Acid Variants (SAAVs). **Methods:** In the 144 LC/MS/MS raw files from human hippocampal tissues of control, epilepsy and Alzheimer Disease (AD), we identified the novel proteoforms with a workflow including Integrated Proteomic Pipeline (IPP) which consists of three different search engines, MASCOT, SEQUEST and MS-GF+, statistical evaluation tools, DTASelect and Percolator, and ProteinInferencer combined a homemade program for the normalization of three types of scoring from different search engines. **Results and Discussion:** With a less than 1% False Discovery Rate (FDR) at the protein level, the eleven detected peptides mapped to four Translated long non-coding RNA Variants (TRVs) against the customized databases of GENCODE lncRNA, which also mapped to coding-proteins at different chromosomal sites. We also identified four novel ASVs against the

customized databases of GENCODE transcript. The target peptides from the variants were validated by tandem MS fragmentation pattern from their corresponding synthetic peptides. Additionally, a total of 128 SAAVs paired with their wild-type peptides were identified with FDR < 1% at the peptide level using a customized database from neXtProt including non-synonymous single nucleotide polymorphism (nsSNP) information. **Conclusion:** Among these results, several novel variants related in neuro-degenerative disease were identified using the workflow that could be applicable to C-HPP studies. All raw files used in this study were deposited in ProteomeXchange (PXD000395).

Keywords: LC-MS/MS, single amino acid variants, translated lncRNA variants, alternative splice variants

P01.23 Mass-Spectrometry Based Blood Serum Peptidome Analysis of Athletes under Physical Overstrain

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Introduction and Objectives: Professional athletes are able to overcome the physical activities that significantly exceeds the allowable limit for the average person. Through many years of training these people produce in their body the ability to adapt quickly to physical stress and operate for a long time under the stress. Investigation of the effect of physical overstrain on the organism of professional athletes could be a clue to the molecular processes of adaptation in the body. Human blood serum analysis based on mass-spectrometry technologies has been widely carried out. A number of different methods of sample preparation and isolation of peptides have been developed and applied in different studies. **Methods:** Simple, effective and reproducible method for peptide isolation from human serum has been developed for further use with mass spectrometry based analysis of blood plasma/serum peptidome. It involves the following stages: preliminary fractionation of serum using weak cationic exchange (WCX) BioGel and reverse phase solid phase extraction (RP-SPE). Comprehensive LC-MS/MS analysis has been carried out on ABSciex TripleTOF 5600+. **Results and Discussion:** Our high-performance method enabled the identification of more than 5000 unique endogenous peptides derived from about 1000 human proteins. These peptides allowed us to examine in detail the peptidome component of human serum. Most of the peptides originated from common human plasma proteome but some turned out to be non-plasma proteins produced in stress conditions. **Conclusion:** Identification of statistically reproducible sets of blood plasma/serum peptides have been carried out on the bases of the developed fractionation strategy. Comparative analyses of the lists of the identified peptides revealed a number of potential peptide markers specific for high physical stress and exhaustion. This work was supported by the RSF (project No.14-50-00131).

Keywords: mass-spectrometry, Human serum peptidome, Athlete

P01.24 Proteomics of Ocular Diseases

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Introduction and Objectives: Aravind Medical Research Foundation is an institute dedicated to carry out research in eye diseases to reduce the burden of blindness. We have adopted a comprehensive proteomics approach to understand the pathophysiology of eye diseases as well as to identify diagnostic, predictive and prognostic biomarkers. Currently, these approaches have facilitated the study of fungal keratitis, primary open angle glaucoma, diabetic retinopathy. Furthermore, our research efforts contribute to the audacious goal of Human Eye Proteome Project – "to characterize the proteome of the human eye in health and diseases". **Methods:** Eye fluids and tissues were collected employing standardized methods adhering to the Declaration of Helsinki, snap-frozen and stored at -80° C until analysis. Both gel and mass spectrometry based bottom-up proteomics approach was employed to achieve deep proteome coverage in these highly complex clinical samples. **Results and Discussion:** Comparative proteome analysis of tear from mycotic keratitis patients and healthy individuals revealed that inflammation of the cornea is primarily mediated by neutrophils as well as activation of complement pathways. Further, glycoproteomics of tear identified infection-induced changes in glycosylation of tear proteins. Towards identification of prognostic biomarkers for POAG, in-depth profiling and comparison of aqueous humor proteome revealed the regulation of many proteins, interestingly differential levels of two isoforms of transferrin. To understand the signaling mechanisms underlying the pathophysiology of DR, in addition to comparison of vitreous humor proteomes, vesicular proteomes have also been profiled. Mass spectrometry approaches complemented by 2D-gel based comparative proteomics served as a valuable tool to identify the differential regulation of proteins at the level of proteoforms. **Conclusion:** Significant proteomics efforts are being made to understand the pathophysiology of important eye diseases, two of which are in the WHO priority eye diseases. Being associated with an eye hospital, our approaches will be further expanded to other ocular diseases thereby contributing to B/D-HPP.

Keyword: eye proteome, mass spectrometry, eye fluids/tissues

P01.25 Alternatively Spliced Homologous Exons Are Highly Enriched at the Protein Level

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Introduction and Objectives: Alternative splicing of messenger RNA is thought to be one means of generating the protein diversity necessary to carry out cellular functions. Studies have estimated that practically all multi-exon human genes undergo alternative splicing. The presence of alternatively spliced transcripts in the cell has strong support from EST and

cDNA evidence. However, there is limited support for the translation of these alternative transcripts into protein isoforms. Evidence for alternative isoforms from large-scale mass spectroscopy experiments has been patchy and contradictory. A careful analysis of the peptide evidence is needed to fully understand the scale of alternative splicing detectable at the protein level. **Methods:** We carried out a rigorous analysis of the peptide evidence from eight large-scale proteomics experiments to assess the scale of alternative splicing. These eight datasets covered over 100 distinct tissues and cell lines. We filtered the peptides in order to generate as reliable a set of peptides as possible. **Results and Discussion:** We identified just 282 splice events from 12,716 genes. Most would have relatively modest effects on protein structure and function; very few would damage or cause the loss of conserved Pfam functional domains. The most striking result was that more than 20% of the splice isoforms we identified were generated by substituting one homologous exon for another. These homologous exons were remarkably conserved - all evolved over 460 million years ago - and eight of the fourteen tissue-specific splice isoforms we found were generated from homologous exons. **Conclusion:** Our results suggest that most genes have a single dominant protein isoform, while the preference for splice isoforms with whole functional domains indicates that not all annotated alternative transcripts are converted into stable proteins. The combination of proteomics evidence, ancient origin and tissue-specific splicing for isoforms generated from homologous exon substitution events strongly suggests important cellular roles for these isoforms.

Keywords: Human Proteome, Homologous exons, Functional domains, alternative splicing

P01.26 Bioinformatic Approach for Analyzing Missing Proteins in neXtprot Database

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Introduction and Objectives: NeXtProt is web-based protein knowledge platform to support research on human proteins. NeXtProt (release 2015-04-28) lists 20060 proteins, among these proteins, 3373 proteins (16.8 %) lack credible experimental evidence of protein expression and identification at protein level. therefore they were considered "missing proteins" in such database. Accordingly, the rationale behind this work is to investigate the probable relevant causes that made these proteins not detected at protein levels as well as proposing a surrogate approaches to overcome this obstacle. **Methods:** Bioinformatics workflow has been developed to analyze these "missing" proteins and their digested tryptic peptides by applying several bioinformatics analysis on them. This workflow aimed to analyze missing proteins through predicting their physicochemical properties and investigating existence, neighbors and distribution of their tryptic cleavage sites. Moreover, analyzing their digested tryptic peptides to investigate the uniqueness of them. **Results and Discussion:** Predicting the physicochemical properties of missing proteins showed that approximately sixty percentages of missing proteins are basic proteins, which may require ETD fragmentation technology for better detection of these proteins. Approximately Seventy percentages of missing proteins which contain Tansmembrane segments (TMS) were hydrophobic, which means that the exposed regions (loops) of this proteins are short and may not produce any tryptic peptides. Also, tryptic cleavage sites were absent in six missing protein isoforms, the pattern of [(Lys or Arg)Cys] in the missing proteins is 1.5 fold than the same pattern in identified proteins. tryptic peptides analysis showed that approximately fifteen percentages of missing proteins didn't contain any unique tryptic peptides . We have investigated missing proteins by bioinformatics approach. Most of these proteins are basic, 30% contain TMS and approximately 25% are hydrophobic, 15% were digested into only shared tryptic peptides Also, we supposed tolerated experiments, concerning different aspects to identify these missing proteins at protein level.

P01.27 MI-PVT: Michigan Proteome Visualization Tool

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Introduction and Objectives: The Human Proteome Organization (HUPO) initiated a Chromosome-centric Human Proteome Project (C-HPP) for annotating protein-coding genes from all chromosomes. The expression of different proteins is important for analyzing patterns across different tissues, but the heterogeneity of proteomic data in the public domain is a major limitation. **Methods:** Recently, we have published a web-based Michigan Proteome Visualization Tool (MI-PVT) to visualize proteins expression across human chromosomes and tissues (http://guanlab.ccmb.med.umich.edu/mipvt). Initially, we used Human Proteome Map (HPM) data of 30 different tissues and cell types and also provided an option to upload user data. **Results and Discussion:** We are planning to use more large-scale proteomic data in future and also this tool will be integrated with other available chromosome-centric browsers. This tool is useful for biologists to explore protein expression patterns across different chromosomes and tissues both. We also implemented our tool IsoFunc for annotating functions of splice isoforms. **Conclusion:** We believe that C-HPP community will use this tool for studying their data as well as for scrutinizing public data.

Keywords: MI-PVT, C-HPP, HUPO, Chromosome

P02: POSTER SESSION - SAMPLE PREPARATION FOR PROTEOMICS

P02.01 Evaluation of a 96-well Immunodepletion Platform for Multiplex MRM Assay of Proteins in Human Plasma

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Introduction and Objectives: Immunodepletion has recently been implemented as a sample fractionation strategy in clinical LC-MS workflows for plasma proteins. High-abundant protein depletion is traditionally performed serially in LC column formats. The depletion capacity of column formats is ideal for discovery workflows as it allows for larger sample volume processing, producing hundreds of micrograms of depleted protein per sample. As the field moves towards clinical applications where increasingly larger numbers of samples are analyzed using targeted strategies, immunodepletion formats that provide higher throughput and require lower plasma volumes will be needed. In the current study, we evaluate a 96-well format for plasma depletion using Seppro IgY14 resin and a volatile buffer system for use in a multiplex MRM assay of proteins in human plasma. **Methods:** Five human proteins and corresponding stable isotope labeled protein internal standards were spiked into human plasma from 20 ng/mL to 10 ug/mL. The plasma samples were depleted of high-abundant proteins in a 96-well format. The depleted fractions were dried and digested in the collection plate. Nine process replicates were generated for each sample. MRM analysis was performed with 3 peptides per protein. LOD's in depleted plasma were compared to the LOD's of the proteins in non-depleted plasma.

Results and Discussion: Stable isotope labeled EPO was spiked into plasma from 10 ng/mL to 10 ug/mL. After depletion, EPO was detected at 1 ug/mL, but was not observed in any of the non-depleted samples. We are currently evaluating 5 proteins spiked from 20 ng/mL to 10 ug/mL and comparing the LOD's to non-depleted plasma spiked from 1 ug/mL to 100 ug/mL. Well-to-well and cycle-to-cycle variabilities will also be evaluated. **Conclusion:** A 96-well format has been developed for high throughput plasma depletion. Preliminary results indicate improved sensitivity when used prior to MRM assay of proteins in human plasma.

Keywords: Depletion, MRM, quantification

P02.02 Sample Preparation for Low Amount Cumulus Samples

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Introduction and Objectives: A major prerequisite for reproductive biotechnologies is the selection and maturation of oocytes. Proteomics analysis of the cumulus oophorus, accompanying the oocyte throughout life, is an interesting tool to investigate cumulus-oocyte-complex metabolism during the final maturation steps noninvasively for the oocyte. The big challenge of this approach is the highly reduced sample amount available for the analysis. The focus of this study is to establish a sample handling protocol for shotgun proteomics of equine cumulus complexes, which in future can be applied for low sample amounts in general. **Methods:** The combination of sonoreactor aided cell lysis procedure with Filter Aided Sample Preparation (FASP) showed to be suitable for handling of low sample amounts. These techniques were optimized by adjusting the lysis conditions (intensity, frequency, time) of the sonoreactor and the clean-up steps of the FASP-protocol to give a reproducible and sensitive method to process limited sample amounts. The optimized method was used on an experiment with equine cumulus samples. Subsequently the samples were analyzed by reverse-phase LC MS/MS using an Orbitrap Fuison mass spectrometer and further processed by a Lable-Free-Quantitation (LFQ)-pipeline. **Results and Discussion:** Preliminary tests with a small sample set proved the ability of the optimized workflow to be reliable, reproducible and sensitive enough to investigate the cumulus proteome of oocytes. Overall approximately 2000 proteins could be identified by shotgun proteomics of which a set of hundreds were seen in all the analyzed samples giving evidence for a reliable and reproducible workflow. In a further step the data were evaluated by our LFQ-pipeline giving some of interesting candidates to discriminate between different matured cumulus complexes and by this stating the sensitivity of the given workflow. **Conclusion:** With our effort we could establish a method that in future will allow to perform sensitive complete proteomics analysis on research projects with very limited amounts of sample.

P02.03 Phosphopeptide Enrichment Strategies for Mass Spectrometry Analysis

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Introduction and Objectives: Phosphorylation is a critical post translational modification that modulates functional activity of numerous proteins. Immobilized metal affinity chromatography (IMAC) and immobilized metal oxide chromatography (IMOC) are two techniques commonly used to specifically enrich low abundant phosphopeptides from proteolytic digests. In this study, we evaluated and optimized different buffer systems to increase the yield and specificity of phosphopeptide enrichment using IMAC and IMOC resins from unlabeled and TMT reagent-labeled protein digests. **Methods:** HeLa cell lysates were reduced and alkylated and digested with trypsin for phosphopeptide enrichment using various Fe-NTA IMAC or TiO2 resins. Protein digests from cells treated with different cell cycle inhibitors were also labeled with Thermo Scientific™ TMT10plex™ reagents before phosphoenrichment and high pH reverse phase fractionation. Peptide yields were measured using a Thermo Scientific™ Colorimetric Peptide Quantiation kits. 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 and perform phospho site localization. **Results and Discussion:** Both IMAC and IMOC are affinity enrichment techniques which bind phosphorylated peptides. Specificity of phosphopeptide binding has been shown to vary greatly with resin type, ligand concentration, metal type, and buffer pH. Here, we evaluated different buffer systems to increase the yield and specificity of phosphopeptide enrichment for Fe-NTA IMAC and TiO2 IMOC resins. Buffers with different organic solvent and acid concentrations were optimized to increase phosphopeptide enrichment to >97% specificity. Phosphopeptide yield was also assessed for unlabeled and TMT reagent-labeled protein digests to determine if different buffer systems increased recovery of TMT-labeled phosphopeptides. **Conclusion:** Overall, our optimized buffer systems increased the yield and specificity of phosphopeptide enrichment using IMAC and IMOC resins from unlabeled and TMT reagent-labeled protein digests.

Keyword: phosphopeptide enrichment, IMAC, Titanium, TMT 10plex

P02.04 STAGE-Diging: A Novel In-Gel Digestion Processing for Proteomic Samples

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Introduction and Objectives: Proteomics based on high-resolution mass spectrometry has become a powerful tool for the analysis of protein abundance, modifications and interactions. New generation mass spectrometers and UPLC are able to cover approximately an entire cell proteome in one run, but sample preparation, in terms of time and sample recovery is still a critical step. In-gel digestion has proven to be beneficial for shotgun approaches, mainly by providing a simple, reproducible and cost-effective procedure for sample pre-fractionation, which significantly improves proteome depth and dynamic range. Complementary, in solution digestion is faster and less laborious but cannot be applied to a large variety of sample types in particular when removal of contaminants or a highly efficient denaturation method is required. Moreover, SDS-page provides a visual quality control of the samples even though in gel digestion typically employs multistep sample-preparation that are subject to sample loss, high variability and extensive time requirements. **Methods:** Here we present a modification of the in-gel digestion method, called STAGE-diging. Briefly, an entire gel lane is processed in a single, enclosed stage-tip. This approach was compared with the well-established procedures for sample preparation. To avoid biological and technical variability, the same sample was processed in parallel with the two approaches. STAGE-diging efficiency was tested both on high and low complexity samples and on 2 different mass spectrometers. **Results and Discussion:** The results show that the STAGE-diging approach reduces sample handling, decreases the analysis time and improves protein identification and quantification. Moreover, shorter instrument time allows performing multiple replicates that produce wider proteome coverage.

Conclusion: STAGE-diging can speed up sample preparation maintaining all the benefits of standard in-gel digestion. It has been successfully used to characterize proteins in complex mixtures but also in interactomics profiling, covering different dynamic ranges of protein amount and complexity.

Keywords: stage-tip, novel approach, in-gel digestion, complex protein mixtures

P02.05 The SingleShot Workflow: Parallel Analysis of Changes in Gene and Protein Expression

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Introduction and Objectives: Traditionally, different sample preparation strategies are applied to monitor mRNA and protein abundance. An ideal sample preparation strategy would allow for the parallel analysis of both analytes by splitting aliquots from an identical preparation. Recently, the SingleShot workflow was introduced by Bio-Rad Laboratories, a fast and easy sample preparation protocol that allows for the analysis of RNA (and DNA) and protein from the same cell population. The challenge during the development of the SingleShot protocol was to be as effective as RIPA buffer, considered the gold standard in protein analysis for efficient cell lysis and protein solubilization. **Methods:** Here we compare HeLa cells incubated both with SingleShot lysis buffer and RIPA buffer for 5 and 15 min on ice. A protein assay was performed to measure protein yield. The protein solutions were then analyzed by SDS-PAGE and western blotting. The expression levels of various key proteins representing different cell locations were measured in both sample types. **Results and Discussion:** The comparison of both buffers is based on three parameters: (1) yield, as measured by protein assay; (2) qualitative protein pattern, as monitored by SDS-PAGE and (3) protein expression levels of various key proteins representing different cell locations, as measured by western blotting. Normalized yield show no significant difference between SingleShot and RIPA buffer, and the pattern of protein distribution as viewed by SDS-PAGE is very comparable. The expression levels of important key proteins were measured by western blotting, and signal intensities from SingleShot and RIPA preparations were equivalent. **Conclusion:** The unique SingleShot approach is able to integrate important Genomics and Proteomics workflows. It allows for the parallel monitoring of mRNA and protein levels based on an efficient and uncomplicated sample preparation procedure. Protein samples obtained with the SingleShot cell lysis are also compatible with other analytical methods, e.g. 2D PAGE, mass spectrometry and ELISA.

Keywords: SingleShot workflow, Stain-Free technology, Western blotting, Sample Preparation

P02.06 An Efficient Platform for Basic Reversed-Phase Off-Line Fractionation Enables Deep Proteome Coverage

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Introduction and Objectives: To maximize proteome coverage, off-line peptide fractionation with high pH reversed-phase chromatography prior to LC-MS/MS analysis has shown great promise. This strategy introduces a highly orthogonal workflow with the downstream low-pH reversed-phase chromatography in-line with the mass spectrometer. However, degradation of column performance has been observed over time when fractionating samples with ammonium hydroxide (pH~10) in the running buffers. Here, we show that comparable results can be achieved by fractionating complex peptide mixtures with a buffer system composed of ammonium bicarbonate (pH=8). **Methods:** 1mg of tryptic HeLa peptides was subjected to basic reversed-phase off-line fractionation. A total of 70 fractions were collected and automatically concatenated into 14 fractions. Samples were acidified with formic acid prior to concentration in a speed-vac. 1ug from each concatenated peptide fraction was analyzed by nanoflow LC-MS/MS on QExactive instruments using 1h gradients. All LC-MS/MS data were analyzed using the MaxQuant software suite using the Andromeda search engine and requiring an FDR<0.01 on both peptide and protein level. **Results and Discussion:** We analyzed offline peptide reversed-phase fractionation at different pH and found that the results are very comparable for HeLa proteomes fractionated at pH8, 9 or 10. From 14 fractions analyzed by 1h gradients we identify more than 110,000 unique peptides in all three conditions, when analyzed with the fast sequencing speed on a QExactive Plus instrument. We identify more than 140,000 unique peptides utilizing the very rapid sequencing speed (<50ms per HCD spectra) on the latest generation QExactive HF instrument. By optimizing the fractionation gradient, we are able to fractionate very low amounts of input material in microgram range and identify close to 200,000 unique peptides resulting in more than 10,000 unique proteins (Protein groups) in less than one day. **Conclusion:** Basic reversed-phase off-line fractionation enables ultra-deep proteome coverage of >10,000 proteins in less than one day of LC-MS time.

Keywords: Orbitrap, Peptides, deep proteome coverage, Basic reversed-phase off-line fractionation

P02.07 A High-Performance, Scalable and Universal Phosphoproteomics Platform

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Introduction and Objectives: Tremendous advances in MS-based proteomics has revealed the pervasive nature of protein phosphorylation, with >200,000 phosphorylation sites identified to date. The widespread utility and application of existing approaches to quantifying signalling in vivo and en-masse is however hampered by poor scalability of enrichment and labelling workflows, large input-material requirements, and inadequate coverage of key signaling networks, particularly in the context of tissue samples. Here, we set out to develop and refine a phosphoproteomics pipeline that was scalable, without compromising performance. Such a pipeline would enable processing of large sample numbers in parallel, and without using proprietary reagents to ensure its widespread applicability. **Methods:** Not applicable.

Results and Discussion: Here we describe a high-performance, scalable and extensible phosphoproteomics workflow that streamlines the study of large signalling networks. To evaluate our platform in cells and tissues we measured the phosphoproteomes of mouse liver cell lines, where replicate single-shot measurements quantified ~20,000 distinct phosphopeptides in one day. In liver, brain and kidney tissues, half-day measurements together quantified 24,000 phosphopeptides. Deep phosphoproteome coverage in the absence of fractionation was facilitated by high enrichment specificity (>95% of identified peptides phosphorylated). Compared with our previous studies in the same systems we achieved 3x the depth, despite using 10x less material and 1/3rd of the measurement time. **Conclusion:** The rapid and generic phosphoproteomics platform described here requires minimal input material and measurement time without compromising depth, making large-scale signaling studies much more practical. Just as cheap and rapid sequencing technologies have driven an explosion of data in the genomics fields, we envisage an acceleration of MS-

driven signalling studies providing rich and complex insights about cellular network function in physiological and patho-physiological contexts.

Keywords: phosphoproteomics, signal transduction, Sample Preparation

P02.08 A Novel Approach for Fabricating Functionalized Plate for Sample Enrichment and MS Analysis

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Introduction and Objectives: Miniaturized sample preparation methods based on solid phase extraction (SPE) have been developed to purify and concentrate trace samples prior to mass spectrometry (MS) analysis. These miniature methods include micropipet SPE tips, microtiter SPE plates, functionalized-magnetic nanoparticles, on-target SPE MALDI target and miniaturized/microfabricated chip-based SPE plates. However, many of these methods were prepared by complex protocols and are not a disposable coating which may bring sample carryover effect. In this study, we introduced a novel approach to fabricate functionalized MALDI-target for TOF analysis or on plates for offline peptide/protein purification, which can be subjected to nanoLC-MS/MS or MALDI-TOF analysis. **Methods:** Protein,or peptide samples were dissolved in loading buffer and loaded onto the C18 spots. The spots were then washed with the ddH2O solution to remove salts, urea or SDS. For on-probe MALDI-TOF analysis, CHCA solution or SA solution was directly applied to the C18 spots, and after matrix/analyte co-crystallization, the C18 plate was sent into the MALDI ionization source for MALDI-TOF analysis. For glycosylated peptide analysis, glycosylated and peptid mixtures dissolved in ddH2O were loaded onto the hydrophilic spots. After sample dried, the spots were then washed with 5 μl 95% ACN/0.1% TFA solution to remove non-glycopeptides. **Results and Discussion:** The C18 plate demonstrated the effective purification of peptides or proteins from high contents of detergents, urea and salts with good sample recovery. The hydrophilic plate shows enrichment performance of glycopeptides. The capacity of the ~2.8 mm diameter C18 spot was estimated to be ~10 μg of BSA digests. For high density lipoproteins samples, the hydrophobic plates provide a rapid approach with reduced sample volume for desalting of KBr compared to dialysis method. **Conclusion:** Our developed particle coating approach could be used for modifying plates with various functionizd beads for more broaden applications.

Keywords: glycopeptide, On-plate, Enrichment, desalting

P02.09 Comparison of Protein Extraction Efficiencies of Dried Blood Spots and Dried Plasma Spots

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Introduction and Objectives: Dried Blood Spots (DBS) are a well established, valuable tool for the clinical diagnostics of metabolic dysfunctions in newborn infants and for population based research. They can be easily adapted to the clinical routine. Storage and transportation are much easier for DBS than for blood or blood plasma samples (Spooner, M. et al. 2009, Anal Chem 81, 1557-63; McDade, T.W. et al:, 2007, Demography,44, 899-925). Due to increased interest in Blood Proteomics for clinical diagnostics interest in DBS for proteomics applications raised (Sleczka, B.G. et al. 2012, Bioanalysis, 4, 29-40; Kehler; J. et al., 2011, Bioanalysis, 3, 2283-90; Chambers, A.G. et al., 2013, Mol Cell Proteomics, 12, 781-91). **Methods:** Fresh blood and plasma samples were spotted onto clinical DBS carriers. Extraction of proteins from DBS was performed based on published protocols (Chambers et al., 2012, J.Mol.Cell.Prot, 12,781-791). Extracted proteins were analyzed by SDS-PAGE and digested to be analyzed by LC-ESI-MS/MS and LC-ESI-HighRes-MS. Peptides were identified by automatic MASCOT search (MS/MS data). For comparison of extraction efficiency specific peptide signals were quantified based on extracted ion chromatograms (EIC) or differences identified by principal component analyses. **Results and Discussion:** The different types of samples showed the expected differences. Dried plasma spots are easier to handle in the analytical lab as cellular proteins are easily depleted by centrifugation. Nevertheless centrifugation of blood samples is not optimal for clinical routine but is not applicable in population based research, where participants have to sample blood in their daily environment. Fresh blood spotted directly from a pricked finger tip to the carrier might be the preferred sample for future optimization studies. Furthermore, this procedure is identical to the procedure used in the final application. **Conclusion:** Specific extraction protocols for a small panel of diagnostic protein biomarkers will be the sampling method of choice for clinical diagnostics and population based research.

Keywords: Plasma proteomics, Dried Blood Spots, Sample Preparation

P02.10 Strong Cation Exchange Separation of Peptides Using a Spin Column Format (ProTrap XG)

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Introduction and Objectives: The development techniques to address the need for high throughput analysis of proteins and their peptide fragments is an industry within the field of proteomics unto itself. 2D-gel electrophoresis, 2D-LC and a variety of other techniques have all play a role at addressing these issues. Often automated separation alternatives are limited by solvent choice.The development of the ProTrap XG (a spin column) provides an oppourtunity to streamline the offline approach by adapting the core principles of MudPIT. This allows the researcher to tailor the separation to a specific proteome by performing the SCX separation ahead of LC/MS. **Methods:** Separation of peptides using strong cation exchange, prior to clean up and secondary separation in LC-MS. **Results and Discussion:** Not Applicable
Conclusion: Not Applicable

Keywords: ProTrap, Bottom-up separation, LC-MS

P02.11 Quantitative Peptide Assay for Optimized and Reproducible Sample Preparations

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Introduction and Objectives: New advances in mass spectrometry (MS)

enable comprehensive characterization and accurate quantitation of complete proteomes. Despite the rapid advances in analytical instrumentation and data processing, quality of the generated data to a large extent depends on the upstream sample preparation techniques. In this study we utilized a peptide quantitation assay to monitor the peptide concentration at each step of a typical bottom-up proteomics workflow, including labeling with Tandem Mass Tag™ (TMT™) reagents and off-line fractionation steps. **Methods:** A549 human cells grown at different conditions were lysed and digested. Concentrations were normalized before labeling samples with TMT 6plex™ labeling reagents, followed by high PH reverse phase fractionation. The peptide concentrations were measured in between each of above experimental step. The fractionated samples were separated using a 50cm length column followed by the detection on the Thermo Scientific™ Q Exactive™ Plus mass spectrometer. **Results and Discussion:** While the protein concentration was routinely measured by the BCA Protein Assay Kit before the digestion, the true peptide concentration after the digestion could vary substantially due to the different digestion efficiency and separate sample handling. We observed up to 20% difference in protein identifications (assessed in triplicates) based on the initial protein assay when injecting 200ng of samples and analyzing by LCMS. The peptide assay indicated 30% variations in the peptide concentrations, which explained the discrepancy in protein identifications. With the normalization, 200ng of sample load for the triplicates only resulted in 5% variability in protein identifications, a significant improvement in reproducibility. Similarly, the measurement of peptide concentrations in the cell digests from different conditions allowed the accurate mixing for TMT labeling, and subsequent sample fractionation. The peptide concentration in each fraction was measured and sample load for each MS analysis was adjusted accordingly. As a result, consistency and reproducibility of all MS data improved significantly. **Conclusion:** not applicable

Keywords: assay, reproducible, peptide

P02.12 A Systematic Investigation Reveals an Unexpected Side Modification Caused by Lysine Guanidination

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Introduction and Objectives: Amine is the most reactive group in peptides and proteins once the sulfhydryl group is protected. Today there are numerous chemical derivatization approaches on the basis of the amine reactivity, including guanidination, dimethylation, acetylation and so on.Among these methods, guanidination is distinct from others since it occurs specifically at the ε–amine of lysine. So far it has been applied in broad proteomics studies to enhance the ionization efficiency and to selectively protect the ε–amine of lysine. It is believed that the reaction should be carried out at pH > 10 to ensure the deprotonation of ε–amine; and in practice the high pH is often achieved by the addition of $NH_3.H_2O$, NaOH or Na_2CO_3. Although several obvious advantages had been claimed using $NH_3.H_2O$ as the reaction buffer, including the easy removal of $NH_3.H_2O$ by evaporation and avoiding the use of metal ion which often interferes mass spectrometry detection , the sodium buffer seem favored in practical application. We evaluated the two buffers and suggested the optimal conditions. **Methods:** not applicable
Results and Discussion: not applicable
Conclusion: We reported for the first time an unexpected side modification, + 57.02 Da, resulting from the well-established guanidination reaction. We carried out a systematic study and discovered that the side modification is buffer dependent and specifically occurs on Lys. Taking advantage of the highly accurate mass spectrometer and bioinformatics tools, we successfully deciphered the element composition of the increased mass as C_2H_3NO, and proposed a reaction mechanism for the side modification. we evaluated the influence of the side modification in large-scale proteomics studies and consequently suggested the optimal reaction conditions for Lys guanidination. Our study also suggested that more attentions should be paid to derivatization methods even for some widely used methods.

Keywords: Guanidination, side modifiction, methyl formimidate derivative

P02.13 High pH Reversed-Phase Peptide Fractionation in a Convenient Spin-Column Format

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Introduction and Objectives: Many biologically relevant changes in the proteome occur at the mid-to-low range of the protein abundance scale. Off-line fractionation of complex peptide mixtures from sample digests enables deeper proteome sequencing through increased protein identifications and sequence coverage. High pH reversed-phase fractionation enables peptide fractionation orthogonal to low pH reversed-phased separation and does not require desalting. In this study, we assessed peptide/protein identification numbers, fractional resolution and reproducibility of high pH reversed-phase fractionation in a spin column format. **Methods:** Protein extracts from HeLa lysates were digested sequentially with Lys-C and trypsin and peptide quantitation was performed using a Thermo Scientific Pierce™ Colorimetric Peptide Quantitation Assay. Portions of the digested samples were labeled with Thermo Scientific Tandem Mass Tag™ (TMT™) reagents. Pierce High pH Reversed-Phase Fractionation Kits were used to fractionate both native and TMT-labeled digest samples by an increasing acetonitrile step-gradient elution. Fractions were dried and re-suspended in 0.1% formic acid prior to LC/MS analysis on Thermo Scientific Orbitrap Fusion™ Tribrid™ mass spectrometer. All fractionations were performed in triplicate, with sample loads ranging from 10-100µg, as determined by quantitative peptide assays. **Results and Discussion:** We assessed different column matrices, fill amounts and step gradients for column-to-column fractionation reproducibility, peptide fractional profiles, peptide fractionation resolution, and unique peptide/protein identification numbers. Prior to LC/MS analysis, injected sample volumes were adjusted to deliver 1µg of sample material on-column. With the optimized format and fractionation protocol, we routinely identified ~4,300 protein groups using a single two-hour LC gradient which increased to ~7,500 protein groups upon combining the data from all eight fractions (two-hour LC runs each). This resulted in greater than 100% increase in unique peptide identifications. This increase in identifications was observed for 10-100µg sample amounts of both native and TMT-labeled samples. **Conclusion:** The spin column format provides a convenient, reproducible, orthogonal fractionation for deeper sequencing of complex proteomes.

Keywords: tandem mass tag, Orbitrap Fusion, High pH, Fractionation

P02.14 Methodology Development for Quantification of Tightly Adsorbed Proteins

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Introduction and Objectives: The purpose of this study is to investigate the non-specific protein adsorption of protein mixtures on polypropylene surfaces, in an attempt to quantify protein sample loss in everyday proteomics experiments. Till now, research on protein adsorption was limited to study of few model proteins. Proteomics however is the study of all proteins present in the sample, and protein adsorption in context of proteomics has lacked investigation. Non-specific protein adsorption of proteomics sample to the vial surfaces, prior to analysis leads to non-uniform loss of proteins, which is a hindrance particularly to the detection and quantification of low abundance proteins. We aim to investigate the adsorption spectrum of all the proteins in a complex mixture. The holistic information derived from this work would help us in understanding adsorption phenomenon and designing techniques to reduce non-specific sample loss in proteomics for sensitive and accurate detection of low abundant proteins. For these reasons we optimized a Direct Protein Analysis method to quantify differential adsorption pattern of proteins in a complex mixture. We used milk proteins as a model for complex protein mixture. **Methods:** We studied milk protein adsorption on polypropylene vials, and used SDS-PAGE based approach for fast and quantitative characterization. To accurately and sensitively characterize the tightly adsorbed proteins, we optimizedstrippingefficiency.Ourdevelopedprotocolisabletoachieveaccurate and reproducible quantitative measurement of different proteins adsorbed from complex mixture by incorporating the corresponding internal standards. **Results and Discussion:** We characterized the reproducibility and accuracy of quantitation for this method. By using this method we were able to observe differential adsorption of different milk proteins.

Conclusion: We successfully developed a simple, fast, reproducible and accurate method to quickly access differential protein adsorption in complex mixtures. Using this method, we hope the sample loss in proteomics can be better understood and prevented in the future.

P02.15 Automated Protein Digestion to Reduce the Sample Preparation Bottleneck

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Introduction and Objectives: Today using targeted quantitative proteomics techniques such as MRM analysis, protein panels can be quantified across a broad dynamic range with very high reproducibility. With SWATH™ Acquisition, similar reproducibility can be obtained at much higher multiplexing of proteins. While these techniques have increased the reproducibility and scale of protein analysis, sample preparation remains a key bottleneck as the number of samples increases. Numerous processing steps are required, each susceptible to technical variation. Here, we have used automation to improve the throughput and day-to-day reproducibility of the protein digestion portion of the sample preparation workflow. **Methods:** To increase throughput and reduce technical variation, we have implemented an automated peptide preparation protocol on a liquid handling workstation (Biomek NX^{P}) coupled with an MRM workflow using either a QTRAP® 6500 or a TripleTOF® 5600+ system. Denaturation, reduction/alkylation and digestion were the steps included, total workflow time was ~5-6 hours, depending on # of samples processed. A large number of proteins/peptides were monitored to ensure that good general digestion was occurring. Two different automation workstations were used to confirm the method transferability and multi-day experiments were performed on each to confirm method reproducibility. **Results and Discussion:** First the protocol was validated by performing a very careful manual digestion across 24 wells using MRMs to 150 peptides. Using this benchmark, the automation protocol was optimized to achieve similar or better performance. For off-deck digestion using separate incubators, on average, ~80% of peptides monitored had raw peak area CVs < 10% as monitored by LC/MRM analysis. Next steps will be to optimize the use of on-deck heating (incubators integrated on workstation) and assess variance. Use of internal standards could also be incorporated for specific workflows to further reduce variance and will be assessed. **Conclusion:** The automation method provided excellent reproducibility for digestion across multiple days on multiple workstations.

Keywords: SWATH, automation, digestion, MRM

P02.16 Protein Fractionation Using a Dissolvable Acrylamide Gel and Its Application to Top-Down Proteomics

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Introduction and Objectives: Electrophoresis using a polyacrylamide gel matrix enables high-resolution pre-fractionation of protein samples prior to mass spectrometry. However, the effective recovery of gel-separated proteins in the intact form remains a challenging issue. Here, we developed an effective method to recover gel-separated intact proteins using a bis-acrylylcystamine (BAC)-crosslinked polyacrylamide gel, which could be useful for sample pretreatment in top-down proteomics. **Methods:** All protein samples were initially reduced with dithiothreitol and then alkylated with acrylamide. Reductively alkylated protein samples were then applied to a 4% acrylamide stacking gel that was cast over a 12.5% BAC-polyacrylamide gel. After gel electrophoresis, the separated proteins were visualized by reversible negative staining. Target protein bands were excised from the gel, and each gel piece was dissolved by adding Tris-(2-carboxyethyl)phosphine (TCEP). After precipitation of the acrylamide filament with acetonitrile, the supernatants were subjected to MS analyses. **Results and Discussion:** A piece of the BAC gel was gradually degraded during incubation with TCEP. Dissolution was achieved within 20 min, which, in turn, released the gel-separated proteins. Complete dissolution of the gel matrix with TCEP treatment has the advantage that reproducible protein recovery can be achieved regardless of the protein length. Recovery rate of bovine serum albumin from the gel was high (>60%), and the recovered proteins were successfully analyzed by matrix-assisted laser desorption/ionization and electrospray ionization mass spectrometry. Although the use of sodium dodecyl sulfate in the process of gel-dissolution with TCEP generally enhanced protein recovery, detergent-free recovery, which is suitable for MS analysis, is also effective. **Conclusion:** A novel method for pre-fractionation of protein samples by using a dissolvable BAC polyacrylamide gel was established. Highly efficient protein recovery methods will be useful for sample pretreatment in top-down proteomics.

Keywords: bis-acrylylcystamine, Gel electrophoresis, Top-down proteomics

P02.17 Low Cost Semi-Automated In-Gel Tryptic Digest for High-Throughput Proteomics

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Introduction and Objectives: Peptide generation by trypsin digestion is commonly the first step of mass spectrometry-based proteomics experiments, including 'bottom-up' discovery and targeted proteomics using multiple reaction monitoring. For quantitative proteomics experiments and those involving large numbers of clinical samples, manual trypsin digest and the subsequent clean-up steps leads to variability even before the sample reaches the analytical platform. While specialized filter plates and tips have been designed to facilitate sample processing, the specialty reagents and equipment required may not be accessible or feasible. The objective of this study was to develop a cost-effective, semi-automated in-gel tryptic digest method using standard 96-well microplates with a laboratory liquid handler. **Methods:** An existing manual in-gel digest methodology was transferred to a small foot print laboratory liquid handler (Bravo), using standard 96 well microplates. Tip positioning and solvent addition procedures were designed to avoid stabbing the gel piece and to minimise tip usage. The methodology has been routinely used in our laboratory. To formally evaluate the methodology, we compared the results of robot digest versus manual digest for (1) a simple mixture of 7 proteins, and (2) a complex sample separated into 8 gel bands. **Results and Discussion:** The liquid handler assisted digest consistently performed as well, if not better, than manual digest. The protocol was designed to reduce costs and wastage. We show that we can minimize the number of tips required without risk of cross sample contamination. We are also able to avoid using specialised plates by modifying the locations of where reagents are dispensed and collected to avoid picking up gel pieces. **Conclusion:** The simplicity, reproducibility and cost-effectiveness of our semi-automated protocol makes it suitable for routine in-gel tryptic digest, as well as high throughput processing of large clinical sample cohorts.

Keywords: robotics, in-gel digest, liquid handler

P02.18 Trypsin Coated Polymer Nanofibers and Its Application to Protein Digestion under the Pressure Cycle

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Introduction and Objectives: Trypsin (TR) is an enzyme, which selectively cleaves the protein sequence and make peptide fragments. This process, called protein digestion, is one of the key steps in proteomic analysis. After trypsin digests protein sample into peptide fragments, they can be analyzed via MALDI-TOF or LC-MS/MS. The current technology of protein digestion in proteomic analysis is time-consuming, tedious and not-automated due to the poor stability and autolysis of trypsin. To improve the efficiency of the protein digestion process, trypsin was immobilized and stabilized on polystyrene-based nanofiber (NF) with enzyme coating approach (EC-TR/NF). **Methods:** Polystyrene-based nanofiber was synthesized by electrospinning polystyrene and poly(styrene-co-maleic anhydride). EC-TR/NF was fabricated via simple two-step process, which consists of attachment of seed enzymes onto the surface of NFs and the crosslinking of trypsin molecules with seed enzymes. **Results and Discussion:** EC-TR/NF effectively prevented both denaturation and autolysis of trypsin and its high stability might be resulted from the multipoint covalent linkages formed among the trypsin molecules.

Highly-stable EC-TR/NF was employed for the digestion of protein samples. During the protein digestion process, external energy, such as pressure cycle, was applied to reduce the processing time. As a result, rapid digestion of protein was achieved while EC-TR/NF maintained its proteolytic activity under the application of external energy due to its stability. **Conclusion:** EC-TR/NF can be an appealing option not only for the protein digestion platform with high performance, good stability and long lifetime, but also for other applications where the poor stability of enzyme is a critical drawback.

Keywords: Trypsin, Protein digestion, Polymer nanofiber, Enzyme coating

P02.19 Improved Speed & Reproducibility of Protein Digestion Using Novel Sample Preparation Technology

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Introduction and Objectives: Reproducibility is a fundamental requirement of quantitative peptide mapping workflows as it enables users to confidently assign data differences to the sample, and not the methodological conditions used. Currently overnight in-solution trypsin digestion of proteins is used during peptide mapping; however this protocol requires a number of steps, which can differ between laboratories, making method transfer and data analysis between user groups problematic. Additionally, due to the number of steps required, in-solution digestion can increase the potential for user error. As a result, this methodology often leads to variations in the chromatographic profile and complicates the adoption of robust, generic workflows. Here we describe a workflow including novel, rapid and precise digestion of Cytochrome C, followed by micro-elution solid phase extraction (SPE) clean-up and analysis with next-generation UHPLC and high resolution mass spectrometry detection (UHPLC-HRMS). **Methods:** Four well characterized peptides, derived from cytochrome C, were used for assessment of the novel digestion procedure. Four exogenous peptides were also spiked in post digest, which allowed determination of the reproducibility of the digestion, and an independent assessment of the clean-up procedures.

Results and Discussion: Recovery and precision were assessed and compared between SPE and filtration; greater levels of recovery and precision were observed with SPE. Some selectivity differences between the two techniques are discussed, as well as reproducibility of both the digestion and clean-up method. Comparison of the different sample preparation techniques determined that when speed and method simplicity is required size exclusion filter plates were optimal, however when higher accuracy and precision are desirable SPE is more appropriate. The workflow described allows for the introduction of fast, generic, and robust analytical methods suitable for a high throughput, environment.

Conclusion: Excellent levels of recovery and precision were observed giving a high throughput and reproducible workflow that can be applied to non-targeted, semi-targeted or targeted quantitative environments.

Keywords: digestion, Peptides, micro-elution, Reproducibility

P03: POSTER SESSION - REGENERATIVE MEDICINE AND STEM CELLS

P03.01 Proteomic Analysis of Infantile Haemangioma

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Introduction and Objectives: Infantile haemangioma (IH) is the most common tumour of infancy, characterised by an initial proliferation followed by spontaneous involution often leaving a fibro-fatty residuum. IH has been considered a tumour of the microvasculature, however, recent data has demonstrated a critical role of stem cells in the biology of this enigmatic condition. This study quantitatively profiled the proteomes of IH tissue at its three phases of development. **Methods:** Total protein was extracted from snap-frozen proliferating (n=2), involuting (n=2), and involuted (n=2) IH samples, trypsin digested, C18 purified, and analysed in quadruplicate by LC-MS/MS. Raw MS/MS data were searched against the UniProt complete human proteome database using Proteome Discoverer™ V1.4. Scaffold 4.0 was used for protein identification relative quantitation. Network and GO analyses were performed using String 10.0. **Results and Discussion:** 764 proteins were identified, of which 682 were common to all three phases of IH. The relative abundance of 66 proteins was significantly greater in proliferating compared to involuting and involuted tissues ($p<=0.05$). Only 8 and 4 proteins were significantly more abundant in involuting and involuted tissues, respectively. The most statistically significant GO Biological processes relating to the proteins in proliferating IH tissues that had the highest relative abundance increases included antigen and immune system processing, and regulation of defence response. Several proteasomal proteins were included in this group. Nucleotide, RNA, and protein binding were the most statistically significant GO Molecular functions among the same group of proteins. **Conclusion:** The data of this study suggests proteasome dysregulation during the proliferation of IH and highlights the complex interplay of proteasomes in stem cell biology. Better understanding of these proteasomes and their role in the patho-aetiology of IH may lead to the identification of novel targets and more effective and safer treatment of IH.

Keywords: Infantile haemangioma, stem cells, proteasome

P03.02 Candidate Surface Biomarkers of Transformed Mesenchymal Stem Cells by Quantitative (Glyco)Proteomics

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Introduction and Objectives: Multipotent mesenchymal stromal/stem cells (MSCs) are a key component of the tumour environment and play a role in enabling tumour growth. Genetically transformed MSCs may represent the initial cell of sarcoma development. There are currently no reliable methods for discriminating between normal and tumour-promoting human MSCs (hMSCs). Here we aimed at identifying cell-surface membrane (glyco)protein markers that are specific to normal and tumourigenic hMSCs and could serve to distinguish each class. **Methods:** We used immortalized/transformed hMSC cultures (n=3) shown to initiate mixoid liposarcoma (MLS) in vivo as a model of human sarcomagenesis and directly compared their membrane protein profile to normal hMSC cultures (n=3) using quantitative multiplexing proteomics and glycoproteomics. LC-MS analysis was conducted on an Orbitrap Fusion using SPS-MS3. **Results and Discussion:** Quantitative proteomic analysis of cell fractions containing non-nuclear membrane/organelle proteins identified ~2700 unique proteins common to all 6 hMSC cultures. Differences in protein abundance levels between each of the 3 normal hMSC cultures (~20-110 proteins with levels greater than two-fold change) and between each of the 3 transformed hMSC cell lines (~5-120 proteins with levels greater than two-fold change) were found. However, there were profound differences in protein abundance levels between normal and tumourigenic cell classes. The tumourigenic hMSCs exhibited 454 differentially regulated proteins, where 252 proteins displayed a two-fold increase in quantity and 202 proteins displayed a two-fold decrease in quantity. For quantitative glycoproteomics, ~450 unique glycoproteins were identified. Preliminary examination showed specific glycoprotein glycoform alterations between hMSC classes. Cellular pathways analysis of (glyco)proteins showing significant quantitative differences between normal and tumourigenic hMSC classes is expected to yield cell-surface membrane proteins that may have utility as biomarkers, as well as non-nuclear membrane and luminal organelle proteins that may shed further light on the biological mechanisms altered in the MLS-initiating cells. **Conclusion:** not applicable

Keywords: glycoprotein, stem cell, Orbitrap, membrane

P04: POSTER SESSION - NEW TECHNOLOGICAL ADVANCES IN PROTEOMICS

P04.01 HiRIEF LC/MRM-MS: Toward Increased Coverage of the Human Plasma Proteome

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Introduction and Objectives: MRM or multiple reaction monitoring based proteomics is the gold standard for mass spectrometry based quantitative proteomics, however to date the most comprehensive analyses of the human plasma proteome have quantified only roughly 300 of more than 10,000 proteins. This highlights the need in the proteomics community for new methodologies that can increase the sensitivity and breadth of our MRM based analyses. Novel separation techniques are highly desirable to further reduce sample complexity and allow for better sensitivity and coverage of the plasma proteome. To this end we utilized high-resolution isoelectric focussing (HiRIEF) of tryptic peptides in conjunction with LC/MRM-MS to compare against other leading separation technologies. **Methods:** Briefly, equimolar mixtures of over 1500 stable isotope-labeled peptide standards (SIS) corresponding to over 600 proteins were spiked in buffer or tryptic digests of human plasma. Samples were separated by HiRIEF in broad, narrow, and ultra-narrow pH ranges (pH 3-10, pH3.7-4.9, and pH 4.00-4.25 respectively). SIS+buffer containing fractions were subjected to nanoLC-MS using a Thermo Orbitrap Fusion to create fraction specific MRM methods. Retention times were scheduled by LC/MRM-MS analysis of SIS+Buffer samples, and endogenous peptides from plasma+SIS samples were detected

by LC/MRM-MS on an Agilent 6495 triple quadrupole mass spectrometer. Data was analysed using Skyline v3.1 and software developed in-house. **Results and Discussion:** HiRIEF LC/MRM-MS analysis of SIS spiked plasma samples enabled the detection of 663 endogenous peptides corresponding to 358 proteins. These results suggest the HiRIEF method is comparable with other leading 2D LC-MS methodologies including high pH prefractionation, however it remains to be determined how many of these detected proteins are quantifiable. **Conclusion:** These preliminary results suggest that HiRIEF may serve as an effective means of reducing sample complexity and increasing sensitivity and coverage in quantitative analyses of the human plasma proteome.

Keywords: MRM, high-resolution isoelectric focussing, quantitative proteomics, Fractionation

P04.02 Accuracy and Sensitivity of Proteome Quantitation Using TMT - An Evaluation of Recent Developments

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Introduction and Objectives: Proteome quantitation using isobaric chemical tags, such as tandem mass tags (TMT), provides multiplexed parallel comparison of differential expression. Previous studies indicate that when a precursor ion is selected for fragmentation, co-isolation of background interfering ions results in distorted reporter ion ratios, and thus significant errors in quantitation. This study presents the first report of direct measurement of interference and compares the accuracy and sensitivity of TMT quantitation using various mass spectrometry approaches. **Methods:** Fifteen non TMT-labeled standard peptides were spiked into TMT sixplex-labeled peptide mixtures of Neurospora crassa whole cell protein digests. The sample was fractionated and then analyzed on an Orbitrap Fusion mass spectrometer with either a MS2 or a recently developed MultiNotch MS3 approach. Proteome Discoverer was used for identification and quantitation of TMT-labeled peptides. The non TMT-labeled standard peptides were analyzed manually to record the reporter ion intensities from background interference. **Results and Discussion:** From the TMT-labeled Neurospora crassa digest, more than 3500 protein groups and 21000 unique peptides were identified using MS2 methods with 97%-98% of the peptides quantifiable. An 8%-10% loss in the number of identified proteins was observed with MultiNotch MS3 methods. TMT reporter ions in the spectra of the non TMT-labeled peptides provide a true measurement of interference since these reporter ions were completely from the co-isolated background interfering ions. The percentage of quantified peptides having lower than 20% interference was 82.9% using the MultiNotch MS3 method, while it was only 4.7% using the MS2 method. However, decreasing isolation width of MS2 method from 2 to 0.7 improved quantitation accuracy such that this percentage increased from 4.7% to 24.8% without loss of sensitivity. Distributions of interference in TMT quantitation with both methods will be compared. **Conclusion:** Compared to MS2, the MultiNotch MS3 method was distinguished in reducing background interference with minimal cost of identification and quantitation sensitivity.

Keywords: Multiplexed Proteome Quantitation, TMT, MultiNotch, interference in quantitation

P04.03 TMT 10-Plex Quantitation by Travelling Wave IMS-QTof Mass Spectrometry

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Introduction and Objectives: Isobaric quantitative LC-MS proteome analysis of peptide mixtures involves fragmentation of isolated precursors modified with mass tag labelling reagents. Peptides modified by these tags produce characteristic low mass reporter reporter fragment ions ratios for relative quantitation. TMT reagents contain different numbers and combinations of heavy labelled carbon and nitrogen, with the 10plex adaptation comprising pairs separated by merely 6 mDa. Precise quantitation of TMT 10plex modified peptides is demonstrated on a travelling wave IMS-QTof mass spectrometer. **Methods:** Tryptic MPDS Mixture1 and HeLa peptide samples were labelled with TMT 10plex reagent. MPDS was labelled with equal reagent amounts and HeLa with varying amounts. Samples were introduced into the mass spectrometer either by direct infusion or by separation using nanoscale LC chromatography. Both single scan MS and Data Directed Acquisition (DDA) were used on a hybrid travelling wave IMS-QTof mass spectrometer operating in positive mode with enhanced low mass resolution. Acquisition speeds of up to 20 Hz were employed to demonstrate TMT 10 plex reporter ion MS2 quantitation. **Results and Discussion:** Initial experiments were performed to determine that the low mass resolution of the hybrid IMS-QToF instrument was sufficient to distinguish between labels that differ in mass by approximately 6 mDa, (i.e. pairs of tags described nominally as TMT 127 to TMT 130). Fragment ion spectra exhibited the expected reporter ion ratios independent of acquisition speed. Moreover, the results illustrated that the oa-ToF mass analyser was operating with sufficient resolution for the 10plex reagent. Labelled peptides of a HeLa cell line were separated using a 90 min LC gradient and analysed using DDA targeting charge states of 2+ and higher. **Conclusion:** Over 25,000 species were identified for MS2 using optimised collision energies, based on precursor mass and charge, generating balanced reporter and sequence fragment ion MS2 spectra.Good agreement of quantitation measurements with the experimental design is demonstrated.

Keywords: Ion Mobility, TMT 10-plex, DDA

P04.04 ELISA-PLA: A Sensitive and Specific Protein Post Translational Modification Detection Method

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Introduction and Objectives: The sensitive and specific detection of low-abundance proteins and their post-translational modifications (PTM) remains a challenge. Conventional ELISA is not sensitive enough to detect low-abundance protein PTMs and can result in nonspecific detection. Here, we introduce a sensitive and specific ELISA proximity ligation assays (ELISA-PLA). **Methods:** In ELISA-PLA, specificity is achieved by the simultaneous and proximate recognition of target molecules using multiple probes, and sensitivity is achieved by rolling circle amplification (RCA). The method has been used to detect the GFP protein and the phosphorylation level of ERK1/2 in a chronic pain rat. **Results and Discussion:** Comparing with conventional ELISA, ELISA-PLA improves the limit of detection in the analysis of the GFP protein by twofold in magnitude. ELISA-PLA was successfully used to distinguish different phosphorylation levels of ERK1/2 in the naïve rat brain and in the chronic pain rat brain. We used p-ERK1/2 (Tyr204 in ERK1 or Tyr187 in ERK2) antibody or p-Y antibody as a second primary antibody in ELISA-

PLA and found that the phosphorylation level of ERK1/2 was up-regulated in a chronic pain rat in both analysis. Accordingly, for PTM proteins lacking a corresponding site specific antibody, ELISA-PLA can be used to investigate the overall PTM level with the help of site-independent PTM antibody. **Conclusion:** In conclusion, ELISA-PLA is sensitive and specific protein detection technique, which is easy to be conducted and commercialized. It is capable of large-scale detections of low abundant proteins and PTMs in complex biological specimens.

Keywords: post-translational modifications, ELISA-PLA, site-independent PTM antibody

P04.05 Extending the Quantitative Information of the Glioblastoma Proteome Using SuperQuant

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Introduction and Objectives: To improve the current knowledge on glioblastoma (GBM) biology, we performed quantitative proteomics combined with a new data processing tool termed SuperQuant. Shotgun proteomics of GBM cells generate complex peptide samples with frequent co-fragmentation of multiple peptides ions. SuperQuant expands on the complementary approach (matching b,y-ion pairs) of identifying co-isolated peptides. We study the efficiency of SuperQuant to extend the quantitative information from MS1 type quantitative measurements. **Methods:** Human GBM spheroid cultured in differentiated, migrating and spheroid states in three biological replicates were cultured and harvested. Peptide samples were dimethyl labeled and mass spectrometry experiments were conducted using a Dionex Ultimate 3000 nano-LC coupled to an Orbitrap Fusion (ThermoFisher) mass spectrometer. Survey scans (m/z 400-1,200) were recorded with 120,000 resolution followed by CID-MS/MS (up to 3 seconds) with 15,000 resolution (isolation width 2 Th). An algorithm for SuperQuant was implemented in C# (Visual C# 2013, .NET Framework 4.5.50938) and compiled as node with Proteome Discoverer 2.0. **Results and Discussion:** Despite the general lower intensity of co-isolated peptides SuperQuant increased the number of PSM, peptide and protein identifications by 36%, 21% and 11%, respectively. This translated into 10% and 6.5% additional quantified peptides and proteins with SuperQuant compared to non-processed data. Compared to the standard analysis, SuperQuant allowed for the quantification of 23%, 85% and 122% more differentially regulated proteins for spheres, differentiation and migration, respectively. Considering all regulated proteins, 210 proteins were found differentially regulated using SuperQuant compared to 128 proteins from standard analysis; 54 proteins were found regulated only with standard analysis, 74 were detected using both strategies and 136 (106%) were exclusive for SuperQuant. Ingenuity Pathway Analysis suggests that the gene products of PTN, PTPRZ1, CRK and RAC1 play an important role in the migration regulation. **Conclusion:** SuperQuant adds additional quantitative proteome information from shotgun proteomics data of glioblastoma cells.

Keywords: data processing, quantitative proteome analysis, Glioblastoma cell line, Mass spectrometry

P04.06 Improved Peptide Identification Using Variable Window SWATH Acquisition, UPLC and DIA-Umpire

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Introduction and Objectives: Data Independent Acquisition (DIA) has achieved increased attention over the past few years as a competitive method to analyze and quantify peptides in complex samples. In particular, the SWATH-MS™ method can achieve comprehensive MS/MS of all peptides in a sample within an LC timescale. DIA-Umpire was recently shown as an effective software tool that enables conventional database search strategies to be applied to SWATH datasets with performance rivaling Data Dependent Acquisition (DDA) datasets. Here we show the improvements in peptide identification success using DIA-Umpire from SWATH data acquired with variable-sized isolation windows and longer gradient UPLC. **Methods:** Samples were prepared by affinity purification (AP) of FLAG-tagged EIF4A2 and MEPCE expressed in HEK293 cell lines. Digested K562 human lysate was purchased from Promega. Nano-LC-MS data was collected using an Eksigent Ultra or 425 HPLC and an AB SCIEX 5600 or 6600 TripleTOF instrument. SWATH methods that varied in precursor isolation window sizes ranging from 1Da-200Da were used to generate DIA data. **Results and Discussion:** Narrow window SWATH acquisition methods address the challenge of multiplex spectra in DIA by improving specificity and decreasing the number of precursors in each window for co-fragmentation. Acquisition methods that cover the same mass range with smaller SWATH windows (ranging from 3-25 Da) result in an increase in the number of peptides that can be identified through DIA-Umpire analysis of human lysate samples. Analysis of data from this SWATH approach enables identification of lower abundance interactors for EIF4A2 and MEPCE that were not initially identified. **Conclusion:** The use of variable window SWATH acquisition with longer gradient UPLC results in an increase in the number of peptides that can be identified by DIA-Umpire.

Keywords: SWATH, DIA, Identification, UPLC

P04.07 Behavioral and Proteomic Analysis of Stress Response in Zebrafish (Danio rerio)

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Introduction and Objectives: Zebrafish have been a popular model organism in genetics and developmental research for decades and have more recently attracted the interest of scientists studying behavior as well. The zebrafish stress system appears to be quite comparable to the hypothalamic-pituitary-adrenal (HPA) axis in mammalian models. **Methods:** Twenty eight adult wild-type zebrafish (50:50 male/female,age 6−9 months) were housed in an Aquaneering table-top housing rack, with a recirculating filtration system using mechanical, biological, and chemical filtration. Animals were divided randomly into three groups of 8−10 subjects each: experimental (EXP), unpaired (UNP), and no shock control (NOS). Subjects in all groups were given 16 trials, distributed across 4 days with four trials each day. beside behavioral testing, protein were extracted and labeled and multiplexed after trypsin digestion and TMT labeling and analyzed with MudPIT technology. **Results and Discussion:** Behavioral results demonstrate a hyperactivity

response to electric shock and a suppression of activity to a stimulus predicting shock. On the basis of the quantitative changes in protein abundance following shock exposure, eight proteins were significantly upregulated (HADHB, hspa8, hspa5, actb1, mych4, atp2a1, zgc:86709, and zgc:86725). These proteins contribute crucially in catalytic activities, stress response, cation transport, and motor activities. This behavioral proteomic driven study clearly showed that besides the rapid induction of heat shock proteins, other catalytic enzymes and cation transporters were rapidly elevated as a mechanism to counteract oxidative stress conditions resulting from elevated fear/anxiety levels. **Conclusion:** Conclusion: To our knowledge, this is the first study to directly examine the effects of stress on both the behavior and the whole-body proteome of zebrafish. The overall pattern results is consistent with elevated fear/stress levels in experimental groups compared to normal control, with some indication of additional effects based on shock predictability.

Keywords: MudPIT, stress, TMT, quantitative proteomics

P04.08 Protein Post-Translational Modifications of Mouse Kidney Using OFFGel Prefractionation

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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.

Keywords: PTM, MudPIT, Kidney, OFFGel Prefractionation

P04.09 Development of LC-Electrochemistry-MS for Disulfide Mapping: Application to Notch3 Protein Fragments

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Introduction and Objectives: Disulfide bonds (DSBs) are important for the stabilization of the structure and biological function of proteins. Alterations in the number of cysteines and DSBs, e.g. due to genetic mutations, may affect protein function as is the case for the Notch3 protein in Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL, a hereditary stroke disorder). Therefore, the characterization of DSBs experiences a growing interest. In classical bottom-up proteomics, the use of reducing and alkylating agents results in a loss of information regarding DSBs. A combination of electrochemistry (EC) and top-down proteomics allows for disulfide mapping, but has only been applied to peptides and small proteins. Methodologies applicable to larger and more complex proteins are needed. **Methods:** In the current project, EC has been implemented in a bottom-up proteomics workflow through direct coupling of an electrochemical cell to a LC system and an ESI-FTICR MS instrument for online reduction and characterization of DSBs in a protein digest. Proteins were digested without prior reduction and alkylation, and at low pH to minimize disulfide reshuffling. The protein digests were first analyzed with LC–EC–MS without electrochemical reduction to detect the disulfide-linked peptides from the digest. In the second analysis, the DSBs were reduced to detect the disconnected peptides that can then be linked back to the disulfide-linked peptides based on retention time and MS2 data. **Results and Discussion:** The approach was evaluated with a standard protein, ribonuclease B (14 kDa) of which all 4 DSBs were characterized with LC–EC–MS. The methodology was then applied to a wild type Notch3 protein fragment (20 kDa, 15 DSBs). Despite low concentration and polymer contamination, several Notch3 peptides were detected following reduction of the DSBs. **Conclusion:** Online LC–EC–MS is a powerful strategy for the characterization of protein DSBs. Work is ongoing to identify the disulfide-linked peptides in the Notch3 protein fragment.

Keywords: Protein disulfide bonds, Electrochemistry, LC-MS

P04.10 Targeted Tissue-Enriched Proteomics in Kidney Tissue

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Introduction and Objectives: Mass spectrometry-based proteomics is developing in the direction of clinical application. Therefore, reliable quantification methods for absolute protein concentration determination are indispensable tools for future application. Here, we performed the absolute quantification of the protein by using a new method (Immuno-SILAC. **Methods:** 26 proteins whose polyclonal antibodies and well-known stable isotope-labeled recombinant protein fragments (heavy PrEST) were available were selected as target proteins from the transcriptome data, which we have previously identified the kidney enriched proteins based on transcriptomics, literature and now used clinical biomarker. A human normal kidney tissue was used as sample. The heavy PrESTs were added to the kidney lysate and the mix was digested by trypsin. Immuno-affinity enrichment of the digested peptides from sample and heavy PrESTs was carried out with corresponding polyclonal antibodies prior to mass spectrometry analysis. The concentration of the target proteins were calculated based on the ratio between their intensity. **Results and Discussion:** By enriching the target peptides using antibodies, the complexity of the sample was reduced and the peptide derived from the antibody reagent could be quantified by only a 15-minute HPLC gradient for sufficient peptide separation. 8 proteins could be quantified as copy number per 1 μg kidney tissue by Immuno-SILAC. This mass spectrometry-based data was integrated with our reported kidney transcriptomics and antibody-based proteomics data to make kidney “omics” dataset.

Conclusion: The combination of immune-based methods with mass spectrometry detection has become an indispensable tool in the emerging field of tissue based proteomics and clinical application.

Keywords: target proteomics, Immuno-SILAC, kidney proteomics

P04.11 Molecular Dissection of a Proteome

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Introduction and Objectives: The development of proteome wide specific binding reagents is a major goal in biological research. Simplification of highly complex proteomes, by affinity reagents will enhance our understanding of areas where analytical capabilities are currently limiting findings. Affimers, developed as antibody-alternative affinity reagents, are engineered combinatorial proteins possessing three variable interaction sites. The aims of this research were to develop an Affimer pull-down assay compatible with mass spectrometry and to identify binding partners of individual Affimers. **Methods:** Affimers had been pre-selected for the pull-down assay based on differential fluorescence signals between control and sepsis human serum from microarray data. An affinity (Affimer) pull-down assay was developed to identify binding partners of unique Affimers. Affimers were bound to magnetic agarose beads by virtue of the His-tag. The Affimer beads were blocked with casein to reduce non-specific binding. Blocked Affimer beads were incubated with human serum and washed to remove non-specific binding proteins. Interacting proteins were visualised with SDS-PAGE and identified using liquid chromatography tandem mass spectrometry (LC-MS/MS) of on-bead tryptic digests. **Results and Discussion:** The binding partner of the positive control Affimer was successfully identified by LC-MS/MS. Consistent binding of 30 serum proteins to Affimer-bead complex was observed across multiple pull-down assays. Specific Affimer protein binders can therefore be identified as distinct from 'background' proteins. **Conclusion:** The work describes the implementation of a mass spectrometry compatible pull-down assay. These findings demonstrate the potential of an Affimer pull-down mass spectrometry assay and with further development, the possibility for decreased background signals and significant target enrichment.

Keywords: Affimers, Mass spectrometry, Protein binders, Protein arrays

P04.12 The Thermal Stability of the Human Proteome

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Introduction and Objectives: The thermal shift assay (TSA) measures ligand induced protection of protein structure against thermal denaturation. Traditionally, it has been applied to purified proteins but, recently, the idea has been generalized to enable protein-ligand binding studies in cell lysates or intact cells (CETSA). When combined with quantitative mass spectrometry (MS-CETSA), the method now enables the systematic analysis of protein-drug interaction studies on a proteome wide scale. This work presents the characterization of the thermal denaturation behaviour of more than 10,000 proteins in human cancer cells and identifies unexpected targets of cancer drugs. **Methods:** K562 leukaemia cells were lysed in PBS and lysates were heated in narrow intervals across a wide temperature range (35°C-95°C) in the presence or absence of drugs. Denatured proteins were pelleted by ultracentrifugation. Supernatant proteins were digested with trypsin and labelled with TMT. After pooling, samples were fractionated by hSAX prior to LC-MS analysis. Acquired data was processed with MaxQuant and custom R scripts. **Results and Discussion:** In MS-CETSA, each sample is encoded by one TMT label which allows pooling up to ten proteomes each heated to a different temperature. By performing seven interlaced experiments, the melting and aggregation behaviour of >10,000 proteins was monitored between 35°C and 95°C in the presence or absence of cancer drugs. The melting point of a protein is a fundamental biophysical property and the characteristics determined in this work correlated with important functional categories allowing deeper biochemical insights into the human proteome. **Conclusion:** This work not only represents a fundamental study in the characterisation of the thermal denaturation behaviour of the human proteome, but is also an example for an ambitious approach to measure biophysical properties of proteins at a large scale. Wilhelm et al. Nature 2014 Savitski et al. Science 2014

Keywords: Thermal Denaturation, Mass spectrometry, Tandem Mass Tags

P04.13 Zeptomole Detection of Serum Biomarkers Using Surface Plasmon Resonance Imaging (SPRi)

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Introduction and Objectives: Surface Plasmon Resonance imaging (SPRi) is a label free surface–sensitive optical detection method for biomolecular interactions in real time with high throughput. Serum biomarkers for neurological disorders, cardiovascular diseases, and cancer are often in low abundance in bodily fluids presenting many challenges for their detection. **Methods:** The availability of an ultrasensitive detection platform that can profile multiple biomarkers simultaneously is a potentially powerful method for the diagnosis of diseases and monitoring of subsequent therapeutic treatments. In the present work, a technology platform is introduced that integrates SPRi and aptamer technology with nanomaterials and microwave-assisted surface functionalization. **Results and Discussion:** This unique combination and integration makes it possible for the SPRi biosensor to detect C-reactive protein (biomarker) in spiked human serum at ultrasensitive level (fg/ml or attomolar). **Conclusion:** The preliminary results are encouraging and show promise in extending the platform to detect an array of biomarkers in complex biological fluids.

Keywords: biomarker, aptamer, nanoparticle, biosensor

P04.14 High-Density Protein Microarrays for Antibody Validation and Autoimmunity Profiling

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Introduction and Objectives: There is a great need in high-throughput affinity proteomics for greater capabilities for characterization of affinity reagents and for profiling of complex biological samples. We have therefore established a high-density protein microarray platform with

a large set of antigens for characterization of antibodies produced within the Human Protein Atlas (HPA) and to constitute a unique resource for proteomic profiling of autoantibodies in autoimmune disease contexts. **Methods:** We have produced high-density protein microarrays of recombinant human protein fragments representing more than 12.000 unique Ensemble Gene IDs. We extended here the standard validation and evaluation format and characterized a set of polyclonal antibodies produced within the Human Protein Atlas on microarrays of three different sizes, ranging from the routine format of 384 antigens to 11.000 and 21.000 antigens. We also screened serum samples from secondary progressive multiple sclerosis patients to profile the autoantibody repertoires in these samples. **Results and Discussion:** The results show that large-scale protein microarrays are an important addition to the Human Protein Atlas toolbox for antibody characterization and demonstrate that these near proteome-wide scale microarrays allow for high-throughput analysis of serum for identification of possible autoantibody targets in the context of autoimmune conditions. **Conclusion:** We have demonstrated that microarrays with large antigen content are useful for highly multiplexed profiling of the autoimmune repertoire in serum against a very large fraction of the human proteome in a fast and efficient manner by profiling serum samples from secondary progressive multiple sclerosis patients. We also demonstrate the feasibility of using high-density microarrays with large antigen content for mapping the off-target interactions of affinity reagents and how the results from low-density (21x384) arrays correlate to high-density (1x 21.000) arrays. This can be an indispensable tool in the validation and characterisation of affinity reagents and can add important information in the pooled knowledge of an affinity reagent.

Keywords: Protein microarray, Human Protein Atlas, Antibody validation, Autoimmunity profiling

P04.15 Electrochemical Detection of Ovarian Cancer Biomarker HE4 Based on μQLISA Principle

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Introduction and Objectives: Simple, sensitive and selective method for quantitative determination of low levels of ovarian cancer tumor marker human epididymal protein 4 (HE4) is presented. This technique is based on microvolume quantum dot-linked immunoassay (μQLISA) including the fast magnetic separation and sensitive electrochemical detection. **Methods:** Magnetic microparticles with covalently attached specific monoclonal anti-HE4 IgG are used as solid phase in binding the target molecules of HE4. Captured antigen is then detected by CdSe/ZnS QD-labelled polyclonal anti-HE4 IgG. QD-immunoconjugate was prepared using functionalized magnetic microparticles specific for our purpose. Square wave anodic stripping voltammetry (SWASV) was applied for Cd^{2+} detection released by acid hydrolysis of QD label. Gained electrochemical signal corresponds to HE4 level in the sample. **Results and Discussion:** Development of presented μQLISA based electrochemical biosensor for HE4 detection consisted of optimization steps including preparation of magnetic immunosorbent for HE4 capturing, preparation of QD-immunoconjugate and electrochemical detection adaptation. Established device enables the HE4 quantification with detection limit on 20 pM level. All measuremetns obtained with standard HE4 protein were confirmed also with standard human serum spiked with known additions of HE4 represented the complex sample. **Conclusion:** Developed miniaturized immunosensor enables detection of low levels of tumor marker HE4, which is important in ovarian cancer diagnostics especially in early stage of disease when the therapy prognosis is promising. QDs conjugates are auspicious tools for diagnosis of bioactive proteins. Acknowledgements: This work was supported by the Czech Science Foundation grant no. 15-16549S.

P04.16 Systematic Exploration of Subcellular Redox Status by Methionine-Containing Peptide Enrichment

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Introduction and Objectives: Photofrin-mediated photodynamic therapy (PDT) causes oxidative damage of cellular constituents in target cells including methionine (Met) oxidation of proteins, where the cell fate can be modulated by the subcellular location of Photofrin. **Methods:** Here, we developed a new Met-containing peptide enrichment protocol combined with SILAC-based quantitative proteomics approach to explore the global change of Met oxidation of proteins in PDT-treated epidermoid carcinoma A431 cells preloaded with Photofrin at different subcellular sites (plasma membrane, ER/Golgi or ubiquitous distribution, designed as condition I, II and III, respectively). We also explored potential Photofrin-binding proteins in A431 cells using affinity purification coupled with LC-MS/MS. **Results and Discussion:** We identified a total of 622 severely oxidized Met-peptides corresponding to 406 proteins from the three PDT conditions (219, 89 and 424 Met-peptides for condition I, II and III). Proteins of cell surface, plasma membrane, ER, Golgi and endosome represent the most susceptible targets for Photofrin-PDT in all three conditions. “PMGXMXSFD” was deduced as the most frequently oxidized Met-peptide sequence. We also identified 398 highly potential Photofrin-binding proteins and confirmed the binding of EGFR and cathepsin D with Photofrin. The enzyme activities of both proteins could be significantly reduced by Photofrin-PDT in vitro. **Conclusion:** These results uncover the global change of Met-peptide oxidation in cells triggered by Photofrin-PDT-mediated oxidative stress originated from distinct subcellular sites, as well as numerous potential Photofrin-binding proteins. It provides an insight into the molecular targets through which Photofrin-PDT can elicit diverse effects on target cells.

Keywords: Methionine oxidation, Photodynamic therapy, subcellular oxidative stress, Methionine peptide enrichment

P04.17 Sensitive Approach to Identify HLA-DR Peptides Facilitating Individual Patient Characterization

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Introduction and Objectives: Autoimmune disorders are multifactorial syndromes affecting about five percent of the population in Europe and North America. There is a strong association with disease susceptibility and HLA-DR alleles, which present potential autoantigens to the immune system and trigger a response. Therefore the identification of HLA-DR peptides from patients and healthy controls is a valuable tool to characterize autoantigens.

Our previously published method required 800x10^6 pooled bronchoalveolar lavage (BAL) cells from sarcoidosis patients and 78 peptides could be identified. Since BAL from healthy never-smokers contains 10-15x10^6 cells, there is a need for increased sensitivity. Here we established a methodology that enables to analyze the peptidome of individual patients and healthy controls. **Methods:** Cells were collected by BAL. Crude membranes were isolated by differential centrifugation and solubilized using non-ionic detergents. HLA-peptide complexes were immunoaffinity purified and peptides acetic eluted. Peptides were analyzed using on-line nanoLC-MS/MS (Q-Exactive) and identified by searching the human complete proteome database using the Mascot search engine. **Results and Discussion:** The novel approach resulted in a 44-104 fold increased sensitivity compared to the previous study, enabling identification of 43-101 peptides from 10x10^6 cells. As expected, the number of identified peptides was dependent on the amount of material, using 85x10^{6}cells as a starting point, 695 peptides could be identified. The identified peptides show HLA-DR characteristics such as forming overlapping clusters potentially corresponding to epitopes and peptide length distributions with a median of 15. Differences in peptide repertoires correlated to the HLA type of the patients. **Conclusion:** This study presents a highly sensitive approach to identify HLA-DR bound peptides from low cell numbers. This enables generation of peptide profiles (peptidomes) from individual patients and healthy controls, in order to determine disease specific peptides. These peptides could lead to new insights into disease etiology and also give knowledge about how to prevent or manipulate autoreactive T-cell responses.

Keywords: peptidome, Mass spectrometry, autoimmune disease, autoantigens

P04.18 Click-MS: Tagless Protein Enrichment Using Bioorthogonal Chemistry for Quantitative Proteomics

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Introduction and Objectives: Quantitative mass spectrometry-based approaches have greatly contributed to the understanding of protein-protein and protein-DNA interactions. Currently, most approaches use epitope-tagging of proteins of interest in order to enrich them and their interactors from complex mixtures. These tags are usually fused to the N- or C-terminus and have the potential to perturb protein functionality due to their size. Unnatural amino acids (UAAs) harboring azide or alkyne moieties have been site-specifically incorporated into proteins. The incorporation of these UAAs in combination with chemoselective bioconjugation reactions and quantitative mass spectrometry-based proteomics has the potential to circumvent problems related to conventional epitope tagging. **Methods:** Using amber suppression technology, we site-specifically incorporate p- azidophenylalanine in a protein of interest. This single amino acid-substitution harbors an azide moiety, which is used to conjugate the protein covalently to beads by the Cu(I)-catalyzed azide alkyne cyclo-addition (CuAAC) or strain-promoted azide alkyne cyclo-addition (SPAAC). **Results and Discussion:** Here, we introduce Click-MS, an enrichment method based on site-specific incorporation of UAAs and bioorthogonal chemistry. We show that the covalent bond formed by click chemistry enables a single-step purification from crude lysates to near purity, allowing for protein-protein interaction screening but also comprehensive post-translational modification (PTM) identification. The method is perfectly compatible with standard mass spectrometry sample preparation and quantitative proteomics workflows. **Conclusion:** The site-specific incorporation of UAAs enables a robust, single-step purification of proteins of interest to near purity. Since the introduced azide is very small, it only minimally, if at all, affects protein structure in contrast to existing epitope-tagging approaches. In principle, any amino acid in a protein can be substituted by the UAA, which makes the method very flexible and allows for a comprehensive investigation of protein interactions and PTMs.

Keywords: Bioorthogonal chemistry, quantitative proteomics, protein-protein interactions

P04.19 Why to Use Ultra-High Resolution Quadrupole Time of Flight Instruments 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 in the shortest possible time. 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 analyzed with nano-flow UHPLC and a CaptiveSpray ion source connected to the impact II (Bruker Daltonik). For data processing the MaxQuant software package was used (Nature Biotechnology 26, 1367 - 1372 (2008)). **Results and Discussion:** Proteomic capabilities were evaluated on a UHR-TOF instrument and results show that the high MS/MS acquisition speed is very suitable for large sample amounts (e.g. 5μg of tryptic HeLa digest) resulting in more than 4,000 protein identifications during a 90 min gradient. Preliminary quantitative data of 200 ng E.coli lysate spiked with iTRAQ labeled peptides were investigated with regard to the theoretical ratios of these proteins at 10:10:5:5:2:2:1:1. A reference amount of 4 ng was used providing a quantification accuracy of all four proteins at 10:10.2:5.5:5.4:3.3:3 .5:2.1:2.8. Additionally, label-free quantitation results as well as identification numbers in very low sample amounts will be shown, further supporting UHR-TOF capabilities for proteomics applications. Obtained data clearly reveal that hardware changes leading to higher sensitivity at fast acquisition speed and an increased resolution are beneficial for proteomics applications. The improvements result in higher identification rates and a very accurate quantification making the impact II a good choice for proteomics applications. **Conclusion:** Improvements to several hardware components allow identification and quantification of complex proteomics samples with very high dynamic range.

Keywords: QTOF, quantitation, impact II, shotgun proteomics

P04.20 Towards a “Load and Play” Solution for Parallel Reaction Monitoring Assays

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Introduction and Objectives: Targeted quantitative proteomics studies are gradually shifting from low resolution SRM to high resolution PRM methods, using quadrupole orbitrap instruments. The use of internal

standards to perform quantification by isotope dilution can be further leveraged to drive the acquisition, as recently described [1]. The approach was refined to carry out large scale screens or to follow pathways. [1] S. Gallien, SY. Kim, B. Domon. Mol. Cell Proteomics (2015), in press **Methods:** The analyses were performed on a quadrupole-orbitrap instrument (Q-Exactive HF, Thermo Scientific). Internal standards were synthetized and mixed at defined concentrations. Spectral libraries of reference synthetic peptides were created. The IS-PRM application was developed using the instrument programming interface (API). **Results and Discussion:** A new PRM workflow for quantitative analyses was developed. The acquisition method taking into account the m/z values and elution times of the precursors relies on the on-the-fly detection of the internal standards added to the samples; this to drive in real-time the measurement of endogenous peptides by optimizing the acquisition parameters. The creation of spectral libraries using synthetic peptides is an essential element of the approach as it is use to identify the analytes when they elute. The data processing method, also based on the spectral library was used to confirm identity and qualify fragments. The relevant traces were uploaded to Skyline to perform the quantification while reducing the volume of data. A proof-of-principle of IS-PRM was performed by quantifying 600 endogenous peptides representing 338 proteins in plasma and urine samples. It was applied to monitor the MAPK, WnT and mTOR signaling pathways in lysates of lung cancer cell lines. **Conclusion:** A complete automated solution was established to design, execute and, process data of PRM experiments; it was applied to systematic quantify peptides in biological and clinical samples.

Keywords: quantification, PRM, High resolution, Targeted

P04.21 Human Proteome Array Revealed That hnRNP K Binds and Affects the Accumulation of Mature miR-122

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Introduction and Objectives: MicroRNAs regulate the expression and accumulation of mRNAs. While the proteins required for miRNA biogenesis have been identified, little is known about how other miRNA-binding proteins could influence miRNA function. **Methods:** We probed a human proteome chip containing ~17,000 unique proteins with miR-122 and identified 40 proteins that can bind miR-122, including hnRNP K. In vitro kinetic study showed hnRNP K effectively binds to miR-122 with fast on and off rate. **Results and Discussion:** The hnRNP K recognized miR-122 mainly through pyrimidines in the central and 3' portion. In liver hepatocytes, miR-122 was found to co-immunoprecipitated for hnRNP K whether replication was present or not. Next-generation DNA sequencing of the miR-122 revealed that only mature miR-122 was bound by hnRNP K. Moreover, siRNA knockdown of hnRNP K in human hepatocytes reduced the levels of the miR-122. **Conclusion:** These results suggest that hnRNP K binds and affects the accumulation of miR-122.

Keywords: Protein chip, HCV, Protein-microRNA interaction, High throughput screening

P04.22 A New Approach for Better Identification of Cellular Peptide Using Tandem Mass Spectrometry

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Introduction and Objectives: In animal systems, cell-cell communication events are mainly mediated by peptides. However, unlike animals, most of the plant signaling peptides are still undiscovered. The major challenges for the detection of signaling peptide in plants is due to their low concentration, dynamic expression and most of the plant peptides are production from the hydrolysis of precursor proteins. To date, the MS-based proteomics approach was developed to detect and quantify the peptides in a highly sensitive and efficient manner. However, it is still difficult to detect the signaling peptides, because the enzymes for hydrolysing proproteins the are mostly unknown, all of the possible peptide cleavage rules need to be considered during the peptide identification. This non-enzyme specific database search can result high false identification rate, thus reduce peptide identification sensitivity. In this study, we proposed a new approach for better identficaiton of the celluar peptide. The performance and the the application of this approach was stuidied and discuessed. **Methods:** The cellular peptide extracted from Tomato was subjected to the LC-MS analysis and followed by the protein database search with the consideration of all possible cleavage possibilities. The 1st to the 10th peptide matching scores for each of the MS/MS spectrum was evulated for the new peptide matching score. **Results and Discussion:** In this study, we analyzed several factors in peptide identification, including mass accuracy and matching score. We discovered that the score distribution of truth and false positive can be well separated by correlating the 1st and subranked matching scores. With liquid chromatography-tandem MS analysis of 285 known peptides, the number of correctly assigned cellular peptides was 25% enriched with the use of new algorithm in compared with the use of best matching score. **Conclusion:** This method does not require complex computational steps and can significantly improve the sensitivity for novel peptide hormone discovery.

Keywords: Peptide Hormone, peptidomics

P04.23 mzDB: A File Format Optimized for the Efficient Analysis of Large LC-MS/MS and SWATH-MS Datasets

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Introduction and Objectives: The analysis and management of MS data, especially those generated by data independent mass spectrometric 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. To overcome these issues we developed mzDB, an efficient file format for large MS data sets. **Methods:** This new format relies on the SQLite software library and consists of a standardized, 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. **Results and Discussion:** 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 (a binary and open-source format). Both C++ and Java implementations will be released under permissive license, providing tools to convert XML formats to mzDB and programming libraries that could be used by existing pipelines. mzDB can be easily accessed by the SQLite C library and its drivers (available for all major languages), and browsed with existing dedicated GUIs. We also developed the mzScope Java tool, an mzDB-dedicated viewer for interactive MS data visualization and very fast signal extraction. **Conclusion:** The mzDB format described here can boost mass spectrometry data analysis, offering unprecedented performance in terms of efficiency, portability, compactness, and flexibility.

Keywords: SWATH, raw data, standards, LC-MS

P04.24 A Comprehensive Database and Search Method for Large-Scale Metaproteomics

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Introduction and Objectives: The field of metaproteomics aims to characterize extremely heterogeneous mixtures of proteins from diverse sets of microorganisms. Mass spectrometry-based proteomics experiments rely on accurate matching of experimental spectra against a database of sequences for proteins that are potentially present in a sample. Current proteomic search methods are unable to efficiently access the enormous proteomic search spaces necessitated by metaproteomic data analysis, leaving large numbers of spectra unassigned and many proteins and functionalities unidentified. **Methods:** We designed a broadly applicable metaproteomic analysis method that consists of several high-performance, scalable databases of protein and peptide information and coupled it to "Blazmass," a new, rapid proteomic search engine. Our metaproteomic search space, termed "ComPIL," is currently comprised of ~82 million protein records and includes all publicly available microbial protein and peptide sequences. We have incorporated this method into an extensible, open-source web application that allows for easy data analysis and visualization. **Results and Discussion:** A major concern with large search spaces is peptide identification accuracy. Using HEK293 and B.fragilis lysates, we show that spectral assignment using ComPIL-Blazmass identifies the same peptide for 98-99% of spectra when compared to smaller, single proteome-focused database searches. We applied ComPIL-Blazmass to several human gut microbiome proteomic samples, showing that roughly 4x to 5x as many high quality peptide identifications were performed with the new method compared to a previously used search database comprised of ~50 bacterial proteomes. These new peptide identifications represent a large number of new ontological functionalities. **Conclusion:** The ComPIL-Blazmass method allows for efficient analysis of proteomic search spaces derived from an input FASTA files of any size. We believe the method will be beneficial for complex, unculturable systems such as the human microbiome and for personalized proteomics projects where the identification of protein sequence variation is important.

P04.25 SWATH-ID: An Instrument Method Which Combines Identification and Quantification in a Single Analysis

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Introduction and Objectives: Proteins spectral library creation often requires users to run different types of analysis on the samples, reducing throughput of the technique and introducing variations between runs. Here we will introduce a new data-independent acquisition approach, "SWATH-ID", to achieve both accurate quantitation and reliable identification of a wide range of species from a single analysis. **Methods:** In "SWATH-ID", the data acquisition allows parent ions to transmit without fragmentation as well as their fragments within the same MSMS accumulation period as the small precursor isolation window (3 Da) applied. The outputs extracted of each SWATH spectra containing both precursors and fragments are used to generate the ID library with which the same SWATH data is processed for quantitation analysis. Samples of E.coli lysate were analyzed using a Nano-LC system and a prototype time-of-flight instrument. **Results and Discussion:** This approach significantly improves the parent ion intensity without substantially affecting the detection of fragment ions or their quantitative properties. With the 2 ms parent-ion accumulation in the 20 ms MSMS, an average increase in parent-ion intensity was 6.58X and the average decrease in fragment-ion intensity was only 1.18X, as compared to that in the standard 20 ms SWATH. The quantitation quality in "SWATH-ID" was well maintained, showing that 88% of confidently identified peptides had CVs of 20% or better. This approach provides more reliable and accurate information on peptide parent ions, leading to a significant increase (~56%, 2000+ peptides) in peptide identification yields compared to those with the standard SWATH. With the same peptide confidence threshold, the "SWATH-ID" and IDA analysis gave similar peptide identification yields, while "SWATH-ID" provided better identification reproducibility. **Conclusion:** The "SWATH-ID" shows capability of parent ion detection enhancement during MSMS acquisition, resulting in improved peptide identification coverage to the extent of being comparable to IDA-based analysis without compromising of SWATH quantitation quality.

Keywords: quantitation, SWATH, Peptides, Identification

P04.26 An Alternative Mass Spectrometry View of the Proteome

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Introduction and Objectives: Comprehensive LC-MS based mass spectrometry studies of yeast and other model proteomes have been extensively performed. As such, the identification and quantitation of modifications, especially those relating to chemical and post-translational modifications, as well as processing and point mutations, are now the main areas of interest. The ability to detect and identify these modifications within the cell cycle is still a major challenge to modern bottom-up proteomics since LC-MS experiments are challenged by sample complexity and the specificity of the workflow. **Methods:** Enzymatically digested yeast was separated over ninety minutes from 0-35% on an M-Class LC system with a 75 μm x 25 cm BEH C18 column packed with 1.7 μm particles in fluid registration with a hybrid IMS-QTof MS platform. Data were acquired using a multi-mode acquisition method. Briefly, ions were acquired in low-energy followed by an elevated energy scan interspaced with 6-8 DDA acquisitions all in a 1 second cycle time. The isolation widths for the DDA acquisitions varied between 3 to 20 amu with the quadrupole isolation window following the previously described distribution of m/z. The DIA data were processed

using ProteinLynxGlobalSERVER and the DDA data using Mascot Distiller. **Results and Discussion:** The application of a multi-mode acquisition approach used in combination with hybrid IMS-QTof MS platform improved selectivity substantially , thereby increasing the yeast proteome coverage in a single LC-MS experiment by 2.1 fold for peptides and 1.7 fold for proteins, compared to data DDA and DIA methods. The added specificity of multi-mode acquisitions reduced AUC variance by 40%, improving quantitative accuracy and precision greatly. **Conclusion:** The combination of travelling wave IMS with oa-TOF MS, and the increased selectivity of the multi-mode acquisition workflow, provided a significant increase in protein amino acid sequence coverage and number of identified PTMs.

Keywords: mulit-mode acquisition, IMS-QTof MS, DDA, IM-DIA

P04.27 Immunoaffinity-MS Platform for Antibody Screening and Native Protein Analysis in Biological Fluids

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Introduction and Objectives: Monoclonal antibodies (mAbs) that bind the native conformation of proteins are indispensable reagents for proteomic analysis. Immunoaffinity techniques coupled with mass spectrometry (IA-MS) promise reliable quantification of low abundance antigens and delineation of protein interaction networks. Here, we describe an IA-MS platform for production and characterization of mAbs against native forms of proteins, production of mAb libraries for a variety of antigens in biological samples and development of quantitative assays for specific protein isoforms. **Methods:** Monoclonal antibodies were generated by injecting mice with: (i) yeast recombinant TEX101, (ii) KLK6 protein immunopurified from ovarian cancer ascites and (iii) seminal plasma which had undergone a 3-step chromatographic fractionation. Polystyrene microtiter plates and various forms of agarose beads (e.g. NHS-activated sepharose beads, magnetic beads, and MSIA™ pipette tips) were used for mAb immobilization. Antibodies were then incubated with biological fluids containing the antigens of interest (such as seminal plasma and ovarian cancer ascites), followed by shotgun or SRM mass spectrometric analysis. **Results and Discussion:** IA-MS facilitated the development of monoclonal antibodies and immunoassays against native forms of challenging protein targets, such as membrane-bound testis-specific TEX101 protein (Korbakis et al. Mol Cell Proteomics, 2015, Epub Mar 26). Furthermore, IA-MS protocols and filtering criteria were optimized for target identification of mAbs produced by injecting mice with complex biological fluids (e.g. identification of RibonucleaseT2 in seminal plasma) (Korbakis et al. J Proteomics 2015,114:115-24). Finally, in-house mAbs were used for immunopurification of KLK6 from ovarian cancer ascites, leading to the production of novel mAbs and immunoassays for the ascites-specific KLK6 isoform. **Conclusion:** IA-MS is a powerful tool for the screening and characterization of mAbs, as well as development of assays for the measurement of native forms of proteins in biological fluids.

Keywords: Monoclonal antibodies, immunoaffinity, Mass spectrometry

P04.28 Increased MS Protein Identification Rates Using 75 cm Long Nano LC C18 Separation Columns

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Introduction and Objectives: Deep proteomics ultimately aims for comprehensive proteomic analysis. To achieve this, the sensitivity and speed of the mass spectrometers has been greatly increased over the last decade. Other strategies to improve sequence coverage that are being tested in the field of bottom up proteomics include the implementation of longer columns and shallower gradients for improved peptide separation. Here we carried out a systematic investigation into the effect of increasing column length on peak capacity and ultimately on the peptide and protein identification results. **Methods:** Prokaryotic and eukaryotic cell digests were used for comparative analyses. Chromatographic separation was performed on EASY-nLC systems using linear gradients. Acclaim PepMap C18 separation columns of different lengths (25, 50, 75 cm) were evaluated for their peak capacity and the resulting effect on the peptide identification rates. Data was acquired using a Q Exactive HF mass spectrometer. Consecutive data analysis was done with Proteome Discoverer using Sequest and MASCOT search algorithms. **Results and Discussion:** Increasing gradient duration and column length resulted in higher peak capacities and as a result, in higher peptide identification rates. The 75 cm columns clearly outperformed the shorter 50 cm and 25 cm separation columns when analyzing complex samples and using identical gradients. The use of an optimized gradient on a 75 cm column further improved the peptide identification rate. **Conclusion:** The improvement in identification rates afforded by the 75cm columns will allow scientists to dig deeper into their samples and thus enable them to reliably identify proteins of low abundance.

Keywords: EASY-nLC, 75cm column, Q Exactive HF, UHPLC

P04.29 A Novel MS1-Based Strategy for Accurate Proteomic Quantitation with Extremely Low Missing Value

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Introduction and Objectives: We describe a novel MS1-based strategy for accurate and extensive proteomic quantification, with extremely-low-level of missing-value and the capacity of quantifying numerous technical/biological replicates. Robust sample treatment and LC/MS approaches were established to enable reproducible analysis; quantitative features were acquired in a data-independent manner with high-field Oribtrap Fusion and a locally-developed informatics package. This method can accurately quantifies >5000 proteins without any missing data in >40 biological replicates. **Methods:** A robust surfactant-aid precipitation/on-pellet-digestion(SOD) method was developed to treat and digest tissue or cellular samples; peptides were resolved with high efficiency and reproducibility by long-column chromatography, and detected with a high-field Orbitrap Fusion. High-resolution MS1 signal was procured and aligned, then subjected to filtering, peak detection, normalization, aggregation and statistics using a locally-developed algorithm. **Results and Discussion:** The SOD method showed high and reproducible peptide recovery from samples. The extensive and reproducible separation and sensitive Orbitrap analysis laid foundation for robust and comprehensive proteomic quantification. Additionally, high-resolution MS1 detection and optimal peak alignment were found critical to achieve reliable matching and low missing data. The quantitative performance was evaluated using human

cell lysates spiked with E. Coli extracts at four different levels (1-3 folds, 5 groups and N=4/group). High quantitative precision (median-CV<10% intra-group) and accuracy (<15% median-error) were achieved using the optimized pipeline. Only 0.024% of all proteins showed missing value in one of the 20 replicates. We applied this strategy in two applications requiring the analysis of a large number of biological replicates. First, investigation of the effects of three chemotherapy regimens on pancreatic cancer cells via a 5-time-points study. Almost 5000 unique proteins were quantified in all 45 samples without any missing data. Second, we investigated the temporal changes in lung proteomes at 11 time points post influenza virus infection (44 animals), and ~3800 proteins were successively quantified without missing value. **Conclusion:** not applicable

Keywords: large-scale, missing-value, quantitative proteomics

P04.30 Deep, Single Shot Human Cell Line Protein Profiling Using DIA and Spectronaut on a Q Exactive HF

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Introduction and Objectives: Quantitative protein profiling using LC-MS provides valuable insight into biological processes or enables the discovery of novel biomarkers. Close to 100% coverage of the expressed proteome in human cell lines can be achieved using extensive sample pre-fractionation. However, long measurement times render pre-fractionation impractical if many samples are to be profiled. Here, we used deep single shot data-independent acquisition (DIA) on a Q Exactive HF mass spectrometer to profile three human cell lines. **Methods:** Samples for three human cell lines, HeLa, HEK-293 and Jurkat, were prepared using the FASP protocol. Biognosys' HRM Kit was spiked into each sample for automatic calibration of mass and retention time. A Thermo Scientific EASY-Spray column of 75μm ID and 50cm length, packed with 2μm particles, was coupled to a Thermo Scientific Q Exactive HF mass spectrometer. Shotgun runs with 3 hour nonlinear gradients were searched using MaxQuant and Proteome Discoverer and a spectral library was generated using Spectronaut. DIA methods were designed with Spectronaut such that an average number of 6-7 data points per peak was acquired. Targeted analysis of DIA runs and quantitation was performed with Spectronaut. **Results and Discussion:** DIA runs with gradients of 1, 2 and 3 hours were acquired for the HeLa cell line. 37,912/5,729, 55,901/6,743 and 69,819/7,235 peptides/proteins were identified in 1-3 hours respectively with median CVs of 13/13, 10/9 and 10/7 % for peptides/proteins respectively. We compared two different methods with different resolution settings on MS2 (30k/60k) and different numbers of precursor windows (30/20) to balance the number of data points per peak. Both methods performed very similar in terms of IDs (7,189/7,235 protein groups) but the 60k method had significantly improved CVs (15 / 10 %). **Conclusion:** The EASY-Spray/Q Exactive HF instrument setup combined with Spectronaut is a robust and powerful setup for routine, single shot and deep quantitative protein profiling.

Keywords: Q Exactive, software, data independent acquisition, SWATH

P04.31 A Robust SRM Assay for UMOD and Albumin in Urine

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Introduction and Objectives: Chronic kidney disease (CKD) evolves over years and inevitably leads to end-stage renal disease. A common UMOD variant is associated with lower levels of urinary UMOD and protects against CKD. Urinary albumin is a diagnostic marker for renal damage due to its association with proteinuria. Urine presents challenges for quantitative proteomics: it has a wide and highly variable range of total and individual protein concentrations and contains compounds that interfere with standard protein assays and MS. **Methods:** Urine sample preparation was optimized by testing the effect of various denaturants, trypsin digestion conditions, and desalting conditions on the accuracy and reproducibility of UMOD and albumin multiplex SRM quantification. β-galactosidase was incorporated at the initial step as a protease digestion and peptide recovery control. Signature peptides for UMOD and HSA having transitions with strong and consistent SRM signals were identified empirically because the peptides suggested by discovery MS, publically available databases, and predictive methods (e.g. Skyline) were inconsistent. The final multiplexed MRM assay targets 4 UMOD peptides, 2 albumin peptides, and 3 β-galactosidase peptides along with their corresponding ^{15}N-labeled internal standards. LLOQs ranged from 0.5-14.1 μg/ml. The ULOQ for all peptides was >446.4 μg/ml. **Results and Discussion:** The optimized MRM assay was tested on 42 urine specimens from Atherosclerosis Risk in Communities (ARIC) study. There was a high correlation between the concentration of uromodulin measured by MRM for all 4 peptides and ELISA data (mean r^2=0.93). In contrast, there was little correlation between the concentrations of urine and albumin, suggesting that UMOD and albumin enter the urine by different mechanisms in CKD patients **Conclusion:** We developed a robust MRM assay for UMOD and albumin.

Keywords: MRM, Validation, signature peptide, assay development

P04.32 Data Aquisition and Processing to Apply Inter-Peak Isotope Spacing for Turnover Analysis

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Introduction and Objectives: Metabolic labeling with deuterium has been used to monitor turnover rates for proteins, lipids, DNA, and RNA in humans as well as model systems. Turnover is measured as time-dependent deuterium incorporation into newly synthesized molecules, using mass spectrometry to monitor changes in isotope distribution. The increase in deuterium changes the relative ratio of isotope abundances and a within-pattern isotopic mass spacing that is different than a simple increase of a neutron mass (mass defect). Simulations of the isotope pattern suggest that the changes in inter-peak distances due to changing ratios of mass defect incorporation into each isotope position (i.e. increasing ^{2}H vs. ^{13}C) should provide turnover information similar to changes in isotope abundance. A series of metabolically labeled blood samples were created as per the reference: Price, MolCellProt. 2012, 1801-1814 **Methods:** We monitored the isotope patterns of hundreds of peptides throughout the labeling period, using either QToF (Agilent 6530) or Orbitrap (XL and Fusion) mass analyzers. Multiple post-processing software methods were used on each data set to test for process-dependent accuracy and precision versus theory for relative peak abundance and inter-

peak distances within peptide isotope patterns. Thermo and Veritomyx supported this study by donating instrument and analysis time, respectively. **Results and Discussion:** We present evaluations of mass analyzers, instrument parameters (resolutions from 10K to 480K) and multiple software post-processing tools. We find that increasing mass resolution does not directly improve the accuracy of isotope patterns. Optimization of instrument parameters and post-processing data analysis allowed productive application of the within-pattern peak spacing changes for more robust measurements of in vivo protein turnover. The optimized workflow yields up to seven metrics of turnover per peptide. **Conclusion:** Comparison against the theoretical isotope pattern provided a gold standard to evaluate whether each permutation increased or decreased accuracy. Using the optomized workflow, kinetic proteomics studies can gain additional confidence by leveraging inter-peak distance information.

Keywords: Kinetic Proteomics, Metabolic Labeling, isotope pattern, data processing

P04.33 Ultrafast, High Sensitive microLC/MS/MS for Peptide Quantitation in Highly Targeted Assays

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Introduction and Objectives: Peptide quantitation by LC/MS/MS using MRM is rapidly growing in many research areas. Combining LC/MS with targeted sample preparation allows the development of highly specific protein assays. While nanoLC/MS would give the best sensitivity possible, the low throughput and inherent low robustness of nanoLC prohibits its use in high-throughput environments. MicroLC/MS (3-50 µL/min) can still offer substantial sensitivity improvements over conventional LC/MS, while maintaining sample throughput and robustness. In this study, we introduce a fast separation microLC/MS method for peptide quantitative analysis of samples with a limited number of peptides (<30). **Methods:** The system used consisted of an Eksigent nanoLC 425 gradient system in microLC mode, coupled to a QTRAP® 6500 system (SCIEX) in MRM mode. MicroLC columns of 0.3 and 0.5 mm ID, with lengths of 10-150 mm, packed with a variety of phases were used. MultiQuant™ Software (SCIEX) was used for quantitation. **Results and Discussion:** Compared to using NanoLC/MS/MS the microLC method has an approximately 10 fold higher throughput. Gradient run times between 0.5 and 15 minutes were explored using columns with different lengths and ID's. For the simple mixtures of up to 30 peptides, a 10 min method using a 0.5mm x 10cm column at 6 uL/min was chosen. Reproducibility on a 4 peptide mixture across 20 replicates was better than 5%CV and the retention time RSD is 0.11%. For quantitation, good linearity was observed for the calibration curves of all analytes with correlation coefficients (R^2) better than 0.99. The lower limit of quantitation was determined to be 100 amol on column for peptide 1 and 50 amol for peptide 2. **Conclusion:** Preliminary data shows that high-throughput peptide quantitation with good sensitivity can be achieved using microLC/MS/MS. Further exploration will be done on more column types and methods to determine best practices for high-throughput peptide quantitation using microflow.

Keywords: nanoLC, peptide, quantitation, microLC

P04.34 Compression and Rapid Visualisation of Quantitative LC-MS Data Based on Raw Signal Decomposition

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Introduction and Objectives: It is critical that efficient compression and visualisation tools are available to facilitate archival, quality control, verification, validation, interpretation and sharing of raw MS data and the results of MS analyses. Currently MS data is stored as contiguous spectra. Recall of individual spectra is quick but panoramas, zooming and panning across whole datasets necessitates processing/memory overheads impractical for interactive use. **Methods:** In order to tackle these issues, we leverage our seaMass technique [Liao et al., IEEE ISBI 2014, http://dx.doi.org/10.1109/ISBI.2014.6868123] for raw signal decomposition. LC-MS data is modelled as a two-dimensional surface through selection of a sparse set of weighted B-spline basis functions from an over-complete dictionary. By ordering and spatially-partitioning the weights with an R-tree data model, efficient streaming visualisations are achieved. In a recent publication [Zhang et al., Proteomics 2015, http://dx.doi.org/10.1002/pmic.201400428], we describe our core MS1 visualisation software. Here, we present this framework and new tools for SWATH/MSE and to handle centroided data, plus an approach packaging mzML and seaMass decompositions within an HDF5/netCDF4 container for realising a highly compressed format with rapid 2D visualisation capability. **Results and Discussion:** seaMass is dependent on the desired compression ratio set through parameter l. With l = 0.06, ToF data is compressed to ~5% original size, with mean absolute error per data-point ~1.5 ion counts. By additionally storing the difference delta between the reconstruction and original dataset, lossless archival can be achieved. With our R-tree model, visually complete overviews take seconds to appear, zoomed regions much quicker, and streaming visualisation across a network or the Internet is possible. **Conclusion:** Our platform enables the possibility of implementing novel visualisation schemes integrating results and raw data across complete experiments, which would greatly facilitate QC, verification, validation and expert interpretation of MS analyses, beyond that of what we demonstrate here. The open-source software is available from http://seamass.net/viz/.

P04.35 A High-Efficient Yeast BiFC Approach for Genome-Wide Interactome Mapping

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Introduction and Objectives: Genome-wide yeast two hybrid (Y2H) screening and affinity-purification coupled with mass spectrometry (AP-MS) have been extensively used for mapping the interactome of various species. However, only limited coverage was obtained for most organisms, which partially due to the labor intensive and high cost of current technologies for protein-protein interaction research. We aims to develop an improved BiFC (bimolecular fluorescence complementation) that suitable for genome-wide interactome study. **Methods:** A yeast BiFC technology was developed and tested in yeast cells. The positive yeast colonies was collected by FACS (Fluorescence-activated cell sorting). The positives were sequenced by NGS (Next generation sequencing) method. **Results and Discussion:** We developed a yeast BiFC approach coupled with next-generation sequencing and used it to investigate the interactome of a well-known tumor suppressor, p53. This method was amenable to high-throughput screening through FACS. More than 2000 positives were obtained within a single screening. The identity of the positives were sequenced by NGS method. A quantitative scoring method was applied to

discriminate true positives and false positives. The p53 network contains several dozen interactions from a genome-wide screening assay, which covered 3% of known partners of p53 from BioGrid database. The confidence reliability was evaluated by a bioinformatics tool (PRINCESS), which suggest that the novel interactions have higher confidence than random interactions. The data reliability from the screening by this yeast BiFC method was further validated by mammalian BiFC and co-immunoprecipitation assays. **Conclusion:** A high-efficient yeast BiFC method was developed, which is suitable for genome-wide protein-protein interaction network mapping.

Keywords: BiFC, FACS, NGS, Interactome

P04.36 A Comparison of Three-Dimensional Strategies in Mining the Human Plasma Proteome

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Introduction and Objectives: Human plasma is easily available clinical sample and its constituents reflect the status of the body, which makes it an ideal choice to develop noninvasive test. Thus comprehensive proteome analysis of human plasma is critical. However the wide dynamic range and immense complexity of the plasma protein composition pose the obstacles and impede the transfer of proteome information to clinical research. Various methods including immuno-depletion, protein equalization and hyper-fractionation have been developed to obtain the depth of identification at cost of a lot of time. **Methods:** The optimal strategy is expected to improve the efficiency of human plasma proteome profiling. In this study, we compared five three-dimensional strategies combing high-abundance proteins (HAP) depletion (the 1st dimension) and protein fractionation (the 2nd dimension), followed by LC-MS/MS analysis (the 3rd dimension) to profile the human plasma proteome. The features of the five strategies are discussed. **Results and Discussion:** Strategy A using proteome equalizing method showed its bias towards the enrichment of basic and low-molecular weight proteins. Strategy B by tandem immuno- removal increased the efficiency of HAP depletion, whereas more off-target proteins were subtracted simultaneously. The added step of the deglycosylation before high-pH RPLC separation in strategy D did not increase the proteins ID number as expected. The results of strategy E showed oversampling of HAP and less identification number. Finally, combined single Seppro IgY14 immunodepletion, high-pH RPLC fractionation and LC-MS/MS analysis, strategy C generated the largest dataset, containing 1544 plasma protein groups and 258 newly identified proteins in a 30-h-machine-time analysis. **Conclusion:** Strategy C as the preferred method is recommended. The analysis of the data supports the reliability of the identification results. The characterization of 20 cytokines with the concentration range from sub-ng/milliliter to micrograms/milliliter demonstrates the sensitivity of the current strategy.

Keywords: human plasma, immunodepletion, proteome equalizing, deglycosylation

P04.37 Functionalized Polymer Material for Enrichment and In-Situ Digestion of Membrane Proteins

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Introduction and Objectives: Membrane proteins (MPs) are one of promising target for drug discovery because of the unique properties in physiological processes. Due to their low abundance and extremely hydrophobic nature, the analysis of membrane proteins is still a great challenge. In our work, an effective and in-situ method was developed to enrich and digest of MPs by adopting as-made tresyl- functionalized porous polymer material. **Methods:** Organic solvent-assisted (methanol) and surfactant-assisted (SDS) solubilization were performed to extract MPs. A certain amount sample was mixed with the polymer material. After incubated for 2 h, the material was retained by separation. Then, redundant tresyl groups were blocked. Next, trypsin in NH4HCO3 buffer (25 mM, pH 8.0) was added with a final enzyme/substrate ratio of 1:40 (w/w), and the mixture was incubated at 37 °C for 2 h. After the digestion, the mixture was centrifuged. Then the supernatant collected was directly detected by MS analysis. **Results and Discussion:** With tresyl groups, the material can effectively immobilize membrane proteins via covalent bonding on the surface. The material became a facile carrier to enrich MPs from the rat liver in detergents and organic solvents owing to its outstanding binding capacity and excellent biocompatibility. A total of 700 membrane proteins were identified by nano-LC-ESI-MS/MS in 4% SDS, and similar results were achieved in the 60% methanol solution. **Conclusion:** Due to the simplicity and the low cost of the method, it is expected that it can be applied for rapid and high throughput MS analysis of large-scale biological molecules.

Keywords: LC-MS, polymer material, Covalent bonding, membrane proteins

P04.38 High Retention Time Precision and Mass Accuracy LCMS Platform for Deep Label-Free Proteome Profiling

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Introduction and Objectives: Deep liquid-chromatography mass-spectrometry (LC-MS) based high-throughput profiling of biological samples becomes a routine practice to discover biomarkers for early diagnosis, prognosis and treatment follow-up of various disease. Label-free and stable isotope labeling quantification are popular approaches to identify differentially expressed proteins in complex biological samples. The latter allows multiplexing and is less dependent from chromatography reproducibility. In contrast, label-free quantification provides higher dynamic range and requires lower number of sample preparation steps. In this study we have used an LC-MS/MS platform, which provides high retention time reproducibility and high mass-accuracy for deep quantitative profiling of human proteome. **Methods:** HELA cell lysate digest was spiked with different amounts of Pierce™ 6 Protein Digest and was separated on Easy-Spray™ PepMap C18 column (75 μm i.d. x 500 mm) with UltiMate 3000 RSLCnano LC system controlled with Chromeleon™ 7.2 software and coupled to a Q Exactive™ HF-MS. Reversed phase Acclaim Pepmap RSLC C18 cartridge (300 μm i.d. x 5 mm) was used as trap column or served as guard column for direct injections. Peptide identification was performed with Mascot, Sequest, and SearchGUI tools. Sieve™ and TAPP were used for retention time alignment and label free quantification. **Results and Discussion:** Robust and accurate label-free proteome quantification is a challenging task that requires data with small retention

time shifts, high mass accuracy and advanced alignment algorithms to increase a number of peptides that can be relatively quantified beyond the peptides that are successfully identified. In this study we have assessed the protrein quantification performance of LC-MS/MS system for complex biological samples using long gradient (180 min), high retention time reproducibility LC system and high mass accuracy MS. **Conclusion:** Our LC-MS/MS system with advanced data analysis algorithms allows achieving deep protein quantification of human cells. **Keywords:** nanoLC-MS, Label-free quantification, mass accuracy, retention time precision

P04.39 A QPrEST Derived Peptide Resource for Targeted Proteomics

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Introduction and Objectives: Targeted proteomics methods are attractive alternatives for precise protein quantification in complex mixtures. Here, high-quality spectral libraries, acting as templates for the target signal extraction from MS/MS fragment spectra, is a prerequisite for successful target identification. Several initiatives are aiming to construct comprehensive libraries covering the complete human proteome. In the presented study, we investigated if the resource of more than 40,000 individually purified recombinant human protein fragments from the Human Protein Atlas (www.proteinatlas.org) enables the generation of a proteome wide spectral library for targeted assay development. **Methods:** A subset of the Protein Epitope Signature Tag (PrEST) library consisting of 21,364 individually purified protein fragments were pooled in sets of 384 or 768 and thereafter trypsin digested. Each pool was analyzed using an Orbitrap Velos and a Q-Exactive HF operating in DDA mode. Raw MS-files were searched by Sequest against a human protein database (uniprot.org) and the resulting library was refined by only allowing proteotypic peptides. Also, some peptides were verified by spiking in corresponding pre-quantified isotopically labeled QPrEST standards into five different cell lines prior trypsin digestion and subsequent PRM analysis. **Results and Discussion:** The investigation revealed that the majority of all PrESTs with available tryptic peptides were successfully identified. The poster will show that PrESTs with available proteotypic peptides in this investigation yielded at least one peptide suitable for targeted assay development. Using QPrESTs as internal standards, fifteen proteins were quantified ranging from 45 million copies per cell (BLVR) down to 19 thousand copies per cell (TERF2IP) in RT4. **Conclusion:** We provide insights into the applicability of individually purified recombinant human protein fragments originating from the Human Protein Atlas as a resource for development of novel targeted assays. QPrESTs are suitable and precious internal standards for target proteomics and by performing the screening of digested fragments, good responding proteotypic peptides can be identified.

Keywords: Spectral library, Targeted proteomics, Absolute quantification, Recombinant Protein Standards

P04.40 Ultrasensitive Proteome Analysis by On-Line Cell Digestion and Nano-LC-MS Identification

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Introduction and Objectives: Single cell will provide crucial information about cellular heterogeneity and dynamic change. Analysis of single cell proteome is still a big challenge. Great effort was made to develop approach to analyzing a proteome of limited number of cells. Previously, a method to directly digesting proteins in living cells into peptides for mass spectrometric identification [Anal Bioanal Chem (2015) 407:1027-1032] was developed in our lab. In this presentation, we demonstrate an on-line method for ultrasensitive proteome analysis. **Methods:** Living cells was directly introduced into an injection loop installed on a switch valve, where the cells were digested into peptides in solution with trypsin and guanidine hydrochloride. Then the digested peptides was enriched and trapped onto a trapping column. And the peptides were switched into a nano LC-MS system for identification. **Results and Discussion:** We applied such a method to analyze the proteome of 100 cells. Totally more than 800 proteins were identified in three duplicate runs. Proteins whose copy numbers were lower than 1000 were identified from the sample. It was estimated that a detection limit around 200 zmol was achieved. Gene ontology analysis revealed that proteins from different cellular compartments were well-represented. **Conclusion:** Proteome of 100 cells was analyzed with an ultrasensitive detection. Protein within 1000 copies would be possibly identified.

Keywords: proteome analysis, Ultrasensitive, nano LC-MS, whole cell digestion

P04.41 A New Method to Control Ratio Distortion in Isobaric Labeling Experiments

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Introduction and Objectives: The multiplexing capabilities of isobaric mass tag based protein quantification, such as Tandem Mass Tags (TMT) or Isobaric Tag for Relative and Absolute Quantitation have dramatically increased the scope of Mass Spectrometry-based proteomics studies. Not only does the technology allow for the simultaneous quantification of multiple samples in a single MS injection, but its seamless compatibility with extensive sample pre-fractionation methods enable comprehensive study of complex proteomes. However, reporter ion based quantification has often been criticized for limited quantification accuracy due to interference from co-eluting peptides and peptide fragments. Here, we investigate the extent of this problem and propose a simple method to compensate for the observed ratio compression. **Methods:** We evaluate the accuracy and precision of TMT-based quantification in a 2-proteome-sample mixture and compare to the application of a Label-Free quantification method (LFQ). Next, we describe a method to improve TMT quantification accuracy relying on a 6-protein calibration mixture spiked into the samples subject to relative quantification. The calibration mixture data is exploited to predict interference levels in all identified TMT spectra, implementing a multiple linear regression model. **Results and Discussion:** Comparing LFQ to TMT-based quantification showed that, despite evident fold change underestimation, the latter method allowed for better discrimination between changing and constant proteins, resulting from its markedly higher replicate quantification precision. However, sometimes measuring the exact magnitude of protein abundance changes in biological systems upon perturbation is critical, e.g. if comparing relative changes on the transcriptome level to those on the proteome level.

Here, a standard MS/MS isobaric labeling workflow would be suboptimal. To address this issue, we developed an effective ratio adjustment method. **Conclusion:** We propose a calibration mixture dependent ratio adjustment approach, compatible with any isobaric labeling method. Our workflow requires only little additional sample preparation, while dramatically improving the overall ratio accuracy, without reducing the number of quantified proteins.

Keywords: TMT, Label-free quantification, multiplexing

P04.42 Probing Plasma of Patients with Cardiovascular Disease Using a 120-plex Immuno-MRM Assay

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Introduction and Objectives: Recent advancements in immunoaffinity enrichment of peptides followed by multiple reaction monitoring mass spectrometry (SISCAPA or iMRM) include the use of automation and multiplexing to increase sample and analyte throughput, respectively. Here we describe the development of a 120-plex iMRM assay, assess its performance and demonstrate its utility for detecting biomarker candidates of cardiovascular disease. **Methods:** 120 polyclonal antibodies were screened and selected from our inventory of anti-peptide antibodies generated for a number of biomarker studies. Plasma was drawn from five cardiovascular disease patients before, during and after a therapeutic procedure called planned myocardial infarction (PMI). These samples were digested using a Bravo LT liquid handling robot, immunoaffinity enriched using the 120-plex AssayMAP protein G cartridges and analyzed by scheduled LC-MRM-MS. **Results and Discussion:** The 120-plex iMRM assay had a median LOD of 71.5 amol/μL, median LLOQ of 214.6 amol/μL and a CV of 13.9% at the LLOQ. Forty-seven peptides for a total of 42 proteins were detected in patient plasma in 4 logs of concentration range (3 ng/mL to 10 ug/mL). Of these, the concentration of 15 proteins was determined to change significantly over the timecourse. Hierarchical cluster analysis of these proteins identified four timecourse trends. Temporal trends and levels of proteins previously measured by iMRM in a 20-plex magnetic bead assay format were maintained in the 6-fold higher plex assay. **Conclusion:** These data suggest that iMRM assays can be developed and successfully applied at plex levels beyond 100 antibodies-per-plex and achieve similar assay performance to much smaller plex level assays. Previously confirmed trends in PMI proteins were observed using the higher plex antibody cartridge configuration as were novel trends for proteins not previously associated with planned myocardial infarction.

Keyword: multiple reaction monitmass spectrometry, immunoaffinity enrichment, biomarkers, plasma

P04.43 Development of a Reference Peptide Library for Targeted Serum Proteomics Using a Cell-Free System

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Introduction and Objectives: Targeted proteomics using selected reaction monitoring (SRM) assay is a powerful approach for accurate quantification of serum proteome dynamics. However, large-scale assay development is greatly limited by the difficulty in preparing reference standards for targeted proteins. Here, we generated a reference peptide library by high-throughput production using a wheat germ cell-free system (WG-CFS) to develop a large-scale SRM assay for human serum proteome. **Methods:** To achieve large-scale synthesis of the reference stable isotope (SI)-labeled peptides for assay development, we designed His-tag-fused peptide concatamers by combining the sequence information of selected proteotypic peptides, and synthesized SI-labeled concatamers using WG-CFS. Synthesized peptide concatamers were digested with trypsin and subjected to tandem MS (MS/MS) analysis using a QTRAP 5500 triple quadrupole MS. **Results and Discussion:** Based on the obtained MS/MS information, we established a set of SRM assays that targeted the selected peptides. Interference in human serum was further verified for each transition by SRM measurements using a tryptic digest mixture of human serum proteins spiked with synthesized reference peptides. Highly reliable information was obtained on the final assays for human serum proteome (1,058 peptides representing 786 proteins). **Conclusion:** The strategy we described here makes the large-scale synthesis of reference peptides possible once the template for in vitro transcription is established. SI-incorporation efficiency of synthesized peptides was approximately 97%, which is sufficient for its use as an internal standard for targeted protein quantitation.

Keywords: SRM/MRM, Serum proteomics, wheat germ cell-free system

P04.44 A Cross-Platform Comparison of Data Independent Acquisition Methods for Proteomic Analysis

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Introduction and Objectives: Traditionally the global proteomic analysis were conducted using automated data dependent acquisition (DDA) mode, which suffers from poor reproducibility among large sample cohorts due to the under-sampling issue innate from shotgun proteomics. Recently, data-independent acquisition (DIA) strategies have been introduced which allow reproducible detection and quantification of thousands of proteins at constant sensitivity across samples. The cross-platform reproducibility has however not been assessed to date. In this study, we firstly generated the platform dependent large spectral library by applying peptide fractionation and then we aim to evaluate the identification and quantitative performance of DIA on five different high resolution mass spectrometers. **Methods:** An off-line high pH reversed-phase fraction strategy was optimized. RAW264.7 (incubated with or without LPS treatment) and Hela cell lysate were digested and separated using 10mM ammonium formate in buffer A and B (pH=10). 78 fractions were collected and pooled into 26 fractions for shotgun proteomics. LC-MS/MS analyses were performed on Q-Exactive Plus and Orbitrap Fusion with DDA and DIA modes. Similar analysis are conducted on other three platforms using Agilent 6550 Q-TOF, Sciex TripleTOF 6600 and Waters SYNAPT G2. **Results and Discussion:** Totally 8502 and 9327 proteins from Hela lysates were identified by Q-Exative plus and Orbitrap Fusion respectively to generate the spectral library for follow-up DIA analysis. Pilot DIA analysis was conducted on Q-Exactive plus and 2014 proteins in the spectral library can be quantified by DIA. Similar DIA analysis will be conducted on the other three Q-TOF mass spectrometers on Raw264.7 cells following LPS treatment. **Conclusion:** Fractionation by high pH RP-HPLC is proved to be an efficient tool for improving proteome coverage and thus enabling building of sufficient spectral library for DIA analysis. DIA analysis on different mass spectrometer platforms will be evaluated and performance comparison will be presented.

Keywords: Shotgun, High pH fractionation, DIA, Spectral library

P04.45 Quantitative Profiling of Cyp P450s Using DIA and Processed Using Cloud Computing

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Introduction and Objectives: 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 P450s, 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. SWATH® acquisition is a data-independent MS acquisition 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. SWATH acquisition can facilitate even highly homologous proteins to be discriminated refining our understanding of enzyme function. **Methods:** Mice were exposed to inducers of P450s, 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 system (SCIEX, USA). Individual samples were then processed and LC-MS data were acquired using SWATH acquisition. SWATH data and its respective ion library was uploaded to the BaseSpace® cloud environment using the CloudConnect™ microapp and the data was processed using OneOmics™ project applications. **Results and Discussion:** Using the various algorithms and visualizations available in OneOmics project, we were able to differentiate 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 allowing for the possibility to identify and quantify peptides unique to Cyp2C50 and Cyp2C54 despite the fact that the proteins share 92% sequence identity. **Conclusion:** The use of OneOmics™ allowed for quick and rapid analysis of the large data set, giving quantifiable data and further expanding informatics by also showing levels of up and down regulation of the proteins within biological networks.

Keywords: SWATH, OneOmics, TripleTOF6600, P450

P04.46 Low Attomole Limit of Quantification on the Orbitrap Fusion Lumos Tribrid Mass Spectrometer

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Introduction and Objectives: A major challenge in Proteomics is the quantification of low abundance proteins and peptides of biological relevance such as transcription factors. The quantification of these analytes is complicated by the large dynamic range observed for cellular protein expression. Strategies have emerged to overcome the matrix effect and quantify low level targets such as Selected Reaction Monitoring (SRM). With the development of new generations mass spectrometry platforms providing high resolution and multiple detector versatility, new strategies can push the limit of quantification. We compared various strategies using the new Orbitrap Fusion Lumos Tribrid mass spectrometer. **Methods:** To ascertain the quantitative performance limit on an Orbitrap Fusion Lumos Tribrid for various contemporary peptide quantification methods, a set of stable isotope labeled synthetic peptides were spiked in various concentrations spanning from 1 attomole to 100 femtomole per microliter into a high complexity matrix of HeLa digest. Targeted and untargeted quantitation methods such as PRM (Parallel Reaction Monitoring), WiSIM (Wide Isolation Selected Ion Monitoring) and DDA (Data Dependent Acquisition) were performed by using an Easy nLC 1000 and 75umx50cm PepMap column. Label free quantification of the results was undertaken using Skyline and Proteome Discoverer. **Results and Discussion:** Limit of quantitation (LOQ) down to 1 attomoles (CV<15%) while maintaining linearity were observed when using PRM, which proved to be the most sensitive tested techniques. Several orders of dynamic range were observed from the quantitation results of the DIA method which exhibited limits of quantification down to 100 attomoles. Meanwhile, the sensitivity of the ion trap analyzer and the use of high resolution greatly benefited quantitation using the WiSIM method. Finally, the DDA method proved to be the least sensitive but was necessary for generating a high quality spectral library. **Conclusion:** The Orbitrap Fusion Lumos Tribrid provides accurate low attomole LOQ for peptides spiked into a matrix in under 30 min.

P04.47 DOSCAT: Double Standards in Proteomics for Mass Spectrometry and Quantitative Western Blotting

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Introduction and Objectives: Absolute quantification of proteins is becoming increasingly important in biological research, for example in systems biology and biomarker development. The current gold standard for quantification is selected reaction monitoring mass spectrometry (SRM-MS). However, this is inaccessible to many users and can lack the sensitivity to detect the lowest abundance proteins. Alternatively, antibodies are routinely used to quantify proteins through western blotting (WB) with great sensitivity. Currently there is little overlap of these techniques due to the need for different calibration standards. We now introduce DOSCATs, novel dual-purpose calibration standards for quantification of target proteins in either or both quantitative platforms. **Methods:** A DOSCAT gene was designed and synthesised to encode an artificial protein that is a concatenation of tryptic peptides and antibody epitopes for five target proteins in the NF-κB signalling pathway. The gene was expressed heterologously in E.coli in the presence of stable isotope labelled amino acids and purified via the His-tag. A known concentration of DOSCAT was spiked into SK-NAS cell lysates pre- and post- 24hr TNFα stimulation in triplicate, and target proteins were analysed either by SRM-MS or by automated WB using the Wes platform (Protein Simple, Santa Clara, USA). **Results and Discussion:** Three target proteins were quantified using SRM-MS and four using WB. Both platforms offered comparable precision (CVs < 20%). Proteins were not quantified due to either low abundance or a poor antibody. Relative quantification was almost identical for both techniques, for example WB measured an 8.8-fold increase in RelB post TNFα stimulation and SRM-MS a 9.6-fold increase. Absolute quantification by SRM-MS and WB were broadly comparable; for example, p65 in unstimulated lysate was quantified at 175,000 (+/-13,000) copies/cell by WB and 131,000 (+/-4,000) copies/cell by SRM-MS. **Conclusion:** This demonstrates the utility of DOSCATs as multiplexed, dual purpose standards supporting seamless integration of the two major platforms for proteome quantification.

Keywords: Quantitative western blotting, Selected reaction monitoring mass

spectrometry, Calibration standards, DOSCAT

P04.48 Identification of Peptide Fragments of Gut Microbiota Proteins in Human Blood Serum

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Introduction and Objectives: The number of microorganisms living inside a human intestine is about 100 trillion cells, a number ten times greater than the total number of human cells in the body. Furthermore, the number of microbiota genes is about 100 times larger than the number of host genes. Gut bacteria have a strong influence on human physiology and nutrition. It has been recognized that the human intestine microbiota play a role in the development of the host immune system. The processes underlying regulation of gut microbiota by host immunity is much less studied. **Methods:** We have carried out comparative LC-MS/MS analysis of serum samples of 10 patients with leukemia before chemotherapy, 7 and 14 days after the beginning of the course as well as of 10 healthy donors. Optimal procedure for sample preparation has been worked out to achieve high-performance isolation of human serum peptides. The reliability of the identification of the peptides was confirmed by a number of bioinformatics approaches, and with the help of synthetic analogues. **Results and Discussion:** LC-MS/MS experiments resulted in identification of more than 5000 unique peptide fragments of human proteins and about 500 peptides related to microorganisms belonging to the human intestinal microbiota. Through careful analysis of serum peptidome we were able to study changes in representation of gut microbiota during chemotherapy. For leukemia patients we observed noticeable changes associated with decrease in the number of Proteobacteria, Firmicutes, Bacteroidetes and Actinobacteria. **Conclusion:** We developed a high-performance method of mass-spectrometry based analysis of human serum peptidome, which allowed to study representation of microorganisms in the human intestine. Further steps to verification of the obtained results will be discussed. This work was supported by the RSF (project No.14-50-00131).

Keywords: Human serum peptidome, Gut microbiota, Bioinformatics

P04.49 Retention Time Independent SWATH Acquisition Scoring

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Introduction and Objectives: The convention in SWATH acquisition and recent DIA [Data Independent Acquisition] analysis methods is to use a spectral library of peptides with known experimental LC elution times and spectra to localize and confirm the presence of the peptides in MS data. Here we present an algorithm which localizes the target peptides without requiring a spectral library. This algorithm is significant as by removing the requirement for a peptide library it now becomes possible to probe SWATH acquisition data for discovery as well as validation. **Methods:** Using in silico digestion and fragmentation we detected peptides based on the premise that a common signal is present in the XICs. Using this premise we mathematically transformed the data to create a score which has maxima where the selected fragment XICs contain the same signal. This maxima and the corresponding MS/MS are then used to calculate a second score for the peak. This second score consisted of a score between the spectra and the theoretical spectra, a score based on coherence of the apex of the XICs as well as conventional scores common to OpenSWATH. The resulting overall score was then ranked against those of decoy peptides in order to estimate the FDR rate. **Results and Discussion:** Using two datasets we compare the built-in SWATH acquisition 1.0 software algorithm with our RT independent algorithm with and without retention time. The results demonstrated that with RT we are able to find targets but that it also found a significant subset without needing RT. This method propels SWATH acquisition into the discovery space, significantly broadening the applicability of the SWATH acquisition method. **Conclusion:** This algorithm finds peptides in SWATH/DIA data without spectral library data making it possible to use SWATH data for discovery.

Keywords: SWATH, DIA, Bioinformatics, Algorithm

P05: POSTER SESSION - TOP DOWN PROTEOMICS AND MACROMOLECULAR COMPLEXES

P05.01 Middle-Down HDX-MS for Structural Characterization of Antibodies at Single Residue Resolution

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Introduction and Objectives: Higher-order structural characterization is challenging for large proteins. Hydrogen/deuterium exchange (HDX) monitored by mass spectrometry (MS) is a powerful tool for this purpose. Peptide-based 'bottom-up' HDX-MS has no protein size limit, but it suffers from limited spatial resolution and incomplete sequence coverage. The 'top-down' approach overcomes these problems through analyzing intact proteins, but its success decreases rapidly as protein size increases. Here we present the development of a new 'middle-down' approach that combines the advantages of both bottom-up and top-down. **Methods:** HDX of the antibodies was performed under "exchange-in" conditions. Subzero temperature HPLC was achieved using a temperature controlling system. MS data were acquired on a Thermo Scientific Orbitrap Fusion mass spectrometer equipped with ETD. **Results and Discussion:** Antibodies were digested into a limited number of specific fragments under cold acidic conditions, followed by HPLC separation and online ETD fragmentation. The back-exchange was kept low by using our subzero-temperature HPLC strategy (Pan et al, JACS, 2014, 136:13065). The specific fragments obtained this way have molecular weights of 12-25 kDa. They covered 100% of the light chain and 95.3% of the heavy chain, representing a total coverage of 96.8% for the whole antibody. These fragments were of perfect size for online ETD fragmentation, which reached a spatial resolution of less than two residues and complete coverage was achieved for every fragment. The effect of deglycosylation on the structure of HER is uncovered by combining this method with HDX, and it is located on two residues in the middle portion of the heavy chain. A mechanism is proposed to explain why the antibody without glycans displayed diminished receptor binding as well as a diminished immune response. **Conclusion:** A new middle-down approach has been developed to combine the advantages of top-down and bottom-up HDX-MS.

Keywords: electron transfer dissociation (ETD), Glycosylation, Top-down proteomics, Mass spectrometry

P05.02 Metaproteomic Analysis, next Generation Environmental Assessment

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Introduction and Objectives: Metaproteomics techniques could provide a wide resolution of proteins from a complex ecosystem. The quantitative metaproteomic analysis provides a functional overview over several level of complexity: at the community level, how the communities respond to the exposure, stress or disease; at the molecular level, which protein families have been expressed; and at the biochemical level, which enzymatic reaction or macromolecular machines have acquired a relevant role to maintain the community in equilibrium. Thus, metaproteomics analysis could offer a molecular snap shot of the eukaryote or prokaryote communities at a certain time and place. This alternative methodology has been applied to analyze the functional role of the microbial community in food quality, in bioremediation, characterizing how microbial species could remediate toxic metal contamination in soils and ground waters, in bioenergy,]or most extensively in human health and personal medicine, identifying how microbial species impact/control disease vs health in organs. However, metaproteomic analysis has not yet explored to implement traditional environmental assessment. **Methods:** Metaproteomics data would provide a representation of the populations at different taxonomic levels on the bases of the taxon-specificity of a tryptic peptide list. **Results and Discussion:** As a probe of concept, we presented the metaproteomic analysis of Baltic Sea soil exposure to propranolol for 6 weeks in a microcosm and the effects of salinity changes have also been evaluated. We tested if a metaproteomic-based assessment can estimate: i) variation in the abundance of protein families, ii) changes in biodiversity, and iii) changes in the equilibrium of a marine soil ecosystem after exposure at a stressor. **Conclusion:** Environmental assessment has traditionally focused on evaluating the environmental quality by measuring abiotic components. The fundamental goal of a metaproteomic environmental assessment is to deliver a robust evaluation the environmental impact at population, individual, cellular and molecular level from one single, comprehensive and cost-efficient methodology.

Keywords: metaproteomics, environmental assessment, microbiome, biodiversity

P05.03 Interactome Analysis of Key Protein Complexes Responsive to DNA Damage and Replication Stress

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Introduction and Objectives: To maintain genomic stability, cells have developed sophisticated signaling pathways and repair mechanisms which abate the DNA damage effects and reduce DNA replication stress. H2AX, the KU70/80 heterodimer, and RPA complex together play an essential role in the initial phase of DNA damage response. Specifically, they are involved in sensing DNA lesions, recruiting and maintaining necessary DNA-repair complexes at damaged sites, and are needed for the activation of DNA-repair kinases from the phosphatidyl-inositol-3 kinase family: ATM, DNA-PK and ATR. Depending on the specific stress stimuli, either one of these kinases is preferentially activated. The kinase signaling cascades then spread the signal to their downstream pathways, leading to DNA repair and cell survival. Moreover, these kinases can also in turn phosphorylate H2AX, KU70/80, and RPA complexes to modulate their functions. The aim of the project was the analysis of the H2AX, KU70/80, and RPA DNA damage response complexes by affinity purification/mass spectrometry. **Methods:** We generated three stable HeLa cell lines inducibly expressing streptavidin-hemaglutinin (SH)-tagged proteins of interest and a control cell line expressing SH-tagged GFP. We then used affinity purification (AP) of SH-tagged proteins followed by targeted, quantitative proteomics (SWATH-MS and S/MRM) to identify proteins interacting with H2AX, RPA1, and KU80 respectively and quantify their relative abundance in identified complexes in response to different DNA-damaging agents. Using specific inhibitors, we evaluated the dependency of these interactions on PI3K family kinases. **Results and Discussion:** We identified several proteins interacting with H2AX, RPA1, and KU80. By comparing to a list of proteins co-purified with SH-tagged GFP, we succeeded to remove unspecific interaction contaminants. Analysis of dynamic changes in protein composition of identified complexes using S/MRM is ongoing and detailed overview of protein-complex interaction networks identified will be given. **Conclusion:** Using AP-MS we characterized proteins dynamically interacting with key protein complexes in response to DNA damage and replication stress.

Keyword: DNA damage response, protein-protein interactions, quantitative proteomics, AP-MS

P05.04 Cold Vaporization of Tissue with a Picosecond Infrared Laser for Top down Proteomics

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Introduction and Objectives: Tissue homogenization is a critical step in proteomics. Recently we have shown that proteins can be desorbed by cold vaporization from tissues by irradiation with a picosecond infrared laser (PIRL) without changing their exact chemical compositions (Kwiatkowski et al. 2015, Angew Chem Int 54: 285-8). It is hypothesized that the release of proteins from tissues offers a more close view to the in-vivo state of proteins and gives a better access to native proteoforms (respective protein species). **Methods:** Mammalian tissues were either irradiated with PIRL and the ablation plume was condensed by cryo-trap and in parallel aliquots subjected to a classical protein extraction applying lyophilisation and protein extraction with a lysis buffer containing protease- and phosphatase inhibitors. Samples with comparable total protein amounts from PIRL condensates and supernatants (classical protein extraction) were applied to one dimensional- (1DE) and two-dimensional electrophoresis (2DE), bands respectively spots were cut out from the gels, subjected to tryptic digestion and subsequent analysis of the tryptic peptides by liquid chromatography (nano-UPLC) coupled to tandem mass spectrometry (orbitrap mass spectrometer). Resulting data were processed and interpreted with OpenMS Proteomic Pipeline (TOPP) applying the search engines OMSSA and XTANDEM) as well as Proteome-Discoverer software. **Results and Discussion:** Bands of 1DE and patterns of 2DE of the condensate of the PIRL tissue condensates contained more proteins with larger molecular weights whereas on the gels of the classical protein extracts bands and spots in the lower molecular weight dominated. In addition the comparison of the patterns of the 2DE comprised a larger number of proteoforms in the samples from the PIRL condensates. Thus, extraction of proteins from tissues by PIRL guarantees minimized

proteolysis and enzymatic removal of posttranslational modifications. **Conclusion:** Extraction of proteins from tissues by PIRL is giving a more realistic view on proteoforms in-vivo and therefore well suited for top down proteomics.

Keywords: in-vivo proteome, proteoforms, protein extraction

P05.05 Optimizing Top down Analysis of Proteins on an Orbitrap Fusion Lumos Tribrid Mass Spectrometer

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Introduction and Objectives: Top down analysis enables measurement of intact protein masses, including post-translational modifications, and verification of protein sequence via fragmentation in the mass spectrometer. Here, we optimize intact protein characterization on an Orbitrap Fusion Lumos MS. Optimal parameters for intact protein fragmentation using ETD, HCD, and CID were determined based on the analysis of intact protein standards and were applied to top down analysis of the oncogenic protein TROP2, a transmembrane glycoprotein highly expressed in epithelial cancers. TROP2 function is regulated by intramembrane cleavage, though not all cleavage sites are known. Aberrant cleavage may drive tumorigenesis as accumulation of the intracellular domain in the nucleus can drive proliferation, transformation and self-renewal. **Methods:** Protein standards were purchased from Sigma Aldrich. Protein standards in 0.1% Formic Acid, 1:1 water/Acetonitrile solution were directly infused at 5ul/min into a Thermo Scientific™ Orbitrap Fusion™ Lumos mass spectrometer using an Easy-Max NG ion source. Full MS or SIM scans for the intact proteins and MS/MS spectra were acquired in low pressure mode. For LC/MS analysis, proteins were desalted using a Proswift™ RP monolithic column and MS/MS spectra were acquired by averaging over 3-10 microscans. Resulting spectra were averaged over the LC peak. Data was processed using ProSightPC™ 3.0 software. **Results and Discussion:** Improvements in ETD detection limits and intraspectral dynamic range enabled higher sequence coverage for standard proteins on LC time scale. For carbonic anhydrase, ETD fragmentation provided >30% sequence coverage (averaging 10 microscans at 120K resolution), and yielded >55% protein sequence coverage (averaging 200 microscans at 240K resolution). Combining results from the CID, ETD and HCD experiments generated >70% sequence coverage for carbonic anhydrase. Similar optimizations for Enolase resulted in >30% sequence coverage via ETD fragmentation alone. Our LC/MS analysis of TROP2 identified multiple deamidation sites as well as confirmed an N-terminal glutamine to pyroglutamate modification. **Conclusion:** not applicable

P06: POSTER SESSION - CANCER PROTEOMICS

P06.01 avβ6, Plasminogen and Latent TGF-β Drive Colorectal Cancer Aggression

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Introduction and Objectives: The αvβ6 integrin, urokinase-type plasminogen activator receptor (uPAR) and transforming growth factor-β1 (TGF-β1) are crucial proteins involved in the progression of colorectal cancer (CRC) towards metastasis. Commonly upregulated in epithelial cancers, αvβ6 enhances metastatic cell attributes including proliferation, invasion and the epithelial-mesenchymal transition. αvβ6 is suggested to interact with uPAR and the latency-associated peptide of TGF-β1, promoting the activation of the latent TGF-β1 (L-TGFβ1) and plasminogen (Plg) zymogens. αvβ6 interacts with ERK2-P through a unique C-terminal tail, promoting the plasminogen activation (PA) cascade and mitogen-activated protein kinase (MAPK) signalling pathways. The present study investigated whether αvβ6 expression enabled CRC cells to activate zymogen members of these proteolytic and growth factor pathways, inducing proteomic and phenotypic changes necessary to facilitate pro-metastatic transformation **Methods:** Triton X-114 phase partitioning with label-free Immobilised pH Gradient Isoelectric Focusing (IPG-IEF) was employed to compare the membrane proteomes of CRC subclones transfected with a β6 integrin overexpression vector against the 'empty' mock control following treatment with L-TGFβ1 and/or Plg. Cell proliferation, migration, invasion assays and ERK1/2, SMAD2 and Akt1/2/3 signalling activity studies were performed to determine whether these zymogen treatments enhanced metastatic phenotypes in an αvβ6-dependent manner. **Results and Discussion:** Membrane proteomic comparison of cell lines has so far identified a significant change in the expression of 337 proteins resulting from zymogen treatment, including the significant upregulation of pro-metastatic proteins such as galectin-1, Bcl-2-associated transcription factor 1 and members of the tumour necrosis factor receptor superfamily. β6-overexpressing cells treated with L-TGFβ1 and/or Plg were significantly more proliferative, invasive and maintained higher ERK1/2 signalling activity following treatment compared to control cells. **Conclusion:** This study provides evidence of significant αvβ6-dependent changes to the membrane proteome of pre-metastatic CRC cells following zymogen treatment, significantly increasing proliferation, invasion and a "switching" from SMAD to ERK1/2 signalling.

Keywords: β6 integrin, colorectal cancer, TGF-β activation, Plasminogen activation

P06.02 Quantitative Mass Spectrometry Reveals Markers for Colorectal Tumors

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Introduction and Objectives: Colorectal cancer is common in elderly individuals and is a major cause of cancer-related deaths worldwide. Although a vast array of genomic and epigenomic changes related to

colorectal tumorigenesis have been identified, the consequences of these alterations on protein effector molecules have not been comprehensively explored. In this study, we apply quantitative proteomic techniques to exhaustively analyze the proteome of human colorectal tumors. **Methods:** Using iTRAQ 8-plex labelling, OFFGEL electrophoresis, and LC-MSMS, we investigated the proteome of 30 colorectal adenomas and paired normal mucosal samples prospectively collected during colonoscopy, and that of normal (HCEC) and cancerous (SW480, SW620, CACO2, HT29, CX1) colon epithelial cell lines. High-throughput selected reaction monitoring assays were developed using stable isotope internal standard peptides, and employed to reproducibly monitor the abundance of candidate tumor markers from the shotgun study in a large independent group of 72 tissues samples comprising tumor and adjacent normal mucosa lesions: 19 adenoma and normal mucosa pairs; 17 adenocarcinoma and normal mucosa pairs. **Results and Discussion:** A total of 4325 and 2017 non-redundant protein families were identified and quantified in tissue and cell lines, respectively. Principal component analysis distinguished adenomas from normal mucosa, and cancer cell lines from HCEC cells. Two hundred twelve proteins displayed significant adenoma-related expression changes (q-value<0.02, mean fold change +/-1.4), including a key enzyme of the polyol pathway. The SRM assays we developed were applied to uniquely and confidently monitor the abundance of 25 proteins in 72 tissues samples comprising neoplastic and adjacent normal mucosa lesions. We confirmed the statistically significant upregulation (fold change>1.3, adjusted P value<0.02) of nine proteins in both adenomas and cancerous colorectal tumors. **Conclusion:** Although benign, adenomas exhibited novel protein changes some of which were documented only in advanced colorectal cancers. The proteins we verified with SRM are candidate tumor markers for early-stage colorectal cancer.

Keywords: colorectal cancer, proteomics, selected reaction monitoring

P06.03 Multiplexed Immunoassays for Cytokine Profiling in 3D-In Vitro Tumor-Stroma Cell Culture Models

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Introduction and Objectives: 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 PEG 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. **Methods:** Multiplexed Sandwich Immunoassays for different cytokines and matrix metalloproteinases were used to characterize a 3D in vitro tumor-stroma cell culture model with four different cell types (tumor cells, fibroblasts, neutrophils, macrophages). **Results and Discussion:** Cytokine profiles and some matrix metalloproteinases were measured in cell culture supernatants of the complex 3D-in vitro tumor stroma cell culture model. First results showed that different cytokine profiles were detected for different treatments of the cell culture. Nevertheless, at the moment the results are based on a low number of samples only. The sample number has to be increased to allow the definition of statistically valid biomarker profiles from our data. **Conclusion:** Cytokine and matrix metalloproteinase profiles might be useful tools to analyze the complex interaction of fibroblast and immune cells with tumor cells in defined 3D- in vitro cell culture tumor models.

Keywords: 3D tumor model, multiplexed immunoassays

P06.04 Data Independent Quantitation of the Rab GTPase Family

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Introduction and Objectives: The monomeric G-proteins, the Rab GTPases, are members of the Ras-related superfamily. Membrane localization is achieved by two geranylgeranyl lipid anchors at their C-terminus. In the active GTP-bound form, Rab GTPases interact with down-stream effector proteins, which are necessary in intracellular membrane traffic. More than 60 different human Rab proteins are involved in this complex vesicular transport process. Due to this crucial role, Rab expression is tightly regulated and kept at low levels. Aberrant expression of Rab GTPases has been linked to cancer development. Therefore, strategies for screening Rab proteins and testing the efficiency of newly developed cancer drugs must be provided. **Methods:** For mass spectrometric investigation of Rab proteins, we utilised density centrifugation for cell compartment enrichment and co-immunoprecipitation. After tryptic digestion, enriched samples were separated using one hour RP-gradients with a nano LC 400 with cHiPLC system (Eksigent, part of SCIEX), connected to a TripleTOF 6600 mass spectrometer (SCIEX) for data dependent acquisition (DDA) and SWATH. Co-immunoprecipitations were additional analysed with an LTQ Orbitrap Velos system (Thermo Scientific) via an 18 hours MudPIT-experiment. **Results and Discussion:** By using a step density gradient, Golgi membranes were enriched from homogenised cancer cells. In these enriched Golgi membranes approximately 25 different Rab GTPases were identified with higher abundance, compared to cytosolic cell fractions where only 10 different Rab proteins were detected. Direct enrichment by co-immunoprecipitation with appropriate bait proteins revealed approximately 40 different Rab proteins. The SWATH-MS data correlated well with a spectral counting method conducted with an LTQ Orbitrap Velos. **Conclusion:** Using two different enrichment strategies, we have shown that the identification and quantitation of almost two-thirds of all known human Rab proteins can be achieved from cancer cell line lysates. This approach has utility for screening of Rab proteins in different cells to investigate the impact of newly developed cancer drugs targeting Rab GTPases.

Keywords: Rab GTPases, protein enrichment, Data independent quantitation

P06.05 Proteomic Analysis of Circulating Extracellular Vesicle for Diagnostic Biomarker of Breast Cancer

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Introduction and Objectives: Cancer cell-derived extracellular vesicles (EVs) containing proteins are linked to the disease pathogenesis in various cancers. In this study, we investigated whether a protein in EVs secreted from a representative invasive breast cancer (BC) cell line, MDA-MB-231, contribute to invasion through EV-mediated mechanism in breast cancer. **Methods:** Circulating EVs, isolated from the plasma of 10 patients with early-stage BC, and 5 healthy controls, were analyzed using LC-MS/MS. We focused on the Del-1 protein based on the fact that this protein is highly expressed in MDA-MB-231 EVs, located on extracellular matrix, and involved in the integrin signaling pathway as well as cancer progression. Enzyme-linked immunosorbent assays (ELISAs) were used to measure Del-1 in plasma samples from healthy controls, patients with BC, BC patients after surgical resection, patients with benign breast tumors, and patients with non-cancerous diseases, in two cohorts. **Results and Discussion:** Here, we report Del-1 protein on EVs, which are sufficient for enhancement of BC cell invasion and for acceleration of lung metastasis at the initial stage of cancer mouse models. This invasion is most likely mediated via the integrin-FAK signaling cascade in cancer cells. In human patients with breast cancer, the levels of Del-1 on EVs are significantly elevated at the early stage of breast cancer, as determined by ELISA with high sensitivity and specificity, but return to almost normal after removal of the tumor. Taken together, these results identify a new function of Del-1 on EVs in cancer cell invasion and its utility as an early diagnostic biomarker of breast cancer. **Conclusion:** In this study, we discovered a new function of Del-1 on EVs by which invasion of BC cells is mediated through integrin-FAK signaling pathway. These EVs could be a potential biomarker for early diagnosis of BC and offer a new therapeutic target for treatment of breast cancer.

Keywords: Developmental endothelial locus-1, Early diagnosis, Extracellular vesicles, Breast cancer

P06.06 Development of a Risk Stratification Test to Discern Aggressive and Non-Aggressive Prostate Cancer

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Introduction and Objectives: The aim of our research project is to develop a Risk Stratification Test (RST) capable of differentiating aggressive from non-aggressive prostate cancer in patients diagnosed with localized prostate cancer. We intend to prequalify a set of 5-10 informative glycoprotein biomarkers for immunoassay development and clinical validation in large cohorts. **Methods:** We focus on the quantitative evaluation of 52 preselected glycoproteins for their prognostic value to determine prostate cancer aggressiveness in blood samples. The biomarker candidates are monitored in the context of a unique set of clinically defined samples from the proCOC cohort using a combination of data-dependent (DDA), data-independent (DIA) and parallel reaction monitoring (PRM) workflows. Glycoprotein capturing on the proCOC blood samples is performed by hydrazide chemistry and the glycosite-containing peptide fraction is analyzed on a Thermo QExactivePlus mass spectrometer in DDA/DIA/PRM mode. For all biomarker candidates heavy labelled SpikeTides for proteotypic glycopeptides were synthesized and used to generate reference spectra and to define the retention time window. **Results and Discussion:** In initial experiments using DDA analysis we detected 24 proteins out of our pre-defined biomarker candidate set. We recorded spectra in DIA/SWATH mode to establish a pipeline for automated data analysis and quantitation via DIA-Umpire, aiming towards a complete digital data matrix for relative quantitative biomarker determination. Statistical analysis will be performed in order to prioritize and select biomarkers for independent validation. Validation of a set of 5-10 biomarkers will be performed via PRM on an independent serum set of more than 200 patients. **Conclusion:** The successful development of Risk Stratification Test would provide doctors and patients with localized prostate cancer with a tool supporting them in their decision making in-between prostatectomy and active surveillance.

Keyword: biomarker, prostate cancer, glycoprotein captuer, DIA

P06.07 Large-Scale Analysis of Melanoma Tissue Samples

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Introduction and Objectives: Malignant melanoma is an aggressive disease defined by cancer of pigment producing skin cell – melanocyte. Lack of knowledge about mechanism underlying melanoma progression results in unfavorable prognosis and high mortality rate. Studying protein expression levels in melanoma tissue samples can provide with valuable information concerning progression of disease and drug resistance. Present study focuses on defining most efficient methodology for following large-scale analysis of melanoma tissue samples. **Methods:** Frozen tissues obtained from a local biobank were lysed with urea and enzymatically digested with trypsin or trypsin/Lys C. Buffer exchange or dilution were used to reduce urea concentration prior to digestion. The tissue digests were analysed directly or following strong cation exchange (SCX) fractionation by nanoLC-MS/MS on Q-Exactive Plus (Thermo Scientific). Statistics and data analysis were performed in Proteome Discoverer v 1.4 (Thermo Scientific) and in Matlab R2014b (The MathWorks). **Results and Discussion:** The most efficient methodology for large-scale analysis of melanoma samples was defined. The power of the offline separation (SCX) for maximising efficiency of shotgun analysis was demonstrated. Two different strategies for sample cleanup before enzymatic digestion were compared and the value of replication was explored. The most efficient approach involved buffer exchange before enzymatic digestion with trypsin and chromatographic separation in 120 min gradient. The number of identified proteins was increased to 9307 proteins when SCX-RP separation of peptides was utilised. **Conclusion:** The difficulties associated with analysis of complex tissue samples is resolved in the present work by introducing multidimensional separation of peptides by charge (SCX) and hydrophobicity (RP). It allows to identify 74% more proteins than with one-dimensional approach. Combination of optimised workflow and state-of-art instrumentation opens possibility for efficient and controlled analysis of large sample cohorts. Protein expression level data will be collected in a local melanoma database and used to deepen knowledge about various disease subtypes and drug resistance.

Keyword: melanoma, shotgun proteomics, sample preparation, database

P06.08 Quantitation and Evaluation of Candidate Biomarkers of Pancreatic Cancer in Plasma Using MRM Method

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Introduction and Objectives: Pancreatic cancer is one of the most highly aggressive and lethal of all solid malignancies. Systematically discovering and validating new biomarkers for early diagnosis of pancreatic cancer is under urgent demand. Targeted mass spectrometry (MS) through multiple reaction monitoring (MRM) has emerged as an alternative to affinity-based measurements of defined protein sets with faster and cost-efficient assay development, high sensitivity and high reproducibility. **Methods:** We previously analyzed differential expressed proteome of serum from healthy people, people bearing benign pancreatic diseases and pancreatic cancer people using TMT and ITRAQ labeling coupled with LC MS/MS method, then we performed the targeted proteomics approach, MRM method, to validate the overexpressed proteins. **Results and Discussion:** Through restrict data analysis, 1822 proteins were identified with high abundant protein depledted in the 2DLC-MS/MS study . we find that 29 proteins were overexpressed in pancreatic cancer patients' serum. Based on these, we further quantify and validate these proteins in huge sample herd, evaluate their sensitivity and specificity, analyze them and chose a combination of new biomarkers with development prospect. **Conclusion:** MRM was applied to verify proteins up regulated in both tissue and serum of pancreatic cancer.APOE and PIGR was finally chosen for conbined diagnosis which showed a sensitivity of 91.2% and specificity of 89.1% in 80 serum samples.

Keyword: pancreatic carcinoma iTRAQ MRM

P06.09 Functional Characterization of a Novel NF1-Related Protein TCTP by the Interactome Analysis

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Introduction and Objectives: Neurofibromatosis type 1 (NF1) is an autosomal dominant disease that predisposes individuals to developing benign neurofibromas and malignant peripheral nerve sheath tumors (MPNSTs). Owing to the lack of information on the molecular mechanism of NF1-associated tumor pathogenesis, biomarkers, and 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, we identified a novel NF1-related abnormal network, "Translationally controlled tumor protein (TCTP)-mediated oncogenic signaling". To further analyze the function of TCTP in detail in NF1-associated tumor, we identified the TCTP-interacting proteins by affinity purification and mass spectrometry (AP-MS). **Methods:** We constructed the plasmid expressing Flag-tagged TCTP, and transfected it into NF1-defficient MPNST cells. Using anti-flag antibody beads, 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), followed by in-silico protein-protein network analysis. **Results and Discussion:** The AP-MS and in-siico protein-protein network analysis revealed that TCTP significantly interacts with the proteins related to protein translation and stress respose. Especially, TCTP strongly binds to the translation elongation complex (EFC) consisted of elongation factors and valyl-tRNA synthetase, which were also found to be up-regulated in NF1-deficient cells. Moreover, amino acid substitution of TCTP Cys28 to Ser caused significant reduction of its binding activity to EFC, suggesting that Cys28-mediated structure of TCTP is important for the functional relation to the translational machinery in MPNST cells. **Conclusion:** These findings indicate that TCTP is functionally implicated in the tumorigenesis and progression of NF1-associated tumors via the TCTP-translation elongation complex formation and protein translation regulation. Kobayashi et al. Mol Cell Proteomics 2009 and 2013, J Biol Chem 2014.

Keywords: NF1, TCTP, affinity purification

P06.10 Hypoxia-Induced Alternative Splicing Proteomics in Cancer Cell Lines

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Introduction and Objectives: Alternative splicing can significantly increase the biodiversity of proteins from the same genome. According to recent studies in cancer biology, adapting to low oxygen by changing gene expression and inducing alternative splicing are vitally important for cell survival and tissue development, especially in HIF or non-HIF induced genes. In contrast to the transcriptional response induced by hypoxia, which is mainly mediated by members of the HIF family induced proteins. However, there are only few studies investigating alternative splicing proteome due to without available method for MS data analysis. Therefore, we performed a new MS-based method for the proteome-wide analysis of hypoxia-related changes of alternative splicing in human cancer cell lines. **Methods:** The human breast cancer cell lines as experimental model were incubated under hypoxia condition 1% O2 for 48 h. For protein identification, Tryptic peptides were analyzed using Waters SYNAPT G2 Q-TOF MS coupled with a nano UPLC. MS database searches were performed on a novel protein database. For quantification, first, peptide were labeled with Isobaric tags for relative and absolute quantitation reagent. Then, combined peptides were fractionated by high pH RP chromatography, and analyzed by LC-MS/MS. We constructed a novel peptide databases which contain many different types of alternative spliced isoform through bioinformatics tool. **Results and Discussion:** We perform an alternative splicing proteome analysis techniques to identify and quantify alternative splicing isoform. Nowadays, we characterize splicing proteins under hypoxia and reoxygenation condition with human cancer cell line (MCF7) and validate our novel peptide sequence dataset results using transcriptomic databases derived from RNA-seq technology. By means of our newly developed method, the hypoxia-induced alternative spliced proteome can lead us to further understand how organisms response to hypoxia. We believe that such an integrative systems approach is important to development and validation of alternative splicing isoform for the further research steps. **Conclusion:** not applicable

Keywords: alternative splicing, Hypoxia, mass spectrometry-based proteomics, cancer proteomics

P06.11 Discovery of Pulmonary Disorder Biomarkers by In-Depth Proteomic Analysis of Pleural Effusions

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Introduction and Objectives: Pleural effusion (PE), a tumor-proximal body fluid, may be a promising source for biomarker discovery in human cancers. Because a variety of pathological conditions can lead to PE, characterization of the relative PE proteomic profiles from different types of PEs would accelerate discovery of PE biomarkers specifically used to diagnose pulmonary disorders. **Methods:** The high-abundance protein depletion followed by GeLC-MS/MS were used to generate PE proteomes from six types of PEs, including three malignant PEs (MPE, from lung, breast, and gastric cancers), one lung cancer para-malignant PE (PMPE), and two benign diseases (tuberculosis and pneumonia). Spectral counting was utilized to semi-quantify PE protein levels. Principal component analysis (PCA), hierarchical clustering (HCL), and Gene Ontology (GO) were applied to analyze the differential levels and functional profiling of proteins in each type of PE. The protein levels in PE and cancer cells were determined by ELISA and Western blot, respectively. **Results and Discussion:** We identified/quantified 772 proteins from six types of PEs. PCA, HCL, and GO of cellular process analyses revealed the distinct protein profiling in each PE type. Three potential markers, MET, DPP4, and PTPRF, were further verified by ELISA using two independent cohorts of PE samples. The protein levels of these potential biomarkers were significantly higher in lung cancer MPE than in benign diseases or lung cancer PMPE. We also observed that the PE protein levels were more clearly discriminated in effusions in which the cytological examination was positive. Western blotting analysis further demonstrated that MET overexpression in lung cancer cells would contribute to the elevation of soluble MET in MPE. **Conclusion:** We show the first comprehensive, label-free, quantitative proteomic study of six types of exudative PEs. We propose that the PE protein levels would provide a useful adjunct if combined with cytological evaluation in diagnosis of lung cancer with pleural cavity metastasis.

Keywords: quantitative proteomics, biomarker, hepatocyte growth factor receptor, pleural effusion

P06.12 A Proteomic Investigation for Detection of Early Stage CRC Biosignatures

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Introduction and Objectives: The current methods widely deployed for colorectal cancer (CRC) screening are grossly inadequate on sensitivity and specificity ground. 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. This study was designed to meet the longstanding unmet clinical need for cancer screening and surveillance using minimally invasive techniques. **Methods:** Presence of potential biomarkers are being investigated in clinically stage CRC (Duke's A, B, C, D) and control (i.e., unaffected) EDTA plasma samples using commercially available MARS 14 as well as MQ-patented immune based depletion methods. Detection of low abundance novel biomarkers are expeted using state-of-the-art LC-MS instrumentation housed in Australian Proteome Analysis Facility. **Results and Discussion:** The detailed results will be presented at the upcoming 14th Human Proteome Organization world Congress in Vancouver which would specifically provide useful information to researchers about a longstanding unmet clinical need for early stage CRC detection. **Conclusion:** We believe these findings will enable to establish new, improved and volume-sparing plasma biomarkers/biomarker signature panels.

Keyword: Cancer, Plasma, Proteomics, Biomarker

P06.13 Cell Phase Specific Proteomic Profiling of Newly Synthesized Proteins Using SILAC-TMT Quantification

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Introduction and Objectives: Temporal analysis of protein synthesis contributes enormously to the molecular characterisation of cellular phenotypes and defines the key pathways. Cellular senescence has a tumor suppressive function through growth arrest however there is evidence for a procarginogenic role due to altered secretory activities that can contribute to tissue microenvironment changes (Senescence-associated secretory phenotype-SASP). This is particularly germane to Oncogenic H-RAS-V12-induced senescence (RIS), characterized by specific global alterations in gene expression. To better understand the translational mechanisms involved in senescence we profiled the levels of newly synthesized proteins in distinct cell phases in a 4-hydroxytamoxifen-inducible RIS (ER:Ras) model in IMR90 human diploid fibroblasts using orthogonal SILAC-TMT labelling to gain an insight into temporal changes in protein expression responsible for the onset of senescence. **Methods:** IMR90 cells cultured in medium containing isotopically light arginine (R) and lysine (K) were pulse labelled with isotopically heavy R and K at key phenotypic phases following RIS induction: growing, mitotic, transition, senescent and quiescent phases. Cells at each phase were lysed and the total protein digested, TMT labelled and analysed by mass spectrometry. **Results and Discussion:** 7,000 newly synthesized proteins were identified and quantified of which about 6% showed differential expression at each one of the cellular phases. Gene ontology analysis showed mainly over-representation of cell-cycle and extracellular proteins. In the context of the senescence-associated secretory phenotype three interleukins namely IL-1α, IL-1β and IL-11 were found up-regulated in the senescence cells. **Conclusion:** We have characterized cell-phase specific protein expression upon senescent transformation increasing our molecular understanding of the SASP phenotype. The combination of pulsed SILAC and TMT-labelled proteins opens the opportunity for rapid time-dependent identification and quantification of changes in proteins responsible for phenotypic progression.

Keywords: senescence, TMT-SILAC, Mass spectrometry, protein synthesis

P06.14 Immuno-MALDI for Quantifying Akt1 and Akt2 Phosphorylation in Colorectal Cancer

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Introduction and Objectives: Epidermal growth factor receptor (EGFR) and vascular endothelial growth factor (VEGF) signaling pathways play a critical role in colorectal cancer (CRC) and are targeted by novel therapeutic agents. However, these targeted treatments only work in a minority of patients. Biomarkers to predict a patient's response to specific treatments are lacking or only partially successful, and currently there are no reliable methods to quantify the activity of these pathways. We therefore set out to develop immuno-MALDI (iMALDI) assays to measure the phosphorylation degree of RAC-alpha serine/threonine-

protein kinase (Akt1) and RAC-beta serine/threonine-protein kinase (Akt2) in CRC tissues as predictive biomarkers for targeted therapies. **Methods:** The iMALDI approach is based on tryptic digestion of the endogenous target proteins, followed by affinity-enrichment of specific tryptic peptides and analogue stable-isotope labeled standard (SIS) peptides, and absolute quantitation using MALDI-TOF (Bruker Microflex LRF). **Results and Discussion:** We quantified non-phosphorylated Akt1 in 100 μg of EGF-induced and non-induced MDA-231 breast cancer cell lines, SW480 and HCT116 colon cancer cell lines, and in ~180 μg breast cancer tumor lysates. All samples analyzed fall within the linear range (0.08 fmol Akt1/μg – 20 fmol Akt1/μg, or 4.4 pg Akt1/μg to 1114 pg Akt1/μg) of the Akt1 assay. Precision for triplicate digests was found to be consistently <10%. In addition, non-phosphorylated Akt2 has been quantified in 100 μg MDA-231 breast cancer cell lysate. **Conclusion:** We have developed iMALDI Akt1 and Akt2 assays for quantitation of non-phosphorylated Akt1 and Akt2 target peptides. The next steps will involve sensitivity optimization, determination of phosphorylation degree in cancer cell lines and tumor samples (either by targeting phosphopeptides, or by applying our phosphatase-based phosphopeptide quantitation (PPQ) method), and automation of the sample preparation.

Keywords: iMALDI, phosphorylation, colorectal cancer

P06.15 SWATH-MS Profiling of NSCLC Cell Lines: Defining Mechanisms of Erlotinib Resistance

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Introduction and Objectives: Although significant advances have been made in the development of targeted therapies for lung cancer in recent years, acquired resistance limits long term use of these agents. One targeted therapy, erlotinib, an epidermal growth factor receptor (EGFR)-targeting tyrosine kinase inhibitor is used as standard-of-care treatment in non-small cell lung cancer (NSCLC) in patients with sensitising EGFR mutations. However, patients inevitably develop resistance. We are creating a protein profile to identify protein pathways associated with the development of resistance to erlotinib. **Methods:** Two NSCLC cell lines were compared by SWATH-MS analysis: a parental NSCLC cell line sensitive to erlotinib (HCC827, contains a deletion in EGFR exon 19) and its matched resistant subline (HCC827_ER). Erlotinib resistance was established using 17 cycles of 100μg/mL erlotinib. Cell lines were treated with an IC50 dose of erlotinib or mock treated, prior to a 3-day incubation period. Protein mapping of each cell line was performed using the Sequential Windowed data independent Acquisition of the Total High-resolution Mass Spectra (SWATH-MS 2.0) algorithm, conducted on the AB Sciex 6600 TripleTOF mass spectrometer. Bioinformatic analysis to identify significantly differentially expressed proteins will be performed using the AB Sciex OneOmics platform (beta version). **Results and Discussion:** This analysis will identify differentially expressed proteins from pathways that are associated with induced erlotinib resistance. **Conclusion:** The success of targeted agents in molecularly defined subsets of patients with NSCLC has radically changed paradigm in treatment and improved patient outcomes. However, this success is limited by the emergence of therapy resistance. Increasing understanding of the mechanisms involved in the acquisition of resistance is crucial for the creation of rational strategies to overcome resistance.

Keywords: non-small cell lung cancer, SWATH-MS, erlotinib

P06.16 Optimizing MS Based Methods to Characterize MHC-I Peptidome in Response to Cellular Stimulation

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Introduction and Objectives: Recent developments in the field of cancer immunotherapy has shown the great potential as a therapeutic approach in fighting cancer. Many of these therapeutics work by directing and eliciting cytotoxic T lymphocyte responses toward tumor associated antigens presented on the cell surface by MHC Class I (MHC-I) molecules. Because of the significant role antigenic peptides play in cancer immunity, there is great interest in developing analytic techniques to characterize the cancer specific MHC-I peptidome. Recent advances in mass spectrometric instrumentation and bioinformatic algorithms for searching and predicting affinity of MHC-I peptides have enabled the identification of immunogenic T cell epitopes (Yadav et al, 2014). Here, we investigate methodologies for profiling MHC-I peptides. **Methods:** Granta cells (2E8 cells) were immune-precipitated using a pan anti-HLA antibody. Samples were analyzed on a Fusion MScoupled to a Waters nanoACQUITY UPLC. Two different search algorithms were employed to compare their performance and complementarity. Data was also searched against two databases, Uniprot and a cell line specific transcriptome generated FASTA database to assess identification of neo antigens. Global protein expression profiling was performed on Granta cells using high pH reversed phase fractionation followed by LC-MS/MS to determine correlations between presented peptides and protein expression level. **Results and Discussion:** Mascot and PEAKS search algorithms yielded very different number of peptide spectral matches (PSMs) for the Granta immune-precipitated samples. We investigated the difference in PSM observations; comparing the peptide sequences identified to the scans matched between the two search algorithms. The use of a personalized cell line database generated from transcriptome data allowed the successful identification of several mutant peptide sequences. **Conclusion:** Optimization of the MS-based methodology for identifying MHC-I peptides enhanced our lab's ability to characterize the peptide repertoire. The results from this study suggests drug treatments can alter the repertoire and abundance of presented antigens.

Keywords: Mass spectrometry, MHC class I peptides

P06.17 Discovery of Biomarkers for Oral Cancer by Proteomic Profiling of Tumor Interstitial Fluids

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Introduction and Objectives: Patients with Oral cavity squamous cell carcinoma (OSCC) are frequently first diagnosed at an advanced stage, leading to poor prognosis and high mortality rate. Early detection of OSCC using body fluid-accessible biomarkers may help improve the prognosis and survival rate of OSCC patients. Tumor interstitial fluid (TIF) is a proximal fluid enriched with cancer-related proteins that is therefore

a reservoir suitable for cancer biomarkers discovery. In this study, we aimed at identifying biomarkers for OSCC by proteomic profiling the paired tumor (TIF) and adjacent noncancer (NIF) interstitial fluids. **Methods:** The paired TIFs and NIFs from ten OSCC patients were profiled using one-dimensional gel electrophoresis coupled with the nano-liquid chromatography-tandem mass spectrometry (GeLC-MS/MS) approach. The relative expressions of identified proteins between paired TIFs and NIFs were evaluated using label-free spectral counting method. The salivary levels of two potential biomarkers were determined using a beads-based immunoassay and a sandwich ELISA, respectively, in 55 OSCC patients, 55 health individuals, and 55 patients with oral premalignant diseases (OPMD). The tissue expression of one potential biomarker in OSCC patients was evaluated through immunohistochemistry (IHC) staining. **Results and Discussion:** By spectral counting, we found that 111 proteins were up-regulated in at least six OSCC TIFs compared to their respective NIFs. The biomarker candidates were prioritized based on their gene expressions in OSCC tissue collected from public domain and their predicted secretion pathways Two candidate proteins were selected and verified that their salivary levels were significantly higher in OSCC patients compared to health and OPMD individuals. Moreover, the IHC results showed that the expression of one candidate protein in OSCC was higher than those in adjacent non-cancerous epithelium and correlated with pathological T, N, and overall stage as well as survival of OSCC patients. **Conclusion:** These results collectively indicated that analysis of TIFs is a feasible strategy for cancer biomarkers identification.

Keywords: proteomics, cancer biomarker, oral cavity squmous cell carcinoma, tumor interstitial fluid

P06.18 Molecular Consequences of PC1/3 Inhibition in Macrophages and Application in Immunotherapy

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Introduction and Objectives: The prohormone convertase 1/3 (PC1/3) is an enzyme playing an important role in the processing within the regulated secretory pathway in the nervous system. Recently, a possible implication of PC1/3 in innate immunity has been highlighted. These studies have reported a role of PC1/3 in Toll-like receptor immune response in macrophages. A dysfunctional phenotype characterized by uncontrolled cytokine secretion without stimulation has been observed in PC1/3 knock-out mice and in NR8383 macrophages cell line. Here, using lentivirus strategy, we investigated the consequences of PC1/3 down-regulation in NR8383 on the intracellular impact and its biological application to cancer therapy. **Methods:** A proteomic approach (shotgun and FASP) has been undertaken in order to obtain a global vision of secreted and cellular proteins overtime. Results were validated by ELISA and confocal microscopy. **Results and Discussion:** Under sterile conditions, we demonstrated that PC1/3 inactivated macrophages express an M1-like phenotype characterized by filopodia extensions, pro-inflammatory chemokines and cytokines secretion (IL-6, CXCL10, TNF-α) and TLR4 Myd88 dependent signaling activation. Under LPS challenge, PC1/3 KO cells secrete through store-operated calcium entry increase, a cocktail of pro-inflammatory factors including alarmins and chemokines. This secreted factors have been shown to attract naïve T helper lymphocytes (Th0) which favors cytotoxic response. These factors are also able to inhibit viability and resistance of breast and ovarian cancer cells (SKBR3 and SKOV3). Under inhibitory conditions using IL-10, PC1/3 KO cells still continue to produce inflammatory cytokines. **Conclusion:** These data established that inhibition of PC1/3 increase pro-inflammatory response giving an M1-like phenotype to macrophages. This strategy may be used as a potential immune therapy for awaking intratumoral macrophages.

Keyword: prohormone convertase 1/3, macrophages, cytokines, immunotherapy

P06.19 Acidosis-Induced Proteome Pattern of AT-1 Prostate Carcinoma Cells

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Introduction and Objectives: Under various pathological conditions, such as inflammation, ischemia or in solid tumors, physiological parameters (local oxygen tension, extracellular pH) show distinct abnormalities, e.g., hypoxia or acidosis, in these tissues. In this study, we investigate the impact of extracellular acidosis on the proteome of a prostate cancer derived tumor cell line. To obtain first comprehensive results on alterations of the cellular phenotype during acidosis, cells were grown at different pH conditions and protein patterns were compared by LC/MS/MS. The identified, differentially regulated proteins were verified on the mRNA level and by functional tests. **Methods:** AT-1 rat prostate carcinoma cells were exposed to pH 7.4 (control) or pH 6.6 (acidosis) for 24 h and 48 h. For isobaric labeling, TMT-10 plex reagents (Thermo Fisher Scientific) were used according to the manufacturer's protocol. Samples were analyzed using an Ultimate 3000 RSLC nano-HPLC system coupled to an Orbitrap Fusion mass spectrometer (Thermo Fisher Scientific). In a first experiment, samples were analyzed with a combined CID/HCD MS/MS strategy for peptide identification and reporter ion quantification. In a second experiment, MS^3 data were used for reporter ion quantification. **Results and Discussion:** Comparative quantification was obtained for more than 3000 proteins. At pH 6.6, four proteins (GSTA3, NQO1, CRABP2 and NBC3) were significantly differentially up-regulated, while three further proteins (MT-2A, Glut-1, FPPS) were significantly down-regulated by at least 50% at one or both time points. We evaluated the expression levels of all seven identified proteins by quantitative real-time PCR. The mRNA levels of all up-regulated proteins were found to be increased. The enhanced expression was most pronounced for NBC3 (6.3 fold) and NQO1 (4.5 fold). Consistent with the alteration of the protein levels, the levels of MT-2A, Glut-1 and FPPS mRNA became diminished remarkably over time. **Conclusion:** Acidosis–induced proteome patterns were obtained for the first time a for tumor cell type.

Keyword: acidosis, tumor proteome, isobaric labeling

P06.20 Proteomic Analysis of Tumor-Associated Macrophages in an In Vivo Tumor Microenvironment

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Introduction and Objectives: In solid tumors, the tumor microenvironment is an essential part of each developing neoplasm. In this microenvironment,

malignant cancer cells co-reside with stromal cells including fibroblasts and immune cells. Tumor-associated macrophages (TAMs) are one of the most prominent infiltrated leukocytes present in neoplastic tissues and are able to influence tumor progression by allowing the establishment of an attractive environment for tumor growth and metastasis. Despite the fact that the tumor promoting features of macrophages are investigated extensively, to our knowledge an analysis on the level of the proteome is currently missing. **Methods:** In this study, we applied peptide-centric tandem mass tag (TMT)- based quantitative proteomics to compare the protein expression profiles of TAMs with control macrophages (peritoneal macrophages and bone-marrow derived macrophages). The macrophages were isolated via fluorescence activated cell sorting (FACS) from orthotopic non-small cell lung adenocarcinomas. 18 macrophage samples (six independent biological samples per experimental group) were randomly divided over the three TMT sixplex experiments. Each of these sixplex experiments was fractionated offline in 10 separate fractions before further LC-MS/MS analysis on a LTQ-Orbitrap Velos platform using a parallel CID-HCD approach and data-dependent acquisition. Analysis and data normalization of the identified and quantified proteins present in the 30 LC-MS/MS runs was performed with the CONSTANd algorithm. **Results and Discussion:** In this experiment, 1250 unique proteins were identified, of which 1178 proteins were quantified with all six TMT reporter ions. After CONSTANd normalization, hierarchical clustering of the identified and quantified peptides present enabled a clear distinction of the experimental groups based on expression profiles. Differential expressed proteins responsible for the discriminating potential of different experimental conditions were found to be involved in oxidative stress and several metabolic processes. **Conclusion:** Proteomics strategies allow to provide additional insights in the biology of TAMs.

Keywords: macrophages, Tumor microenvironment, quantitative proteomics, CONSTANd normalization

P06.21 Identication of Tyrosine-Phosphorylated Proteins Upregulated during EMT Induced with TGF-B

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Introduction and Objectives: Epithelial–mesenchymal transition (EMT) is a morphogenetic process related to migratory ability and invasive potential of epithelial cells, and acquisition of EMT may be involved in transition and invasion of cancerand cell fibrosis. Transforming growth factor-b (TGF-b) plays a central role in the regulation of EMT and exerts pleiotropic effects through binding to receptors type I and II. TGF-b is often overexpressed in tumor tissues, and it facilitates cancer progression through a diverse repertoire of downstream effects, including enhancement of cell motility and invasion, both of which are involved in the process of EMT. When TGF-b is added to the culture medium for epithelial cells, cell adhesion molecules such as E-cadherin are down-regulated, and the cells adopted a fibroblast-like morphology. Therefore, proteins specific to EMT induced by TGF-b represent candidates of therapeutic biomarkers. In this study, we performed relative quantitation of tyrosine-phosphorylated peptides in TGF-b–treated human alveolar adenocarcinoma cells (A549) and untreated A549 cells. We detected remarkable up-regulation of three up-regulated tyrosine-phosphorylated proteins and identified them by LC-MS/MS. All of them are proteins known to have various functions important for signal transduction and EMT. Next, we performed multiple reaction monitoring (MRM) mass-spectrometric analysis on these three proteins, and compared them between good- and poor-prognosis stage I adenocarcinomas. These tyrosine-phosphorylated proteins were confirmed to be up-regulated in TGF-b–treated A549 cells and stage I adenocarcinoma patients with poor prognosis. These proteins represent possible candidates of prognostic prediction markers for lung adenocarcinoma.
Methods: not applicable
Results and Discussion: not applicable
Conclusion: not applicable

Keywords: lung adenocarcinoma, tyrosine phosphorylation, EMT, TGF-b

P06.22 Comprehensive Transcriptomic and Proteomic Analysis of Retinoblastoma

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Introduction and Objectives: Retinoblastoma (RB) is a rare and intraocular cancer found in children. It originates due to the inactivation of RB1 gene through epigenetic modification or gene mutation. Although previous gene expression studies have defined the transcriptional profiles but proteomic signatures of RB have not been studied. Integrative analysis with transcriptome and proteome will helps in identifying therapeutic targets and also biomarker discovery for RB. **Methods:** For transcriptome analysis GeneChip primeview gene expression array chips were used. For quantitative proteomic analysis samples were iTRAQ labelled, SCX fractionated and analyzed on Orbitrap mass spectrometer. **Results and Discussion:** We identified 5154 genes were deregulated at the transcriptome level and 1429 proteins were deregulated at the proteomic level. Overlap of the transcriptome and proteome identified 367 deregulated genes/proteins.. We identified IGF2BP1which was found in 3.8 folds up regulated in transcriptome and 4.8 folds upregulated in proteome and performed functional studies. IGF2BP1 is a mRNA stabilizing molecule which stabilizes the mRNAs of growth factors such as c-MYC, IGF, ACTB. siRNA mediated knockdown studies of IGF2BP1 in Y79 Retinoblastoma cell lines showed a decreased tumor progression and decreased migration in vitro. **Conclusion:** This study gives a comprehensive map of deregulated genes/ proteins in retinoblastoma. Our data further suggests that IGF2BP1 is a key regulatory molecule which can used as therapeutic target for the treatment of retinoblastoma.

Keyword: Retinoblastoma, Transcriptome, Proteome

P06.23 Proteomic Analysis of the Resection Margin at Different Distances of a Gastric Cancer

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Introduction and Objectives: Gastric cancer presents a high rate of malignancy, being the second in cause of death worldwide. Cancer tissue, together with morphologically apparent healthy tissue (resection margin) is removed by surgery; yet, recurrence is higher than 50%. Resection margin is a region of interest for understanding disease biology. We investigated the proteomic profiles of biopsies of gastric cancer and resection margins at different distances using histopathology analysis combined with iTRAQ-8 plex proteomic approach. **Methods:** Four biopsies (tumor area, resection margin of 1, 3 and 5 cm) of two patients diagnosed with histological type adenocarcinoma were collected from Gaffrée and Guinle Hospital, Rio de Janeiro, Brazil. Biopsies were divided in two parts, one for proteomic analysis and the other for histopathology analysis performed by hematoxylin and eosin stains. Samples were trypsin digested, labeled with iTRAq 8-plex. Aliquots were submitted to strong cation exchange and hydrophilic interaction-LC prior the reverse phase chromatography/ tandem mass spectrometry. **Results and Discussion:** Analysis disclosed 2107 proteins. Detailed examination evidenced gastrokines (isoform 1 and 2) and trefoil factor (isoform 1 and 2) as less abundant proteins; their lower expression is correlated with a poor prognosis in this type of cancer. Proteins such as thymosin beta 10, glutathione S-transferase and protein S100 were found in increased amounts at different distances of the resection margin suggesting that molecular modifications related to oncogenesis are occurring even in the regions free of disease according to the histopathology analysis (i.e. resection margin of 3 and 5 cm). **Conclusion:** Conclusions: This work builds on our previous data that point to a panel of proteins correlated to gastric cancer and that the resection margin does not present a “healthy proteome” as suggested by histopathology

Keywords: quantitative proteomics, Gastrokines, Trefoil factor, Gastric cancer

P06.24 Tracing Growth Factor Driven Resistance to EGFR Inhibition by Chemical- and Phosphoproteomics

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Introduction and Objectives: The epidermal growth factor receptor (EGFR) is an oncogenic driver of various types of cancer. Inhibition of EGFR by small molecule tyrosine kinase inhibitors has shown some success in cancers with EGFR activating mutations. However, tumors with high expression of wild type EGFR are often resistant or show only moderate sensitivity to EGFR inhibition. Oncogenic bypass mediated by mitogenic stimuli secreted by the tumor microenvironment is a common resistance mechanism. To investigate resistance mechanisms mediated by growth factor stimulation, we used the EGFR overexpressing cell line A431 that shows modest sensitivity to gefitinib and is prone to mitogenic stimuli. **Methods:** We used an Fe-IMAC column for comprehensive phosphopeptide enrichment and the Kinobead technology for affinity enrichment of kinases. Enriched phosphopeptides and kinases were measured and quantified by label-free mass spectrometry on a Q-Exactive Plus and an Orbitrap Elite, respectively. Pathway mapping tools were used to visualize bypass signaling and to rationalize combination treatments. **Results and Discussion:** The results show that the growth factors HGF, FGF2, IGF-1, EGF rescue cells from killing by the EGFR inhibitor gefitinib. KEGG pathway mapping of phosphorylated proteins revealed changes in IGF1-R/INSR signaling and for all growth factor rescues we found significant changes on IRS1/IRS2, IGFBP3 and GRB7 phosphorylation sites. Druggable targets of this pathway include MEK/ERK or the PI3K/AKT/mTOR as well as IGF1-R/INSR. Combination treatment of gefitinib and MEK inhibitor trametinib was able to neutralize the rescue of all growth factors and IGF1-R/INSR inhibitor OSI-906 diminished growth factor mediated rescue. However, the PI3K/mTOR inhibitors XL-147 and BEZ-235 were not effective to circumvent growth factor driven rescue. **Conclusion:** Our findings show the power of phosphoproteomics and chemical proteomics for the discovery of drug resistance mechanisms leading to a rational combination treatment targeting MEK and EGFR to suppress oncogenic bypass signaling.

Keywords: drug resistance, EGFR, phosphoproteomics, Chemical Proteomics

P06.25 Proteomic Analysis of the Transcriptional Program Induced by TWIST in Human Fibroblasts

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Introduction and Objectives: The basic helix-loop-helix (bHLH) transcription factor Twist1 is known to be a key player in tumor metastasis by inducing Epithelial-Mesenchymal Transition (EMT). Expression of Twist1 has been associated with invasion and poor survival in different tumors. However, the factors and pathways by which Twist1 promotes cancer development and metastasis are mostly unknown. As the expression of Twist1 is manly localized in the tumoral stroma, we aimed to further characterize the role of Twist1 in human fibroblasts. **Methods:** We stably transfected BJhTert fibroblasts with a lentiviral vector encoding for Twist1. We conducted an iTRAQ quantitative assay of the whole cell extracts or alternatively nuclear fractions. Proteins were trypsin digested and resulting peptides were labeled. 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:** Twist1-expressing fibroblasts developed active fibroblasts characteristics, such as hyperproliferation, increased ability to migrate, actin cytoskeleton alignment, collagen gel contraction and remodeling of the extracellular matrix. By using MS-based quantitative proteomics, we obtained a total of 198 proteins whose expression was directly or indirectly controlled by Twist1 overexpression, including several transcription factors and co-factors such as PRRX1, STAT1, MYT1L or HMGA2. Besides, we found enrichment in proteins whose expression is a typical marker of activated fibroblasts such as VIM, LEPRE1, TAGLN, S100A4, PALLD and several collagens. Bioinformatic analysis showed TGF-β, cell proliferation, matrix metalloproteinase and angiogenesis among the top predicted biological networks deregulated by Twist1 expression. **Conclusion:** Twist1-expressing fibroblasts developed activated fibroblasts characteristics such as hyper-production of extracellular matrix components and expression of typical markers. Our proteomic screening 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.

Keyword: Twist1, EMT, activated fibroblasts, cancer invasion

P06.26 SWATH Quantitation Using Ion Libraries from Multiple Cancer Cell-Types Identifies Drug Resistance

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Introduction and Objectives: SWATH-MS enables accurate and parallel quantitation of numerous proteins. The current success of SWATH depends crucially on retention time stability and ion libraries (IL) complexity especially with extensive IL are becoming increasingly available in public repositories. However, the impact on quantitation of IL derived from multiple cell-types remains to be tested. Here, we evaluate SWATH-MS using IL from local acquisition and repository-based IL and assessed SWATH-MS of cancer cells derived from different cell-types. **Methods:** To evaluate IL extension, yeast digests were spiked (2, 5 and 10%) into 1 μg digested human K562 cell lysate and acquired within 2h nanoLC in 60 variable window SWATH-MS on a TripleTOF 6600 (SCIEX). Multiple cell-type IL was generated from 2h nanoLC-MS/MS analysis of lysate digests from HEK293, HeLa, SW1736, Colo205, SW480, SW620 and 6 patient-derived melanoma cell lines. Each cell line was then acquired under equal nanoLC conditions with 100 variable window SWATH-MS. **Results and Discussion:** SWATH-MS data were extracted using IL from comprehensive local acquisition (~3000 human, ~2000 yeast proteins) and from merging local IL with human library downloaded from SWATHAtlas. Results were classified in true or false positives and negatives. For local IL p-values <0.05 and fold-change >1.5 maximised TP/TN and minimised FP/FN for detecting protein expression changes greater 2-fold. However, for extended IL, more stringent cut-off criteria need to be used to minimise FP/FN. Multiple cell-type IL generation (4800 proteins) revealed 3700 proteins quantifiable by SWATH-MS across cancer cell lines. Hieratical clustering demonstrated separation between melanomas and carcinomas, but not between genotype. However, SW1736 and two melanomas expressed proteins absent in other carcinomas and the remaining melanomas. Correlation with cell viability after MEK inhibition (AZD6244) reveals drug resistance as common feature detectable from phenotype profiling. **Conclusion:** Multiple cell-type IL SWATH-MS of cancer cell lines discriminates between cell-types and identifies common cellular processes associated with drug sensitivity.

Keywords: SWATH data independent acquisition, Ion Libraries, Drug Sensitivity

P06.27 Verification of Serological Biomarkers for Lung Adenocarcinoma by Targeted Mass Spectrometry

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Introduction and Objectives: Lung cancer is the leading cause of cancer mortality worldwide. Although many biomarkers have been identified for lung cancer, the low specificity and sensitivity bring out the urgent need for more candidate biomarkers. Here, we attempted to conduct a multiple reaction monitoring (MRM)-based targeted analysis to evaluate the potential utility of a list of candidate proteins for lung cancer diagnosis. **Methods:** A total of 1249 transitions of 420 peptides representing 102 candidate proteins from our previous study and literature were first screened by MRM analysis in the pooled plasma samples, remaining 78 proteins in the list. Relative quantification of these 78 proteins was further performed in 60 individual plasma samples from patients in 3 stages and paired healthy control subjects. Additional 9 transitions of the 3 peptides from β-galactosidase were also monitored as internal standard. The Wilcoxon test was used to assess the difference in expression levels of the biomarker between the patient and the control of a pair. **Results and Discussion:** Over the triplicate runs, the intensity of the monitored peptides had CVs of <20%. Moreover, the retention time of the target peptides exhibiting CVs below 10%. Ultimately, 9 proteins were found to be able to distinguish patients from controls. Further combination of 5, 3, and 2 candidate marker proteins has improved sensitivity indiscriminating ability as well as a merged AUC value of nearly 1.00 in stage I, II, III groups versus controls, respectively. The alterations of gelsolin, galectin-1, and actin, cytoplasmic 1 between different stages were further confirmed in other set of plasma samples by commercially available ELISA kits. **Conclusion:** Our result has highlighted several possible markers for lung adenocarcinoma and the proposed protein panels append further validation in a larger cohort for evaluating their potential use in clinical applications or development of therapeutics.

Keywords: biomarkers, plasma, multiple reaction monitoring (MRM), lung adenocarcinoma

P06.28 YBX1 Induces Oncogenicity via Release of Angiogenic Factors into the Tumour Microenvironment

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Introduction and Objectives: Epithelial-mesenchymal transition (EMT) describes a morphogenetic program which confers mesenchymal cell properties such as reduced cell-cell contact and increased cell migration and invasion, to epithelial cells. Here we utilize molecular biology, tumourigenic models and proteomic profiling to investigate the role of the pleiotropic transcription/splicing factor and RNA-binding protein nuclease-sensitive element-binding protein 1 (YBX1/YB-1) in increasing the oncogenic potential of epithelial cells. YBX1 is a member of the cold shock protein family of proteins, and a master transcription factor regulating an assortment of genes controlling cellular proliferation and development. **Methods:** Characterization of epithelial MDCK cells expressing YBX1 ($MDCK^{YBX1}$ cells) revealed the onset of EMT, with $MDCK^{YBX1}$ cells exhibiting cytosolic E-cadherin relocalization, increased cell scattering and expression of Snail1 and Twist, and anchorage-independent growth. Most significantly, $MDCK^{YBX1}$ cells established viable tumour xenografts, and immuno-histochemical staining indicated murine vascularization by CD31+ endothelial cells. **Results and Discussion:** Using extensive proteomic profiling and LFQ we analysed the cellular and tumour xenograft lysates of $MDCK^{YBX1}$ to explore underlying proteins that may confer tumourigenic properties in comparison with epithelial, and oncogeneic-H-Ras lysates. Further, to investigate regulation of the inter-cellular microenvironment, we profiled the total secretome (containing soluble and extracellular vesicles) of $MDCK^{YBX1}$ cells using comparative antibody arrays and relative proteomic profiling. YBX1 expression elevated release of secreted factors known to enhance angiogenesis (TGF-β, CSF-1, NGF, VGF, ADAM9 and ADAM17), compared to epithelial cells. Importantly, treatment with $MDCK^{YBX1}$ cell-derived secretome increased recipient 2F-2B endothelial cell motility. **Conclusion:** This study defines YBX1 as an oncogenic enhancer that can regulate tumour angiogenesis via differential expression of secreted

modulators into the extracellular microenvironment. Studies are underway to determine the molecules which induce a carcinogenic microenvironment to directly promote EMT and neovascularization, towards design of therapeutic strategies that may reverse EMT and limit cancer progression.

Keywords: cancer, microenvironment, epithelial-mesenchymal transition, secretome

P06.29 Detection of Aggressive Prostate Cancer Subforms by LC-MS and MALDI Imaging

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Introduction and Objectives: Prostate cancer (PCa) is today's third most common cancer diagnosed in Europe. One major problem in PCa management is the assessment of cancer progression risk and thereby the decision for a suitable therapy. There is an urgent need for markers which allow an early prognosis and personalized therapy for each patient. The aim of our work was to identify protein markers which help to predict tumor progression in PCa. Therefore, different state of the art techniques were combined to gain an overall reflection of the proteome of the aggressive and non-aggressive PCa to identify new targets for risk assessment in PCa management. **Methods:** The discovery phase was performed using microdissceted fresh frozen tissue from aggressive (n=8) and non-aggressive (n=6) tumors and the corresponding tumor free tissues. Samples were analyzed in technical triplicates and evaluated by the Progenesis software. Additionally, we performed MALDI imaging experiments on formalin fixed paraffin embedded (FFPE) material. A tissue microarray (TMA) was used comprising 215 patients (42 aggressive and 173 non-aggressive tumors). SCiLS lab software was applied to discriminate the different groups from each other. **Results and Discussion:** We revealed a number of interesting candidate proteins, like for example prostate stem cell antigen, which allow differentiation between aggressive and non-aggressive tumor forms. For the MALDI imaging approach a cross validation accuracy of 0.83 could be achieved by using the linear discriminant analysis and a training and test set. Distinction between tumor subtypes of the same grade and stage is challenging and further verification of promising candidates in body fluid is needed to confirm their suitability as reliable biomarker. **Conclusion:** The determination of aggressive and non-aggressive prostate cancer tumors was successfully achieved using LC-MS and MALDI imaging and further validation will be performed.

Keywords: aggressive tumor, biomarker, prostate cancer, MALDI imaging

P06.30 In-Depth Proteome Characterization of Intraductal Papillary Mucinous Neoplasm Pancreatic Cysts

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Introduction and Objectives: Intraductal papillary mucinous neoplasm (IPMN) is a benign tumor that grows within the pancreatic ducts characterized by the production of thick mucinous fluids by the tumor cells. IPMN is the most important precursor lesion for pancreatic cancer that is the fourth most common cause of cancer deaths. Pancreatic cyst fluids are commonly utilized in the medical field to diagnose the severity of IPMN. However, proteomic research of these has been limited so far, because of the complexity of samples, the multifocality of IPMN, and immaturity of proteomic techniques. **Methods:** We investigated in-depth proteome of pancreatic cyst fluids from IPMN by using our novel proteomic strategy combined with removal of contaminants, filter-aided sample preparation, peptide fractionation based on high-pH, and high-resolution Orbitrap LC-MS/MS. Signature proteins were quantified using targeted proteomic strategies such as parallel reaction monitoring and data-independent acquisition. **Results and Discussion:** In this study, approximately 2000 proteins were identified in soluble part of pancreatic cyst fluids. This figure is the largest among the worldwide pancreatic cyst fluids proteomic researches. Interestingly, our dataset contained several pancreatic tumor markers. In addition, targeted proteomic analysis of signature proteins was performed in small cohort. These results suggest that our proteomic platform is suitable to discover biomarker candidates that demonstrate a disparity in quantity among the fluids from IPMN and other pancreatic cyst lesions. **Conclusion:** Despite the difficulty of handing pancreatic cyst fluids, we identified a considerable amount of unique proteins in small volume of samples. To date, our study has the highest depth in comparison with previous pancreatic cyst fluids proteomic studies. In addition, signature proteins were verified using targeted proteomic strategies. Interestingly, gene ontology analysis demonstrates that significant numbers of proteins identified in soluble parts are related to immune responses and inflammatory responses that are relevant to pathogenesis of IPMN.

Keywords: Targeted proteomic analysis, biomarker, Intraductal Papillary Mucinous Neoplasm, Pancreatic cyst fluids

P06.31 Epithelial to Mesenchymal Transition Induced by SNAI1 Operates throughout Epigenetic Mechanisms

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Introduction and Objectives: Epithelial to mesenchymal transition (EMT) is a process that naturally occurs during embryogenesis and tissue repair, but it is also involved in cancer metastasis. EMT induces complex cellular and microenviromental changes, resulting in loss of epithelial phenotype and acquisition of mesenchymal properties, which in turn promotes invasive and migratory capabilities of cells. EMT can be triggered by several factors such as extracellular matrix components, growth factors and transcription factors.

With the aim to uncover new molecular mechanisms that EMT modulates at the protein level, we performed a proteomic analysis of the breast adenocarcinoma cell lineage MCF7 induced to EMT stimulated by SNAI1 overexpression. **Methods:** The study was based on a quantitative proteomic analysis of subcellular fractions enriched in cytosolic, nuclear or membrane proteins obtained by differential centrifugation. MCF7 cell extracts were mixed with MDA-MB231 breast cancer cell line labeled with heavy lysine by SILAC, which was used as a reference for quantitative analysis. Subsequent intact protein fractionation by SDS-PAGE was used to improve proteomic coverage. Data was collected using high-resolution mass spectrometry coupled to liquid chromatography (LC-MS/MS) in a Orbitrap Elite instrument. **Results and Discussion:** Through bioinformatics tools, we identified signaling pathways and sets of proteins altered during EMT induction that are directly related to important cellular processes, such as cell adhesion, cytoskeleton remodeling, cell cycle, endocytosis and oxidative phosphorylation. From a network analysis of interacting proteins, the epigenetic regulator histone deacelitlase I (HDAC1) was highlighted as an important node. Inhibition of HDAC1 induced EMT and increased the levels of SNAI1, thus revealing that HDAC1, which epigenetically regulates gene expression, represents an important point of control of EMT associated to SNA1. **Conclusion:** With this proteomic study, we uncover a direct link between EMT induction by SNAI1 and HDAC1, highlighting their importance as potential key regulators of cancer metastasis.

Keywords: Histone Deacetilase I (HDAC1), Epithelial-Mesenchymal Transition (EMT), Cancer Metastasis, Intact Protein Fractionation

P06.32 Large Scale Systematic Proteomic Quantification from Non-Metastasis to Metastasis Colorectal Cancer

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Introduction and Objectives: A systematic proteomic quantification of formalin-fixed, paraffin-embedded (FFPE) colorectal cancer tissues from stageⅠ to stageⅢC was performed in large scale. 1017 proteins were identified with 338 proteins in quantitative changes by label free method, while 341 proteins were quantified with significant expression changes among 6294 proteins by iTRAQ method. We found that proteins related to migration expression increased and those for binding and adherent decreased during the colorectal cancer development according to the gene ontology (GO) annotation and ingenuity pathway analysis (IPA). The integrin alpha 5 (ITA5) in integrin family was focused, which was consistent with the metastasis related pathway. The expression level of ITA5 decreased in metastasis tissues and the result has been further verified by Western blotting. Another two cell migration related proteins vitronectin (VTN) and actin-related protein (ARP3) were also proved to be up-regulated by both mass spectrometry (MS) based quantification results and Western blotting. Up to now, our result shows one of the largest dataset in colorectal cancer proteomics research. Our strategy reveals a disease driven omics-pattern for the metastasis colorectal cancer. **Methods:** not applicable
Results and Discussion: not applicable
Conclusion: not applicable

Keywords: proteomic, quantification, colorectal cancer, large scale

P06.33 HSP60-Silencing Disrupts Mitochondrial Proteostasis and Promotes Tumor Progression

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Introduction and Objectives: The molecular chaperone heat shock protein 60 (HSP60) play a crucial role for the maintenance of mitochondrial integrity and protein homeostasis. Our recent studies showed that HSP60 was up-regulated under oxidative stress, suggesting that the up-regulation of HSP60 protects proteins from ROS damages. Identification of HSP60-mediated protein interaction network is important to understand functions and action mechanisms of HSP60 in normal and stressed cells. **Methods:** In the present study, we have established multiple stable knockdown cell lines in which HSP60 expression was knockdown by HSP60-directed shRNA. TMT-labeling based quantitative proteomics was carried out to identify the differentially expressed proteins between HSP60 knockdown and control cells. Immunoprecipitation-mass spectrometry was employed to identify binding partners of HSP60. **Results and Discussion:** Down-regulation of HSP60 expression leads to multifaceted changes in cell morphology, cellular ROS level, and cell metabolism. Quantitative proteomic analysis show that components of respiratory complex I are substrates of HSP60 which are down-regulated in HSP-KN cells to enhance ROS production and activate AMPK pathway. Activation of AMPK associated signaling transduction reprograms metabolic pathways leading to enhanced glycolysis. The high level of ROS drives HSP60-KN cells to undergo EMT. In xenograft experiments, HSP60-knockdown cells grew faster in nude mice than do control cells. These results propose that HSP60 silencing plays a crucial role in promoting tumor progression via ROS-AMPK pathway. **Conclusion:** HSP60 knockdown-mediated disruption of mitochondrial proteostasis and excessive ROS generation drive metabolic reprogramming and enable cells to undergo EMT process, suggesting that HSP60 is an important target for therapeutic intervention in cancer.

Keywords: HSP60, mitochondrial proteostasis, ROS, EMT process

P06.34 Protein-Chain Quantitation by Targeted MS for Exploration of the Cancer-Thrombosis Connection

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Introduction and Objectives: Venous Thrombosis (VT) occurs in one per thousand per year ending with death of 12%–20%. It is also a major cause of mortality in cancer patients. Current methods for evaluating VT are based on ELISA and activity assays of various coagulation-related proteins. Although they provide high sensitivity and accuracy, these methods require a high volume of plasma and are costly. Here we report the preliminary results from a multiplexed targeted proteomics (LC/MRM-MS) evaluation of the concentrations of 31 coagulation-related proteins in diseased and control subjects. **Methods:** In a blinded study we analyzed plasma samples from 25 healthy controls, 25 cancer patients, and 25 thrombosis-and-cancer patients. The samples were obtained from a previous well-characterized and analyzed cohort where the concentration of 15 of the 31 targeted proteins where evaluated using ELISA or activity assays. Using stable-labeled internal standard (SIS) peptides, we measured the concentration level of 2 to 3 proteotypic peptides per protein, with a total of 79 peptides representing all 31 proteins. Few of the targeted proteins have multiple chains or an

activation peptide. We developed a multiplexed LC/MRM-MS method, and evaluated the peptide concentrations using single point measurements. **Results and Discussion:** Our unsupervised cluster analysis was able to group 17 out of 25 thrombosis-and-cancer patients. After sharing the patient meta-data, different outliers were explainable by the medications the patients were taking. In few cases, we were able to evaluate the concentrations of two protein chains (e.g., Coagulation factor V, or a chain and an activation peptide as in Vitamin K-Dependant Protein C). Interestingly, in these cases ELISA showed good correlation with the peptide concentration from one chain, but poor correlation with the other chain or the activation peptide. **Conclusion:** Measuring the concentration of activation peptides or surrogate peptides for specific protein chains allows better insight into the biological differences between healthy and control subjects.

Keywords: multiple reaction monitoring (MRM), Targeted proteomics, protein chains, thrombosis

P06.35 Characterization of the Interactome of RSK Isoforms to Decipher Their Roles in Cancer Cells

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Introduction and Objectives: The Ras/MAPK (mitogen-activated protein kinase) pathway plays a key role in transducing extracellular signals to intracellular targets involved in cell growth, survival and proliferation. Inappropriate regulation of this pathway leads to a variety of diseases, including cancer and diabetes. This pathway regulates the activation of the RSK (p90 ribosomal S6 kinase) family of protein kinases, which is composed of four isoforms (RSK1-4). The RSK kinases are differently expressed amongst cell types and organs, suggesting isoform-specific biological functions. While a number of phosphorylation substrates have been identified for RSK1 and RSK2, the exact functions of RSK3 and RSK4 remain elusive. In an attempt to characterize the isoform-specific functions of the RSK, we have used complementary proteomics-based approaches to determine the specific interacting partners of RSK1-4. The general objective of this project is to characterize these novel interactions and determine their relevance in RSK-dependent signal transduction, especially as it relates to cancer. **Methods:** For each RSK isoform, we have performed affinity-based purifications of binding partners and analyzed their identity using liquid chromatography-based mass spectrometry (LC-MS). In addition, we have used a proximity-based labeling approach to identify potential binding partners in cells. Our results were combined to validate potential binding partners, which were then validated using more classical approaches, such as co-immunoprecipitation. **Results and Discussion:** Both proteomics approaches have resulted in the identification of several potential binding partners for the RSK isoforms. Whereas some proteins appeared to bind all RSK isoforms, most specifically bound to certain isoforms. Notably, several proteins were identified using both proteomics approaches, suggesting that they are bone fide binding partners. **Conclusion:** Using two different and complementary proteomics approaches, we have identified cellular partners for each RSK isoform. This step is crucial to understand and identify the role of the RSK protein kinases, particularly with respect to their described roles in cancer.

Keywords: Cancer cell signalling, Protein kinase, mitogen-activated protein kinase (MAPK), 90 kDa ribosomal S6 kinase (RSK)

P06.36 Verification of Biomarkers in Uterine Aspirates by LC-PRM to Improve Diagnosis in Endometrial Cancer

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Introduction and Objectives: Endometrial cancer is the most common gynecological malignancy among women, accounting for approximately 74,000 deaths annually worldwide. Uterine aspirates are biofluids in direct contact with the endometrium that are collected by minimally invasive procedures. We evaluated by targeted MS analysis in parallel reaction monitoring (PRM) mode the potential of using uterine aspirates as a source of biomarkers for endometrial cancer. **Methods:** A list of 506 proteins was selected from the literature and refined based on their biological relevance and experimental detection by shotgun LC-MS/MS in four uterine aspirates. Fifty four candidates were quantified in the soluble and solid fractions of uterine aspirates and their paired endometrial tissues, coming from 7 endometrial cancer patients and 7 controls by LC-SRM. The most promising candidates were further quantified in the supernatants of uterine aspirates of an independent cohort of 20 controls and 20 patients with a quadrupole-orbitrap mass spectrometer operated in PRM mode. **Results and Discussion:** From 54 proteins measured by LC-SRM, 19, 21 and 20 candidates were detected as differentially expressed ($P < 0.01$ and a fold change ≥ 2) between tumoral and control in supernatant and solid fraction of uterine aspirates and endometrial tissues, respectively. The supernatant fraction was selected for further verification phases as it provided the most robust and reproducible results. These results were confirmed by analyzing supernatant samples of 20 controls and 20 endometrial cancer patients by LC-PRM. The systematic quantification analysis of 51 proteins was carried out, and the results showed a significant improvement in selectivity and sensibility compared to previous results obtained by LC-SRM. **Conclusion:** Uterine fluids are a promising source of biomarkers for an earlier and minimally invasive diagnosis of gynecological diseases such as endometrial cancer. The quantification by LC-PRM acquisition dramatically increase the robustness and the confidence of the peptides quantification.

Keywords: parallel reaction monitoring, Endometrial cancer, biomarkers, Uterine aspirates

P06.37 TP53-Mediated Regulation of Exosome Proteins and microRNA

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Introduction and Objectives: Tumor cells secret the exosomes, which are membranous vesicles 30-100 nm size with the plasma membrane, impacting on the tumor microenvironment. However, little is known about the role of mutant TP53-mediated regulation of exosomes including proteins and microRNA in colon cancer. We investigated the different expression profiling of proteins and microRNA in exosomes in the TP53 mutant, TP53 knock-out and wild-type colon cancer cells. **Methods:** Exosomes were isolated from the culture supernatant of cells by a new nanomaterial in the TP53 mutant, TP53 knock-out and wild-type HCT116 cells. Proteins composition profiling of exosomes were analyzed using iTRAQ-LC-MS/MS, on the other hand, microRNA expression of exosomes were identified using Agilent miRNA microarray.

Results and Discussion: For proteomics analysis, we found that there are 144 differentially expressed proteins in exosome between TP53 wild-type and TP53 mutant HCT116 cells, 480 differential proteins compared with TP53 wild-type and TP53 knock-out HCT116 cells. Most of the differential proteins are involved in metabolism, cellular processes, immune processes, apoptosis, stimulus response, reproduction and cell adhesion. Furthermore, several candidate proteins derived from exosomes including Atg13、CAB39L、HSP90, were validated by western blot assay in TP53 wild-type,TP53 mutant and TP53 knock-out HCT116 cells. For microRNA analysis, we found that there are 139 significantly different expression profile of microRNA in exosome, which identified 103 up- and 35 down-regulated microRNA more than 2 fold change, compared with TP53 wild-type and TP53 mutant HCT116 cells, 208 different expression profile of microRNA including 142 up- and 66 down-regulated microRNA between TP53 wild-type and TP53 knock-out HCT116 cells. **Conclusion:** TP53 mutation and knock-out may impact on multiple proteins and microRNA involved in different signaling pathways, and further regulate to load into sorting exosomes.

Keywords: exosome proteome, TP53, colon cancer, iTRAQ-LC-MS/MS

P06.38 Quantification of Mutant SPOP Proteins in Prostate Cancer Using SRM-MS

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Introduction and Objectives: Speckle-type POZ protein (SPOP) is an E3 ubiquitin ligase adaptor protein that functions as a potential tumor suppressor, and SPOP mutations have been identified in ~10% of human prostate cancers. However, it remains unclear if mutant SPOP proteins can be utilized as biomarkers for diagnosis, prognosis or targeted therapy of prostate cancer. To address this issue, selected reaction monitoring (SRM) mass spectrometry assays have been developed for quantifying mutant SPOP proteins. **Methods:** SRM assays for wild-type SPOP protein and 11 prostate cancer-derived mutations were developed. The presence of multiple lysine residues in the mutation regions precludes the use of trypsin for enzymatic digestion. Arg-C was selected instead due to its superior performance in generating mutation site(s) containing SPOP peptides more suitable for SRM analysis comparing to other proteases (e.g., Asp-N). **Results and Discussion:** Although in general the resulting Arg-C peptides are longer and more hydrophobic than typical tryptic peptides, all the SRM assays showed a linear dynamic range of more than two orders of magnitude. The limit of quantification for the mutation site(s) containing peptides range from 10 to 100 fmol/mg of total protein in the cell lysate. Applying these SRM assays to analyze 293T cells with and without expression of three most frequent SPOP mutations in prostate cancer (Y87N, F102C or F133V) led to confident detection of all three SPOP mutations in corresponding positive cell lines but not in the negative cell lines. Expression of F133V mutation and wild-type SPOP was at much lower levels comparing to that of F102C and Y87N mutations, which agrees with RT-PCR results. It is unknown if this is related to activity of the SPOP protein. **Conclusion:** SRM enables multiplexed, isoform-specific detection of mutant SPOP proteins in cell lysates, which holds great potential in biomarker development for prostate cancer.

Keywords: selected reaction monitoring, quantitation, SPOP, mutation

P06.39 SRM Verification of Circulating Ovarian Cancer Biomarkers at Early Detection Research Network (EDRN)

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Introduction and Objectives: Developing blood-based tests is extremely appealing for early detection of ovarian cancer in which the majority of women are diagnosed at a late stage when frontline therapy is less effective. To date there are no FDA-approved biomarkers for ovarian cancer screening. To address this limitation, 165 proteins previously identified as candidate ovarian cancer biomarkers at participating EDRN sites were evaluated for their ability to discriminate serous ovarian cancer patients from patients with serous benign ovarian disease. **Methods:** Both in silico approaches and quantitative tandem mass spectrometry analyses of pooled plasma from serous ovarian cancer cases and serous benign ovarian disease controls were used to prioritize candidate biomarkers and initially triage candidates by differential expression, leading to 61 proteins that have sufficient evidence to warrant further evaluation. **Results and Discussion:** 32 of the 61 proteins were evaluated using selected reaction monitoring mass spectrometry (SRM-MS) assays. The other 29 proteins could only be detected using high-pressure high-resolution separations with intelligent selection and multiplexing (PRISM)-SRM, due to the required analytical sensitivity. Because of its low prevalence, early detection of ovarian cancer requires very high specificity (≥99.6%), achievable when a blood test at 98% specificity is followed by trans-vaginal ultrasound. Therefore, sensitivity was estimated at 98% specificity for all candidates by quantifying candidates in serum from serous ovarian cancer cases (n=20) and serous benign ovarian disease controls (n=20). 11 best performing candidates were further analyzed by PRISM-SRM in another 30X30 sample set. WFDC2, K1C19, MUC16, A2GL, CBPA4, and SPON1 proteins were identified as potential members of a panel of ovarian cancer biomarkers. **Conclusion:** In summary, this multi-pronged, antibody-independent approach enabled 61 candidates to be rapidly triaged to six candidates that warrant further evaluation in longitudinal pre-diagnostic plasma or sera from cases detected in screening studies and matched controls for the early detection of ovarian cancer.

Keywords: Ovarian cancer, EDRN, SRM, circulating biomarker

P06.40 Elucidating Metastatic Changes during Ovarian Cancer Progression by LCM Proteomics

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Introduction and Objectives: Ovarian cancer is the deadliest gynecological cancer worldwide with a mortality rate of more than 60% within 5 years. This poor outcome results primarily from diagnosis at a late-stage when the primary tumor has already disseminated throughout the peritoneal cavity. Understanding the complex changes that occur during disease progression in the tumor and the surrounding microenvironment are consequently of enormous clinical value and may help to identify novel treatment modalities. **Methods:** not applicable

Results and Discussion: Our study encompasses the quantification of in total >7500 proteins across normal, pre-invasive, primary and metastatic tumor and stroma sites isolated from an estimated range of less than 5.000 to 50.000 microdissected cells. We have identified novel stromal proteins involved in the metastasis to the omentum, the primary site of OvCa metastasis. Interestingly, this novel stroma-derived protein signature is highly conserved across patients. Ongoing functional follow-up experiments in-vitro highlight the importance of the selected candidate proteins for ovarian cancer metastasis. **Conclusion:** Discovery based proteomics applied to FFPE biobank specimens has the great potential to pinpoint compartment-resolved proteomic changes occuring during disease progression . Given the excellent availability of FFPE specimens, this approach could be applied to a variety of clinical questions in future.

Keyword: Clinical proteomics, cancer progression, FFPE

P06.41 Exosome Omics Study for Molecular Backgrounds of Drug Response towards Personalized Medicine

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Introduction and Objectives: Exosomes are high-priority in the biomarker studies, because exosomes are stable in blood stream, and play functionally important roles in carcinogenesis and cancer development. Exosomes have been investigated in cancer research, and their clinical utilities were suggested based on the expression and function studies. However, the clinical significances of exosomes were not fully established yet, and further investigations are required for clinical applications. In this study, we aimed to identify proteins and miRNAs in the exosomes, which correspond to drug sensitivity and treatments. **Methods:** Using tissue cultured cell lines, the response to treatments with TKIs and conventional chemotherapy drugs was examined, and the cell lines were grouped according to their IC50 values. The exosomes were recovered from the conditioned medium, and extracted by ultra-centrifugation. The proteins in the recovered exosomes were separated by SDS-PAGE, and subjected to LC-MS/MS (Orbitrap XL). Pathway analysis was performed using IPA software. The expression level of miRNAs was globally monitored by miRNA microarray experiments (3D-Gene, Human miRNA Oligo chip, TORAY, Japan). **Results and Discussion:** We examined the response of sarcoma cells to drug treatments. The cell lines exhibited significantly different response to the treatments, and the degree of treatments was evaluated by IC50 values. On the basis of response to treatments with typical TKIs, we grouped the cell lines of identical histological origin into two groups. We detected miRNAs and proteins with unique expression pattern to drug treatment and sensitivity. **Conclusion:** We hypothesize that the integrative global expression studies of miRNAs and proteins in exosomes are a novel intriguing approach to biomarker development. We obtained interesting data by this approach, and our hypothesis is worth discussing in the presence of experimental data.

Keyword: exosome synovialsarcoma pazopanib

P06.42 Drug-Centric Proteomics for Molecular Backgrounds of Drug Response towards Personalized Medicine

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Introduction and Objectives: To identify the biomarker candidates to stratify the patients according to drug response, we employed "drug-centric proteomics", and focused on the proteins of drug targets. We examined tyrosine kinase inhibitors (TKIs), because TKIs were firstly developed and applied to cancers such as leukemia, lung adenocarcinoma, and gastrointestinal stromal tumor, and various TKIs have been widely utilized in many types of cancers. **Methods:** Using tissue cultured cell lines, the response to treatments with TKIs was examined, and the cell lines were grouped according to their IC50 values. The effects of TKIs treatments on the expression level as well as auto-phosphorylation status of all membrane type tyrosine kinases were examined by western blotting and antibody arrays, respectively. Using BioPlex assay system, the level of 71 proteins in the conditioned medium was measured before and after the treatments. The level of mRNA was also examined before and after the treatments using DNA microarray experiments. **Results and Discussion:** The examined cell lines exhibited significantly different response to the treatments, and the degree of treatments was evaluated by IC50 values. For the typical TKIs, we grouped the cell lines into two groups according to their sensitivity to the treatments. The comparative studies on these two cell line groups revealed distinct molecular backgrounds. It was noteworthy that certain membrane proteins, which were considered as molecular targeting proteins, were released from the cells in response to treatments. The unique profiles of membrane type TKs and mRNAs were obtained and integrated to interpret the biological significances of the proteins released from the cells. **Conclusion:** We hypothesize that the drug target proteins, i.e. drug-centric proteomics, is an effective way to develop biomarkers for personalized medicine. We obtained intriguing data by this approach, and our hypothesis is worth discussing in the presence of experimental data.

Keyword: Sarcoma, Tyrosine kinase inhibitor, Biomarker, Personalized medicine

P06.43 Discover the Interactome of the Potent Oral Cancer Oncoproteins by Quantitative Proteomics Analysis

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Introduction and Objectives: Oral squamous cell carcinoma (OSCC) is the most common head-and-neck cancer with increased mortality for years. Despite recent improvement in surgical, radiotherapy, and chemotherapy treatment, the long-term survival rate of OSCC patients with pN-positive (pN+) was still poor (~40%). Recently, we found that overexpression of OC-80L protein in tumor cells of nodal metastatic OSCC tissue specimens by quantitative proteomics and immunohistochemical assays, which is associated with cervical metastasis and poorer prognosis of OSCC patients. And, the OSCC cells with OC-80L overexpression showed significantly increased migration and invasion abilities. However, the molecular mechanisms of OC-80L in the regulation of migration and invasive processes are unclear. Here, we conduct the quantitative proteomics analyses to uncover the interaction proteins of OC-80L for better understanding the network of OC-80L involved in cell movement organization as well as tumor progression. **Methods:** By using immunoprecipitation and quantitative proteomics

analyses, the aberrant protein expression and interaction in cells with or without OC-80L expression are explored. The potential candidates regulated by OC-80L are verified by Ab-based methods as well as cellular activity assays. **Results and Discussion:** We have successfully captured Flag-tagged OC-80L protein from total protein extracts, and determined its associated protein complex by SILAC-assisted quantitative proteomics. More than 2200 proteins were quantified. About thirty of them with OC-80L/control ratios higher than two were selected as potential interacting candidates of OC-80L. Those selected protein candidates are further verified by immunofluorescence, Western blotting, as well as cellular activity assays. **Conclusion:** OC-80L is a protein with a role in cytoskeletal reorganization and can be phosphorylated by various kinases, e.g. Erk1/2 and CKII. Systematic quantitative proteomics analyses provide us a great opportunity to specifically discover the OC-80L protein complex for further exploring their roles in the regulations of cytoskeletal reorganization and cellular malignancy.

Keywords: Oral Cancer, Interactome

P06.44 A Multiplexed Cytokeratin MRM Panel for the Classification of Lung Cancer Patient Pleural Effusions

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Introduction and Objectives: Lung cancer is the most common cancer in men and the fourth most frequent cancer in women. It is classified into small-cell lung cancer (SCLC) and non-small cell lung cancer further subtyped into adenocarcinoma (ADC) and squamous cell carcinoma (SCC). Additionally, less frequent subtypes and a series of metastatic cancers exist. Proper classification is critical for correct treatment choice, usually involving highly invasive procedures and histopathological analysis. In immuno-histopathology, cytokeratins have long been used clinically as markers for cancer diagnosis, prognosis and therapy monitoring, however, often with low analytical and diagnostic specificity. We propose the minimally invasive analysis of pleural effusion fluid that accumulates due to lung cancer and other ailments for these clinically proven biomarkers with the addition of high analytical specificity provided by targeted mass spectrometry analysis of specific cytokeratin peptides. The objective was to assess the classification ability of a multiplexed cytokeratin panel on pleural effusions from lung cancer patients and those suffering from benign disease. **Methods:** Cytokeratin levels were measured by multiple reaction monitoring (MRM) with stable-isotope-labeled standard peptides for increased assay specificity and sensitivity. Pleural effusions from 124 patients suffering from lung cancer (SCLC, ADC, SCC), pleural mesothelioma, metastatic cancers, bacterial infection, and benign causes were screened for 37 proteins using 148 peptides. **Results and Discussion:** A finalized panel of 16 cytokeratins and two other proteins was used to build a classifier that could successfully categorize pleural effusions with bacterial infection (AUC 0.90), those with lung cancer from the rest with an AUC of 0.80, and all cancer patients from benign-causes (AUC 0.74). Furthermore, we could classify the ADC subtype patients with an AUC of 0.71 from all other groups. **Conclusion:** We show that a multiplexed cytokeratin MRM panel can classify patient pleural effusions as a less invasive procedure and promises usefulness in clinical practice of lung cancer.

Keyword: lung cancer, pleural effusion, MRM, cytokeratin

P06.45 Quantitative Analysis of AKT/mTOR Pathway Using Immunoprecipitation and Targeted Mass Spectrometry

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Introduction and Objectives: The PI3K/AKT/mTOR pathway plays a central role in tumor progression and anti-cancer drug resistance. Quantitative measurement of AKT/mTOR pathway protein expression and post-translational modification (PTM) status is necessary for classifying disease states, monitoring cancer progression and determining treatment response. Immunoprecipitation mass spectrometry (IP-MS) is increasingly used to enrich, detect and quantify low abundant proteins and PTMs. Here, we used an improved IP-MS workflow to enrich single or multiple AKT/mTOR pathway proteins simultaneously for targeted MS quantitation. **Methods:** Serum starved A549 and HCT116 cells were stimulated with EGF or IGF-1 to activate AKT/mTOR pathway signaling. Pathway targets were enriched via an improved IP workflow using Pierce Protein A/G and Streptavidin magnetic beads and eluates were processed using in-solution digestion for LC-MS analysis. Targeted SRM MS assays were developed for quantitation of AKT/mTOR pathway target peptides including EGFR, IR, IGF1R, IRS1, AKT2, AKT1, PTEN, PIK3CA, PIK3R1, mTOR and p70S6K. Multiple targets were also immunoprecipitated simultaneously and quantitated by a multiplexed targeted SRM assay. **Results and Discussion:** In this study, we validated numerous antibodies for IP-MS with both Pierce Protein A/G and Streptavidin magnetic beads. Enrichment of total and phosphorylated forms of EGFR, AKT1, AKT2, mTOR, GSK3beta, p70S6K and PTEN resulted in quantitation of low to sub nanogram target levels in two cell lysates by targeted MS. We also combined target antibodies to enrich multiple AKT/mTOR pathway protein targets for a single IP-MS analysis. This multiplex enrichment and targeted assay can be used for simultaneous detection and quantitation of AKT/mTOR pathway proteins in other cancer cell lines or tissue samples. **Conclusion:** Improved IP-MS workflow enables multiplexed enrichment, detection, and quantitation of PI3K/AKT/mTOR pathway proteins and PTMs at sub to low ng/mL concentrations.

Keywords: immunoprecipitation, pathway, Multiplexed

P06.46 Single-Shot Proteomics Profiling and Quantification of Human Prostate Cancer Cells

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Introduction and Objectives: Rapid and highly sensitive single-shot proteomics, which enables the identification of thousands of proteins in a few hours without any prefractionation, holds considerable potential to radically simplify proteomic studies of very complex samples. The objective of this study is to develop a rapid, highly sensitive, and robust single-shot proteomics method and apply it to quantitatively analyze the proteomes of three widely used human prostate cancer cell lines with different metastatic potentials. **Methods:** We systematically optimized the proteomic workflow through evaluating ten sets of conditions: 1) lengths of LC columns and gradients, 2) time windows for dynamic exclusion, 3) LC flow rates, 4) mass windows, 5) monoisotopic precursor selection, 6) intensity thresholds for precursor ion fragmentation, 7) numbers of data-dependent MS/MS, 8) loading amounts, 9) methods for protein digestion, and 10) LC gradient times with the optimized settings. Subsequently, the single-shot proteomics was coupled with label-free quantitation to compare the proteomes of LNCaP, DU145, and PC3 cells. **Results and Discussion:** Our single-shot proteomics method enabled

the identification of about 5,000 proteins from a single 200-min LC-MS/MS analysis of 2 μg LNCaP lysates. A label-free quantitative single-shot proteomics analysis of LNCaP, DU145, and PC3 cells profiled over 6,500 protein groups, among which 738 were identified as differentially expressed. Bioinformatic analysis revealed cell invasion and migration as the most differentially activated processes, providing a potential molecular basis for the different metastatic potentials of the three cell lines. **Conclusion:** In summary, we developed a novel single-shot proteomics method that enables rapid and deep proteomic profiling and robust label-free quantification, and applied the method to generate a quantitative proteomic map of over 6,500 proteins in human prostate cancer LNCaP, DU145, and PC3 cells. Our single-shot proteomics method and comprehensive quantitative proteome database for human prostate cancer are expected to be valuable for future proteomics and prostate cancer studies.

Keywords: prostate cancer, Single-shot proteomics, label-free quantitation, metastasis

P06.47 Proteomic Profile of EGF-Stimulated Ovarian Cancer Cells during Epithelial to Mesenchymal Transition

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Introduction and Objectives: Ovarian cancer (OvCa) is the sixth most common cancer in women and the most lethal among gynecological malignancies. The search for biomarkers correlated with OvCa has become essential to early detection and mortality decrease. Based on this, the epithelial to mesenchymal transition (EMT), an initial step of metastasis, has become an interesting process to elucidate biomarkers correlated with aggressive tumor phenotype. Although different stimuli, such as cytokines, growth factors and miRNAs have been suggested as inducers of EMT, the detailed mechanisms involving epidermal growth factor (EGF) remain unclear. In this study, we evaluated if and how EGF induces EMT in ovarian cancer cell line Caov-3. **Methods:** Caov-3 cells were stimulated with EGF and EMT markers evaluated by phase contrast microscopy, qPCR, western blotting and phospho kinase array. Quantitative proteomic analysis using SILAC was performed by mixing Caov-3 and Caov-3/EGF cells extracts with Caov-3 cells labeled with heavy lysine, which was used as a reference for quantitative analysis. Subcellular fractionation enriched with cytosolic, nuclear or membrane proteins fractions was obtained by differential centrifugation. Data was collected using high-resolution mass spectrometry coupled to liquid chromatography (LC-MS/MS) in a Orbitrap Elite instrument. Regulated proteins were validated using targeted proteomic pipeline based on Skyline software. **Results and Discussion:** EGF-induced EMT was confirmed by morphological changes in Caov-3 cells, downregulation of E-cadherin and upregulation of fibronectin and N-cadherin. Increased phosphorylation of ribosomal protein S6, Erk1/2 protein and Src kinase activity was observed during EGF-induced EMT. Proteomic analysis showed clear regulation of ribosomal proteins (RPS20, RPL7, RPL17, RPL24, RPL37A), elongation factors (EEF1D, EFTUD2), cell adhesion molecules (ITGA2, ICAM1) and mitochondrial components (ETFB, ETFA, COX5A). **Conclusion:** Our findings suggest modifications of different pathways involved in EGF-induced EMT OvCa as revealed by a range of altered relevant proteins, which can be used as potential biomarkers for prognosis or therapeutic target.

Keywords: epithelial to mesenchymal transition (EMT), epidermal growth factor (EGF), Proteomic profile, Ovarian cancer

P06.48 Proteomic Identification of Malignant Pleural Mesothelioma-Related Molecules

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Introduction and Objectives: Malignant pulmonary mesothelioma (MPM) is a form of tumor that develops from the protective lining that covers the lung and usually caused by exposure to asbestos. MPM is an aggressive and treatment-resistant tumor and steadily increasing. Thus MPM is considered to be an alarming worldwide health problem. Long-term exposure to asbestos leads to accumulation of alterations of NF2, p16 and p14, however, the underlying mechanisms of disease progression need to be clarified for identification of novel molecular targets. **Methods:** We employed proteomic technologies to obtain protein expression profiles of human MPM tissues. **Results and Discussion:** We identified more than 1000 proteins expressing in MPM tissues and found a dozen of upregulated proteins in MPM compared with normal mesothelial tissues. We further conducted functional analyses of candidate MPM-related molecules and found the potential targets of MPM. **Conclusion:** Our findings demonstrate that the combined proteomic technology is an efficient strategy for global proteomic analysis to discover novel biomarkers.

Keywords: expression profiling, malignant pleural mesothelioma, biomarker

P06.49 Interactome-Wide Analysis Reveals the Actin-Regulating Function of TNFAIP2 in NPC Cells

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Introduction and Objectives: TNFAIP2 (TNF-α-inducible protein 2) was originally identified as an angiogenic and proinflammatory factor, and its expression can be induced by proinflammatory cytokines, e.g. tumor necrosis factor (TNF)-α. TNFAIP2 has also been suggested to play a role in antivirus responses and notably, in formation of a distinct type of actin-based membrane protrusions, which allow direct physical communications between remote cells. However, the molecular basis for the function of TNFAIP2 remains largely unknown. Recently, TNFAIP2 is shown to be overexpressed in nasopharyngeal carcinoma (NPC) and this overexpression is significantly correlated with poor survival of patients. We further revealed that TNFAIP2 associates with actin filaments, particularly at the cell membrane, and induces actin remodeling, cell motility and cell invasion in NPC cells. Since NPC is a highly metastatic cancer common in Taiwan, elucidating the way by which TNFAIP2 modulates the actin-dependent cellular processes will accelerate to understand the role of TNFAIP2 in cancer progression. **Methods:** To explore the function of TNFAIP2 in the view of protein-protein interactions, we performed co-immunoprecipitation (co-IP) coupled with SILAC-based quantitative proteomic analysis to systematically identify the interacting partners of TNFAIP2 in NPC cells. **Results and Discussion:** After data normalization, 1332 proteins were quantified in co-IP experiments. By using the median+1SD as a cutoff value, 120 candidates were identified as potential TNFAIP2-interacting proteins. Gene ontological analysis highlighted the biological processes, in which these 120 proteins are mainly involved, are cytoskeleton arrangement, oxidation-related, and RNA-related processes. Among the 120, 14 proteins with reported roles in actin-related functions were selected for the further validation. The interaction of TNFAIP2 with these 14 proteins was confirmed in individual. **Conclusion:** This study revealed for the first time the interacting proteins

of TNFAIP2. Characterization of the relationships between these protein-protein interactions will advance the understanding of the function of TNFAIP2 in NPC pathogenesis.

Keywords: TNFAIP2, Interactome, nasopharyngeal carcinoma, actin

P06.50 Profiling of Autoantibodies for the Stratification of Prostate Cancer

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Introduction and Objectives: One of the main challenges in Prostate cancer (PCA) research remains the lack of disease specific and outcome-related biomarkers. The finding that the immune system produces autoantibodies against tumor-associated antigens suggests this as a possible source of biomarkers. Counting on two types of high-throughput array platforms, and a collection of several thousands human antigens produced within the Human Protein Atlas, the aim of our study was to explore the autoantibodies repertoire in PCA patients to identify antigens that could serve new prognostic autoimmune biomarkers. **Methods:** A hypothesis-free discovery phase was performed on planar protein microarrays. Eighty plasma and serum samples - Low and High-stage PCAs - were tested for reactivity towards 3,700 fragments from human proteins antigens (approximately 100 aa length) representing 3,300 unique proteins. The second phase was conducted with 232 antigens on suspension bead array (SBA), representing 190 unique proteins selected by discovery phase and supplemented by candidates found in the literature. One-thousand-twenty plasma and serum samples from 3 PCA study-sets were tested for differential immunoreactivity levels in High versus Low T-stages and Gleason scores and Cases versus Controls. Reactivity frequencies in the two groups were compared by Fisher´s exact test. Targets with P-value<0.05 were selected and further ranked by reactivity difference and signal intensities difference in the two groups. **Results and Discussion:** From the untargeted discovery phase, differences in immunoreactivity were significant for 167 out of 3,700 antigens. These antigens plus 65 additional were selected for validation on SBA. The most substantial difference was found for High versus Low T-stage comparison, suggesting to select 30 candidate biomarkers (1% of discovery) for further validations. **Conclusion:** Our findings demonstrate that autoantibodies could represent a precious source of biomarkers for PCA patient stratification. Moreover, this study is another example of how combining independent high-throughput immunoassay platforms can improve the stringency for proposing biomarkers for validation.

Keyword: Affinity proteomics, Prostate cancer, Autoimmunity, Biomarkers

P06.51 Secretome of Naïve Senescent Mesenchymal Stromal Cell Promotes Senescence in ARH-77 Myeloma Cells

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Introduction and Objectives: Mesenchymal stromal cells (MSCs) differentiate in mesodermal derivatives, support hematopoiesis, contribute to the homeostatic maintenance of many organs and tissues and modulate inflammatory response. All these activities are accomplished mainly by secretion of a plethora of cytokines and growth factors. MSC secretome is profoundly affected by senescence phenomena. Indeed, several studies have demonstrated that senescent cells secrete dozens of molecules, for which the term 'senescence-associated secretory phenotype' (SASP) has been proposed. SASP contributes in preventing cancer progression. Cancer cells, however, can misuse these secreted factors for their growth and survival. **Methods:** We decided to analyze the effect of the MSC senescent secretome on the biology on ARH-77 myeloma cancer cells. We collected conditioned media from replicative senescent MSCs obtained from human bone marrow and adipose tissue. We also obtained secretomes from MSCs treated with genotoxic stressors (acute senescence). In another set of experiments, we grew replicative or acute senescent MSCs with ARH-77 before collecting their conditioned media. We performed LC-MS proteome analysis to identify factors that are presents in SASP of replicative and acute senescent MSCs. **Results and Discussion:** Of interest, the several types of naïve secretomes induced, with a different extent, senescence of ARH-77 cells with an increase of persistent DNA damage foci in the cancer cell nuclei. The secretomes of cancer-primed senescent MSCs showed a reduced ability in inducing senescence or apoptosis of ARH-77 cells. We aim to identify how cancer-priming affects secretome composition. In senescent naïve MSCs we identified about 400 proteins. Of those, more than 20% were senescent-specific since were not present in young MSCs. Priming with ARH-77 partially modify the composition of senescent secretome. **Conclusion:** For the first time, we evidenced that naïve senescent MSCs may have different effect on tumor progression compared with cancer-primed MSCs, which have been suggested to promote cancer growth.

Keywords: cancer, secretome, proteome, senescence

P06.52 New Workflows Combine Albumin Depletion and On-Bead Digestion, for Quantitative Serum Proteomics

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Introduction and Objectives: Serum proteomics can be challenging for two reasons: 1) the presence of highly abundant proteins and 2) a proteolytic resistant sample type. We consider the efficiencies gained by an optimized workflow of albumin depletion, low abundance enrichment, and optimized on-bead digestions, to identify and quantify proteins from sera using iTRAQ labeling. We demonstrate these new workflows support quantitative investigations and highlight several proteins that were observed to be up and down regulated from lung and breast cancer sera. **Methods:** The workflow follows the basic AlbuVoid sample prep method. In brief, the serum is prepared by adding a binding buffer, applied to the AlbuVoid beads and washed. All steps are perfomed within a microfuge spin-filter format. The albumin is selectively voided out, while the low abundance serum proteome is retained on the bead. After the final wash, reduction, alkylation and Trypsin digestion all take place on the bead. The tryptic peptides were then sent for LC-MS/MS analysis, using a singular 3 hour gradient. **Results and Discussion:** Although perfect specificity and complete digestion of proteomes is often assumed and certainly desirable, unfortunately it is not realistic. We demonstrate that highly efficient on-bead digestion workflows can support labeled quantitative investigations of different sera samples. We observed 200 total proteins, several of which were up or down regulated in the cancer sera samples. **Conclusion:** The data reported here supports the importance of digestion conditions as such conditions can impact both missed cleavages,

and non-specific cleavages. When non-specific cleavages occur, higher proteolytic background can obscure sequence-rich features leaving many proteins unaccounted for. For quantitative applications, peptides should not be subject to inconsistent or inefficient proteolysis. Therefore, the speed and efficiency of AlbuVoid™ on-bead digestions can improve workflows and minimize many of the inconsistencies of proteolytic hydrolysis during the generation of serum or plasma peptides.

P06.53 Statistical and Pathway Analysis of Protein Data

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Introduction and Objectives: Proteomics has taken either one of two tacks: discovery approach where data-dependent-acquisition used to obtain MS/MS data on every possible peptide in sample/s or targeted approach where MRM is used on a known list of peptides. The discovery approach suffers from the daunting requirement to sort out the biologically meaningful peptides and proteins using data analysis; the targeted approach requires substantial pre-knowledge of relevant peptides and proteins to create an MRM method. We propose the use of a proteomics, statistical analysis, and pathway visualization tool suite to delve further into the biology. **Methods:** Human glioblastoma multiforme (GBM) tumor and epilepsy specimens were and tissues were snap-frozen in liquid nitrogen within 30 minutes of removal and stored at -80°C. The homogenized tumor tissue proteome was fractionated via SDS-PAGE. The in-gel tryptic digested peptides were analyzed in triplicate in data-dependent mode on HPLC-Chip/Q-TOF MS. The MS/MS data was processed by protein identification software. Peptide and protein identifications and their abundances were brought into statistical and pathway analysis software package. Differentially expressed proteins were visualized on relevant pathways. **Results and Discussion:** Protein identification was performed on GBM and epilepsy samples by searching MS/MS spectra against UniProt human database and search results were validated at 1% FDR. The protein database search results identified about 14K protein groups across entire data set. These proteins and their peptides as well as abundance information were placed in an XML-based format for import into a statistical package. Statistical analysis of GBM and control samples identified about 370 differentially expressed proteins. These proteins clearly discriminated tumor from control samples using PCA. This conclusion was reinforced by viewing data in heat map of sample-to-sample correlation. Differentially expressed proteins were searched against human pathways and visually inspected to develop hypotheses about biological function. **Conclusion:** Statistical and pathway visualization software allowed classifying GBM tumor into different categories.

Keywords: Pathways analysis, glioblastoma (GBM), Differential proteomics, Statistical analysis

P06.54 Ubiquitination Profiling Identifies Sensitivity Factors for IAP Antagonist Treatment

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Introduction and Objectives: Inhibitor of apoptosis (IAP) proteins play critical roles in cellular survival by blocking cell death, modulating signal transduction and affecting cellular proliferation. IAP proteins have been implicated in human malignancies because of their elevated expression levels, anti-apoptotic activity and the ability to engage survival signaling. IAP antagonists are efficient at inhibiting tumor growth and inducing cell death, but only in a subset of tumor cell types. Currently there is no explanation for this selective sensitivity to IAP antagonist treatment. Here we used IAP antagonists as a tool to modulate IAP E3 ligase activity with the goal to identify substrates. **Methods:** Two IAP antagonist-sensitive breast-cancer cell lines (EVSA-T and EFM192A) and one resistant cell line (KPL4) were treated for 24h with vehicle, Gemcitabine, IAP antagonist (BV6) or the combination. To induce IAP-mediated ubiquitination, sensitive cells were treated with BV6 for 0, 5 or 20 min. Lysates were subjected to proteolytic digestion and ubiquitin-remnant peptides enriched using anti-K-GG immunoaffinity purification followed by MS analysis. **Results and Discussion:** We profiled ubiquitination sites on thousands of substrate proteins and identified two major groups of substrates whose ubiquitination status changed upon IAP antagonist treatment. The first group constitutes mitochondrial proteins, especially mitochondrial cell death regulators CYC and PGAM5. The ubiquitination of mitochondrial proteins is probably not specific to IAP antagonist treatment, as it was not observed in short BV6 treatments. The second group involves regulators of NF-kB signaling whose ubiquitination was preferentially observed in IAP antagonist sensitive cells following long and short BV6 treatments. The importance of NF-kB regulators for IAP antagonist-induced cell death and signaling was functionally confirmed. **Conclusion:** Our data identify ubiquitination of NF-kB proteins and, particularly, RIP1 as indicators of sensitivity to IAP antagonists and pave the way for future development of biomarker(s) for IAP antagonist anti-cancer treatment.

Keywords: inhibitor of apoptosis (IAP), IAP antagonist, RIP1, ubiquitin

P06.55 A Novel DIA Workflow for More Sensitive and Accurate SILAC Quantitation for Protein Kinome

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Introduction and Objectives: Protein kinases represent one of the most tractable drug targets in the pursuit of new and effective cancer treatments. Workflows have been developed to incorporate targeted kinome enrichment with differential profiling as a means to identify response factors that may be key to understanding biological states and/or drug interaction. To increase the confidence in determining relative abundance values, stable-isotope labelling (SILAC)-based mass spectrometry quantitation is used to provide a global internal standard for normalization. Current LC-MS based data acquisition strategies, however, still lack capabilities for comprehensive sampling for all heavy and light peptide analogs. In addition, quantitative determination solely from precursor mass measurements may be compromised by background interference for isobaric co-eluting peptides. We present a new DIA-SILAC approach that addresses these challenges and provides improved quantitative results on these SILAC-kinase samples. **Methods:** Isolation of endogenous protein kinases from cell lines was carried out using multiplexed-inhibitor beads and quantitative mass spectrometry (Q-MIBs). A super-SILAC metabolic labeling technique was employed to assess relative quantitation differences across the kinome. LC-MS data acquisition was performed using a Q Exactive mass spectrometer using a hybrid MS and narrow window DIA acquisition scheme. All data was processed using Pinnacle software and quantitation was determined based on both MS and DIA data.

Results and Discussion: Utilization of narrow DIA windows enabled comprehensive product ion spectral acquisition throughout the entire gradient. The width of the DIA window was set to isolate the heavy and light peptide pairs facilitating relative quantitation confirmation using precursors and product ion values. Using this DIA workflow, has increased our kinome coverage by nearly 20% over pre-existing DDA methods where we are routinely capturing >300 protein kinases per run/per cell line. **Conclusion:** We present a novel acquisition and informatics strategy that provides a more comprehensive and accurate quantitation on SILAC samples.

Keywords: DIA, Kinase, kinome, SILAC

P06.56 Omega-3 Fatty Acid-Enriched Diet Favors a Reduction of Murine TRAMP-C2 Prostate Tumor Growth Compared to Omega-6 Fatty Acid-Enriched Diet

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Introduction and Objectives: Inflammation is one of the contributing factors to prostate cancer (PCa). The potential anti-inflammatory effects of omega (ω) 3 fatty acids (FA) on PCa microenvironment still remain to be explored. The goal of our study was to compare, in the TRAMP-C2 murine PCa model, the effects of dietary ω3 vs ω6 FAs on tumor growth and intratumoral immune response. **Methods:** Groups of 10 C57BL/6 mice were fed with ω3 or ω6-enriched diets. After 4 weeks of diet, $2x10^6$ TRAMP-C2 cells were injected sub-cutaneously in all mice. Tumor growth was measured every other day. Mice were sacrificed when the tumor volume reached 2 cm^3. Plasma, red blood cells (RBC), and tumors were collected at sacrifice. The FA profiles of RBCs and tumors were determined by capillary gas-liquid chromatography. Cytokine profiles of plasma and tumor were determined using Luminex assays. Tumors were dissociated and analyzed for immune cell infiltration by flow cytometry. **Results and Discussion:** Tumors of mice of the ω6 group were found to grow faster than those of the ω3 group (p=0.0003). FA profile analyses showed substantial incorporation of ω3-FAs in the tumors of ω3 vs ω6 mice (100-fold). A hemopoietic growth factor, GM-CSF was detected in the tumors of half of the ω3 mice but of none of the ω6 mice. Eosinophil recruiting cytokine (Eotaxin) was observed in the tumors of most of the ω3 mice but in only one of the ω6 mice. IL-1b, IL-13, and, MIP-1b were expressed at significantly higher in the ω3 group (fold change: 1.5, 1.4, and 1.6 & p=0.0041, 0.0130 and, 0.0220 respectively). Tumor infiltrating lymphocytes were significantly more abundant in ω6 mice compared to ω3 mice. **Conclusion:** Our results show that dietary ω3 FAs help to reduce the growth of prostate tumors. This could be achieved by favoring a more effective immune response.

Keywords: prostate cancer, omega-3/6, Immune responce

P06.57 Proteomics Analysis of Cisplatin Resistance in Ovarian Cancer Cells

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Introduction and Objectives: Cisplatin is widely used in the treatment of ovarian cancer, but most patients acquire the drug resistance that compromises the efficacy of drugs. Understanding the mechanism of drug resistance is important for finding new therapeutic targets. The aim of the present study is to identify the differentially expressed proteins between drug-resistance and control cells and to explore functions of vimentin in drug resistance. **Methods:** A2780 cells were incubated with cisplatin by stepwise increasing cisplatin concentrations to establish a cisplatin-resistant ovarian cancer cellline A2780-DR. Quantitative proteomics was carried to identify differentially expressed proteins between A2780 and A2780-DR cells. To explore functions of vimentin in drug –resistance, the vimentin-directed shRNA was designed and transfected into A2780 cells using a lentivirus transfection system. The vimentin knockdown cells were characterized and the differentially expressed proteins between the vimentin-knockdown and control cells were identified. **Results and Discussion:** In the present study, we established a resistant ovarian cancer cell line with a resistance index of 6.64. Quantitative proteomic analysis identified 340 differentially expressed proteins between A2780 andA2780-DR cells, which involve in diverse cellular processes, including metabolic process, cellular component biogenesis, cellular processes, and stress responses. Down-regulation of Ras-related Protein Rab 5C-dependent Endocytosis led to cisplatin accumulation in cisplatin-resistant cells. We also found that the expression of vimentin were down-regulated in drug-resistance A2780-DR and SKOV-3/DDP cells compared to control cells. Vimentin silencing in ovarian cancer cells leads to reprogramming cells to cancer stem cell-like state, and a prolonged G2 arrest. **Conclusion:** Rab 5C-mediated endocytosis and vimentin silencing contribute to drug resistance in ovarian cancer and vimentin is a potential target for treatment of resistance ovarian cancers.

Keywords: Cisplatin Resistance, Ovarian cancer, vimentin, reprogramming

P06.58 SWATH-MS and Proseek® Oncology Proteomic Biosignatures of Dukes' Staged Colorectal Cancer

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Introduction and Objectives: Current methods used for colorectal cancer (CRC) screening are grossly inadequate on both sensitivity and specificity grounds, whilst morbidity and mortality remain high due to low compliance with uncomfortable tests (e.g., FOBT, FIT and/or colonoscopy). Minimally invasive blood tests may overcome this issue. All blood-based cancer biomarkers in use clinically today are in plasma in low concentration (i.e., pg-ng/mL) and are thought to reflect microenvironment changes or release from cancer tissues, followed by inevitable dilution in 4.5-5.5 litres of blood. **Methods:** Here, we report SWATH-MS and Proseek® Oncology data as part of a program evaluating numerous biomarker discovery platforms on a particular set of CRC plasmas. **Results and Discussion:** Firstly, expression of 92 potential cancers biomarkers were measured in pooled CRC Dukes' staged (i.e., A-D and controls) EDTA plasmas utilizing Olink's PEA based Proseek® Multiplex Oncology I kit, with duplicates asayed using Bio-Plex Pro™ human cytokine 27-plex immunoassays. Expression of CEA (a diagnostic biomarker for CRC) was significantly elevated in Dukes' stages C and D, whilst IL 8 and prolactin expression changed significantly between control, localised and spread CRCs. Secondarily, we employed SWATH-MS a DIA method that allows complete and permanent recording of fragment ions of detectable peptide precursors from pooled plasmas (n=20/stage) previously immunodepleted (i.e., MARS-12 + patented IgY ultradepletion) from the same CRC patients with age-, sex-matched control plasmas. **Conclusion:** Results aimed at early detection of CRC and signature differences between Dukes' stages will be discussed.

Keyword: biomarkers, cancer, SWATH-MS, multiplex

P06.59 Verification of Biological Insights Gained from Global Proteomic Analysis Using SWATH and SRM

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Introduction and Objectives: It is generally accepted that the outcomes of genetic alterations in cancer are not linear, but are instead manifested as network-related changes. Our group previously conducted a comprehensive proteomic characterization of ovarian high-grade serous carcinomas as part of the Clinical Proteomic Tumor Analysis Consortium (CPTAC) effort to systematically identify proteins that are derived from alterations in cancer genomes and related biological processes. To orthogonally verify the biological insights gained from our initial discovery-phase study, we used sequential window acquisition of all theoretical ions (SWATH)-MS wherein digital records were acquired for all the detectable components of the ovarian tumor tissues. Targeted mass spectrometry-based selected reaction monitoring (SRM) assays were also developed to further verify a subset of the candidates from the discovery-phase study. **Methods:** 106 clinically annotated ovarian high-grade serous carcinomas previously characterized by The Cancer Genome Atlas (TCGA) were processed for proteomic analysis. Quality control 1DLC-MS/MS runs and SWATH analyses were conducted on a 5600+ Triple-TOF mass spectrometer. SWATH data were acquired using a variable window acquisition strategy. Data were analyzed and filtered using PeakView and SWATH 2.0. Data quality, protein fold change and pathway analyses were conducted using OneOmics applications (Sciex). **Results and Discussion:** The reference ion library generated from a fractionated pool of the ovarian carcinoma-derived peptides enabled the SWATH-based quantification of an average of 1,968 ± 360 proteins covering a dynamic range of 4 orders of magnitude in each tumor. Verifying the results from our discovery-phase study wherein proteins involved in histone acetylation displayed co-expression patterns differentiating homologous recombination deficiency (HRD) positive from negative tumors, SWATH-MS and SRM analysis indicated a higher relative abundance of histone H4 K12,K16 acetylation in non-HRD vs. HRD tumors. Additionally, proteins involved in translational elongation were significantly enriched in HRD tumors. **Conclusion:** SWATH and SRM verified protein changes associated with genomic alterations in ovarian high-grade serous carcinomas.

Keywords: cancer, SWATH, verification, targeted mass spectrometry

P06.60 Discovery of Metastasis-Related Biomarkers in Lung Cancer by Quantitative Tissue Proteomics

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Introduction and Objectives: Lung cancer is the leading cause of cancer death worldwide. Non-small cell lung cancer (NSCLC) is the most common type comprising ~80% of all lung cancers. The stages as well as lymph nodes (LNs) involvement in lung cancer is highly correlated to prognosis and mortality. Thus, it is emergent to identify and characterize the stage and/or metastasis-related biomarker for lung cancer diagnosis/prognosis and rational design of therapeutics. **Methods:** We used iTRAQ-based quantitative proteomics strategy to perform the differentially expressed tissue proteome from paired lung adenocarcinoma tumor tissues with different extent of LNs involvements. The potential biomarkers were selected based on network analysis, integrated databases, functional classification and literature search. The clinical significance of potential metastasis-related biomarkers was verified by Western blot and immunohistochemistry staining (IHC). The gene knockdown and overexpression followed by cell survival, migration and invasion assays were used to examine the biological function of potential biomarkers in NSCLC progression. **Results and Discussion:** We identified 99 candidates with 1.5-fold increased in distant metastatic or LNs involvement cancer tissues compared with non-LNs involvement cancer tissues. Three potential novel biomarkers were verified by Western blotting and IHC. We observed that the protein levels of LC-001 and LC-002 were overexpressed in cancer tissues as compared with adjacent normal tissues. The LC-001 and LC-002 levels in tumor cells from metastatic lymph nodes were significantly higher than those detected in primary tumor cells. The higher LC-001 level in NSCLC tissue was associated with poorer prognosis. Moreover, both LC-001 and LC-002 modulated the migration and invasion of NSCLC in vitro. **Conclusion:** Our results collectively suggest that differential lung cancer tissue proteome is a useful source for metastasis-related biomarkers discovery in NSCLC.

Keywords: biomarker, non-small cell lung cancer, metastasis, quantitative tissue proteome

P06.61 NetGestalt CPTAC Portal: Empowering Biologists with Cancer Proteogenomic Data

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Introduction and Objectives: Recent completion of the deep proteomic characterization of the TCGA breast, colorectal, and ovarian tumor cohorts resulted in the first large human tumor datasets with complete molecular measurements at the DNA, RNA and protein levels. The goal of the project is to develop a computational framework that enables biologists to effectively leverage the vast amount of the NCI's proteogenomic data from CPTAC and TCGA to gain novel insights in cancer biology. **Methods:** We developed a web application, NetGestalt CPTAC portal, which is publicly available from the NetGestalt website (http://www.netgestalt.org). **Results and Discussion:** The portal includes genomic, epigenomic, transcriptomic, proteomic, and clinical data for the TCGA breast, colorectal and ovarian tumors, as well as existing knowledge on pathways and networks, giving a total of tens of millions of data points. Experimental data are organized on the basis of tumor types, data types and data processing levels. Specifically, data for individual samples were summarized at the gene level to generate gene-by-sample matrices. For selected clinical and genomic attributes, appropriate statistical analyses were performed to generate test statistics, nominal p values, and False Discovery Rates (FDRs) for individual genes. Based on pre-defined thresholds, significant genes were identified. Through the web portal, data from different tumor types, analytical platforms, and processing levels can be easily accessed and analyzed in the contexts of human genome, protein-protein interactome,

kinome, and biological pathways. Visualization and analytical features enable a high-level simultaneous overview of multiple genome-wide datasets, visual or statistical identification of chromosomal or network regions of interest, and zooming into specific regions to retrieve detailed information. Users can perform data integration within a single omic data type, across multiple omic data types, and over biological networks. **Conclusion:** The NetGestalt CPTAC portal provides a unique resource for biologists to access and analyze large cancer proteogenomic datasets within biological contexts.

Keywords: cancer, Proteogenomics, data integration, Data Visualization

P06.62 Quantitative Proteomics of Isogenic BAP1-Deficient Cells Lines for Targeted Therapy Discovery

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Introduction and Objectives: BAP1 is a deubiquitylase and tumor suppressor. It is frequently inactivated by somatic mutation in malignant pleural mesothelioma (MPM). BAP1 germline mutation predisposes to cancers including MPM, uveal melanoma and kidney cancer. We are interested in identifying BAP1-dependent pathways that might sensitize these cancer cells to specific therapies. **Methods:** We used rAAV (Horizon Discovery) to engineer BAP1 haploinsufficient or knockout isogenic MeT5A mesothelial cells. Alleles were sequentially targeted with a predisposition point mutation (w-) and a promoter trap (KO), which mimic clinical mutations identified in MPM. Accurate gene targeting was sequence verified, and the cell biology characterised. We profiled the BAP1-dependent proteome by SILAC-mass spectrometry, to reveal lead targets for drug discovery, additionally transcriptomic analysis is in progress. **Results and Discussion:** We generated BAP1w-/+ MeT5A cells and from these successfully produced three independent w-/KO MeT5A clones. The cell lines show sequentially reduced BAP1 protein expression, with expected effects on cell proliferation and cell cycle phase distribution. We found that the w-/KO cells were desensitized to the HDAC inhibitor Vorinostat, as we previously showed in established BAP1 null mesothelioma cell lines (Sacco et al, 2015). Three triple-labeled SILAC-MS experiments compared parental MeT5A and w-/+ cells with each of the w-/KO clones. MaxQuant analysis of the pooled experiments identified over 2500 proteins. Compared with the parental cells, ~15% or ~11% of identified proteins were modulated by more than 1.5-fold in the w-/KO or w-/+ cells, respectively. Analysis of gene ontology terms associated with these proteins reveals alteration in cytoskeletal and glycolytic pathways. **Conclusion:** We have generated a novel and valuable model to analyse the effects of the loss of BAP1. Proteomic profiling suggests BAP1 loss impacts on cancer cell motility and metabolism. We are verifying these findings and assessing druggable targets within our dataset that will be tested for sensitization in BAP1-deficient cells.

Keywords: lung cancer, SILAC, pathway analysis, Drug Targets

P06.63 Studying of Malignant Ascites as a Unique Tumor Microenvironment

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Introduction and Objectives: Cancer cells form complex network of interactions between them and their local environment. Recent studies have shown that after exposure to chemo/radiotherapy during the treatment, apoptotic cells secreted some factors accelerating proliferation of neighboring tumor cells and contributing to their more aggressive phenotype. The general aim of this work was to identify signaling molecules secreted by tumor cells in ascites that could promote cancer cell survival. **Methods:** We performed deep proteomic analysis of ascites samples from patients who had received several courses of chemotherapy prior to ascites collection; cirrhosis ascites were taken as control samples. To confirm the result of proteome analysis we examined the proteins exported from ovarian cancer cells in vitro. **Results and Discussion:** Functional analysis of the ascites proteomes demonstrated that the greatest differences are related to splicing-associated proteins that fulfill their functions in complexes with snRNAs. We checked for the presence of snRNAs in the examined ascites. Noteworthy, all types of minor spliceosome snRNAs were present only in the malignant ascites. Minor spliceosome splices rare U12-type introns; approximately 600 genes containing U12-type introns are known in the human genome. It has been shown that these introns are spliced at a slower rate and less efficiently compared to the major U2-type introns. This suggests that U12-type introns are needed for the proper regulation of the genes that they reside in. Interestingly, U12-type intron-containing genes code some proteins important for cancer development: PTEN, BRCC3, and about 27 proteins involved in MAPK signaling pathway. We showed that the lifespan of mice with transplanted tumors after injection of synthetic U12snRNA was significantly lower in comparison with control groups. **Conclusion:** We assume that the secreted components of spliceosome could promote cancer cell survival by changing mRNA expression levels of minor intron-containing genes. This work was supported by the RSF (project No.14-50-00131).

Keywords: Ovarian cancer ascites, proteomics, Spliceosome, snRNA

P06.64 Insight on Colorectal Carcinoma Infiltration by Studying Perilesional Extracellular Matrix

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Introduction and Objectives: The extracellular matrix (ECM) is a complicated meshwork of proteins and proteoglycans providing architectural support and anchorage to cells and it is a reservoir for cytokines, growth factors and ECM-remodelling enzymes which regulate cell behaviour. In cancer, ECM is considered a major contributor to tumour progression and invasion, as well as angiogenesis and recruitment of inflammatory infiltrate. An important area of future cancer research will be to determine whether abnormal ECM could be an effective cancer therapeutic target. To achieve this goal, it is mandatory to study ECM composition and organization in healthy and cancer environments. **Methods:** We developed an "ex vivo" method to purify ECM from human biopsies. We collected tumour, perilesional area and healthy mucosa from five patients undergoing colorectal cancer (CRC) resection surgery. ECMs were decellularized and subjected to a comprehensive biochemical characterization.

Label-free proteomic profiling experiments were performed in technical triplicates with subsequent protein validation by WB and IHC analyses. **Results and Discussion:** Among the identified 1140 proteins, 51 are significantly dis-regulated ones between tumour and healthy ECM, being perilesional and healthy samples mixed together. Moreover, in the tumour ECM we find altered modulation of matrisome proteins, increased content of hydroxylated lysines and over-expression of lysyl oxidase. **Conclusion:** These ECMs provide competent scaffolds to assess efficacy of anti-tumour drugs in a 3-dimensional setting, closely recapitulating the native microenvironment.

Keywords: extracellular matrix, Clinical proteomics, label-free mass spectrometry, colorectal cancer

P06.65 Quambalarine B Induces Metabolic and Proteomic Rearrangement in Leukemia Cells

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Introduction and Objectives: Quambalarin B is a secondary metabolite produced by basidomycete Quambalaria cyanescens. Recently, it was reported it inhibits fungal growth and it depletes mitochondria of human cancer cell lines (JURKAT, REH and NALM6). In order to uncover mechanism of its activity several biophysical, biochemical and analytical approaches were carried out. **Methods:** The effect of Quambalarin B on growth curves and population doubling levels was measured using propidium iodide staining. The influence of Quambalarin B on cancer cells metabolism was determined monitoring phospho-P70S6 kinase, C-MYC and GLUT-1 levels by western blot. The metabolite profiling was studied utilizing NMR spectroscopy and high-resolution mass spectrometry. Also, MS-based label-free quantification was performed to monitor proteins turnover. **Results and Discussion:** We determined effective concentrations (EC50) for each cell line in presence and absence of low concentration of Quambalarin B. We found that proliferation is significantly blocked after 2-3 days for JURKAT and REH and after 4-5 days for NALM6. Interestingly most of cells were alive with low percentage of apoptotic cells. Since we found significant alterations in metabolic genes expression levels and changes in phospho-P70S6K, C-MYC and GLUT1 levels, we performed metabolomics analysis using NMR spectroscopy and mass spectrometry. These analyses showed strong changes of detected metabolites levels in JURKAT and NALM6 whilst there were almost no changes detected in REH line. **Conclusion:** The differences in response to Quambalarin B among tested cell lines can be explained by the growth dependence on glucose and glutamine – general metabolites utilized by cancer cells to support growth. In contrast to REH and NALM6 JURKAT cells growth strongly depends on glucose although all cell lines are dependent on the presence of glutamine. Moreover Quambalarin B resistant JURKAT cell line was created and it shows low level of C-MYC protein and strongly increased level of YAP - transcriptional coactivator with oncogenic potential.

Keywords: NMR spectroscopy, Mass spectrometry, Label-free quantification, Cancer metabolism

P06.66 Proteomic/Transcriptomic Characterisation of a Prostate Cancer Cell Line Model of Spontaneous EMT

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Introduction and Objectives: Several well characterised clonal progenies of OPCT1 prostate cancer cell line, having varying EMT potential, have been generated. Utilising High Resolution Accurate Mass mass spectrometry and data independent acquisition (SWATH™) along with gene expression microarray we have studied global transcriptional/proteome changes. The major objective being to understand the molecular mechanisms governing this non TGFβ/TF induced phenomenon and further cross-compare with induced systems to identify novel and overlapping pathways. **Methods:** Secreted proteins and whole cell lysate from five clonal progenies of OPCT1 (Two highly epithelial and three highly mesenchymal) were prepared. Following tryptic digestion, samples were analysed using a SCIEX 6600 TripleTof instrument using SWATH™/OneOmics. mRNA isolations of the same cell-lines prepared and labelled cRNA hybridised onto Affymetrix human-U133 arrays. Correlation of differentially expressed genes/proteins were assessed and investigated using iPathwayGuide software and DAVID functional enrichment tool. **Results and Discussion:** 822 secreted proteins of OPCT1 parental, epithelial and mesenchymal clones (n=10) were quantified by SWATH™. 43 were significantly down-regulated and 38 up-regulated in the mesenchymal clone on the basis of a 0.7 log fold-change and $p<0.05$. Similarly, 1697 proteins were quantified in the whole cell lysates of the OPCT-1 parental and clonal progenies, of which 360 were significantly differentially expressed (85% confidence, MLR weight 0.15). Pathway enrichment analysis revealed proteins involved in focal adhesion, ECM interaction, cytoskeletal remodelling, TGF-β and p53 pathway are significantly enriched amongst other unreported ones. Gene expression analysis identified 700 differentially regulated genes (pcorr≤0.01, Fold-change ≥3.0, n=3). **Conclusion:** A model for the investigation of EMT in human prostate cancer was established and revealed heterogeneity in EMT events among distinct clonally derived populations of prostate cancer cells. Gene/protein expression studies identified the overall molecular changes in the model to understand EMT in a non-TGF-β/TF induced system. Several other unreported molecular events were also identified. Secretome profiling of epithelial/mesenchymal progenies identified differential secretory profiles.

Keywords: SWATH, prostate cancer, transcriptomics, EMT

P06.67 Proteomic Analysis of Serum Biomarkers for Gastric Cancer

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Introduction and Objectives: Gastric cancer is one of the most common human cancer types. It is the second leading cause of global cancer death. In recent studies, surgical resection along with chemo-radiation showed significant improvement over surgery alone. However, most gastric cancer patients have advanced diseases at diagnosis. While surgical resection is an effective therapeutic procedure for curing gastric cancer patients, the 5-year survival rate is only about 20% for patients with late stage cancer. Therefore, early diagnosis is beneficial and critical for successful surgical removal of gastric cancers since metastases often occur in the late stages of gastric cancer and greatly reduce the effectiveness of surgery intervention. **Methods:** In this study, we used a strategy for enrichment of multiple

post-translational modifications combined with hydrophilic interaction chromatography or high pH reversed phase fractionation and nanoLC–MS/MS to identify biomarker candidates in serum samples. We furthermore combined this strategy with TMT 10plex labeling in order to compare serum obtained from 10 patients with gastric cancer before and after surgery. **Results and Discussion:** The comprehensive strategy resulted in identification of phosphorylated peptides, sialylated N-linked glycopeptides, Cysteine-containing peptides and non-modified peptides. Several proteins and modified peptides were identified as significantly regulated in all gastric cancer sera from before to after surgery, and among them proteins involved in the LXR/RXR activation pathway, regulating lipid metabolism and inflammation, and in the acute phase response responding to inflammation, supporting the critical role of hormone deregulation and inflammation in cancer. Surprisingly, these proteins have not been previously reported within a network with functions related to gastric cancer. **Conclusion:** LXR agonist treatments disrupted proliferation, cell-cycle progression, and colony-formation in various cancers such as, prostate, breast, colon and many others. Targeting LXR/RXR pathway in gastric cancer might help in the development of new therapies that can minimize the risk of toxicity and reduce the cost of treatment.

Keywords: proteomics, post-translational modifications, biomarkers, Gastric cancer

P06.68 Development of Assays for Measuring CUZD1, a Pancreas-Specific Protein, in Biological Fluids

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Introduction and Objectives: Using proteomics and bioinformatics we have previously identified CUZD1 as a candidate pancreatic ductal adenocarcinoma (PDAC) biomarker. Although highly pancreas-specific, CUZD1 has also been implicated in Ovarian Cancer progression and Crohn's disease. Currently, no validated methods are available for measuring CUZD1 in biological fluids and tissues. Our aim was to develop analytical technology for CUZD1, including specific antibodies, a Selected Reaction Monitoring (SRM) assay and an ELISA immunoassay. **Methods:** The extracellular region of human CUZD1 was expressed in the mammalian Expi293 expression system. Recombinant CUZD1 was purified to homogeneity by ion-exchange-FPLC and used as an antigen for the generation of mouse mAbs. An SRM assay was developed for the selective detection and quantification of CUZD1 and was optimized in supernatant samples derived from the culture of mammalian cells expressing and secreting recombinant CUZD1. Calibration curves were generated to determine the limit of detection (LOD) and limit of quantification (LOQ) of the assay. Finally our SRM assay was used for the quantification of CUZD1 in pancreatic tissue protein extracts. **Results and Discussion:** Our SRM method showed good linearity and reproducibility. The LOD of our assay was 3 ng/mL and the LOQ was 30 ng/mL. The SRM assay was successfully transferred to pancreatic tissue samples, in which the assay was able to measure approximately 38 ng of endogenously expressed CUZD1 in 1 mg of digested protein. **Conclusion:** We are towards the development of a proteomic toolbox for the detection and quantification of CUZD1. Monoclonal antibodies have been generated against recombinant CUZD1 and they will be used in Immunohistochemistry studies, to establish the tissue expression pattern of CUZD1 at the protein level, and for the development of an ELISA immunoassay. Both the ELISA and the SRM assays will used to explore the potential of CUZD1 as a biomarker of PDAC and other pathological conditions.

P06.69 Stroma COL6A3 Is a Prognosis Marker of Colorectal Carcinoma Revealed by Quantitative Proteomics

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Introduction and Objectives: Colorectal cancer (CRC) represents the third most common cancer in males and second in females worldwide. The current study aims to identify stroma specific protein marker(s) of colorectal cancer. **Methods:** We performed a quantitative 8-plex iTRAQ proteomics analysis of the secreted proteins from five colonic fibroblast cultures and three colon cancer epithelial cell lines. We integrated the quantitative proteomics results and public available cancer transcriptomic data to identify cancer stroma enriched secreted proteins, which were further verified using Western blot. A candidate stromal marker was analyzed using tissue-microarray (TMA)- immunohistochemistry (IHC). The association of protein expression with clinicopathological parameters of colon cancer patients and the prognostic significance were analyzed using k square test and Kaplain-Meier survival analyses, respectively. The plasma expression of the candidate marker was analyzed using ELISA and Receiver operating characteristic curve (ROC) methods. **Results and Discussion:** We identified 1114 proteins at 0% FDR, including 587 potential secreted proteins. We further recognized 116 fibroblast-enriched proteins which were significantly associated with cell movement, angiogenesis, proliferation and wound healing, and 44 epithelial cell-enriched proteins. Twenty and eight fibroblast-enriched proteins were up- and downregulated in CRC, respectively. Western blots confirmed the fibroblast-specific secretion of filamin C, COL6A3, COL4A1 and spondin-2. Upregulated mRNA and stroma expression of COL6A3 in CRC, which were revealed by Oncomine analyses and TMA-IHC, indicated poor prognosis. COL6A3 expression was significantly associated with Dukes stage, T stage, stage, recurrence and smoking status. Circulating plasma COL6A3 in CRC patients was upregulated significantly comparing with healthy peoples. ROC analysis revealed that COL6A3 has better predictive performance for CRC with an area under the curve of 0.883 and the best sensitivity/specificity of 83.9%/89.4%. **Conclusion:** COL6A3 was a potential diagnosis and prognosis marker of CRC.

Keywords: proteomics, colorectal cancer, Tumor microenvironment, Fibroblast

P06.70 Development of a Global Phosphotyrosine Proteomic Method for Analysis of Cancer Cell Lines

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Introduction and Objectives: It is widely known that phosphorylation signaling pathway is important for several cellular functions such as cell growth and differentiation. Disruption of the signaling is often related to oncogenesis. Especially, previous studies revealed that abnormality in phosphorylation at tyrosine residues causes tumour. From the aspect of cancer therapy, phosphorylation at tyrosine residues and phosphotyrosine kinase are potential targets in anti-cancer therapy. Thus, understanding of global phosphotyrosine proteomics significantly contribute to cancer biology and drug discovery against cancer. **Methods:** In case of large scale phosphoproteomics with Fe immobilized metal affinity chromatography (IMAC), phosphotyrosines in whole phosphopeptides account for no more than five percent. In previous studies,

enrichment of phosphotyrosines with immunoprecipitation was used in order to improve identification of phosphotyrosine peptides. In this study, we evaluated the several conditions, such as combination of phosphotyrosine antibodies, eluting solvents of phosphopeptides and mass spectrometry to increase the number of phosphotyrosine peptide identification. **Results and Discussion:** We successfully identified more than one thousand phosphotyrosine peptides from a single cancer cell line. Using tyrosine phosphorylation data obtained from several cancer cell lines, we generated a dataset of phosphotyrosine sites and speculated activated tyrosine kinases in the cell lines using kinase-substrates data set. **Conclusion:** A global phosphotyrosine proteomic method developed in this study can be applied for discovery of therapeutic targets and companion markers in personalized cancer medicine.

Keywords: Phosphotyrosine, cancer proteomics, kinase substrate relationship

P06.71 Proteomic Study Revealing Roles of MET and Caveolins in Exosome-Induced Hepatoma Motility

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Introduction and Objectives: Cancer-derived exosomes play various roles in carcinogenesis, such as promotion of motility and invasion. In this study, we aimed to decipher proteome contents of exosomes secreted by human hepatocellular carcinoma (HCC) cell lines, and identify the exosomal proteins that play important roles in exosome-induced tumor motility and invasiveness. **Methods:** Exosomes were harvested from two high motile HCC cell lines (MHCC97L and HKCI-8), one non-motile HCC cell line (HKCI-C3) and one non-tumorgenic immortalized liver cell line (MIHA) from culture medium by ultracentrifugation. The exosome proteins were separated by SDS-PAGE, and subjected to shot-gun proteomic profiling by MALDI-TOF/TOF MS. Protein expressions were confirmed by Western blot. Roles of exosomal proteins in tumor motility and invasiveness were investigated, and confirmed with siRNA knock-down experiments. **Results and Discussion:** A total of 276 proteins were identified in exosomes secreted from the 4 cell lines. 25 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. 158 proteins were only detected in high motile HCC cell lines, including MET proto-oncogene tyrosine kinase (MET), caveolin-1 (CAV1) and caveolin-2 (CAV2). The specific associations of MET, CAV1 and CAV2 proteins with the high motile HCC cell lines (MHCC97L and HKCI-8), but not non-motile HKCI-3 and MIHA cells, were confirmed by Western blot. Exosomes of MHCC97L promoted migration and invasiveness of MIHA cells. Subsequently experiments showed the uptake of exosomal MET, CAV1 and CAV2 proteins by MIHA cells. Knockdown of MET and CAV1 in MHCC97L depleted exosomal MET and CAV1 proteins and abrogated the effect of MHCC97L exosomes-induced MIHA migration. **Conclusion:** This is the first study showing critical roles of exosomal MET and caveolins in exosome-induced hepatoma motility and invasiveness. [This research was supported by a Collaborative Research Fund from the Hong Kong Research Grants Council (ref. no. CUHK8/CRF/11R)]

Keywords: exosome, Hepatocellular carcinoma, invasiveness, motility

P06.72 Novel Protein Biomarkers for Subtype Stratification and Immunotherapy in Pancreatic Cancer

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Introduction and Objectives: Pancreatic ductal adenocarcinoma (PDAC) is a lethal disease with a median overall survival of six months. Recently, three PDAC subtypes (classical, quasi-mesenchymal and exocrine-like) have been proposed based on transcriptomic profiling of microdissected cancer cells from primary tumors and established cell lines. These subtypes exhibit differences in patient survival and drug resistance to conventional therapies. So far no protein biomarkers for the sub-classification of PDAC are known. We aimed to uncover novel pan-PDAC and subtype specific cell surface and secreted biomarkers relevant for the development of diagnostics and/or therapeutics. **Methods:** The identification of differentially expressed proteins on the cell surface was achieved by in vitro biotinylation, followed by streptavidin capturing of the biotinylated proteins and trypsin digestion. For the analysis of the secretome, cells were cultured in serum free stem cell media for 48 hours and organelles and microvesicles were removed by ultracentrifugation. Secreted proteins were digested with trypsin in solution. The resulting peptide mixtures were analyzed by LC-MALDI-MS/MS and label-free quantified using MSQBAT, an in-house developed software. Putative protein biomarkers were selected and validated using SRM, RT-qPCR, confocal immunofluorescence and immunohistochemistry. Additionally, the potential co-localization of two subtype-specific protein biomarkers was evaluated using the proximity ligation assay (PLA). **Results and Discussion:** Twelve primary PDAC cell lines and two control cell lines have been analyzed, resulting in the identification of 3288 proteins. Two protein biomarkers (cadherin-17 and an exo-lectin) were fund to be exclusively expressed in the exocrine-like primary cell lines and were also highly expressed in the original patient tumors. PLA analysis of the two proteins revealed that the two biomarkers co-localize on the surface of exocrine-like cells and could represent potential interaction partners. We have identified and validated novel pan-PDAC and subtype specific protein biomarkers, which could be used for the development of novel diagnosis/ immunotherapeutic tools.

Keyword: Biomarkers, Immunotherapy, Pancreatic Cancer

P07: POSTER SESSION - NEW ADVANCES IN BIOMARKER DISCOVERY

P07.01 Narrow-Range pI Plasma Protein Abundances as Alzheimer Disease Biomarker

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Introduction and Objectives: In-silico analysis of the human blood proteome revealed multi-modality of the pI distribution of proteins with a deep minimum in the narrow range between pI 7.35 and 7.45. Considering that the pH of blood is ≈7.4, the conclusion is that normal forms of human proteins tend to eschew this specific pI region, thus avoiding charge neutrality and, as a consequence, enhanced precipitation. However, abnormal protein isoforms, which are the hallmarks and potential biomarkers of neurodegenerative diseases, are likely to be found everywhere in the pI distribution, including this "forbidden" region. Therefore, we hypothesize that neurodegenerative disease biomarkers are best detected in this narrow pI region.

Methods: To isolate this region, we applied the recently introduced multijunction capillary isoelectric focusing device (MJ-CIEF). A pooled sample of 16 patients with progressive Alzheimer disease (AD) and a pooled sample of 16 healthy donors matched for age and sex were used in this investigation. A mixture of 7 synthetic peptides with known pIs was spiked into the samples to be used as pI markers. For each pooled sample, five replicates, 3 μL each, was desalinated and passed through MJ-CIEF for pI separation. 12 pI fractions were collected, digested, and analyzed by HPLC-MS via a 15 cm analytical column coupled with a Q-Exactive mass spectrometer. **Results and Discussion:** The protein abundances in each fraction were determined by a label-free method, and the average pI of proteins in each fraction was calculated using the abundances of the pI markers. The protein concentration in the fractions around pI 7.4 was compared between AD and healthy samples. On average, 3.6 times higher concentration of proteins was found in patients with diagnosed progressive AD in comparison to the normal samples (p-value = 0.02). **Conclusion:** The results indicate strong potential of the approach to yield prognostic biomarkers for progressive AD.

P07.02 Overcoming the Challenges in Data Independent Acquisition (DIA) via Orbitrap Technology

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Introduction and Objectives: To accurately identify and precisely quantify thousands of proteins per DIA experiment, the completeness and specificity of spectral library, the mass accuracy of the data, and the technical variance in quantitation play important roles. In this work, we utilize Thermo Scientific™ Q Exactive HF™ mass spectrometer for DIA LC-MS/MS experiments to study the urinary proteome, and demonstrate how Orbitrap technology overcomes these DIA challenges. **Methods:** Urinary sample were desalted, trypsinized and were measured multiple times with both data-dependent acquisition (DDA) and DIA experiments on a nanoLC online coupled to Q Exactive series system. **Results and Discussion:** To elucidate the effect of the spectral library on data analysis, the libraries were generated from DDA data of this urine samples, and compared the results to the same DIA samples analyzed with a publicly available human spectral library (1). The urine sample-specified spectral libraries are built based on the multiple DDA runs against database, resulting in > 2000 proteins and ~ 20,000 peptides (1% FDR). During data analysis, the ion chromatograms of multiple fragment ions are extracted and aligned for peptide detection and quantification. To separate the analyte of interest from interferences, a highly accurate mass of the ions is crucial. We applied different mass tolerances for the data analysis (50ppm, 20ppm, 10ppm, and 5ppm). A high technical reproducibility is essential for a precise quantification of biological compounds. We assessed the technical reproducibility of the DIA method by analysis of the same sample multiple times and calculated the CV of peptide and protein quantification using the sample-specific spectral library. **Conclusion:** A higher reproducibility of peptide/protein identification and quantification when using the urinary spectral library. 10 ppm mass accuracy is minimum requirement for an accurate detection of peptides. The median CV was < 10% and >85% of the peptides and >80% of the proteins were quantified with a CV better than 20%.

Keywords: Urinary proteome, Data independent Acqusition, high resolution accurate mass, Orbitrap

P07.03 Quantitative Analysis of the Soluble Proteome of Airway Epithelial Cells in COPD

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Introduction and Objectives: Chronic Obstructive Pulmonary Disease (COPD) is a leading cause of mortality worldwide. Smoking represents a major risk factor for COPD and evidence of significant gender differences with women having a higher risk to develop the disease than men are emerging. We recently showed that there are gender differences in the soluble proteome of alveolar macrophages. In this study, we investigated alterations in the airway epithelial proteome due to early stage COPD, with focus on gender-related differences. **Methods:** The soluble proteome of airway epithelial cells from age- and sex-matched COPD patients (GOLD I-II/A-B), smokers with normal lung function, and never-smoking healthy controls (n=85) were analyzed by DIGE Difference Gel Electrophoresis using an internal standard to normalize for technical variance. Quantitative image analysis and Analysis of Variance (ANOVA) were performed on log transformed data using SameSpots vs4 (Nonlinear Dynamics), and multivariate modeling was performed using SIMCA vs 14 (Umetrics). **Results and Discussion:** Comparison of the soluble proteome of airway epithelium of non-symptomatic smokers and smoking COPD patients showed significant differences between groups and genders: Multivariate modeling showed a separation between groups for the female population, with 7 protein spots being most prominent in driving the separation between female healthy smokers and smoking COPD patients (Female: R^2= 0.71, Q^2= 0.60, CV-ANOVA= 0.03). In the corresponding male population no significant model could be generated, as confirmed by permutation test (Male: R^2= 0.57, Q^2= 0.26, CV-ANOVA= 0.08). **Conclusion:** Our results indicate differences in the airway epithelium of male and female COPD patients, potentially correlating with gender differences in clinical phenotypes. The differing proteins will be identified by liquid chromatography-mass spectrometry, which may give new insight into gender-specific molecular disease mechanisms.

Keywords: chronic obstructive pulmonary disease, proteomics, multivariate modeling, airway epithelium

P07.04 MRM-Based Proteins Diagnostic of Chronic Obstructive Pulmonary Disease Exacerbations

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Introduction and Objectives: In patients with chronic obstructive pulmonary disease (COPD), fixed airflow limitation often results in symptoms such as dyspnea, cough, and sputum production. The periodic worsening of these symptoms is known as acute exacerbations (AECOPD) and such result in considerable morbidity and mortality. The diagnosis of AECOPD, largely made on the basis of clinical gestalt, is fraught with imprecision. The objective of this study was to identify blood-based protein biomarkers that could potentially be used in the clinical setting to diagnose AECOPD.

Methods: Multiple Reaction Monitoring (MRM) mass spectrometry was used to measure 230 peptides, mapped to 128 proteins, in COPD patients' plasma samples. Biomarker discovery was performed in 37 LEUKO study subjects. The biomarker was tested in two cohorts, TNF-a (n=81) and Rapid Transition Program (RTP; n=109). For all three cohorts a plasma sample was collected at time of AECOPD and one at convalescence. Proteins that were statistically different between AECOPD were included in elastic net classification that built the final biomarker model. **Results and Discussion:** A total of 21 proteins were found to have differential levels between AECOPD and convalescent states. Of these, 18 were chosen by elastic net to be in the model. Biomarker scores derived from this model were significantly higher during AECOPD than in the convalescent state (p=0.001). The area under the receiver operating characteristics curve (AUC) for diagnosing AECOPD, estimated by leave-pair-out-cross-validation, was 0.70 in the discovery LEUKO cohort. A similar performance was observed when the biomarker panel was tested in the replication samples, with AUC=0.72 in both TNF-a and RTP. Biomarker proteins were particularly enriched for HDL transport pathways. **Conclusion:** A panel of 18 biomarker proteins can distinguish AECOPD from a convalescent state and may in the future provide a basis for a clinically applicable blood test to diagnose AECOPD.

Keywords: diagnostic biomarker, MRM

P07.05 Evaluation of New Biomarkers of Resistance to Trastuzumab in the Treatment of HER2+ Breast Cancer

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Introduction and Objectives: Trastuzumab is the standard treatment for patients with HER2 overexpressing breast cancer (BC). However half of patients develops resistance. The causes of trastuzumab resistance are poorly understood and elucidating the molecular mechanisms underlying primary or acquired resistance is critical to improve the therapeutic management of BC patients. This retrospective study aims to evaluate whether ADAMs 10 and 17 (A Disintegrin And Metalloprotease) and PTEN (Phosphatase and Tensin homolog) are tissue biomarkers of resistance to trastuzumab treatment. **Methods:** So far 37 tumor tissues were collected from HER2+ locally advanced (Stage IIIA-B) BC patients, treated with the same neo-adjuvant scheme. The pathological complete response (pCR) was used to evaluate treatment outcome. Biomarkers expression were assessed by immunohistochemistry on the initial biopsies. For patients who did not achieve pCR, the evaluation was performed, when possible, on the tissue specimens after surgery. The correlation between biomarkes expression and patients' characteristics (tumor dimension, hormonal receptor and lymph node status, response to treatment) was tested by non-parametric tests. **Results and Discussion:** Preliminary results showed that ADAM10 was expressed in almost half of the patients at low level; a lower expression was found in patients not achieving pCR (p=0.0770, 15 patients). For ADAM17, that was found highly expressed in most of the tissues, no significant difference was detected in relation with tumor characteristic or patients' response. Regarding PTEN, the majority of tissues showed no expression. Intriguingly, in all not responders positive for PTEN expression in the biopsy, a complete loss occurred in the residual carcinoma. **Conclusion:** The study is still ongoing, but these results suggest that ADAM10 could have a role in the mechanisms involved in trastuzumab resistance; to verify if those expressions could be specific features of HER2+ carcinoma the same analysis will be performed on a group of HER2- patients.

Keyword: Breast Cencer, Biomarkers, Resistance, HER2

P07.06 Intelligent Workflows for Proteomics Data Analysis

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Introduction and Objectives: The goals of using scientific workflows rather than taking the data through traditional analysis methods are minimizing the user-interference for the analysis and supporting modularity and reusability. An intelligent workflow should be able to make decisions based on the nature of the data. One way would be using the metadata. In mass spectrometry based proteomics, some metadata is almost always provided in mzXML/mzML files converted from raw data files. However, in order to be able to use this metadata in a wider domain, we need conventions to formalize the knowledge; i.e. an explicit ontology. **Methods:** We built an intelligent workflow using metadata to guide the data processing. The workflow consists of 8 modules in total (with an alignment module being used twice), each performing a discrete analysis step within a well-defined scope. Each module could also be used separately from the overall workflow, as long as the necessary inputs are provided. **Results and Discussion:** By default, the workflow will read the metadata and, using a set of heuristics, select the best, or at least reasonable, parameters for the analysis. However, since most of the metadata and parameter files are in human-readable XML format, users can also impose specific parameters. In order, the modules assembles the input, searches a sequence database using, aligns chromatograms, warps chromatographic time axes, validates the peptide identifications, recalibrates the MS1 data, quantifies the peptides and perform statistical analyses. **Conclusion:** Workflow output will be presented on the poster. Minimal user interaction is a key property of an intelligent workflow. Modularity and reusability are important aspects of the design of intelligent workflows, as these have to keep up with new types of experiments and data generated by mass spectrometers. Metadata plays a crucial role in making intelligent decisions. Hence, ontologies and controlled vocabulary terms should be utilized carefully and consistently.

Keywords: bioiinformatics, scientific workflows, ontologies, Mass spectrometry

P07.07 Respiratory Disease Confirmation via Proteome Analysis of Exhaled Breath Condensate

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Introduction and Objectives: Nonvolatile biomarker profiling in the cooled breath sample called exhaled breath condensate (EBC) is a completely noninvasive procedure which can be performed even in very severe patients and also repeated within short intervals. Therefore, breath testing

is considered to be a potentially ideal candidate for screening purposes. Community-acquired pneumonia (CAP) and chronic obstructive pulmonary disease (COPD) are the most frequently occurring respiratory diseases in the world and are characterized by the leading role of inflammation in their pathogenesis. In this study we tried to identify differences in EBC proteomes of healthy non-smoking group and patients with COPD and CAP in order to evaluate the possibility of using EBC for profiling diseases under consideration. **Methods:** We examined EBC proteomes obtained from 17 patients with COPD, 13 patients with CAP and 23 healthy non-smoking donors. Samples were collected using the Jaeger ECoScreen (VIASYS Healthcare, Germany), freeze dried, treated with trypsin and analyzed by nanoflow LC-MS/MS with a 7-Tesla Finnigan LTQ-FT mass spectrometer (Thermo Electron, Germany). More than 200 different proteins were revealed. **Results and Discussion:** More than 200 different proteins were revealed. It has been demonstrated that the proteomes of each of the groups under consideration differ by specific set of proteins.The results of the proteome analysis were consistent with the clinical picture of the diseases in question. In EBC samples obtained from patients with COPD and CAP cAMP-specific 3',5'-cyclicphosphodiesterases were detected. This result could be a link between analysis of the proteome and lipid composition of exhaled breath condensate in future. **Conclusion:** In this study, we showed that the proteomic analysis of EBC is informative and has great potential as a method for respiratory disease diagnostics, as it allows not only to identify the differences between the "sick-healthy" groups, but also between different diseases, such as COPD and CAP.

Keywords: exhaled breath condensate, chronic obstructive pulmonary disease, FT ICR mass spectrometry, community-acquired pneumonia

P07.08 Discovery of Autoantibody Biomarkers for Oral Cancer Detection Using Protein-Array Analyses

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Introduction and Objectives: Antibodies to tumor antigens possess benefits over other body fluid-accessible molecules as cancer biomarker candidates because they are highly specific, stable, and are easily detected with well-established secondary reagents. Here, we used protein-array analyses combined with bead-based multiplexed immunoassay to identify novel autoantibody (auto-Ab) as biomarker candidates for diagnosis of oral cavity squamous cell carcinoma (OSCC). **Methods:** Serum samples obtained form 96 OSCC patients and 94 control individuals were analyzed by protein-array chip which simultaneously detect auto-Abs expression against 1346 protein targets. Then the differentially expressed auto-Abs between two population were further verified by in-house established bead-based multiplexed immunoassay in both serum and saliva specimens from independent cohort. **Results and Discussion:** Compared with the control group, the levels of 85 auto-Abs have been identified to be elevated in OSCC group in protein-array analyses. Among these auto-Abs, 29 auto-Abs were selected for further verification using 100 serum samples and 208 saliva samples by in-house established bead-based multiplexed immunoassay. Multiple auto-Abs showed significant differences between two groups and would be considered as good biomarker candidates that should be validated using a large cohort samples in the future. **Conclusion:** Protein-array analysis is a quick and powerful tool for discovery of auto-Abs biomarker candidates. Furthermore, its combination with the bead-based multiplexed immunoassay could provide the verification phase of biomarker identification in a high-throughput manner.

Keywords: auto-antibody, biomarker, Oral Cancer, protein-array

P07.09 Tear Proteins of Dry Eye Patients Respond to Environmental Stress

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Introduction and Objectives: Introduction and Objectives: Dry eye disease affects 5-33% of the adult population around the world. Low humidity as found in airflights exacerbates these symptoms. It was previously found that the tear film in these patients contains unregulated inflammatory proteins characterizing the disease. The purpose of this study was to determine the proteomic profile of the tears in response to a desiccating stress. A secondary objective was to determine if a steroid could lessen the effect of a stress on the eye of these dry eye patients. **Methods:** Methods: In an approved study, dry eye patients (n=40) receiving either an antiinflammatory steroid (fluoromethalone=21) FG, or vehicle (n=19) VG, were exposed for 2-hr in an environmental chamber at 23ºC, 5% relative humidity, with airflow (0.43 m/s) directed to the eye. From one eye of 40 subjects 1μl of tears was collected by fire polished pipettes, and subjected to MS/MS^{ALL} with SWATH acquisition enables MRM-based quantitation (high reproducibility with CV% < 20%) of around 700 tear proteins. **Results and Discussion:** Results and Discussion: Initial analysis found that FG was characterized by changes in inflammatory proteins, S100 proteins or those associated with tear functions (lacritin -2.35 fold change). Dendrograms based on the protein levels showed that prior to the low humidity exposure, separation into two groups, FG and VG was clear and that the steroid decreased inflammatory protein levels. However, immediately following the desiccating stress, inflammatory proteins increase less in the FG compared to the VG. After recovery at 24hrs the FG showed a decrease in the levels of inflammatory proteins compared to the VG. **Conclusion:** Conclusions: This study has shown the protective effect of steroid treatment for lower levels of inflammation after a desiccating stress. In addition, it shows the utility of SWATH as a procedure to obtain data even when sample availability is highly constrained.

Keyword: dry eye, desiccating stress, inflammation

P07.10 Systems-Wide Proteome Analysis of Hepatocellular Carcinoma Tissues by Targeted Proteomic Platform

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Introduction and Objectives: The mass spectrometry based quantitative proteomics in clinical samples have been shown to be powerful strategies for the discovery of novel disease biomarkers that can provide diagnostic,

prognostic, and therapeutic targets. While discovery proteomics relying data-dependent acquisition is routinely used in analysis of clinical samples, this strategies presented drawbacks to quantify interesting proteins in complex samples. By contrast, targeted proteomics has emerged to more systematically quantify peptides/proteins with high analytical performance in complex samples. However, pre-clinical and clinical applications of targeted proteomic strategies were limited so far. Here, we present a study of hepatocellular carcinoma (HCC) in tissue specimens using novel quantitative targeted proteomic strategy. **Methods:** In this study, we analyzed hepatocellular carcinoma tissue samples compared with adjacent normal tissues from 6 patients to generate pools of signature proteins. To perform label-free quantitation, we performed pre-defined proteomic strategies including filter-aided sample preparation, single shot MS analysis with long gradients, and high-resolution quadruple Oribtrap LC-MS/MS. For targeted proteomic analysis, endogenous peptides were quantified in 20 pairs of tissues using Super-SILAC mixtures as standard heavy peptides **Results and Discussion:** In total, 5000 protein groups were quantified, of which 1300 proteins were significantly regulated in 6 pairs of HCC liver tissues. Some important proteins and novel proteins that related to HCC pathology were discovered using bioinformatics analysis and network analysis. Approximately 300 peptides corresponding to 150 proteins selected in discovery stage were verified using our tissue targeted proteomic platform in 20 pairs of independent tissues. **Conclusion:** Our tissue targeted proteomic platform will measure the levels of 300 peptides with highly reproducibility from quite small amounts of individual human cancer and adjacent normal tissues. Our platform can easily be implemented in other type of cancer to analyze large numbers of pathologically relevant proteins in clinical specimens using small amount of proteins.

Keywords: quantitative proteomics, Hepatocellular carcinoma, Targeted proteomics, Biomarker discovery

P07.11 Development of Meta-Markers for Recurrence Lung Cancer Using Multiple Reaction Monitoring

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Introduction and Objectives: Recurrence lung cancer shows poor prognosis in cancer related death than primary lung cancer. This is due to the low symptoms and lack of diagnosis tools in early stage with high specificity. To develop clinical diagnostic tools for recurrence lung cancers in early stage, robust meta-markers, which are combination of well-established markers, are on high demand. In this study, quantitative analysis on 195 human patient sera and modeling for generating meta-marker was conducted. **Methods:** Clinical serum samples of 174 lung cancer and 21 recurrent patients that were collected at six time points from pre-surgery to post-24-month. Six targets were selected from our previous results and clinically well-established markers. These 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:** Quantitative analysis on patient samples using MRM showed various serum levels of target proteins in a recurrent time dependent manner. To develop robust diagnostic model, which called 'meta-marker', logistic regression model was used. Finally, we found a best combination of three target proteins reflecting recurrence time points and showing best predictive diagnostic capability. **Conclusion:** 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 early diagnosis in recurrent lung cancer patients.

Keywords: Meta-marker, recurrence, lung cancer, multiple reaction monitoring

P07.12 Affinity Proteomics for the Identification of Biomarkers of Drug-Induced Liver Injury in Humans

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Introduction and Objectives: Drug-induced liver injury (DILI) is a major concern in drug development where it is the 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 1196 serum and plasma samples from 241 individuals across four independent cohorts with antibody suspension bead arrays. **Methods:** 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 287 antibodies targeting 251 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). Findings were further investigated in healthy volunteers receiving heparins (n=281). **Results and Discussion:** By the application of highly multiplexed antibody suspension bead arrays, we have identified two proteins (CDH5 and FABP1) which were elevated in cases compared to controls. In the HV APAP and HIV/TB cohorts, CDH5 was elevated already in baseline samples, indicating a potential predictive use. FABP1 showed significant elevation after treatment initiation. Interestingly, this marker seemed to respond more rapidly compared to ALT in a number of individuals. Both markers were verified in the confirmatory cohort. In the heparin cohort, CDH5 showed relatively stable levels. **Conclusion:** 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.

Keywords: Biomarker discovery, affinity proteomics, Drug-induced liver injury, suspension bead arrays

P07.13 A Biomarker Panel to Rule-Out CT-Scan Lesions in Mild Traumatic Brain Injury

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Introduction and Objectives: Mild traumatic brain injury (mTBI) lesions are detected using CT-scan imaging. The majority of all CT-scans are negative for mTBI patients and moreover, they are harmful to the patients. Blood biomarkers have been investigated for their capacity to reduce the number of unnecessary CT-scans. The most promising protein so far is the S100b with 30% specificity and 100% sensitivity. Three protein have previously been highlighted in brain injury models; GSTP1 (glutathione S-transferas pi), NDKA (nucleoside diphosphate kinase A) and H-FABP (heart-type fatty acid binding protein). Here we investigated if these three proteins individually or in a panel could perform better than s100b and thus reduce the number of unnecessary CT-scan. **Methods:** The plasma levels of S100b, GSTP1, NDKA and H-FABP were measured using immunoassays. The mTBI patients (n=87), GCS 13-15, were recruited within 6h after trauma and dichotomized into CT-positive and CT-negative groups for statistical analyses where Mann-Whitney U test, ROC curves and Panelomix were used. **Results and Discussion:** Plasma GSTP and S100b levels were significantly increased in CT positive patients ($p<0.05$). The best individual performance was obtained by S100b (sensitivity: 100% and specificity 34%). However, a performance of 58% specificity and 100% sensitivity was obtained when combining S100b, GSTP and H-FABP in a panel. **Conclusion:** This study demonstrated that a panel of S100b, GSTP and H-FABP could be useful to rule-out unnecessary CT-scans. A further multicentric study is warranted.

Keywords: mTBI, biomarker, Panel

P07.14 Proteomic Investigation of Biomarkers Associated with Regression of Tumor Diseases after HDT/ASCT

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Introduction and Objectives: There is increasing evidence that autoimmunity can control the tumor in variety of malignancies [1]. In our case we focused on patient with multiple myeloma who developed an aplastic anemia type syndrome together with high titer of anti-carbonic anhydrase I autoantibodies after high dose therapy (HDT) with autologous stem cell transplantation (ASCT). Tumor regression without relapse was observed in this patient [2]. The mechanism of this phenomenon is still unclear, so we continue in screening the patients´ sera to identify other associated interactive protein(s). **Methods:** Firstly, we separated lysates of two different human cell lines (adherent breast adenocarcinoma cells - SKBR3 and suspension erythroleukemia cells - HEL92.1.7) using two-dimensional gel electrophoresis (2-D SDS-PAGE) followed with western-blot (WB) analysis for identification of proteins immunoreactive with applied sera. Detected protein spot(s) were excised from 2-D gel and after in-gel digestion were analyzed by MS and MS/MS (MALDI LTQ Orbitrap XL). Obtained results were confirmed by using commercial antigen(s). **Results and Discussion:** We observed by SDS-PAGE/WB analysis with chemiluminescence detection one abundant protein spot (Mw 48 kDa with basic character). Protein α-enolase was identified by MS analysis, which is known as tumor-associated antigen inducing specific immune responses in cancer patients [3]. **Conclusion:** We hope that this information could be helpful for explanation of the described phenomenon and applicable in various diagnostic and therapeutic approaches. Acknowledgements: This work was supported by the grant of University of Pardubice SGFCHT 04/2015 and by the project of Ministry of Education, Youth and Sports of the Czech Republic CZ.1.07/2.3.00/30.0021 “Strengthening of Research and Development Teams at the University of Pardubice“. References: [1] Nissen C. and Stern M.: Autoimmunity Reviews 9 (2009) 11–16, [2] Lakota J., et al.: Neoplasma 55(6): 488-92 (2008), [3] Capello M., et al.: FEBS Journal 278: 1064-1074 (2011).

Keyword: autoantibodies, tumor regression, carbonic anhydrase I, α-enolase

P07.15 Refinement of PRM Methods to Improve Sensitivity and Accuracy in Peptide Quantification

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Introduction and Objectives: Targeted mass spectrometry quantification based on high-resolution is increasingly adopted to conduct various biological and biomedical studies. Refinements of the parallel reaction monitoring (PRM) methodology were performed to improve quantification accuracy in clinical applications. They relied on the concurrent addition of internal standards (IS) in different isotopic forms and amounts into the samples to control the acquisition. **Methods:** The analyses were performed on a quadrupole-orbitrap instrument (Q-Exactive HF, Thermo Scientific). Internal standards were synthetized in different isotopic forms and spiked into the samples at well-defined concentrations. The mass spectrometer was operated either in conventional PRM mode or using in-house developed acquisition methods. For the latter, the instrument programming interface (API) was used to create application to control the acquisition. **Results and Discussion:** Model experiments have demonstrated some interdependence between the characteristics of a peptide assay (i.e., LOD, LOQ, and linearity range) and the parameters of PRM acquisition, including ion trapping parameters. This was confirmed by the analyses of actual clinical samples, which showed occasionally some inconsistencies with respect to the determination of the actual concentrations of the peptides of interest. Refinements of the conventional PRM methodology were implemented to improve its overall quantification performance. First, the benefits of spiking different isotopic variants of IS into the samples in different amounts were evaluated. This strategy is well adapted to the recently introduced high-sensitive internal standard triggered-PRM (IS-PRM, Gallien et al., MCP, 2015) by allowing to distinguish one IS to drive in real-time the measurement of endogenous peptides, while the second is used for actual quantification. A refined control of the ion accumulation/trapping process further improved the quantification accuracy. The benefits of the refinements will be demonstrated by analyzing lung cancer markers in bodily fluids. **Conclusion:** The PRM method was improved to detect, reliably identify, and quantify peptides in biological samples.

Keywords: parallel reaction monitoring, real-time data analysis, biomarker, Targeted proteomics

P07.16 Biomargin: Protein Biomarkers of Renal Graft Injuries in Kidney Allograft Recipients

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Introduction and Objectives: In renal allograft recipients, histological examination of graft biopsies is the gold standard to confirm graft injuries, but biopsies are invasive and histological grading is not very robust. There is thus a need for robust, non-invasive methods to predict and diagnose acute and chronic graft lesions, to improve patient treatment, quality of life and long-term graft survival. One of the goals of the presented research is to discover urine biomarkers with good diagnostic performance. **Methods:** 130 banked urine samples of patients with different renal graft conditions (normal biopsy controls, antibody-mediated rejection, IFTA (Interstitial Fibrosis/Tubular Atrophy) and T-cell mediated acute rejection) were depleted for albumin and digested into tryptic peptides. All peptides were separated and analyzed using nanoLC-MS/MS in DDA mode (Orbitrap Velos). Peptides were identified using Peaks and Sequest. In house developed software was used for targeted quantification of the confident PSMs at the MS1-level across the 130 LC-MS runs. **Results and Discussion:** On average 2.233 MS1 and 20.440 MS2 scans were recorded for each LC-MS run. Approximately 13% of the MS2 scans resulted in confident peptide-spectrum-matches, with only first ranked peptides maintained in the peptide list. Because of high variation in the LC chromatograms, no commercial software packages could be used for quantification of the peptides because chromatogram alignment did not work. Using in house developed software we could quantify all confident PSMs at the MS1-level. Unsupervised clustering of a subset of the normalized data (Constand) shows very nice clustering of the different conditions. **Conclusion:** Further data analysis will have to show the clustering results for the entire dataset and the most discriminating proteins.

Keywords: biomarker, Urine, kidney allograft

P07.17 Differential Proteomics Identifies CFH Proteolytic Species as Possible Early Cancer Biomarkers

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Introduction and Objectives: Breast cancer is one of the most common cancers among female population worldwide. We have been particularly interested in whether proteolytic species in human blood plasma can be biomarkers for detection of early breast cancers. Thus, a new differential proteomics approach has been implemented to evaluate this hypothesis. **Methods:** We first collected plasma samples from patients before and after surgical treatment. These pairs of samples were then subjected to a modified two-dimensional differential gel electrophoresis (modified 2-D DIGE), comprising fluorescent dye labeling, macroporous reverse phase (mRP) HPLC and reducing/non-reducing SDS-PAGE. The difference protein species were analyzed with LC-MS/MS. Cleavage site-specific antibodies have been produced to perform a large-scale examination of plasma samples. **Results and Discussion:** Modified 2-D DIGE indeed revealed the presence of a group of proteolytic species in some breast cancer patients. Notably, these species disappeared from the plasma after the diseased tissue was surgically removed. From the same mRP fraction, a pair of complement factor H (CFH) derivatives were identified using LC-MS/MS analyses. Based on their molecular masses and peptide coverage maps, their formation is likely due to specific protein cleavage. Through a series of examination, we conclude that proteolytic removal of Arg-341 is likely the molecular mechanism that leads to these findings. While these biomarkers can be specifically detected in breast cancer patients at early and advanced stages, it is quite encouraging that positive detection is shown in 20-25% of patients with stage 0 and 1 diseases. **Conclusion:** Our results show the utility of this novel strategy in detection of cancer-specific proteolysis. The promise of these proteolytic species as early cancer biomarkers is particularly remarkable, since their detection can be applied to establish higher cure rates and thus lead to better prognosis for breast cancer patients.

Keywords: Breast cancer, proteolytic process, Early cancer biomarker

P07.18 Towards Minimally Invasive Diagnosis of Prostate Cancer

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Introduction and Objectives: Today diagnosis for prostate cancer (PCa) is based on PSA, DRE and biopsy. Blood tests are harmless for patients but unfortunately, PSA screening is prone to a high false positive rate. Additionally biopsies are needed to stratify tumors with respect to their aggressiveness to decide for an optimal treatment. Therefore, new biomarkers to detect the disease reliable and to stratify detected tumors are urgently needed. A broader set of biomarkers complementing the existing PSA screen could help to detect PCa reliably in an early stage. Additionally, biomarkers are needed to stratify tumors by their aggressiveness and the most likely prognosis for the patient to decide for a more personalized treatment strategy. Ideally such biomarkers would be detectable so that clinically relevant information could be obtained in a minimally invasive manner. **Methods:** A systematic biomarker study is perfomed on blood samples of PCa patients to identify such sets of biomarkers. Glyco-proteins are known to represent changes in tissue and are therefore the target of the analysis. The novel massively parallel targeted, data-independent acquisition method SWATH-MS is used to measure the glyco-proteome globally in a randomized setup. For SWATH-data-extraction and quantification, the open-source software openSWATH was used. To increase the information contents of plasma analysis, proteins targeted in the plasma-cohort will be derived from tissue proteomic measurements from tumor biopsy samples with known disease outcome. Potential biomarkers will be revealed using statistical pipelines and network-based methods. **Results and Discussion:** Preliminary results showed 200 glyco-proteins from more than 2000 peptides were identified reproducibly among all samples present in the study. The first analysis displayed high correlation between biological replicates and supports the high quality of the systematic study design. **Conclusion:** In a systems biology approach data from longitudinal follow-ups of the patients and from PCa tissue samples will be integrated to validate potential biomarkers and find global coherences.

Keywords: prostate cancer, personalized medicine, biomarkers, SWATH-MS

P07.19 Serum Cytokines, Chemokines and Soluble Receptors for Evaluation of Pulmonary Tuberculosis

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Introduction and Objectives: The needs for rapid, sensitive and reliable biomarkers to detect pulmonary tuberculosis are increasing for tuberculosis control. The aim of this study is to analyze

serum biomarkers for evaluating pulmonary tuberculosis and discriminating pulmonary tuberculosis patients from healthy controls. **Methods:** This study was based on 300 Korean adult populations. To analyze multiple cytokines, chemokines and soluble receptors, unstimulated serum samples from 73 Korean pulmonary tuberculosis patient and 79 healthy controls were tested using luminex multiplexed bead array platform as the first set. Biomarkers were validated for the second independent validation set (83 pulmonary tuberculosis patient and 65 healthy controls). **Results and Discussion:** Among 10 tested cytokines, chemokines and soluble receptors [IFN-r, macrophage inflammatory proteins (MIP-1a), eotaxin, IL-6, IL-8, monocyte chemotactic protein 1 (MCP-1), TNF-a, soluble CD40 ligand (sCD40L), human soluble IL-2 receptor alpha chain (sIL-2Ra) and Lipocalin-2 (LCN2, also known as neutrophil gelatinase-associated lipocalin], eotaxin, MIP-1a, sIL-2Ra, and LCN2 were different significantly in median serum concentration between pulmonary tuberculosis patients and healthy controls ($P < 0.05$). The concentrations of eotaxin and sIL-2Ra were higher in pulmonary tuberculosis patients than controls, while those of MIP-1a and LCN2 were lower. Single cytokines or chemokines differentiated pulmonary tuberculosis and controls with an area under the receiver operating characteristic curves of 0.832 for eotaxin, 0.697 for MIP-1a, 0.816 for sIL-2Ra, and 0.794 for LCN2. Those biomarkers were validated by the second independent validation set. **Conclusion:** Unstimulated serum eotaxin, MIP-1a, sIL-2Ra, and LCN2 could be used as promising immunological markers for diagnosis of pulmonary tuberculosis infection.

Keywords: Tuberculosis, biomarker, cytokine, luminex

P07.20 Identification of Prognostic Biomarkers for Oral Cancer by Low-Molecular-Mass Secretome Profiling

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Introduction and Objectives: The profiling of cancer cell secretomes is considered to be a good strategy for identifying cancer-related biomarkers, but few studies have focused on identifying low-molecular-mass (LMr) proteins (<15 kDa) in cancer cell secretomes. **Methods:** In an effort to discover novel biomarkers/therapeutic targets for oral squamous cell carcinoma (OSCC), we used tricine-SDS-gel-assisted fractionation in conjunction with liquid chromatography-tandem mass spectrometry (LC-MS/MS) to systematically identify LMr proteins in the secretomes of five OSCC cell lines. We then analyzed a number of OSCC tissue transcriptome databases available in the public domain, searching for proteins that are specifically overexpressed in OSCC tumor cells compared to the normal oral epithelium. **Results and Discussion:** A total of 1718 nonredundant proteins were detected in the five OSCC LMr secretomes, as either intact proteins or degraded fragments. Of them, 248 (14.4%) were found to have theoretical intact molecular masses less than 15 kDa, and were thus further classified as "true" LMr proteins. Cross-matching of these 248 LMr proteins with nine OSCC tissue transcriptome datasets dentify 33 LMr genes/proteins that were highly upregulated in OSCC tissues and secreted/released from OSCC cells. Immunohistochemistry and quantitative real-time PCR were used to verify the overexpression of two candidates, HMGA2 and MIF, in OSCC tissues. The overexpressions of both proteins were associated with cervical metastasis, perineural invasion, deeper tumor invasion, higher overall stage, and a poorer prognosis for post-treatment survival. Functional assays further revealed that both proteins promoted the migration and invasion of OSCC cell lines in vitro. **Conclusion:** Collectively, our data indicate that the tricine–SDS-gel/LC–MS/MS approach can be used to efficiently identify LMr proteins from OSCC cell secretomes, and suggest that HMGA2 and MIF could be potential tissue biomarkers for OSCC.

Keywords: Oral Cancer, low-molecular-mass secretome, HMGA2, MIF

P07.21 Absolute Quantification of the Marker Candidates in Ascites Using Multiple Reaction Monitoring

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Introduction and Objectives: Gastric cancer (GC) is a highly lethal malignancy and most often occurs in East-Asia. GC specific biomarker is necessary for early screening and diagnosis of the patients with GC due to the limitation of current method such as gastroscopy. Ascites is a good source of discovering biomarker, because it contains the secreted proteins from malignant cells, growth factors, and cytokines. We validated 6 biomarker candidates (SNU_1, SNU_2, SNU_3, SNU_4, SNU_5, and SNU_6) which were selected by our previous quantitation results using Parallel Reaction Monitoring (PRM) and Multiple Reaction Monitoring (MRM) assay. **Methods:** We performed the absolute quantitation using stable isotope standard (SIS) peptide (>95% purity) in benign (N: 25) and cancer groups (N: 44). For more accurate and stable quantitative assay, we followed the CPTAC assay guideline (CPTAC Assay Development Working Group, Version 1.0), which provides a list of experiments to help users to get more reliable data. Before we performed MRM assay using the individual samples, we first evaluated whether or not our MRM assay was accurate, stable, and reproducible. **Results and Discussion:** In this study, we were completely able to generate response curve, validate repeatability, and assess selectivity for 6 biomarker candidates. From the 69 individual sample assay, we successfully determined the 6 protein endogenous concentration range in ascites. In quantification analysis, 4 proteins (SNU_2, SNU_3, SNU_4, and SNU_5) were quite differentially expressed in control versus cancer groups (AUC value > 0.7). From the stepwise-logistic regression (stepwise-LR) analysis, 2 verified markers (SNU_2, SNU_4) were selected, which resulted in a merged AUC value of 0.935. **Conclusion:** The 4 protein markers and 2-protein marker panel (SNU_2, SNU_4) can be used as baseline data for the discovery of novel biomarkers for detecting gastric cancer and for further validation using a larger panel of proteins.

Keywords: Absolute Quantitation, MRM, Gastric cancer biomarkers, Multi-marker panel

P07.22 SOMAscan™ Assay: A Proteomic Platform That Can Also Detect SNPs

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Introduction and Objectives: Slow Off-rate Modified Aptamers (SOMAmer® reagents) are a novel class of affinity binding reagents made from single-stranded DNA engineered with hydrophobic side chains. These modifications on the 5-position of uracil greatly expand the physicochemical diversity of the large combinatorial SELEX libraries from which they are selected, resulting in binding molecules to more proteins, and with higher affinity, than observed with traditional aptamers. The hydrophobic nature of these interactions results in exquisite shape complementarity

between the reagents and their protein targets. SOMAmer reagents have proven effective as reagents for biomarker discovery, diagnostic products, and research tools. SomaLogic has developed a proteomic assay called SOMAscan™ assay for biomarker discovery that transforms protein concentrations in a biological sample into a corresponding DNA signature that can be measured using any DNA quantification technology. **Methods:** Human plasma measurements were made on the SOMAscan assay, measuring 1129 human proteins in parallel from a single sample. Binding to recombinant proteins was measured using radiolabeled DNA and protein capture on Zorbax resin. **Results and Discussion:** Recent SOMAscan data have suggested that certain SOMAmer reagents can distinguish between proteins resulting from single-nucleotide polymorphisms (SNPs) and wild-type proteins in human plasma. One such protein is the Low affinity immunoglobulin gamma Fc region receptor II-a. We have demonstrated that the SOMAmer reagent selected against the H167R mutant of the protein, resulting from a SNP, does not bind the H167 form. As a result, proteomic measurements in the SOMAscan assay can be utilized to not only detect protein levels, but, in some cases, identify individuals expressing alternate forms of proteins. **Conclusion:** The SOMAscan assay measures over 1000 human proteins in biological samples. For some analytes, the SOMamer reagents are sensitive to protein changes resulting from SNPs. The ability to measure these types of changes in a proteomic assay provides a valuable tool across many research areas.

Keywords: SNP, affinity reagent, Biomarker discovery, FCGR2A

P07.23 Stable Isotope Labeled Peptide Kits for Multiplexed Analysis of Biologically Relevant Protein Sets

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Introduction and Objectives: Targeted proteomics as an efficient approach for protein identification and quantification is dependent on the availability of peptide standards for assay development and protein quantification. Here we present the design, preparation and application of ready to use kits containing stable isotope labeled (SIL) SpikeTides™ peptides (1,2) for the efficient, multiplexed quantification of biologically relevant target classes. **Methods:** To design the new peptide standard kits, proteotypic peptide sequences were selected for a range of protein classes and antigen targets. Non-quantified, stable isotope labeled peptides were prepared by SPOT™synthesis (3) and combined to equal peptide amounts in pools. In addition, absolutely quantified peptides were prepared using a novel and highly accurate procedure based on a UV-active tag that is removed after quantification by tryptic digestion (2). The resulting peptide kits were analyzed by LC-MS in order to detect or quantify proteins in biological samples. **Results and Discussion:** Peptide standard kits were prepared to enable the multiplexed detection and quantification of the following targets: •Cytokines: 12 cytokine kits covering a variety of species •Hormones: A kit covering the majority of human peptide hormones •CEF CD8: A reference kit for immunodominant T-cell epitopes from EBV, CMV and Flu •Cytokines Activation Loops: >300 phosphorylated heavy labeled and unphosphorylated peptides in a single kit to study the kinase activation status •Metabolic Enzymes: 24 enzymes involved in the human central energy metabolism •Histones: Histone peptides covering important PTMs **Conclusion:** The new kits were used in proof of concept studies for the multiplexed quantification of proteins and epitopes in biological fluids and tissues. (1) (a) Picotti et al., Nat. Methods 2010, 7, 43-46. (b) Wilhelm et al., Nature 2014, 509, 582-587. (2) Schnatbaum et al., Non-peer-reviewed application-note in Nat. Methods 2011, 8. (3) Wenschuh et al. Biopolymers 2000, 55, 188-206.

Keywords: Targeted proteomics, Stable Isotope label, Peptide kit, quantitation

P07.24 Efficacy Assessment of a Dietary Functional Food on Diabetic Subjects

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Introduction and Objectives: Persimmon leaf extract (PLE) has shown beneficial health effects in treating allergies, constipation, dermatitis, diuresis, and inflammation. However, little is known about the in vivo effects of PLE on these conditions. We aim to find molecular markers that can be used to evaluate efficacy of PLE dietary supplements based upon the proteomic profiles of saliva, serum, and urine samples obtained from diabetic patients. **Methods:** First, the proteome differences between the healthy and metabolic syndrome subject were analyzed, and second, proteomic analysis was performed to find molecular markers for efficacy assessment of PLE on diabetic patients. Participants were subjected to a randomized crossover study where they were treated with PLE or placebo twice a day for eight weeks, followed by a four-week wash out period before inverting the treatment regimen. Saliva, serum, and urine samples from healthy, metabolic syndrome, and diabetic subjects were analyzed by LC–MS/MS data-dependent acquisition on the Orbitrap Fusion. Data analysis was performed using Comet search engine, MaxQuant, and QSpec software. **Results and Discussion:** Preliminary results identified 443 urinary proteins through comparative proteomics analysis between healthy and metabolic syndrome subjects, with 38 proteins having ≥2-fold increased expression among metabolic syndrome subjects. TPM3, Fibulin-2, APOD, IGFBP6, and Kallikrein-11 were most up-regulated, while 28 proteins, including CA-2, Annexin-AI, TFF-1, and GM2A, showed down-regulation in metabolic syndrome subjects. Notably, Complement C3, which is involved in diabetes and obesity, was found only in the metabolic syndrome patient group, whereas FSTL3 and GPR116 were absent in the metabolic syndrome patient group. **Conclusion:** The proteome of healthy and metabolic syndrome subjects showed distinct differences and we have identified a few candidate urinary proteins for the metabolic syndrome. Further proteomic studies are underway to discover serum and saliva molecular signatures for metabolic syndrome as well as to understand therapeutic potential of PLE in disease amelioration.

Keyword: Biomarkers, Diabetes, Metabolic Syndrome, Persimmon leaf extract, Proteomics

P07.25 SRMAtlas: Towards Defining Tuberculosis Biomarkers

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Introduction and Objectives: Tuberculosis (TB) is an infectious disease caused by Mycobacterium tuberculosis (Mtb). With one third of the world population being infected and 9 million new cases of active disease per

year, TB results in 1.5 million deaths annually. TB vaccines provide limited protection and current TB diagnostics have low specificity or are costly and not used widely. Our goal is to identify and quantify Mtb proteins released into human body fluids to provide molecular markers with adequate sensitivity and specificity that can be used as a triage field test. **Methods:** We used a multi-tiered approach to determine Mtb protein candidates by selecting targets that are i) highly expressed, ii) differentially expressed in response to environmental stresses encountered in the host, iii) secreted or iv) known Mtb antigens. We developed a quantitative targeted approach to evaluate over 500 Mtb proteins using SRM assays based on the Mtb SRMAtlas. Sample preparation methods tailored to different human tissues were developed to screen with established SRM assays. **Results and Discussion:** We developed a prioritized list of over 500 proteins and selected over 2000 peptide surrogates to develop quantitative SRM assays. We determined the best performing SRM assays and deployed these to screen different human tissues from TB suspect patients. Our efforts resulted in positive identification of Mtb peptides with 26 SRM assays. Several SRM assays defined at the limit of detection need further verification utilizing additional fractionation steps. SRM assays to identified Mtb proteins have been optimized to increase their sensitivity and are used to verify these targets in a large sample cohort from different geographic sites. **Conclusion:** We have utilized the SRMAtlas to define highly sensitive SRM assays to identify Mtb proteins in human body fluids from TB suspect patients. These proteins provide the potential for development of a cost-effective molecular triage tests for TB suspect identification.

P07.26 Simple and Efficient Fractionation via Solid-Phase Extraction for In-Depth Proteome Profiling

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Introduction and Objectives: Over the previous decade, mass spectrometry-based proteomics methods have been advanced to identify thousands of peptide. **Methods:** In this study, we evaluated a reversed phase solid phase extraction (RP-SPE) method over a broad pH range for in-depth proteome profiling. Our results demonstrate that a broad pH range RP-SPE is superior to the existing high pH fractionation in the total number of peptides detected when analyzing the same number of fractions under identical LC–MS/MS condition. **Results and Discussion:** Compared with fractionation at high pH, the use of concatenated sequential high–and low-pH RP-SPE fractionation strategy resulted in ~1.4 fold increases in the number of peptide and protein identifications, respectively. In addition to broader identifications, advantages of our broad pH approach include improved protein sequence coverage, reduced sample losses and peptide selectivity as a simplified sample processing without liquid chromatography system(LC) for collecting of each fraction. **Conclusion:** Based on this methodology, clinical samples diagnosed with biochemical recurrence or without recurrence after cancer treatment were used for LC-MS/MS-based discovery and longitudinal evaluation of potential protein biomarkers. Taken together, our results demonstrate that the concatenated RP fractionation strategy is an attractive alternative as a strategy for in-depth proteomic studies such as further clinical biomarker screening.

Keywords: high-pH, low-pH, recurrence marker, cancer

P07.27 Identification of CLIC1 as the Potential Diagnostic Marker for Epithelial Ovarian Cancers

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Introduction and Objectives: Ovarian cancer remains the most fatal gynecological malignant tumor to women. Early diagnosis is the key to increase the survival rate of ovarian cancer patients. The aim of this study was to find the potential diagnostic marker of ovarian cancer. **Methods:** Quantitative proteomics was employed to find the differentially expressed proteins between ovarian cancer tissues and normal ovary tissues, while western blotting and immunohistochemistry were used subsequently to confirm the upregulation of LGALS3BP and CLIC1 in tumor tissues. To understand the role of CLIC1 in ovarian cancer, an ovarian cancer cell line A2780-CLIC1-KN in which the expression of CLIC1 was knocked down was established, and the proliferation rates and resistance to cisplatin of A2780-CLIC1-KN were characterized. **Results and Discussion:** In the present study, we identified 7282 proteins from ovarian cancer and normal ovary tissues by TMT-based quantitative proteomics. 430 proteins were found to be differentially expressed between ovarian cancer and normal tissues, which involve in various cellular processes, including metabolism, developmental process, immune system and biological adhesion. Expression levels of CLIC1 and LGALS3BP in ovarian cancer tissues were higher than those in normal ovary tissues, as confirmed by western blotting and immunohistochemistry. A2780-CLIC1-KN exhibited a slower proliferation rate, increased sensitivity to hydrogen peroxide and cisplatin treatment compared to A2780 cells, suggesting CLIC1 are involved in regulation of cell growth, resistant to oxidative stress and cisplatin in ovarian cancer cells. **Conclusion:** We identified that expressions of CLIC1 and LGALS3BP were up regulated in ovarian cancer patients, which can be potential diagnostic biomarkers and therapeutic target of ovarian cancer.

Keywords: Epithelial ovarian cancer, CLIC1, oxidative stress, diagnostic biomarker

P07.28 Identification of Blood-Based Cancer Biomarkers by Deep Microparticle Proteomics Using PROMIS-Quan

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Introduction and Objectives: Unbiased identification of novel biomarkers in the blood, towards the development of non-invasive diagnostic/prognostic tests, is one of the holy grails in cancer research. However, mass spectrometry (MS) based plasma proteomics is limited by the tremendous dynamic range of the plasma, which leads to masking of 'tissue leakage' proteins by highly abundant plasma proteins. **Methods:** To overcome the dynamic range limitation we examined the plasma microparticle proteome. Microparticles are large vesicles that are constitutively shed from all cell types into the blood, carrying a proteomic signature of their cells of origin. We developed PROMIS-Quan, PROteomics of MIcroparticles with Super-SILAC Quantification, a novel MS-based technology for plasma microparticle proteome quantification. PROMIS-Quan enables a two-step relative and absolute SILAC quantification. First, plasma microparticle proteomes are quantified relative to a super-SILAC mix composed of cell lines derived from different tissues. Next, the absolute amounts of selected proteins of interest are quantified relative to the super-SILAC mix.

Results and Discussion: We reached an unprecedented depth of over 3,000 plasma proteins in single runs of healthy donors. We further tested PROMIS-Quan on two different datasets. The first compared plasma samples of prostate cancer patients and healthy individuals. In total we identified over 5,000 plasma-microparticle proteins, revealed a predictive signature for prostate cancer in plasma, and determined the absolute quantitative changes upon treatment of prostate specific antigen (PSA), a well-known prostate cancer biomarker. The second dataset identified markers in serum samples that can predict response to immunotherapy for melanoma patients. In total we identified over 4,000 proteins and found significantly changing proteins between responders and non-responders to immunotherapy treatment. **Conclusion:** Altogether we propose PROMIS-Quan as an innovative high-throughput platform for biomarker discovery, validation and quantification in both the biomedical research and in the clinical worlds.

Keywords: Biomarker discovery, Plasma proteomics, super-SILAC

P07.29 Affinity Proteomic Profiling Reveals Molecular Subgroups in Systemic Lupus Erythematosus

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Introduction and Objectives: Systemic Lupus Erythematosus (SLE) is a heterogenous systemic autoimmune disorder with a prevalence of about 50 cases per 100,000 in western countries and roughly 10 times higher prevalence in women. It is not widely understood why the prevalence is higher in women nor the heterogenous manifestations of the disease. Additionally, today there is no specific diagnostic test for SLE, classification and stratification of patients are currently based on a selection of different clinical and immunological criteria. Lately, there have been a lot of genetic studies made on SLE revealing many inflammation-related genes as a part of potential pathogenesis. However, few of these targets have been studied on protein level on larger sample collections of SLE patients and/or verified as potential biomarker. The goal of this study is to perform targeted affinity proteomic profiling in a set of 320 SLE patients (with age and gender matched healthy controls) to better understand the disease, to find potential biomarkers and characterize molecular subgroups for certain phenotypes within the disease. **Methods:** A targeted selection based on literature and clinical knowledge, resulted in 281 proteins to be targeted by in total 367 antibodies from the Human Protein Atlas. The antibodies were covalently coupled to color-coded magnetic beads. The resulting bead array was then combined with plasma that had been directly labeled with biotin, and read-out was made by adding a streptavidin-fluorophore, using the Luminex-technology. **Results and Discussion:** By dividing our dataset prior analysis in two comparable groups, we've used the two independent datasets to validate our potential targets. We have found 26 proteins that can significantly distinguish between the 320 cases and 320 controls and 14 additionally proteins that can distinguish between different subgroups of patients. **Conclusion:** Affinity proteomic profiling of SLE sample collection revealed potential biomarkers for SLE and the sub-phenotypes, suggesting different molecular subgroups within the disease.

Keywords: SLE, Antibody Arrays, Protein Profiling, affinity proteomics

P07.30 Characterization of the Differences in Cervical Mucus Composition during the Menstrual Cycle

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Introduction and Objectives: The chemical composition of the cervical mucus (CM) change cyclically throughout the menstrual cycle. Aim of this study was to identify the constitutive compostition of CM of fertile women and the proteomic changes throughout the cycle. **Methods:** CM samples were collected by 5 fertile women before, during, and after ovulation of the same menstrual cycle. An aliquot of the soluble acidic fraction of each sample was subjected to tryptic digestion and analyzed by an Ultimate3000 Nano/Micro-HPLC apparatus equipped with an FLM-3000-Flow manager module, coupled with an LTQ Orbitrap XL hybrid mass spectrometer. Tandem mass spectra were analysed by the Thermo ProteomeDiscoverer 1.4 software, using SEQUEST as search engine. We firstly considered the panel of proteins identified in at least one sample for each menstrual cycle phase. Subsequently we further considered the proteins exclusively identified in each ovulatory phase. Proteins identified by SEQUEST were analyzed using the PANTHER software, to evaluate the GO annotations for molecular function in the common proteins. The differences between pre-ovulatory and ovulatory exclusive proteins and between ovulatory and post-ovulatory exclusive proteins were also evaluated according to GO classes for molecular function. The label-free quantification of common proteins was performed using the Peak Area Calculation Quantification during the bioinformatic analysis by ProteomeDiscoverer software. **Results and Discussion:** 59, 81 and 43 proteins were respectively identified in the pre-ovulatory, ovulatory and post-ovulatory samples. 38 common proteins were identified. 42, 38 and 17 exclusive proteins were identified in pre-ovulatory, ovulatory and post-ovulatory CM. The majority of CM constituents has a catalytic activity, mainly related to hydrolase activity. The label-free quantitative analysis for the common proteins revealed a reduction of antileukoproteinase, after the ovulation, and a peak of haptoglobin at ovulation. **Conclusion:** This is the first application of high-resolution MS-based proteomics for the identification of CM constituents, identifying putative biomarkes of the female reproductive tract.

Keywords: cervical mucus, menstrual cycle, fertility, reproduction

P07.31 Alterations in PBMCs from Bulbar and Spinal ALS Patients with Different Rate of Disease Progression

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Introduction and Objectives: Gel-free Tandem Mass Tag® (TMT®) labelling combined with LC-MS/MS provides a powerful investigative tool to unravel molecular factors linked to phenotypic variability in neurodegenerative conditions, where immune responses can drive disease progression. The aim of this study is to identify and quantify regulated proteins in peripheral blood mononuclear cells (PBMCs) associated with clinical sub-types of amyotrophic lateral sclerosis (including bulbar (B-ALS) and limb onset (L-ALS) cases) and use them to clarify the phenotypic heterogeneity of this uncurable disease. **Methods:** Peptides derived from tryptic digestion of proteins extracted from

whole lysates of PBMCs from B-ALS (n=12) and L-ALS (n=12) sub-divided according to rate of neurological deterioration were labelled with TMT®s and analysed using LC-MS/MS. In-house bioinformatic platforms were used to identify significantly regulated peptides/proteins from the complex dataset. **Results and Discussion:** Unique PBMC protein signatures were found in B-ALS and L-ALS individuals with different disease progression rate. 95 proteins demonstrated a significant difference in abundance between B-ALS and L-ALS. Some of these proteins were mapped to molecular pathways, biological processes and molecular functions which were not identified before. PBMCs proteome was highly enriched in membrane proteins which are likely to play important roles in the immune system response. A specific pathway was identified associated to the rate of disease progression. **Conclusion:** These findings may be useful to predict therapies suitable for patients suffering from bulbar/spinal forms of ALS with different rate of disease progression.

Keywords: LC-MS/MS, Biomarker discovery, ALS

P07.32 In-Depth Analysis of Plasma Proteome for Discovery of Age-Related Macular Degeneration Biomarkers

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Introduction and Objectives: Age-related macular degeneration (AMD) is the leading cause of blindness in worldwide. Early detection and treatment of AMD are essential for preventing blindness and improving quality of life in elderly population. However, no serological markers for the screening and diagnosis of AMD have been validated. Therefore, we identified plasma protein biomarkers for AMD using a large-scale quantitative proteomic strategy. **Methods:** Plasma proteomes from 20 exudative AMD and 20 healthy control patients were comparatively profiled by LC-MS/MS. Proteins existing at statistically different levels were validated by ELISA and Western blot in 233 case-controlled samples. Newly discovered plasma biomarkers were further confirmed using in vivo and in vitro experiments. **Results and Discussion:** In plasma proteome, we identified 320 proteins using this profiling strategy. Among the proteins, vinculin, protein S100A9, triosephosphate isomerase, protein S100A8, protein Z-dependent protease inhibitor, C-X-C motif chemokine 7, and tenascin X showed significantly differential expression in AMD patient plasma compared to control plasma. The AUC for vinculin was 0.871 for discriminating between exudative AMD and controls (n= 201) and 0.879 for discriminating between AMD and controls (n= 233). A proteogenomic combination model using vinculin and two known risk genotypes in ARMS2 and CFH genes additionally provided excellent discrimination of AMD from controls (AUC= 0.916). In addition, the expression level of vinculin was strongly increased in retinal pigment epithelial cells of human eyes and its expression was elevated when exposed to oxidative stress. **Conclusion:** Taken together, Vinculin was identified as a potential plasma biomarker for AMD. The early diagnosis of AMD using novel plasma biomarkers with genetic modeling may enable timely treatment and vision preservation in the elderly.

Keywords: proteomics, plasma marker, vinculin, age-related macular degeneration

P07.33 Discovery and Validation of Biomarker Peptides in Plasma Using Two Types of Stable-Isotope Tags

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Introduction and Objectives: Detailed analysis of proteins and peptides in serum/plasma remains challenging due to the presence of many high-abundance proteins, the extensive complexity caused by posttranslational modifications, and considerable individual variability. In particular, detailed analysis and identification of native peptides is extremely difficult due to the tremendous variety of cleavage possibilities and posttranslational modifications, which results in extremely high complexity. Therefore, widely ranging searches based on peptide identification are difficult. Herein, we established a strategy for discovering and validating the disease-related native peptides in serum/plasma including unidentified peptides using two types of stable-isotope tags. **Methods:** For the discovery study, the strategy combined isobaric tag labeling, amine-reactive 6-plex tandem mass tag labeling and a modified differential solubilization method (DS method) for high-yield peptide extraction [Y. Kawashima, et al. J. Proteome Res. 9, 1694, 2010]. The peptides were separated by nano-flow HPLC and introduced to mass spectrometer, Q-Exactive (Thermo Fisher Scientific). For the validation study, the peptides extracted by the modified DS method were labeled by the isotope tag/dimethyl labeling method and analyzed by LTQ-Orbitrap Discoverer employing conventional HPLC. **Results and Discussion:** We quantitatively analyzed six pooled plasma samples (three pre-surgery and three post-surgery) to discover potential candidate biomarker peptides of renal cell carcinoma. The concentrations of about 30 peptides were found to be altered following surgery. A preliminary validation study was conducted using 77 plasma samples to demonstrate the possibility that even unidentified potential candidate biomarker peptides can be verified using the dimethyl labeling method. The strength of this strategy is the use of isotope tag technologies, which make it possible to explore the deeper peptidome zone with equal accuracy by incorporating additional separation technologies. We believe this strategy will play a prominent role in the discovery of novel biomarker peptides that are indicative of the primary stages of diseases.

Keywords: native peptide, serum and plasma, biomarker, stable-isotope tag

P07.34 An Effective Method for Exploring Tumor-Characteristic Glycoforms of Cell Surface Mucins

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Introduction and Objectives: Clinical tissue sections provide useful information related to various tumors for omics-based biomarker discovery including glycoproteomics. However, current analytical methods targeting O-glycoform, such as mucins of a cell surface protein, corresponding to the characteristics of tumors on tissue sections are limited in terms of sensitively and throughput. Here, we demonstrated an effective method for glycoform analysis of MUC1 on minute fragments dissected from formalin-fixed paraffin-embedded (FFPE) tissue sections with the antibody-assisted lectin microarray. **Methods:** MUC1 was stained on FFPE clinical tissue sections, fragments of the MUC1-positive cells were obtained by laser microdissection (LMD), and the

glycoproteins were extracted. After the immunoprecipitation of MUC1, MUC1 glycan profiling was performed with an antibody-overlay lectin microarray and then lectin-antibody fluorescence double immunostaining was performed to verify the MUC1 glycan profile and the localization of these epitopes. **Results and Discussion:** We successfully obtained the tissue MUC1 glycan profiles from the MUC1-positive area of 2.5 mm^2 in 5 μm thickness using LMD. The differential glycan profiling with multivaliable analysis of 21 cholangiocarcinoma (CCA) and 52 pancreatic ductal adenocarcinoma (PDA) FFPE tissue sections showed clearly distinctive glycoforms. Further statistical analysis indicated the lectins showing a significant increase in either CCA or PDA. These results were also supported by the lectin-MUC1 fluorescence double immunostaining. We indicated that the glycoforms of MUC1 between CCA and PDA are quite different. **Conclusion:** We established an effective method for glycoform analysis of MUC1 corresponding to the characteristics of tumor on FFPE tissue sections. Our approach will provide a new possibility to the glycoproteomics-based research of the tissue sections focusing on O-glycosylation.

Keywords: glycan profiling, O-glycome, MUC1, biomarker

P07.35 Differential Expression of C3a and C5a in Allergic Asthma

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Introduction and Objectives: Allergic asthma is an airway inflammatory disease characterized by airway obstruction, increasing bronchovascular permeability, and airway hyperresponsiveness. Two phenotypes -- early responders (ER), who only experience an acute bronchoconstriction within minutes following the allergen exposure, and dual responders (DR), who also experience a further longer-lasting bronchoconstriction several hours after the initial exposure, are involved in the disease but the mechanism that leads to the two phenotypes remains elusive. Activation of the complement system, which is part of innate immunity, is strongly associated with the disease. Various models have shown that the complement anaphylatoxins, C3a and C5a, play an important role in regulating the allergic response owing to their pro-inflammatory effects. Therefore, we hypothesized that C3a and C5a are differentially abundant in plasma of ERs, DRs and non-asthmatic controls. **Methods:** 14 mild asthmatic and 6 non-asthmatic control individuals participated in our study. Peripheral whole blood samples were collected using EDTA tubes and the samples were further processed to obtain plasma. Quidel MicroVue™ C3a Plus and C5a EIA kits were used to measure C3a and C5a expression levels, respectively. Student's T-test was then used to compare C3a and C5a levels in plasma of ERs, DRs and non-asthmatic controls. **Results and Discussion:** C3a levels were lower in controls compare to ERs ($p < 0.01$) and DRs ($p < 0.05$) but there was no significant difference between ERs and DRs. C5a levels were up-regulated in ERs compare to DRs ($p < 0.01$) and controls ($p < 0.05$). The down-regulation of C5a in DRs may be due to the fact that C5a is protective during allergen sensitization. **Conclusion:** Both C3a and C5a expression levels are associated with allergic asthma. C3a is lower in non-asthmatics compare to ERs and DRs; C5a is higher in ERs compare to DRs and non-asthmatics. Further validation is ongoing and will be presented at conference.

Keywords: Allergic Asthma, Complement System, Late Asthmatic Response

P07.36 Fibulin-5 Is a New Biomarker Candidate for Hepatic Fibrosis

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Introduction and Objectives: Hepatic fibrosis and cirrhosis are still major health problems worldwide. Up to now, highly invasive biopsy still remains the diagnostic gold standard beside many disadvantages. In order to develop non-invasive diagnostic assays for assessment of liver fibrosis it is urgently necessary to identify molecules which are robustly expressed in association to hepatic fibrosis. **Methods:** We analyzed biopsied tissue sample from 95 patients with HBV/HCV-associated hepatic fibrosis using three different quantification methods. We performed a label-free proteomics discovery study to identify biomarker candidates using a subset of the cohort ($n = 27$). In parallel, gene expression data from all available clinical samples was was analyzed ($n= 77$). Finally, we performed a targeted proteomics approach (multiple reaction monitoring) to verify the candidates in samples independent from the discovery approach ($n = 68$). **Results and Discussion:** We identified Fibulin-5 (FBLN5) as a new biomarker candidate for hepatic fibrosis. Furthermore, we confirmed the significance of microfibril-associated glycoprotein 4 (MFAP4), lumican (LUM) and collagen alpha-1(XIV) chain (COL14A1) for diagnosis of hepatic fibrosis using three different quantification strategies. **Conclusion:** To our knowledge, no tissue-based biomarker discovery study for hepatic fibrosis has been performed using a cohort of comparable size. By this means, we add substantial evidence to the diagnostic relevance of the biomarker candidates examined in this study. With Fibulin-5 we present a new biomarker candidate related to fibrosis-associated tissue remodeling.

Keywords: Hepatic Fibrosis, multiple reaction monitoring, Fibulin 5, MFAP4

P07.37 MRM-Based Quantifications of Blood Biomarkers Using a Quantification Peptide-Tagged Fusion Proteins

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Introduction and Objectives: Proteins secreted, released or shed from the cultured cells, collectively termed the secretome, are promising biomarkers because they might be detectable in blood or other body fluids. Biomarker candidates found in secretome analysis must be quantitatively verified in patient samples before progress to clinical validation. Here, we introduce an absolute quantitation method of targeted protein in blood sample by immunoprecipitation-multiple reaction monitoring (MRM) assay without using synthetic peptides corresponding to individual targeted proteins. **Methods:** To easily obtain known concentration of internal standard (IS) proteins, we constructed a secretory expression plasmid vector for recombinant IS-proteins tagged with FLAG-peptide for high-yield

immunoaffinity purification, and with a common peptide sequence for high-sensitive detection by MRM. The plasmid vectors were introduced into the SILAC-labeled 293T cell line. Concentrations of resultant isotope-labeled IS-proteins in culture media could be determined by trypsin digestion after affinity enrichment with anti-FLAG antibody and detection of the common tagged-peptide by MRM. Culture media containing IS-proteins were spiked into serum samples at a certain concentration to determine serum level of target proteins. **Results and Discussion:** Immunoprecipitation with IS-protein by polyclonal antibody against target protein and detection by MRM provide absolute quantitative data of target protein in serum sample, irrespective of IP-capture efficiency. Using this method, we successfully measured serum levels of biomarker candidates for kidney cancer and prostate cancer (4.0-800 pM), which were identified from cancer secretome analysis, and found potential biomarker candidates which should progress to clinical validation. This methodology may be adapted quickly to various secreted protein biomarker candidates, and provide a technique to bridge the gap between discovery and validation platforms. **Conclusion:** not applicable

Keywords: biomarker, MRM, quantification, immunoprecipitation

P07.38 Analysis of Cryosections Enables Disease Classification, Validation and Correlation Clinical Data

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Introduction and Objectives: Endometriosis affects up to 10% of women in their reproductive years with symptoms ranging from severe pain and bleeding to delayed pregnancy and infertility. The disease is characterized by the presence of endometrial-like tissues outside the uterus, typically in the peritoneal cavity and ovaries. We present results from a large study with stakeholders from hospitals, several companies, and patients aimed at identifying markers from clinical tissues. Molecules including hormonal comparisons, mRNA analysis, immunohistochemistry, cytokines in addition to the proteome were measured. We report on the methods and results of comparisons of patient endometrium and ovarian endometrioma tissues, quantitation of those proteins representative for these tissues and on the development and application SRM assays in patient plasma and peritoneal fluid. Additionally, we report on the correlation of proteome measurements with other molecular methods including IHC and microarray analysis of the same tissue material. **Methods:** Sections of 10 μm were digested directly on the surface. Adjacent sections were stained by standard H&E. Comprehensive MS analysis of patient tissues employed: 1) AIMS - for proteotypic peptides based on mRNA expression; 2) DDA of healthy and control tissues; and 3) Directed Mass Spectrometry. Quantitative analysis was based on MS1 data. SRM assays were developed using Skyline and measured on a TQMS. **Results and Discussion:** Quantitative comparison of tissues revealed 214 differentially expressed proteins in endometrium and ovarian endometrioma of endometriosis patients, of which 88 were also differentially expressed by microarrays. Validation of results included IHC, WB and SRM analysis of 168 endometrium and ovarian endometrioma proteins. Updated results of SRM screening in plasma and peritoneal fluid will be presented. **Conclusion:** We provide comprehensive digital maps of the proteome in the pathological state of ovarian endometriosis, a panel of 168 complete SRM assays and new information about which proteins pathways appear to be active during in patients suffering from endometriosis.

Keywords: Endometriosis, Patients, SRM, quantitation

P07.39 Rapid and Automated Quantitation of Protein Biomarkers for Nutrition Status from Dried Blood Spots

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Introduction and Objectives: Nutritional status of patients in hospitals and other settings is not always easy to asses and a number of protein biomarkers have been used for this puprpose. Dried blood spots (DBS) are a convenient and inexpensive method for collection of blood samples for analysis allowing for convenient remote analysis, since DBS can be stored and shipped cheaply under ambient conditions. LC-MRM/MS with stable isotope-labeled standards (SIS) is a powerful strategy for precisely quantifying proteins in biological samples, but requires relatively expensive equipment and a laboratory staff capable of performing the analyses. To enable the widely distributed use of LC-MRM/MS, we have developed an LC-MRM/MS assay from DBS for 5 proteins that reflect nutritional status and will validate the assay on a large cohort of human samples. **Methods:** 18 Surrogate peptides for quantitation of the 5 target proteins were identified using our PeptidePicker software and both the SIS peptides and corresponding endogenous natural form (NAT) peptides were synthesized. To qualify the targets for quantitation, rigorous detectability and interference testing was performed, and this resulted in 10 qualified peptides from DBS. The 5 best performing of these 10 peptides were selected, and quantification of these qualified peptides using standard curves and a concentration-balanced SIS mixture was performed on DBS obtained from healthy volunteer donors. **Results and Discussion:** The protein concentrations determined by LC-MRM/MS were all within the reference intervals for the 5 selected proteins, and the CV's obtained from three replicate measurements ranged from 1.0-8.4%. The stability of these proteins in DBS was also checked, and the remaining concentration of the proteins after two weeks of storage at ambient conditions was >88%. **Conclusion:** A robust quantitative LC-MRM/MS assay with very good CV's for a 5-protein panel indicative of nutrition status has been developed. Validation of the panel on a cohort of 100-200 human samples is planned.

Keyword: Nutrition Blood MRM Quantitation

P07.40 Use of Spectral Deconvolution Centroiding to Find More Robust, Sample-Discriminating Biomarkers

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Introduction and Objectives: Biomarker discovery via LC/MS is often limited by inability to detect and resolve overlapped nearly isobaric species that were also unresolved chromatographically. This study evaluated the ability of mass spectral post-processing with advanced signal processing for peak detection, deconvolution and centroiding (PeakInvestigator™) to reveal additional robust, sample-differentiating, biomarkers. **Methods:** The Fiehn Lab published LC/MS analyses of 3 different food plate homogenates (Davis/Korean-inspired, American, or California diet) in six replicates each, using an Agilent 6550 at 40K resolution in positive

and negative ion modes. The profile data were centroided using both PeakInvestigator and the standard center-of-mass centroiding routinely used by the Fiehn lab. Multi-group XCMS feature analyses were conducted across the 3 food plates from each centroiding method independently. Only the most robust and statistically-significant (p-score < 0.01) sample discriminators (found in all six replicates of a food plate and not found in any single replicate of the other two plates) were included in this study. The resulting sample-specific features were then compared between the two centroiding methods. **Results and Discussion:** PeakInvestigator revealed 88 (3% of total features) new, robust, and sample-discriminating features that were not found with standard centroiding. These features reduce to 28 new monoisotopic biomarkers. Standard centroiding produced 363 (13% of total features) unique sample-discriminating features; however, 98% of these were not monoisotopic, or were below the threshold of statistical significance locally determined by PeakInvestigator. Only five unique monoisotopic biomarkers were reported via standard centroiding, and two of these were classified as "unique" by falling in between wide peak overlaps resolved only by PeakInvestigator. **Conclusion:** Without benefit of dynamic thresholding, standard centroiding can generate numerous statistically-insignificant features as unique sample-differentiating biomarkers. PeakInvestigator centroiding resulted in 28 new robust sample-discriminating biomarkers that were unresolved by standard centroiding.

Keywords: biomarker, deconvolution, centroiding, food

P07.41 More Biomarkers Discovered with Spectral Deconvolution Centroiding (Part 1)

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Introduction and Objectives: Peak overlaps created by isotopomers, multiply-charged species, and intermingled isotopic patterns continue to confound mass spectral biomarker analysis. This problem presents itself in all mass analyzers, at any resolution. In this study we compared a new advanced signal processing method (PeakInvestigator™) to conventional spectral centroiding practice on two LC/MS lipodomic samples from the SATURN Coronary Atheroma study. **Methods:** Raw LC/MS profile data were generated from two human plasma samples on an Agilent 6550, at both 20K and 40K resolutions. The 20K profile data were centroided by both PeakInvestigator and the standard center-of-mass centroiding routinely used by the Fiehn lab. The spectral mass lists produced by these two methods were then compared using XCMS, for prospective discoveries made uniquely by PeakInvestigator. The 40K resolution data were used to validate new chromatographic LC/MS features deconvolved by PeakInvestigator in the 20K data. **Results and Discussion:** A combined total of 978 chromatographic features were identified over both samples and centroiding methods, with 91% of these features being shared in common (similar in both mass and abundance). The remaining 83 features (9%) differed by more than 20% in abundance. Half of these were seen by both methods in at least one sample, differing only in chromatographic peak heights. However, 41 (4.2%), of these chromatographic peaks were revealed only by PeakInvestigator. From the 40K resolution runs 36 (3.7%) of these new features were validated as true positives. Accounting for isotopes, 23 unique monoisotopic true positive biomarkers were revealed by PeakInvestigator versus only 5 false monoisotopic detection events. **Conclusion:** The new advanced signal processing algorithms used in PeakInvestigator resulted in 3.7% more true chromatographic features being revealed from these LC/MS data with a Positive Predictive Value of 88% based on chromatographic features and 82% based on monoisotopic biomarkers.

Keywords: Lipidomics, TOF

P07.42 More Biomarkers Discovered with Spectral Deconvolution Centroiding (Part 2)

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Introduction and Objectives: Peak overlaps created by isotopomers, multiply-charged species, and intermingled isotopic patterns continue to confound mass spectral biomarker analysis. This problem presents itself in all mass analyzers, at any resolution. In this study we compared a new advanced signal processing method (PeakInvestigator™) to conventional spectral centroiding practice on 93 patient samples from the SATURN Coronary Atheroma study. **Methods:** Raw LC/MS profile data were generated from 93 human plasma samples on an Agilent 6550, at both 20K and 40K resolutions. The 20K profile data were centroided by both PeakInvestigator and the standard center-of-mass centroiding routinely used by the Fiehn lab. The differences between these spectral mass lists were determined using XCMS, for prospective discoveries made uniquely by each method. Statistically significant features with p-value < 0.05 and mean fold change > 1.2 were analyzed further using the 40K spectral data. **Results and Discussion:** A combined total of 1645 chromatographic features were identified among all samples using both centroiding methods, with 99% of these features being common to both centroiding methods (independent of significance level). More than half (69.1%) of these features differed in mass or abundance at 95% confidence between the two centroiding methods. Nine (0.6%) of the features were uniquely detected using standard centroiding. All of these were very low in abundance and are below the automated statistically-determined detection threshold of PeakInvestigator. Six (0.37%) features were uniquely revealed with PeakInvestigator (at 95% confidence), with a median abundance 100 times the statistical detection threshold. **Conclusion:** PeakInvestigator uniquely detected six new, real, chromatographic peaks in the LC/MS data set, which standard centroiding failed to detect in any sample. These data also suggest that the nine low abundance features uniquely reported by standard centroiding were statistically undifferentiated from noise.

Keywords: TOF, Lipidomics

P08: POSTER SESSION - PROTEOMICS AT PHARMA

P08.01 Improved Method for Assessing Immunoreactivity Coverage in Detecting Host Cell Protein in Biologics

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Introduction and Objectives: The level of host cell proteins (HCPs) is an important quality attribute for therapeutic monoclonal antibodies. Using ELISA, HCPs are typically monitored in the final drug substance and during process development to assess the effectiveness of the purification procedures. The anti-HCP polyclonal antibody (pAB) used for the ELISA assay must be evaluated for its immunodetection specificity against the total population of HCPs before it can be qualified for use in the HCP ELISA assay. This evaluation is accomplished through development of an immunoreactivity coverage assessment. The current study reports on an optimized and improved method for immunoreactivity coverage assessment with high reproducibility. **Methods:** Commercially available anti–E.coli pAB from Cygnus was labeled with an activated CyDye fluorescent dye. HCP samples were subjected to 2D western analysis. Immunoblot membranes were initially stained with Sypro Ruby. Following imaging, labeled Cygnus anti-E.coli antibody was used for the immunodetection. Membranes were scanned using Pharos FX Plus systems and PD Quest 2D analysis software was used for the spot analysis. Each sample was analyzed in duplicate and under non-reduced conditions. **Results and Discussion:** Two dimensional gel electrophoresis combined with Western blot currently is the "gold standard" for evaluation of the pAB coverage. However, the method has been plagued with numerous limitations, and many parameters need to be optimized due to the overall length and laborious nature of the assay. We have evaluated and optimized several key parameters in the current method for immunoreactivity coverage assessment of Cygnus' antibody used for detection of HCPs in the process development of monoclonal antibody therapeutics. Additionally, we have developed a reproducible and reliable 2D Western method using a combination of blot staining and fluorescent dye labeled pAB. **Conclusion:** The improved method greatly increases sensitivity and reliability when compared with the current method, and offers the potential of being used as a platform method.

Keywords: immunoreactivity coverage, monoclonal antibody, 2D Western blot, Host cell Proteins

P08.02 Comparison of SPRi, Nano-SPRi and ELISA in the Evaluation of rhGH in Crude Samples

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Introduction and Objectives: The concentration of human growth hormone (hGH) varies from high levels of 50–100 ng mL^{-1} to minimum levels of 0.03 ng mL^{-1} in blood. The misuse of recombinant hGH (rhGH) in sports has become a chronic problem. **Methods:** Surface plasmon resonance imaging (SPRi) is an analytical tool that allows you too directly (label-free) monitor and visualize biomolecular interactions by recording changes of the refractive index adjacent to the sensor surface in real time. To increase detection sensitivity, amplified SPRi uses a sandwich assay format and takes advantage of near infrared quantum dots (QDs). After direct SPRi detection of rhGH in spiked human serum, the SPRi signal is amplified by the sequential injection of detection antibody coated with near-infrared QDs (Nano-SPRi). **Results and Discussion:** In this work, the diagnostic potential of direct and amplified SPRi was assessed for measuring rhGH spiked in human serum and compared directly with commercially available ELISA kit. **Conclusion:** Based on our results, the main advantage of the SPRi and Nano-SPRi method is that rhGH concentrations can be measured in a quicker manner than in comparison with ELISA. In reference to sensitivity, ELISA (1 ng/mL) is comparable to SPRi (3.61 ng/mL) but nano-SPRi (9.20 pg/mL) provides a 3 order of magnitude improvement in sensitivity therefore enabling comfortably measurements at the lower biological levels of rhGH 0.03 ng/mL.

Keywords: blood, proteins, kinetics, biomarker

P08.03 Creating Quantitative Protein Maps as Remineable Resources in Pharmacodynamic Studies

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Introduction and Objectives: The burgenoning technology of quantitative proteome maps are of particular use to pharmacodynamics. Here proteome data can be leveraged not just as one experiment but as reusable resource serving multiple projects and purposes. This information, such as tissue distribution and abundance can be of great utility when considering PK/PD modelling, drug design, off target effects and other protein-centric drug considerations. Here we describe, the generation of a "proteome map" to achieve such goals. **Methods:** Human and rodent tissue samples were prepared and analyzed by long gradient UHPLC-MS on a Q-Exactive Plus instrument. Briefly, a variety of tissues were lyzed and digested into peptides, followed by separation on a 25cm C18 column over a 4 hour gradient. The resulting data was analyzed by MaxQuant using the "protein ruler approach" (Wiśniewski 2014) to determine concentrations of proteins. Where known from literature or previous MRM studies, several protein targets were compared against different methodologies. **Results and Discussion:** Proteome maps were generated for several key organs and animal models. By building a comprehensive library of detectable proteins with approximate concentrations, proteins of interests can be queried very early in the drug development stage in silico. Where possible, several proteins of interest were cross referenced against known concentrations from literature or in house immunoaffinity MRM studies. These showed that, as expected, immunoaffinity MRM was ultimately more sensitive but where values were available for both, they were within acceptable accuracy. **Conclusion:** Proteome maps can be highly useful in early stage protein assay development as a rough guide to assess key phamacodynamic concerns a priori. Examples include tissue specificity/off-target effects, abundance and variation both intra and inter species. Having a remineable resource to facilitate these interrogation of protein targets in silico can create efficiencies in both the drug and assay development pipeline.

Keywords: pharmacodynamics, proteome, map

P08.04 Determination of Biorelevant HA Content in Influenza Vaccines Using SEC and Quantitative MS

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Introduction and Objectives: The regulated protein in influenza vaccine is hemagglutinin (HA). Currently, vaccine potency is determined by measuring

activity with the single radial immunodiffusion assay (SRID), which has been correlated with the production of neutralizing antibodies to HA in vivo. This assay requires the production of both reference antigens and polyclonal antibodies raised against HA, which takes several months and can be problematic. In case of an influenza pandemic, where time is of the essence, it is desirable to develop an alternative assay that can operate independently or as a stop-gap for SRID. Size exclusion chromatography (SEC) has been employed to separate HA species under the same conditions employed in the SRID assay. In this work, we have used a protocol where vaccine samples are first separated by SEC (for separation of potent and supotent HA) followed by LC-MS/MS quantitation of the protein components. **Methods:** SEC of influenza vaccines was performed via HPLC with fraction collection. Collected SEC fractions were subjected to tryptic digestion followed by quantitative mass spectrometry measurements on a TSQ Quantum. A measure of potency was determined by comparing the total determined HA content of the sample as analyzed by LC-MS/MS to the MS-determined content of the relevant HA fractions. **Results and Discussion:** We have demonstrated that HA fractions can reproducibly be isolated via SEC followed by MS quantitation. Samples subjected to temperature and pH based stress produce changes in the relative amounts of components in the SEC chromatogram, which can then be quantied via MS means. **Conclusion:** In the work presented here, we have demostrated the combination of SEC for separating infuenza vaccine components with mass spectrometry based quantitation methods. Vaccines subjected to simulated stressing regimens produce unique profiles on SEC. HA of various size factions can sucessfully be quantified by mass spectrometry and a measure of vaccine potency determined.

Keywords: size exclusion chromatography, hemagglutinin, Influenza, Mass spectrometry

P08.05 In Vitro Metabolism of Small Bioactive Peptides in Plasma, Liver and Kidney Subcellular Fractions

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Introduction and Objectives: Knowledge of metabolic pathways is very important in anti-doping research since excretion time of metabolites exceeds excretion time window of the parent drugs as was shown in our previous work [1]. Both in vivo and in vitro approaches are useful for the metabolites prediction. In most cases in vivo approach in human is not possible because most of synthetic peptides have not been approved for human use, and in vitro methods are an alternative which does not require ethic committee approval. **Methods:** We have investigated biotransformation of bioactive peptides with different chemical modification such as amidation (growth-hormone releasing peptides, GHRPs, and Desmopressin), acetylation (Thymosin beta-4 (17-23) fragment, TB-500), unnatural and D-amino acids (GHRPs) inclusion and disulfide bonds (Desmopressin). The various in vitro models were used: human serum, human kidney microsomes, human liver microsomes and liver S9 fraction. Peptide metabolites were obtained during incubation for 24 hours at 37°C. Depletion of most abundant tissue-specific proteins was done using acetonitrile precipitation followed by centrifugation. Supernatants were evaporated, reconstituted and analyzed by nanoHPLC-ESI-MS/MS methods. **Results and Discussion:** The main pathways of proteolytic degradation of GHRPs, Desmopressin and fragment TB-500 were revealed after incubation with subcellular fractions. GHRPs and TB-500 have been readily degraded in human kidney microsomes and liver S9 fraction. Metabolites were detected being formed by cleavage of peptide bond between unnatural and D-amino acid residues. Also, these subcellular fractions demonstrate enzymatic deamidation activity, which leads to deamidation of C-terminal amide groups among all GHRPs. Less metabolites were obtained after incubation of bioactive peptides in human serum. **Conclusion:** Therefore we have concluded that human kidney microsomes and liver S9 fraction are the most appropriate in vitro model system for simulating of peptides metabolism. Acknowledgement. Participation was upheld and budgeted by WAADS (World Association of Anti-Doping Scientists).

[1] Semenistaya, E., Zvereva, I., Thomas, A., Thevis, M., Krotov, G., Rodchenkov, G. (2015) Drug Test Anal., Apr 13. doi: 10.1002/dta.1787.

Keywords: peptides metabolism, subcellular fractions, doping control, LC-MS/MS

P08.06 PEAKS AB – A Reliable Workflow for Monoclonal Antibody Characterization Using MS/MS

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Introduction and Objectives: The development of reliable workflows using liquid chromatography-tandem mass spectrometry (LC-MS/MS) for antibody characterization has become an active research area. Going beyond the determination of the primary sequence, researchers are also interested in post-translational modifications, degradation analysis and variant characterization. To fulfill these requirements, we propose PEAKS AB to provide a reliable workflow for monoclonal antibody characterization from the purified antibody sample to MS/MS data analysis. **Methods:** The PEAKS AB workflow includes: 1. sample preparation: deglycosylation, chain separation, and multi-enzyme digestion 2. high-resolution LC-MS/MS analysis 3. PEAKS-based antibody sequencing: PEAKS de novo, PEAKS DB, SPIDER 4. PEAKS-based antibody characterization: PEAKS PTM, SPIDER, PTM Profiling, Peptide Mapping **Results and Discussion:** A human monoclonal antibody sample was deglycosylated, reduced, the cysteine residues alkylated, and then digested with six different proteolytic enzymes. Peptide mixtures were analyzed using a Thermo Q-Exactive Orbitrap mass spectrometer equipped with a Thermo Easy-nLC UPLC system. For the light chain, six mass spectra files containing 46,576 MS/MS spectra were collected and analyzed using PEAKS 7.5. All spectra were searched against the UniProt database. At 0.1% of FDR at PSM level, the majority of the constant region of the light chain could be found in current database with the coverage of 100%. In contrast, the variable domain containing CDRs was partially covered (61%) by the top ranking protein. By matching de novo only results to database sequences using SPIDER, we found amino acid substitutions and finally identified the full sequence of the target light chain, in which every amino acid is mapped within more than 20 peptides and the confidence level is more than 95%. **Conclusion:** PEAKS AB provides a reliable workflow for comprehensively characterizing monoclonal antibodies. It takes the advantage of both the latest LC-MS/MS technology and PEAKS software to guarantee the quality of the antibody characterization.

Keywords: antibody characterization, Mass spectrometry, de novo sequencing, homology search

P08.07 Robust and Sensitive Nano LC-MS Targeted Quantification of Rituximab™ in Complex Bio-Matrices

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Introduction and Objectives: Monoclonal antibodies (mAbs) are the major element in the fastest growing sector of biopharmaceuticals within the pharma industry and revolutionize the treatment of many diseases. Rituximab™ was the first commercial mAb that was approved for clinical use. Pharmacokinetics of Rituximab™ in patients is a difficult task that is addressed mainly by enzyme-linked immunosorbent assay (ELISA). Currently nano flow LC-MS/MS analysis provides an alternative for proteins quantification in clinics. In this study we developed nanoLC-MS/MS method for quantification of Rituximab™ in complex human bio-samples. **Methods:** The UltiMate 3000 RSLCnano system coupled with Q Exactive HF mass spectrometer was used to quantify Rituximab spiked in HELA cell lysate and in a mixture of high-abundant serum proteins. Acclaim Pepmap RSLC C18 or Easy-Spray (2 μm, 75 μm i.d.) columns were used for peptides separation. Samples were digested using the SMART Digest kit to obtain tryptic peptides. Disulfide bonds were reduced by incubation for 30 minutes at 60° C with 5 mM Tris(2-carboxyethyl)phosphine hydrochloride (TCEP). **Results and Discussion:** The bio-matrices were spiked with Rituximab in proportion from 1:100 to 1:100 000 to estimate the linearity, LOD and LOQ for the following unique Rituximab peptides DTLMISR, FNWYVDGVEVHNAK, VVSVLTVLHQDWLNGK, NQVSLTCLVK, GFYPSDIAVEWESNGQPENNYK. Additionally, MS-tSIM and MS-PRM quantification methods were compared for mAb quantification in complex matrices. In order to reduce the complexity of samples nanoLC gradients with duration from 30 till 180 min were applied for peptides separation. The obtained RSD of peptides retention time were 0.03-0.08% for 30 min gradient and 0.02-0.09% for 180 min gradient. The obtained LOQ values (~1:70000, Rituximab to matrix) are comparable with immunoaffinity technique. **Conclusion:** NanoLC MS/MS targeted analysis of Rituximab is a promising alternative to immunoaffinity based quantification techniques with low LOQ and LOD and wide linearity range in complex bio-matrices.

Keywords: nanoLC, high resolution mass spectrometry, Monoclonal antibodies, quantification

P09: POSTER SESSION - PROTEOMICS OF MICROBES AND INFECTIOUS DISEASES

P09.01 Detecting and Quantifying Dengue Viral Proteins and Virus Maturation by MRM-MS

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Introduction and Objectives: The four serotypes of dengue virus (DENV-1/2/3/4) impose a significant global health burden, with 2.5 billion people living in endemic areas and 390 million cases annually. An important risk factor for developing life-threatening severe dengue is a prior infection with a heterologous serotype; accurate serotype-specific diagnosis is therefore important. Unfortunately, existing laboratory diagnostic techniques are inadequate due to limitations in their specificity, sensitivity, and window of applicability. **Methods:** We have designed and optimized multiple reaction monitoring (MRM)-MS assays targeting DENV proteins, including the structural membrane precursor (prM) and envelope (E) proteins on the virus surface, and the secreted nonstructural protein 1 (NS1). **Results and Discussion:** Using cell culture-derived non-purified DENV in a mock serum sample matrix, we have established sensitivity and reproducibility in the low fmol range for MRM assays targeting sixteen DENV-1/2/3/4 proteotypic peptides. We are currently optimizing additional peptides and obtaining DENV-infected human serum samples to validate our assays and establish biologically relevant lower limits of detection and quantification. We are also developing a novel method of quantifying virus maturation. Proteolysis of prM, mediated by human furin, is a key determinant in the maturation state of DENV progeny, but is enigmatically inefficient. By performing N-terminal acetylation followed by MRM (NTAc-MRM) on the N-terminal peptide of the C-terminal proteolytic product, we can measure virus maturation by differentiating and quantifying furin-cleaved (mature) versus trypsin-cleaved (immature) prM in biological samples. **Conclusion:** MRM-MS directly addresses the limitations of existing diagnostics, with high specificity, high sensitivity, and applicability in any phase of dengue illness. Importantly, this approach is not limited to DENV; any virus with MS-detectable circulating protein levels could theoretically be targeted in this way. This approach also allows high specificity and fmol-scale sensitivity in quantifying viral protein, with many potential applications in tackling globally significant research questions concerning the biology of human viruses. (Funding: NCE/IC-IMPACTS, BCPN)

Keywords: diagnostics, viral infection, virus, MRM

P09.02 Identification of Biological Species Using Spectral Libraries

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Introduction and Objectives: Patterns of tryptic peptides have long been used successfully to identify and compare biological species. Although largely overtaken by DNA-based methods, peptides and mass spectrometry based methods may still have advantages in specific niches. We will here overview such methods, including a new approach using spectral libraries, and discuss their advantages and shortcomings. **Methods:** Query and reference LC-MS/MS data were collected on several types of mass spectrometers, including Bruker amaZon and Thermo LTQ ion traps and Thermo Q Exactive Orbitraps, using settings common for bottom-up proteomics. We used SpectraST to create and query spectral libraries created from similarly prepared reference material. To automate species identification, we constructed a maximally simple Web interface to SpectraST and the analysis scripts. The user only has to upload a dataset and select which reference libraries to search. **Results and Discussion:** Several groups have shown how reference spectra and spectral libraries can be used to identify species in samples of unknown origin. Rather than analyzing peptide sequences, we count how many spectra in the reference library are matched by an unknown sample. The assumption is that data from an unknown species X, regardless of sample source and data quality (within reason), will be most similar to the reference data from species X, as the reference spectra were collected following the same protocol. When X is not in the reference database, the 'unknown' sample generally matched best to the most closely related species in the database. **Conclusion:** In general, the more closely related the bacterial strains, the more data is required to confidently distinguishing between them. We have tried to determine how much data is needed using simulations. Targeted methods here have an advantage, although they need to be carefully designed in

advance. For several genera, the correct species could be identified 90-95% of the time.

Keyword: spectral libraries, bacterial identification, phylogenetics, tandem mass spectrometry

P09.03 MTB Proteome Microarray for Global Studies of Protein Function and Immunogenicity

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Introduction and Objectives: Poor understanding of the basic biology of Mycobacterium tuberculosis (MTB), the etiological agent of tuberculosis, hampers development of much-needed new drugs, vaccines and diagnostic tests. New experimental tools are needed to expedite investigations of this pathogen at the systems level. **Methods:** Here we present a functional MTB proteome microarray covering 95% of the proteome, and a complete ORFome library. **Results and Discussion:** We demonstrate the broad applicability of the microarray by investigating global protein-protein interactions, small molecule-protein binding, and serum biomarker discovery, identifying 59 PknG-interacting proteins, 30 c-di-GMP binding proteins, and 14 MTB proteins that together differentiate between TB patients with active disease and recovered individuals. Results suggest that the MTB rhamnose pathway is likely regulated by both the serine/threonine kinase PknG and c-di-GMP. **Conclusion:** This new resource has potential to generate greater understanding of key biological processes in the pathogenesis of tuberculosis, possibly leading to new therapies for the treatment of this ancient disease.

Keywords: Mycobacterium tuberculosis, proteome microarray, c-di-GMP, serum biomarker

P09.04 Proteome Profiling in the Search for Cerebrospinal Fluid Biomarkers of Pneumococcal Meningitis

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Introduction and Objectives: Bacterial meningitis when left untreated is usually fatal, making it imperative that a swift, accurate diagnostic test is available to clinicians. Current diagnostic techniques rely on microscopy and antigen testing, which can delay diagnosis. A rapid diagnostic assay or point-of-care device based on a specific protein signature would speed the diagnosis and thus dramatically improve the prognosis of patients with bacterial meningitis. This work aims to identify a panel of proteins in cerebrospinal fluid (CSF) associated with Streptococcus pneumoniae infection, with subsequent verification of proteins of interest using quantitative western blotting. **Methods:** Initially, 12 CSF samples (case and control) were analysed using both data dependent (DDA) and data independent (DIA) acquisition workflows. A second batch of samples (case and control, n=16) were then analysed using the same workflow to verify putative biomarkers. Quantitative western blots were performed on a subpanel of selected proteins using an automated western blotting instrument (Wes, Protein Simple) in order to validate the mass spectrometry findings. **Results and Discussion:** A total of 519 proteins were discovered using DDA discovery proteomics, and quantitative data using DIA Hi3 quantification for 161 proteins. Progenesis LCMS was used to determine 202 potential candidates using the DDA approach and 102 using DIA approach with 78 common to both workflows. When repeated using a second cohort of samples, 56 out of 78 candidates were verified. A subpanel of candidate proteins were chosen based on fold changes and quantitative western blotting performed for validation. This confirmed that the protein profiles could be used to clearly distinguish between case and control. **Conclusion:** Discovery quantitative MS complemented by quantitative western blotting has yielded a panel of biomarkers that can discriminate Streptococcus pneumoniae infected CSF from healthy CSF. This has the potential to assist in the rapid diagnosis of bacterial meningitis, resulting in improved disease management and decreased mortality

Keywords: bacterial meningitis, Streptococcus pneumoniae, Quantitative western blotting, biomarker

P09.05 Mechanistic Basis of Phenotypic Drug Resistance in Mycobacteria

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Introduction and Objectives: South Africa has one of the highest incidences of active tuberculosis disease worldwide, with ~900 cases per 100,000 population. Furthermore, the emergence of drug resistant Mycobacterium tuberculosis (M.tb) strains has intensified the need to identify new control measures for tuberculosis disease and to understand in more detail the mechanisms by which M.tb becomes drug-resistant, in order to design chemotherapy to counteract this resistance. Although antibiotics are largely toxic to bacteria at high doses, sub-lethal doses can trigger the expression of stress-response genes that enable bacterial survival. Due to poor penetration of antibiotics, surviving extracellular bacilli persisting in necrotic granuloma centres are particularly at risk of exposure to these sub-lethal drug concentrations. We have used quantitative, differential proteomic analysis of mycobacterial model systems to gain insight into the intrinsic resistance mechanisms employed by M.tb to overcome sub-lethal doses of antibiotic as well as peroxide and nitric oxide stresses. **Methods:** We carried out biologically-replicated time course studies on mycobacterial cells exposed to sub-lethal antibiotic and oxidative stresses to determine the early and late proteomic responses to each stressor that correlate with development of phenotypic resistance. After harvesting the cells, unfractionated total protein extracts were analysed by label-free LC-MS/MS on a Q Exactive. Data was analysed in MaxQuant. **Results and Discussion:** Quantification of >2,800 unique mycobacterial proteins per sample revealed statistically significant changes in the abundance of ~10% of the observed proteome that occur soon after addition of antibiotic or oxidative stressors. Data analysis revealed multiple mechanisms by which mycobacteria with drug susceptible genotypes are able to resist sub-lethal doses of rifampicin, peroxide and nitric oxide, including enzymatic modification of the antibiotic, down-regulation of drug transporter proteins, and stabilisation of the drug target. **Conclusion:** Our study sheds important new light on phenotypic drug resistance in M.tb and suggests persister populations of mycobacteria represent a new frontier for chemotherapy.

Keywords: Infectious disease, Tuberculosis, phenotypic drug resistance

P09.06 Search for a T. Pallidum Antigen Test: Revealing Protein Targets for Diagnostic Test Development

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Introduction and Objectives: Up to 55% of syphilis infections in European countries are caused by reinfections. Since antibody tests detecting the syphilis causative bacteria T.pallidum ssp. pallidum remain positive for life, assessment of both adequate treatment response and reinfection diagnosis depend largely on changes in the titres of 100 year-old non-treponemal tests which are susceptible to fluctuation from a number of conditions. Our objective is to develop an antigen test that will replace the non-treponemal tests in the diagnosis of syphilis infection, reinfection and therapy response. Using proteomics, we identify biomarker candidates that are incorporated into a multiplex assay using multiple reaction monitoring (MRM) in addition to an ELISA. **Methods:** A shotgun approach was utilized to characterize the T.pallidum proteome. DAL-1 strain bacteria were acquired by rabbit inoculation, extracted, purified by Percoll gradient centrifugation and lysed. After trypsinization, peptides were separated by RP-C18 high pH, followed by an offline μCap RP-C18 low pH step as the second dimension. Peptides were analysed by MALDI-ToF/ToF and Orbitrap mass spectrometry. Protein abundance was estimated using spectral counting. Recruitment efforts of syphilis positive patients (N=120) and controls (N=30) are underway via a clinical observational study. **Results and Discussion:** Comprehensive proteomic experiments resulted in the identification of 52% of the T.pallidum proteome. Based on this subset, several promising candidate T.pallidum antigens were identified and 30 corresponding proteotypic peptides (PTP) were characterized. Antigen selection was based on relative protein abundance and low sequence homology with other bacteria. A multiplex MRM assay is currently under development using PTP's of these candidate antigens and testing will be performed on stored plasma and urine samples. **Conclusion:** Development of an antigen test for T.pallidum antigenemia will represent a significant breakthrough for syphilis diagnostics. Characterizing the T.pallidum proteome provides valuable insights into protein expression, function and potential antigen targets for test development.

Keywords: syphilis, antigen test, proteomics

P09.07 Evaluation of DIA Analysis on Orbitrap Fusion for Detection of Bacterial Infection in Human Samples

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Introduction and Objectives: Bacterial infections cause millions of deaths each year, hence the need of rapid and accurate diagnostics. MALDI-TOF analysis for microorganism identification has recently been implemented in microbiology laboratories but this single MS screening requires pure colonies, is unable to distinguish between closely related bacteria species and is non-quantitative. In order to avoid these limitations, we assumed that new proteomic strategies using Data Independent Acquisition (DIA), coupled to spectral libraries containing bacterial strains proteotypic peptides, should allow the detection in a single run of a large number of bacteria species with minimal sample treatment. With high resolution and sequencing speed, the Orbitrap Fusion is well suitable for DIA analysis but the ideal parameters for low abundance peptides detection in complex mixtures remain to be defined. **Methods:** A proteomic standard composed of total human cells lysate spiked with standard peptides at concentrations ranging from 5 fmol to 0.1 fmol per microgram has been used with various DIA methods on Orbitrap Fusion including conventional DIA acquisition and WiSIM-DIA. Parameters like isolation windows size, orbitrap resolution, ion trap filling and maximum injection time were adjusted. Detection of spiked peptides and linearity over the concentration range have been evaluated using Skyline software and benchmarked to another widely used DIA strategy: SWATH-MS on TripleTOF instrument. **Results and Discussion:** Our best DIA method has been applied to the detection of low concentrations of Escherichia Coli spiked in urine samples in order to mimic bacterial infection. For all tested peptides, the limit of detection was below the clinically significant infections level of 1e5 CFU/mL. **Conclusion:** This work showed that DIA analysis on Orbitrap instruments might be an alternative to conventional infection detection strategies in urine, applicable to an unlimited number of bacterial strains, with a single analysis and minimal sample treatment, and which could be extended to other biological samples (blood, cerebrospinal fluid...).

Keyword: DIA - Orbitrap Fusion – Bacterial Infection – Low abundance peptides detection

P09.08 Rapid Discrimination between MRSA and MSSA Using MALDI-TOF Mass Spectrometry

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Introduction and Objectives: Methicillin-resistant Staphylococcus aureus (MRSA) is one of the major pathogens responsible for nosocomial infection. The presence of MRSA in a hospital is detrimental to patients and to hospital management. Thus, rapid identification of MRSA is needed. **Methods:** Here, we report a prospective study of rapid discrimination of MSSA from MRSA using matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) and support vector machine (SVM) analysis in 160 clinical isolates of S. aureus. **Results and Discussion:** The predictive model was trained using 100 S. aureus isolates (50 MSSA and 50 MRSA). The identification rates were 90.0% for MSSA and 87.5% for MRSA in a 10-fold cross-validation SVM. In blind test sets, 60 S. aureus isolates (30 MSSA and 30 MRSA) were correctly classified, with identification rates of 93.3% for MSSA and 86.7% for MRSA. **Conclusion:** The method proposed in this study using a predictive model enables detection in one colony in 5 minutes, and thus is useful at clinical sites at which rapid discrimination of MRSA from MSSA is required.

Keywords: rapid discrimination, MSSA, MRSA, MALDI-TOF MS

P09.09 Leishmania Mitochondria: New Targets for Drug Development

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Introduction and Objectives: Leishmaniasis is a major global health parasitic disease and is spread to humans through sandflies. Leishmania have a complex life cycle alternating between extracellular promastigotes in the insect vector and intra-macrophage amastigotes in the vertebrate host. Differentiation involves significant morphological and biochemical adaptation. As the Leishmania genome is essentially constitutively expressed, the underlying molecular mechanisms responsible for morphological and biochemical changes are likely mediated at the functional protein level. The previously identified A600 family of membrane proteins are required for amastigote replication within the macrophage, and have been localized to the mitochondria. The objectives are to determine the role of A600 proteins in mitochondrial metabolism and whether the A600 proteins constitute a multi-subunit protein complex involved in essential mitochondria function. **Methods:** iTRAQ was used to quantitiate differentially expressed membrane protein profiles of promastigotes and amastigotes. Protein-protein interaction technologies including GST pull down, immunoaffiinty chromatography, and expression of BirA biotin protein ligase fusion proteins were used to determine whether A600 proteins are constituents of multi-subunit protein complexes. **Results and Discussion:** Differential quantitative proteomic profiling of the membrane proteome of promastigote and amastigote life stages identified stage specific proteins involved in cell motility, metabolism, pathogenesis and virulence. A600 knock-out mutants showed a dramatic decrease in mitochondrial redox potential and ATP production correlating with an inability to replicate as intra-macrophage amastigotes. As most mitochondrial proteins form multi-subunit functional complexes, A600 protein-protein interactions are being identified by a variety of proteomic techniques. A600 proteins were shown to be involved in energy metabolism essential for amastigote replication. **Conclusion:** Focusing on differentially expressed membrane proteins essential for pathogenesis will lead to a better understanding of the molecular mechanisms involved in the interaction between pathogens and host macrophages. A600 protein complexes may provide novel targets to disrupt amastigote metabolism offering a novel approach to the development of alternative therapeutic agents against leishmaniasis.

Keywords: quantitative proteomics, Protein-protein interaction, Leishmania mitochondria, drug-developmen

P09.10 Proteomics Analysis of EV71-Infected Cells Reveals the Involvement of NEDD4L in EV71 Replication

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Introduction and Objectives: Enterovirus 71 (EV71) is a human enterovirus that has seriously affected the Asia-Pacific area for the past two decades. EV71 infection can result in mild hand-foot-and-mouth disease and herpangina and may occasionally lead to severe neurological complications in children. However, the specific biological processes that become altered during EV71 infection remain unclear. **Methods:** To further explore host responses upon EV71 infection, we identified proteins differentially expressed in EV71-infected human glioblastoma SF268 cells using isobaric mass tag (iTRAQ) labeling coupled with multidimensional liquid chromatography-mass spectrometry (LC-MS/MS). **Results and Discussion:** Network analysis of proteins altered in cells infected with EV71 revealed that the changed biological processes are related to protein and ion transport, regulation of protein degradation, and homeostatic processes. We confirmed that the levels of NEDD4L and PSMF1 were increased and reduced, respectively, in EV71-infected cells compared to mock-infected control cells. To determine the physiological relevance of our findings, we investigated the consequences of EV71 infection in cells with NEDD4L or PSMF1 depletion. We found that the depletion of NEDD4L significantly reduced the replication of EV71, whereas PSMF1 knockdown enhanced EV71 replication. **Conclusion:** Collectively, our findings provide the first evidence of proteome-wide dysregulation by EV71 infection and suggest a novel role for the host protein NEDD4L in the replication of this virus.

Keywords: enterovirus 71, NEDD4L, IFN-β, iTRAQ

P09.11 Characterization of the Oral Proteome by Metaproteome Analysis

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Introduction and Objectives: Metaproteomics has a great potential to provide information on the microbial behavior and the health status of the human host. In this context, a metaproteomic workflow was developed based on a shotgun LC-MS approach to investigate the pro- and eukaryotic protein composition in complex oral samples like whole saliva and tongue swabs. **Methods:** We analyzed gum stimulated whole human saliva (split into pellet and supernatant) and tongue swab samples of 10 healthy subjects in the age range of 20 to 30 years which received a standardized dental care. Samples were subjected to LC-MS/MS based bottom-up proteomics using a QExactive mass spectrometer (Thermo Scientific). For protein identification MS/MS data were searched against a "Human Oral Microbiom Database" (HOMD) combined with a human Swissprot database. Gene ontology categorization of human proteins was carried out via Protein Center (Thermo Scientific). Taxonomic and functional assignment of bacterial proteins was performed using the Prophane bioinformatics pipeline (www.prophane.de). **Results and Discussion:** The human oral protein profile was most comprehensively represented by the pellet derived from whole saliva, displaying cell derived but also secreted proteins. In contrast, highest diversity of bacterial colonization was observed in tongue swabs. The taxonomical composition in the tongue swab and saliva pellet showed high similarity. As most prominent genera Streptococcus, Rothia, Neisseria, Prevotella, Veillonella and Haemophilus were found in both types of samples. Functionally, the identified bacterial proteins are mostly involved in protein synthesis and energy metabolism. **Conclusion:** The established metaproteomic approach allows a comprehensive analysis of the microbial and the human host proteome in complex oral samples. Based on the microbial protein profile identified, it is now possible to assess the biofilm composition on the tongue and to compare it with the microbial community structure in whole saliva and to analyse the association of the biofilm composition to the oral health status.

Keywords: oral proteome, metaproteomics, LC-MS/MS, microbiome

P09.12 CE-MS for the Detection of Carbapenemases in (Multi-)Drug-Resistant Gram-Negative Bacteria

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Introduction and Objectives: In a time in which the spread of multidrug resistant microorganisms is ever increasing, there is a need for fast and unequivocal identification of suspect organisms to supplement existing techniques in the clinical laboratory, especially using single bacterial colonies. We developed a novel mass spectrometry-based workflow for the rapid and reliable detection of OXA-48 and KPC carbapenemases in clinical isolates. **Methods:** An integrated, high resolution capillary electrophoresis mass spectrometry (CE-MS) platform was developed for sensitive peptide identification. Bacterial cell pellets were lysed and protein extracts were digested with trypsin. The resulting peptide mixtures were analysed by CE-MS/MS and proteins were identified after searching of the spectral data against bacterial protein databases. The method was validated using a set of well characterised OXA-48 and KPC positive clinical isolates. **Results and Discussion:** The CE-MS/MS peptide analysis was optimized with regards to separation window and number of identifications within a one hour analysis, using a ampicillin resistant laboratory E. coli strain due to TEM-1 β-lactamase expression. The method was then applied on a collection of well characterised OXA-48 (n=18) and KPC (n=10) clinical isolates. The minimal inhibitory concentration (MIC) for meropenem in this set ranged from 0.5 mg L^{-1} to more than 30 mg L^{-1}, reflecting the varying levels of resistance. The developed CE-MS was able to identify the presence of OXA-48 and KPC in all 28 isolates. Furthermore, 28 extended-spectrum beta-lactamases (ESBL) were identified in the same analysis, confirming the multi-resistant character of the clinical isolates. Finally, the workflow was tested using single colonies, yielding similar results. **Conclusion:** A method has been developed which allows for accurate determination of the presence of OXA-48 and KPC in clinical isolates. Due to the unbiased nature of the workflow, a number of ESBLs were also revealed for which the isolates had not been previously tested.

Keywords: antibiotic resistance, CE-MS, carbapenemases

P09.13 Decoding Bacterial Adaption Code: How Does Pseudomonas Aeruginosa Adapt to the Cystic Fibrosis Lung?

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Introduction and Objectives: Pseudomonas aeruginosa (PA) is a nosocomial opportunistic pathogen and etiological agent in cystic fibrosis (CF). PA is known for versatile adaptability to host environments. To understand how PA adapts to the harsh microenvironment of the CF host we characterised three novel PA strains isolated from sputum of CF patients. We carried out genome sequencing, proteome profiling and phenotypic analysis in comparison to the PAO1 laboratory strain. **Methods:** We cultured CF isolated strains (PASS) and PAO1 in lung nutrient mimicking medium (SCFM) and minimal medium (M9-glucose). Membrane protein enrichment was performed and proteins labelled using 4-plex iTRAQ mass tags. Samples were fractionated using strong cation exchange followed by nano-LC/MS/MS using TripleTOF® 5600. Identification and quantification of proteins was performed using ProteinPilot™ 4.2. Differential expression pattern of proteins were confirmed using biological and phenotype characterization assays. **Results and Discussion:** Comprehensive membrane proteome analysis revealed 3171 and 2442 proteins across four strains (Global FDR <0.008%) in M9 and SCFM fractions respectively. According to gene ontology and bioinformatics predictions 39% and 37% of identified proteins were designated as membrane proteins in M9 and SCFM fractions respectively. PASS strains portrayed vast heterogeneity in membrane proteome and phenotypes including formation of biofilms, pigmentation, and virulence. We observed differential expression of several key proteins including porins, virulence factors, metal acquisition proteins, drug resistance proteins, motility and adhesion proteins. 68 proteins in relation to drug resistance were identified including MexY, MexX which were up-regulated compared to PAO1. These observations were confirmed by increased resistance of PASS strains to a panel of antibiotics. Proteins involved in chemotaxis, motility and adhesion were downregulated in PASS strains. Confirmatory motility and sputum binding assays confirmed the proteomics data. **Conclusion:** CF isolates of PA revealed diverse modulation in membrane proteome and phenotype used to adapt to the harsh microenvironment of CF patient lungs.

Keyword: Pseudomonas aeruginosa, membrane proteome, cystic fibrosis

P09.14 FUT1 Gene Control Gut Bacteria through Fucosylation of Gut Mucosa Proteins

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Introduction and Objectives: Human health is closely linked to the health of animals, and to the state of the environment we share with all living beings. This connectivity is currently most alarmingly seen as the rise in antimicrobial resistance to antibiotics, which is directly related to the massive use of antibiotics in livestock production. With currently 80 % of all antibiotics being given to farm animals, it is clear that improving farm animal health is mandatory for counteracting the global challenges of antimicrobial resistance. Our study of host pathogen interactions in pig describe how a SNP in the FUT1 gene influences health, growth and early colonization of the piglets gut, clearly demonstrating that the FUT1 gene controls adhesion of both commensal (e.g. Prevotella) and pathogenic (E.coli) bacteria, and that their adhesion is closely correlated to differential glycan structures found on the gut epithelial proteins from the different genotypes. **Methods:** The genotypes of 16 half sibling piglets were confirmed for variation in the FUT1 gene. Animals with A/A in bp308 give rise to Thr/Thr in amino acid position 103, and are null mutants for the fucosylatransferase1 activity, and resistance to E.coli adhesion on gut epithelium. **Results and Discussion:** Next generation DNA sequencing was used to investigate the gut meta-genome. Meta-genome data were correlated to LC-MS/MS mass spectrometry data of the gut membrane protein fractions from different gut segments, and to LC-MS/MS analyses of glycan structures extracted from the gut mucosa fractions. **Conclusion:** Our data provide a means for improving pig gut health and host resitance through genetic selection, and hereby reduce the massive need for antibitotics in the pig production. Moreover, due to the close similarity

between human and pig physiology and pathogens, our research provide an animal models of immediate relevance to human gut health studies.

Keyword: E.coli Metagenomics protegenomics glycoproteomics

P09.15 Proteomic Characterization of HipA-Mediated Mechanisms of Bacterial Persistence in Escherichia Coli

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Introduction and Objectives: Persistence, one of today's major challenges of infection medicine, is a phenomenon in which bacterial cells become temporarily multidrug tolerant by a stochastic switch into slow growth. Due to the low frequencies of persister cells, mechanisms underlying this phenomenon are difficult to study. In E. coli, hipBA toxin/antitoxin locus encodes serine/threonine kinase HipA, whose kinase activity is required for growth inhibition and increased persistence level. When overexpressed, HipA inactivates glutamyl-tRNA synthetase (GltX) by phosphorylation, causing accumulation of uncharged $tRNA^{Glu}$, which triggers attenuation of translation, DNA replication and transcription. A gain-of-function allele with two point mutations in hipA gene, called hipA7, enhances persistence up to 1,000-fold, but, surprisingly, it is less toxic than the wild-type HipA. We aimed to elucidate molecular mechanisms behind the phenotype of hipA7 allele, which are poorly understood. **Methods:** We performed quantitative analysis of the proteome and phosphoproteome of hipA7 E. coli strain by using high resolution QExactive HF mass spectrometer combined with stable isotope labeling by amino acids (SILAC). MS data was processed using MaxQuant software. **Results and Discussion:** We identified 2,148 protein groups and 554 phosphorylation events of which 398 were localized and 354 quantified. Within 29 significantly changing phosphorylation sites, we confirmed phosphorylation of GltX and detected one additional aminoacyl-tRNA synthetase, as well as other potential HipA targets involved in biosynthesis of several amino acids. We are currently examining (phospho)proteome changes in a model which includes induction of persistent state by wild-type HipA and resuscitation by inducing cognate antitoxin HipB. Further insights gained from this study will be presented and discussed. **Conclusion:** This study represents the first comprehensive proteomic investigation of bacterial persistence and provides novel insights into mechanisms of persistence mediated by HipA. Molecular definition of persistent phenotype is of great importance to help developing clinical methods for the eradication of pathogens that cause chronic infections in humans.

Keywords: bacterial persistence, infectious diseases, phopshoproteomics

P09.16 Quantitative Proteomic Analysis Reveals Major Cues for Severe Vivax Malaria

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Introduction and Objectives: Recent reports indicate that Plasmodium vivax can cause severe infections with different fatal complications. In P. vivax malaria, mechanisms that trigger transition from non-severe to fatal severe infections are obscure. In this study serum samples from severe vivax malaria and non-severe vivax malaria patients along with healthy community controls and two other febrile infectious diseases, dengue fever and leptospirosis from three different endemic regions of India were investigated to decipher pathogenesis and identify surrogate markers of severity. **Methods:** Quantitative proteomics (gel-based and gel-free) in combination with ESI-Q-TOF and Q-Exactive mass spectrometry platforms were used in the discovery phase of the study and selected targets were validated by immunoassay-based approaches. **Results and Discussion:** The differentially abundant proteins such as carbonic anhydrase, superoxide dismutase, fatty acid-binding protein, nebulin, and profilin were found to be interesting in context of malaria pathogenesis. Results provided mechanistic insight of progression to severe malaria by modulation of various vital physiological pathways, including oxidative stress, muscle contraction and cytoskeletal regulation, and complement cascades. Strikingly, unlike severe falciparum malaria the blood coagulation cascade was not affected adversely in P. vivax infection. Identified proteins such as CA, SOD, FABP, nebulin, profilin, SAA, HP, Apo A-I and Apo E, which exhibited sequential alterations in their serum abundance with increased severity, could serve as potential markers for disease severity. **Conclusion:** To the best of our knowledge, this is the first comprehensive investigation describing the serum proteomic alterations in vivax malaria patients with different disease severity levels. Investigation of parasite induced alterations in the host proteome and modulation of vital physiological processes in different severity levels of the infection effectively enhanced our understanding regarding disease pathogenesis of vivax malaria and may aid in pattern-recognition of different severe malaria associated syndromes.

Keyword: malaria, infectious disease, biomarker, severe

P09.17 In Vitro Endosomal Recruitment Proteomics Strategy to Study Bacterial Protein Toxin Translocation

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Introduction and Objectives: Methods to identify cytosolic translocation factors (CTFs) facilitating bacterial protein toxin entry from endosomes are limited. Based upon the hypothesis that unique conformations of toxin inserted into biological membranes mediates interaction with CTFs, an in vitro endosomal recruitment purification and proteomics (IVERPP) strategy has been developed to study the mechanism of entry of the diphtheria toxin (DT) catalytic (C) domain using the fusion protein toxin DAB389IL2. **Methods:** Endosomes pre-loaded with various cargoes (e.g. toxin vs control ligand) are partially purified by sucrose gradient ultracentrifugation and then directly used as substrates for affinity chromatography of 13C heavy lysine labeled cytosolic proteins. Proteomic maps of pre-acidified endosomes are then compared to proteomic maps of endosomes post-acidification and post-incubation with the 13C labeled cytosolic proteins. This method allows for qualitative and quantitative analysis of cytosolic proteins recruited to endosomes of various compositions (e.g. +/- toxin, mutant toxin) in settings of various conditions (e.g. +/- ATP, NAD, CTF inhibitors). Proteomic analysis can be further tailored to study concomitant post-translational modifications occurring during endosomal acidification, such as phosphorylation and ADP-ribosylation events. **Results and Discussion:** Preliminary proteomic analysis demonstrates that this is a valid method to observe the recruitment of cytosolic proteins to the endosome. To increase sensitivity and specificity, a second generation IVERPP strategy has now been developed involving the immunoprecipitation of the partially purified endosomes with magnetic beads targeted to the early endosomal marker Rab-5 prior to acidification and 13C labeling. Discussion of preliminary results and the rationale for developing a second generation IVERPP strategy will be presented.

Conclusion: The IVERPP strategy is novel and powerful approach to study endosomal trafficking events including bacterial protein toxin translocation events.

Keywords: Endosome, Toxin Translocation

P09.18 Revealing the Response of the Active Mucosal Microbiota from the Rat Colon to a Change in Diet

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Introduction and Objectives: The intestinal microbiota is a densely inhabited microbial community that provides many functions for the host including the degrading of non-digestible nutrients into useful metabolites, the synthesis of vitamins and the regulation of the immune system. The microbiota is known to evolve over the life time of the host and to respond to different environmental influences. The focus of the study was to observe the response of the mucosal microbiota from a high-fat diet rat model as well as from a chow diet fed diet rat model to a change in diet. **Methods:** To analyse the response of the gut microbiota to a switch in diet 16S rRNA gene pyrosequencing and LC-MS/MS metaproteomic analysis hyphenated with protein-based stable isotope probing (protein-SIP, ^{15}N-fully labelled diet) was performed. **Results and Discussion:** As a result, we were able to decipher the mucosal colon microbiota community structure in regard to taxonomy, enzymatic functionalities and active taxa related to nitrogen utilisation from the feed over a three day period. Microbial active taxa in regard to nitrogen utilisation belonged to the abundant phyla like Firmicutes, Proteobacteria and Bacteroidetes as well as those from low abundant phyla like Spirochaetes, Deinococcus-Thermi and Planctomycetes. In addition, we observed rapid changes in the community composition including a decline of Enterobacteriaceae and Streptococcaceae. Identified proteins were assigned to functional categories of which replication, transcription, signal transduction as well as carbohydrate and amino acid metabolism were overrepresented. **Conclusion:** The integrated data analysis opens the path to understand the complex gut microbiota in more detail using protein-SIP to identify the active taxa for specific substrate utilisation.

Keywords: Protein-SIP, metaproteomics, Gut microbiota

P09.19 Label Free Study for Control of Listeria Monocytogenes to Enhance Food Safety

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Introduction and Objectives: Listeriosis is a disease caused by Listeria monocytogenes. it is an ubiquitous foodborne pathogen extremely hazardous for human population that usually affects high risk patients such as the elderly, immunosuppressed patients and pregnant women. However, it can also affect people who do not have these risk factors. For these reasons it is mandatory to counteract the listeria growth in food, avoiding the use of antibiotics, lantibiotics or chemical compounds. the strategy used is based on the selection of specific strains of starter bacteria (Lactococcus lactis) able to counteract listeria growth. In order to highlight these mechanisms of bacterial competition, the secretome of these two microrganisms in co-culture (BHI first, milk for validation second) has been studied through a proteomic and peptidomic approach. **Methods:** The bacterial secretome of the lactococcus strain with a strong activity in the inhibition of listeria growth has been analysed through 2D electrophoresis, shotgun analysis (UPLC-MS system, Waters) and top-down peptidomics (LTQ-Orbitrap). Data have been validated through MRM analysis (Bruker HCT PLUS, skyline software) both in vitro and in milk in order to resume cheese-making conditions. **Results and Discussion:** Obtained data highlighted, during competition, the higher production by listeria of the moonlighting protein enolase, of Septation ring formation regulator EzrA, involved into cell replication and the lower secretion of Endopeptidase P60. In parallel, during competition, L. lactis produced higher amounts of Secreted 45 kDa protein and switched from lantibiotic Nisin A production to Nisin Z production. **Conclusion:** In competition with listeria, L. lactis, produced higher amounts of Secreted 45 kDa protein with peptidoglycan lytic activity and NisinZ, instead than NisinA, in order to enhance lantibiotic solubility in less acidic environment. The demonstrated properties of this L. lactis strain, using these three complementary proteomics approaches, may help in the additives-free listeriosis prevention. Work supported by Ministry of Health-CCM Milano EXPO 2015 Project

Keyword: Listeria monocytogenes, food safety, bacterial competition, proteopeptidomics

P09.20 Protein Signature during Biofilm Formation in Staphylococcus Aureus Food Isolates

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Introduction and Objectives: Human handling of food products as well as infection/colonization of livestock or farm workers have been described as mechanisms for the contamination of food with S.aureus.Moreover, S. aureus is able to produce biofilm. Low rates of antibiotic transport within biofilms, protective effects of the biofilm matrix, and low rates of metabolic activity within the biofilm interior have all been found to contribute to the persistence of these pathogen. The mechanism and/or process of biofilm formation in S. aureus is poorly understood and the studies on the expression profiles of genes involved in biofilm mechanism are still limited in number. Anyway, S.aureus biofilm on food contact surfaces poses a serious risk of food contamination. The aim of this work is to analyze proteomics of different strain of this bacteria isolated from food with different capacity to produce biofilm in order to elucidate mechanism of biofilm formation. **Methods:** The experiment was conducted with S.aureus strains such as MRSA and MSSA isolated from (food)environments . S.aureus ATCC 35556 (a known strong biofilm producer), S.aureus ATCC 29213 (a known weak biofilm producer) has been used as references strains. S.aureus strains showing ability to produce biofilms will be classified as weak, moderate or strong . Two

independent sets of all experiments will be performed in triplicate. The isolates were then process with both top down and bottom up proteomics, including high resolution narrow 2D electrophoresis coupled with MALDI-TOF analysis. **Results and Discussion:** Mono and 2D electrophoresis were compared from different S. aureus ATCC and isolated strains, together with the capacity to produce biofiilm, and proteins were identified. **Conclusion:** Proteomics analysis through strong and weak producers revealed differencesin the profile of strains cultured in the plactonic form in comparison to the sessile form, and in the profile of different food isolated giving new insights to conteract the biofilm formation.

Keywords: Staphylococcus aureus, food, biofim

P09.21 Immunoproteome Analysis of Bordetella Bronchiseptica by IP-nLC-MS/MS

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Introduction and Objectives: Bordetella bronchiseptica is a gram-negative pathogen and the evolutionary progenitor of Bordetella pertussis, cause of whooping cough in humans. B. bronchiseptica usually causes asymptomatic infections in humans and causes acute and chronic respiratory infection in a variety of animals. This study aimes to identify the antigenic proteins of B. bronchiseptica. The identification of new antigenic proteins may be a resource for further development of a new vaccine candidates. **Methods:** Secreted proteins, membrane-related and hydrophilic fractions of B. bronchiseptica were isolated and treated with B. bronchiseptica whole cell immune sera. Magnetic bead-based immunoprecipitation (IP) method was applied to select the antigenic ones. Then the antigenic proteins were separated by one-dimensional gel electrophoresis and identified by nLC-MS/MS. Gene ontology, cellular location, molecular function, and biological process terms were analysed by using UniProt and protein interaction networks were generated using STRING. **Results and Discussion:** 15 antigenic secreted proteins were identified, of which 11 were reviewed for the first time. By GO analysis, secreted proteins were linked to pathways such as metabolism, genetic and environmental information processing. Also, most of the proteins were located throughout the cytoplasm, outer membrane, periplasm. STRING-generated protein network showed no connectivity between secreted antigenic proteins without 50S ribosomal protein subunit L14 and L22. 41 antigenic membrane related proteins located on plasma membrane, outer membrane or membrane part were detected. Of which, 31 were reviewed for the first time in this study. Membrane-related antigenic proteins were distributed throughout biological procesees; pathogenesis, signal transduction, cell adhesion, biological regulation. 159 antigenic proteins of hydrophilic fraction were identified of which 94 were reviewed for the first time in this study. **Conclusion:** These newly identified proteins may promote a better understanding of the pathophysiology of B. bronchiseptica and also a possible development of new vaccine candidates.

Keywords: Bordetella bronchiseptica, immunoprecipitation, nLC-MS/MS, Immunoproteome

P09.22 SILAC Labelling Dynamics in Staphylococcus Aureus MSSA/MRSA Strains Isolated Form Mastitis

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Introduction and Objectives: Staphylococcus aureus, the main causative agent of nosocomial infections, has become a relevant healthcare problem because of the widespread occurrence of strains resistant to multiple antibiotics, in particular methicillin-resistant S. aureus (MRSA). Although MRSA represents a serious problem for human health, also relevant infections of animal-associated MRSA have been recently identified. Animals involved in food production, their products are therefore a potential reservoir of MRSA for humans (EFSA-Q-2009-00612), and MRSA today has been considered as a potential dangerous zoonosis. The main aim of this preliminary work is to optimize the SILAC labelling strategy in methicillin-resistant (MRSA) and methicillin-sensible (MSSA) Staphylococcus aureus (SA) isolates from mastitis for the next investigation on the host-pathogen subcellular protein dynamics during the phagocytosis of these bacteria. **Methods:** SILAC labelling has been performed on several MSSA and MRSA isolates. Different parameters were taken in account during this task (CFU, growth media composition, labelling time, strain type, genome) in order to correlate this parameters to the incorporation dynamics of the isotopic label into the MSSA/MRSA proteome. Heavy and light protein samples were mixed and separated by SDS-PAGE. Protein bands were digested and peptides analyzed by nLC-MS/MS. Spectra were processed using MASCOT 2.4.1 and MaxQuant 1.5.2.8. Protein functional analysis has been performed using InterProScan 5 **Results and Discussion:** The levels of Isotopic label incorporation and biological and technical reproducibility of SILAC quantitation that has been also correlated to the different parameters mentioned before has been reported. An optimized workflow with the evaluation of the critical key points has been summarized in this work. **Conclusion:** This experimental approach will allow quantitative insights into the bacterial response as well as the turnover of the protein expressed by MSSA and MRSA Staphylococcus aureus during the host-pathogen interaction. Work supported by MIUR under grant "Futuro in ricerca 2013" project code RBFR13PIQE_001 to A.S.

Keywords: Staphylococcus aureus, SILAC, host-pathogen interaction, MRSA/MSSA isolate

P09.23 Proteomics Investigation of Pseudomonas Fluorescens Chromogenic Strains: Insight in Blue-Mozzarella

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Introduction and Objectives: In the last years an high number of mozzarella cheese coming from Italian and German establishments caused complaints in consumers due to unusual pigmentation of dairy products Many microbiological analysis have been performed revealing an ubiquitous non-pathogenic bacteria, Pseudomonas fluorescens, as a causative agents liable for this phenomenon. Some specific strains of this microorganism own gene sequences coding for enzymes, able to produce dyes that can induce anomalous coloration to food. It has been verified

that the occurrence of blue pigmentation in mozzarella cheese samples is however not related to chemical conditions, bacterial concentration, or critical temperature level. Both ribotyping and PFGE methodologies have underlined differences in Pseudomonas fluorescens strains isolated in food coming from different geographical areas. Nevertheless the phylogenetic assessment of bacteria pointed out no correlation between the genetic profile of the microorganism and its chromogenic behavior. the aim of this study was a deeper proteomic investigation of P. fluorescens isolates in order to evaluate how these bacteria could represent contamination source. **Methods:** A shotgun proteomic approach has been performed on isolated Pseudomonas fluorescens cultures coming from samples of mozzarella cheese either showing anomalous pigmentation or not. LC-MSMS experiments have been carried on by nanoHPLC runs and auto MS^n acquisitions on a Bruker amaZon-ETD Ion Trap instrument. Protein identification has been executed either versus Pseudomonas dedicated DBs or on the open-reviewed databases. **Results and Discussion:** Obtained data highlighted several differential expressed proteins in two conditions. Among these, key proteins are, on one hand, the major cold shock protein that increases its level in the anomalous pigmentation, and, on the other, the phosphate starvation-inducible protein that decreases respect to normal condition. **Conclusion:** Proteomics analysis could allowed us to differentiate among chromogenic and non chromogenic strains at the molecular level, widening the knowledge on this bacteria and the molecular mechanism underlying this contamination

Keywords: Pseudomonas fluorescens, Blue-Mozzarella, dairy products, proteomics

P09.24 Investigating HIV-Mediated Dynamics of Cullin RING E3 Ligases by AP-MS and Proximity Biotinylation

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Introduction and Objectives: Human immunodeficiency virus (HIV) is known to hijack cellular components for its replication. A major target is the ubiquitin machinery, in particular Cullin-RING E3 ligases (CRLs). To interfere with antiviral pathways, HIV proteins, Vpu, Vpr and Vif, hijack CRLs to promote ubiquitination and degradation of host restriction factors. Here, we aim to systematically characterize HIV-mediated CRL complex dynamics and potentially identify new ligase-substrate relationships combining affinity purification mass spectrometry (AP-MS) and proximity biotinylation. **Methods:** We generated stable Jurkat T-cell lines expressing tagged CRL complex components. Purifications are performed following infection with wildtype HIV and Vif-, Vpu- or Vpr-deleted HIV mutants to link specific CRL PPIs to each viral protein. To obtain a high coverage of the CRL interactome we combine classical AP-MS for purifying native protein complexes with a novel technique for proximity-dependent biotin identification (BioID). For BioID proteins of interest are fused to the promiscuous biotin ligase BirA* that, upon addition of biotin, biotinylates vicinal proteins which are subsequently purified and quantified by MS. The covalent attachment of biotin allows for identification of transient interactors, such as ligase-substrate interactions, and captures interactions over the time of biotinylation. **Results and Discussion:** We demonstrated that AP-MS and BioID are complementary in identifying high-confidence CRL interactors. Both approaches confirmed a large number of previously reported CRL interactors, including CRL substrate receptors and regulators. Comparing HIV infected with mock-infected conditions, we detected known HIV-mediated dynamics of the CRL complexes, such as Vif-dependent recruitment of CBFB to CUL5 complex for the degradation of APOBEC3G. Furthermore, we identified novel interactors and quantified changes in PPI in the context of HIV infection that are potentially interesting for functional follow up studies. **Conclusion:** This project will provide the first systematic, quantitative CRL PPI network in the context of HIV infection and potentially reveal new ubiquitin ligase-substrate relationships relevant for HIV infection.

Keyword: HIV-host interactions

P09.25 Elucidation of the Ebola Virus VP24 Cellular Interactome

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Introduction and Objectives: Viral pathogenesis in the infected cell is a balance between antiviral responses and subversion of host-cell processes. Many viral proteins specifically interact with host-cell proteins to promote virus biology. Understanding these interactions can lead to knowledge gains about infection and provide potential targets for antiviral therapy. One such virus is Ebola, which has profound consequences for human health and causes viral haemorrhagic fever where case fatality rates can approach 90%. The Ebola virus VP24 protein plays a critical role in the evasion of the host immune response and is likely to interact with multiple cellular proteins. To map these interactions and better understand the potential functions of VP24, label-free quantitative proteomics was used to identify cellular proteins that had a high probability of forming the VP24 cellular interactome. **Methods:** VP24 was fused to GFP and over-expressed in 293T-cells. IPs were performed with a GFP-trap and label free quantitative proteomics used to discriminate background interactions. **Results and Discussion:** Several known interactions were confirmed, thus placing confidence in the technique, but new interactions were also discovered including one with ATP1A1, which is involved in osmoregulation and cell signaling. Disrupting the activity of ATP1A1 in Ebola-virus-infected cells with a small molecule inhibitor resulted in a decrease in progeny virus. **Conclusion:** Quantitative label-free proteomics was invaluable in identifying potential therapeutic targets in the viral pathogenesis of Ebola.

Keywords: label-free proteomics, Interactome,, Ebola, VP24

P09.26 High-Throughput Genetic Analysis of Salmonella-Host Interactions

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Introduction and Objectives: Salmonella directly injects effector proteins into host cells to subvert their function, ultimately leading to disease such as enteritis or enteric fever. Salmonella mutants deficient in the ability to inject effector proteins are dramatically attenuated, indicating the importance of this process, but mutants deficient in individual effectors often have little or no phenotype. To address the hypothesis that effectors may act in concert to exert their effects, we are pursuing

an approach of exhaustive combinatorial knock-out mutagenesis followed by high-throughput cell and animal based assays of virulence. **Methods:** Our mutagenesis approach employs recombineering/ lambda red in combination with pseudo hfr Salmonella to facilitate high-throughput transfer of effector mutations. Cell based assays of Salmonella virulence will use high-content analysis and flow cytometry to assess Salmonella entry into, and replication within cell lines, as well as perturbations of the cytoskeleton and endomembrane system. **Results and Discussion:** Up to this point, we are developing the mutagenesis approach to generate our strain collection for high-throughput phenotypic assays. Assays of actin accumulation at the site of Salmonella entry into host cells using high-content analysis, and surface exposure of MHC class II in infected cells by flow cytometry are in the process of optimisation. **Conclusion:** We intend on pursuing our results by examining genetic interactions between Salmonella and host by using knock-down host cells as well as proteomic analyses as appropriate.

Keywords: Type III secretion, Effector, host-pathogen interactions

P09.27 Endogenous Peptidomics Links Hemoglobin Digestion to Drug Resistance in the Malaria Parasite

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Introduction and Objectives: We recently reported an unexpected connection between chloroquine resistance in the malaria parasite, Plasmodium falciparum, and the accumulation of small peptides within the parasite. These seemingly unrelated phenomena occur when parasites carry drug resistant forms of the vacuolar chloroquine resistance transporter (PfCRT). Despite intensive research, little is known about the native function of PfCRT or why mutations in this protein would affect the levels of endogenous peptides. **Methods:** We employed high-resolution peptidomics to analyze a comprehensive panel of transgenic parasites carrying genetically modified PfCRT proteins. All endogenous peptides found in parasite extracts were mapped to the human and malaria proteomes and the qualitative and quantitative changes in the global peptide profiles were associated with genetic changes in the parasite. **Results and Discussion:** We identified over 500 naturally-occurring peptides ranging in size from 2- to 32-mers. Most of these peptides mapped to α and β hemoglobin in clusters of closely related sequences. Breaks in these sequences corresponded to established protease (plasmepsin and falcipain) cleavage sites and the sub-cleavage of the peptides was consistent with the action of known peptidases. We interpret these data as a comprehensive phenotype of the complete hemoglobin digestion pathway and mapped qualitative and quantitative perturbations in this pathway to genetic changes in PfCRT. Using this strategy, we showed that drug resistance, impaired hemoglobin metabolism, and parasite fitness are closely related phenomena and that several mutations in the PfCRT sequence can have a significant impact on this metabolic pathway. **Conclusion:** These data provide a molecular explanation for the resistance-associated accumulation of peptides and show that PfCRT plays a direct role in vacuolar metabolism. The fitness costs of disrupted hemoglobin metabolism may explain why drug resistant parasites disappear from wild populations following the cessation of chloroquine treatment.

Keywords: Plasmodium, Chloroquine resistance, Hemoglobin catabolism, label-free proteomics

P09.28 Genetic Changes in HIV Effecting Drug Resistance and Immune Response

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Introduction and Objectives: Pakistan has witnessed a transition from low prevalence to a country with concentrated epidemic in high risk groups. Cytotoxic T lymphocytes play an important role in controlling HIV infection while the virus encounters and evade the CTL response through a variety of escape mutations. The anti HIV immunity is influenced by the diversity of host factors particularly human leukocyte antigen (HLA). Objective of this study was to analyze mutations in HIV and association of patient's HLA types with immune response and drug resistance. **Methods:** Blood samples were collected from HIV positive patients followed by DNA extraction which was used in HLA typing and in Nested PCR to amplify the gag and pol gene using specific primers followed by sequencing. Potential proteosomal degradation sites were checked using Netchop and the selected peptides containing the proteosomal degradation sites were synthesized and used in protoeosomal degradation assay. RT and Protease gene Sequences were entered into Stanford HIV Database for drug resistance and mutation analysis. **Results and Discussion:** 93% of sequences have no major or minor mutations. Rest of the sequences only show a major mutation Y115F causing virus to exhibit low to intermediate resistance against lamivudine and emtricitabine. T303V mutation was identified in gag region in most of the Pakistani isolates which is a stronger proteosomal degradation sites as compared to T303T in majority of the global sequences. The in-vitro proteosomal degradation assay also support these results. The HLA types of the patients were relatively diverse but HLA A*68 was found more prevalent than other HLA types. **Conclusion:** HIV-1 in Pakistan is dominated by subtype A-1 with T303V mutation, the infection is relatively new and the virus is evolving under the HLA types and other locally prevailed selected pressures. Majority of the rt sequences did not have any major or minor drug resistance mutations.

Keyword: HIV, PAKISTAN, GENETIC CHANGES

P09.29 Proteogenomic Characterization of Aniline Degrading Bacterium, Burkholderia Sp. K24

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Introduction and Objectives: Burkholderia sp. K24 (formerly known as Acinetobacter lwoffii K24) was a soil bacterium capable of utilization aniline as sole carbon and nitrogen. Genomic sequence analysis reveals Burkholderia sp. K24 has biodegradation gene clusters for various monocyclic aromatic compounds. However, genomic analysis only shows the putative biodegradation pathways. **Methods:** We used LC-based proteomic analysis to identify and confirm the biodegradation pathway enzymes of monocyclic aromatic compounds. Genomic and proteomic results were integrated for the monocyclic aromatic compound biodegradation pathways. **Results and Discussion:** Aniline and p-hydroxybenzoate degradation share the down-pathways of β-ketoadipate pathway. Among the two benzoate degradation pathways, benzoyl-CoA degradation pathway is selectively induced in benzoate culture condition. **Conclusion:** Proteomic result suggest that all monocyclic aromatic compounds are converged into TCA cycles for next catabolic and anabolic pathways. Our result suggest that proteogenomic analysis are useful tools for the interpretation of bacterial biodegradation pathways.

Keywords: aniline degradation, proteogenomic analysis, Burkholderia sp.

K24, monocyclic aromatic compounds degradation

P09.30 Comparative Proteomic Analysis of C. Difficile Clinically Relevant Ribotypes

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Introduction and Objectives: Clostridium difficile is a gram-positive spore-forming anaerobe and causative agent of C. difficile infection (CDI) in humans and animals. Healthcare diarrhoea caused by C. difficile is a global issue with significant mortality and economic burden. The virulence factors have been studied extensively by the means of the genomic based techniques but only limited number of studies was performed on the proteomic level. Taking the advantage of the modern instrumental platforms, the comparative study focused on the relevant clinical C. difficile isolates belonged to different PCR-ribotypes was proposed. **Methods:** The panel of eight C. difficile ribotypes producing A and B toxins (TcdA and TcdB), with or without presence of the gene for binary C. difficile transferase (CDT) and two negative controls (non-toxigenic C. difficile strains) were cultivated anaerobically in thioglycolate broth for five days. Due to the prerequisite of the importance of the secreted fraction in the C. difficile pathogenesis, the precipitates containing secreted proteins from the supernatant of the bacterial culture were studied only. The fraction was digested, separated using a nanoflow liquid chromatography system and analysed by non-targeted high-resolution mass spectrometry (Q Exactive™). **Results and Discussion:** Important peptides involved in C. difficile pathogenesis were identified including toxins TcdA, TcdB and CDT. Moreover, the sensitivity of the applied technique enabled the identification of putative anti-sigma factor (tcdC) at the level of protein. The preliminary estimation of the quantity of TcdA and TcdB differentiated strains with and without presence of the gene for CDT corresponding with the level of their clinical importance. In addition, new candidates for the virulence factors were proposed. **Conclusion:** The employment of the high-resolution mass spectrometry based proteomic approach enabled complex identification of several critical proteins (TcdA, TcdB, CDT and TcdC) involved in the pathogenesis of C. difficile for the first time and prepared the way for the further quantitative analyses.

Keyword: Clostridium difficile Toxins

P09.31 Proteomic Characterization of Vascular Adhesins from the Syphilis Spirochete, Treponema Pallidum

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Introduction and Objectives: Treponema pallidum spp. pallidum is the causative agent of venereal syphilis, a human-specific sexually transmitted infection typified by multi-stage disease and diverse clinical manifestations. Within hours of inoculation, T. pallidum disseminates through the vasculature and gains access to distal host sites where organisms penetrate tissue, placental, and blood-brain barriers; however the molecular mechanisms of T. pallidum dissemination have yet to be fully elucidated. Pallilysin (Tp0751) is a surface-exposed lipoprotein that co-associates with Tp0750, and together these proteins exhibit dual functionality as proteases/adhesins. Pallilysin and Tp0750 play a role in entry of T. pallidum to the vasculature and evasion from intravascular immune factors based on their shared capacity to degrade and associate with host components. Furthermore, pallilysin mediates adherence to endothelial cells under shear flow conditions, demonstrating a specific high-affinity interaction. This study explores the direct interactions of pallilysin and Tp0750 with human endothelial cells to establish roles for each protein in bacterial attachment to the vascular endothelium **Methods:** Affinity chromatography coupled with tandem mass spectrometry analysis was used to identify host endothelial receptors targeted by pallilysin and Tp0750. Membrane proteins isolated from human endothelial cells were incubated with recombinant Tp0750- and pallilysin-cobalt chelate affinity columns and the interacting proteins were identified with mass spectrometry. Results were confirmed through plate-binding assays. **Results and Discussion:** These investigations reveal that Tp0750 binds annexin A2 on the endothelial cell surface, a receptor that plays a role in cell-cell junctional remodeling. Current investigations aim to identify the binding target of pallilysin on endothelial cells using the same methodology, and future work will focus on understanding the functional outcomes of pallilysin- and Tp0750-mediated adherence to the vascular endothelium. **Conclusion:** Proteomic characterization of the interactions of pallilysin and Tp0750 with human endothelial cells will provide insights into T. pallidum dissemination mechanisms, and will uncover targets for syphilis vaccine design.

Keywords: host-pathogen interactions, dissemination, Treponema pallidum, Vascular endothelium

P09.32 Effects of LPS Stimulation in Bovine Uterine Epithelial Cells Model

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Introduction and Objectives: E coli is one of the most frequent bacteria involved in uterine diseases. Lipopolysaccharide (LPS) is a component of outer membrane of Gram-negative bacteria involved in the pathogenic processes leading to post-partum metritis and endometritis in cattle. The aim of this study was to investigate possible changes in protein expression in relation with the proliferative response of bEEC after challenge with E. coli-LPS. **Methods:** Both top down approach and bott up were used to monitor protein expression of uterine cells. Target validation was successful applied to differential expressed proteins. Results from 2-D gel coupled to MALDI-TOF/TOF were very reproducible (same responses between individual cows) and revealed changes in protein profiles very much related (from $p<0.05$ to $p<0.01$) to proliferative phenotypes for more than 5 proteins. **Conclusion:** Differentially expressed proteins were associated to cell proliferation, apoptosis, oxidative stress, regulation of histones, and general cell metabolism pathways. Candidate proteins needs to be confirmed from larger series of individuals and relevant pathways further studied. Work supported by PROLIFIC Project KBBE EU GRANT 311776 7FP, PR and PH

P09.33 Functional Analysis of Gut Microbiome Samples Using Large-Scale Metaproteomics and Activity-Based Probes

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Introduction and Objectives: Existing computational analysis methods are limited by the size of their sequence databases, severely restricting the proteomic sequencing depth and hindering functional analysis of highly complex specimens. **Methods:** Using a broadly applicable metaproteomic analysis method that addresses protein database size limitations by integrating a rapid search engine, Blazmass, with a scalable set of sequence databases derived from genomic libraries, ComPIL, we analyzed several unenriched and probe-enriched human microbiome samples. We developed a novel clustering approach for peptide-to-protein matching and functional annotation whereby redundant protein sequences were collapsed into clusters based on sequence similarity. Each protein cluster was then functionally annotated by propagation of InterProScan annotations. Differential analysis of samples was performed using a power law global error model, and functional analysis was performed using standard gene set enrichment analysis techniques on gene sets built using functional annotations. **Results and Discussion:** Proof-of-principle analysis of a human HEK293 lysate were specific enough to detect human proteins out of a vast library of 80 million proteins, and were sensitive enough to detect proteins from the adenovirus used to immortalize these cells, highlighting the importance of an expanded database including proteins from species not thought to be in your samples. In order to reduce the massive sample complexity of gut microbiome samples, a set of cysteine reactive probes were evaluated. The probes were able to consistently enrich for peptides that were never identified in the unenriched microbiome sample, allowing for a more detailed look at specific functionalities in the samples. Functional analysis confirmed the enrichment of proteins with domains containing nucleophilic cysteines. **Conclusion:** The ComPIL-Blazmass proteomic search method allows for protein identification and functional analysis in highly complex samples potentially containing a large number of proteins and simplifies proteomic analysis by focusing on enzymatic activities.

P10: POSTER SESSION - STRUCTURAL PROTEOMICS

P10.01 A Structural Proteomics Study of Native Alpha-Synuclein in Solution

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Introduction and Objectives: Parkinson's disease is characterized by the death of dopaminergic neurons in the substantia nigra of the brain, characterized by the formation of alpha-synuclein aggregates. The native form of this protein in solution is highly disordered, but a combination of crosslinking and ^{15}N coding of the alpha synuclein protein can provide a collection of inter- and intra-protein crosslinks for use in the elucidation of alpha synuclein structure in solution. **Methods:** ^{15}N labelled alpha synuclein was mixed 1:1 with the unlabelled protein and incubated overnight. Protein samples were then crosslinked separately using a panel of four crosslinking reagents including CBDPS, EDC, PICUP and ABAS, at concentrations determined previously by titration. Crosslinked samples were digested with proteinase K or trypsin, and CBDPS samples were enriched using monomeric avidin. Samples were analyzed on the Thermo Orbitrap Velos. Crosslinks were identified and crosslinked residues assigned using ^{14}N^{15}N DXMSMS Match software. **Results and Discussion:** Analysis of the crosslinking reactions by SDS-PAGE revealed the presence of a crosslinked dimer band. 49 crosslinks were found using all four reagents including four unique inter-protein crosslinks. The micelle-bound NMR structure of alpha synuclein features two alpha helices joined by a short loop and followed by a long, negatively charged tail. Two inter-protein crosslinks found with the zero-length PICUP crosslinker revealed close contacts between Y39 and Y132 and 136, joining the tail of each monomer to the loop of the other. The CBDPS inter-protein crosslink K10-K32 indicates that the loops are likely aligned. Two EDC zero-length intra-protein crosslinks, K43-E105 and E137, join the loop of the monomer to the tail. **Conclusion:** ^{15}N isotopic-coding and crosslinking of alpha synuclein in solution indicated a globular, dimer structure in a parallel arrangement, with the tail of each of the monomers moving back across the alpha helical regions to interact with the loop between them.

Keywords: Crosslinking, isotopic-coding, Parkinson's disease, alpha-synuclein

P10.02 A Crosslinking Study of Fibrin Polymerization

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Introduction and Objectives: Fibrin clot formation plays a central role in thrombus formation. The serine protease thrombin proteolytically cleaves fibrinogen converting it to fibrin, after which fibrin molecules associate both laterally and longitudinally to form fibrin gels that serve as the structural scaffold for blood clot formation. Despite the fact that fibrinogen and fibrin clots have been well studied, questions still remain regarding the protein structural details of clot assembly. Toward a further understanding of these details we used purified fibrinogen to prepare in-vitro polymerized fibrin clots and studied their structure using crosslinking with multiple crosslinking reagents of various reactivities. The multiple structural constraints obtained will allow us to experimentally confirm and to refine structural models of the fibrin protofibril. **Methods:** Fibrinogen was converted into fibrin by reacting with thrombin for 1 hour at 37°C. The supernatant was discarded, and the fibrin gels were washed twice with PBS pH 7.4 (end-over-end) for 1 hour. Fibrin gels were crosslinked, crosslinking reactions were quenched, crosslinked protein was digested with trypsin, pepsin, or proteinase K. Crosslinked peptides were enriched on monomeric avidin beads, separated by HPLC, and analyzed by high-resolution ESI-MS/MS using an Orbitrap Velos Pro FT-MS. DXMSMS Match within the ICC-CLASS Software Suite (Creative Molecules Inc.) was used for identifying crosslinks. **Results and Discussion:** Numerous crosslinks of both intra- and inter-chain origin have been obtained. Some of the structural constraints derived from this data have already shed light on the structure and function of the enigmatic alpha-C domain in fibrin, and have provided previously unavailable structural details of fibrin polymerization. A molecular model of fibrin protofibrils is being constructed that utilizes these new structural constraints. A more precise model is being constructed as more constraints are identified. **Conclusion:** Crosslinking analysis has yielded structural details about the protein-protein interactions and arrangement of fibril and protofibril within

fibrin polymers.

Keywords: polymeric protein complexes, fibril structure, fibrin clot structure, Crosslinking

P10.03 A Feature Analysis of Lower Solubility Proteins in Unstressed and Heat-Shocked Yeast Cells

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Introduction and Objectives: Proteins can misfold and aggregate both in unstressed or stressed conditions such as heat-shock. Failure to eliminate misfolded and aggregated proteins can be deleterious to the cell and is associated with numerous neurodegenerative diseases. In this study, we analyzed features of lower solubility proteins in unstressed and heat-shocked yeast cells to deepen our understanding of potential causes to conformational diseases. **Methods:** Yeast cells were SILAC labeled and low solubility proteins were sedimented by centrifugation. For the analysis of unstressed cells, heavy-labelled low solubility proteins were mixed with equal mass of soluble proteins. In the stressed conditions, low solubility proteins from both light-labelled unstressed cells and heavy-labelled heat-shocked cells were compared. Proteins were then tryptic digested and fractionated offline prior to be analyzed by nLC-MS/MS and MaxQuant. **Results and Discussion:** We first analyzed lower solubility proteins in unstressed cells by normalizing for their abundance. Interestingly, we found that lower solubility proteins were longer, expressed at lower levels and had fewer hydrophobic residues. In addition, these proteins contained more functional related secondary structural features such as low complexity regions, disordered regions and eukaryotic linear motifs. Remarkably, after heat-shock, lower solubility proteins displayed different features and were shorter with fewer polar residues. Our finding indicates that majority of lower solubility proteins found in unstressed or heat-shocked cells are from two different population of proteins. Lower solubility proteins in unstressed cells are potentially involved in formation of functional aggregates, while decrease of protein length and number of polar residues lower proteins' ability to maintain their structure and lead to misfolding and aggregation upon heat-shock. **Conclusion:** Protein susceptible to heat-shock have been associated to decline of proteostasis upon aging. Our results reveal a number of distinct features are associated with lower solubility proteins in stress conditions that may explain why some proteins are more susceptible to aggregation and cause diseases.

P10.04 Structural Proteomic Study of High Density Lipoprotein (HDL)

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Introduction and Objectives: High-density lipoprotein (HDL) facilitates transportation of lipids in the bloodstream. Apolipoprotein A-I and A-II (apoA-I, apoA-II) are the primary protein constituents of HDL, defining its structure and function. The structure of proteins within HDL is difficult to study using conventional methods. Our objective is to obtain a large number of reliable and mutually-consistent structural constraints for the structural modelling of apoA-I and apoA-II HDL particles under differing cholesterol loads, using a combination of advanced protein chemistry and mass spectrometry techniques. **Methods:** HDL samples were surface modified with isotopically-coded amine-reactive reagent pyridinecarboxylic acid N-hydroxysulfosuccinimide ester (PCAS-^{12}C6,-^{13}C6) followed by pepsin digestion for surface modification, digested with pepsin for limited proteolysis, crosslinked with a panel of crosslinking reagents which differ in reactivity and spacer length then digested with trypsin. Digests were analysed by LC/ESI-MS/MS. Top-down HDX was performed on Bruker 12 Tesla FTICR mass spectrometer. **Results and Discussion:** ApoA-I residues representing Y18-K40 and K133-S167 regions were differentially modified by PCAS reagent in native and urea-unfolded HDL indicating buried nature in the native state. Limited proteolysis of the HDL showed a rapid removal of the N-terminus of apo-AI, followed by cleavage in aa107-aa116 region, indicating its surface exposure. With HDX, 110 protons of apoA-I were protected, some of which were located at N- and C- terminal regions, Crosslinking of HDL particles yielded multiple crosslinks of intra- and inter-protein origin. We have obtained reliable and mutually-consistent structural constraints for the structural modeling of apoA-I and apoA-II in HDL particles using multiple structural proteomics approaches. These results will be crucial for improving our current understanding of HDL physiology and for the development of the therapeutic approaches for the treatment of cardiovascular disease. **Conclusion:** The complementary data from multiple structural proteomics methods can be used for the elucidation of HDL protein structure and validation of existing HDL protein structural models.

Keywords: limited proteolysis, surface modification, hydrogen/deuterium exchange (HDX), Crosslinking

P10.05 Investigation of Structural Dynamics of Aquoporin Z at High Osmolar Environment with HDXMS

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Introduction and Objectives: Demand for freshwater is rising constantly and there exists an urgent need to provide freshwater for a large variety of uses both domestically and industrially. The discovery of Aquaporin-based biomimetic membrane for freshwater production has shed light to a possible solution for more freshwater. It is important to understand at molecular level how aquaporin proteins function under harsh process of desalination which subjects the protein to high osmolality not present in its native environment. The current study amins to investigate the structural dynamics of aquaporin Z at high osmolar environment. **Methods:** We employ the use of structural proteomics method namely, amide hydrogen/deuterium exchange mass spectroscopy (HDXMS) to identify conformational/dynamic changes in Aquaporin Z as a read out of differences in deuterium labelling under such extreme osmolarity using inert osmolyte (sucrose). **Results and Discussion:** Our results identify a global decrease in dynamics of the protein with varying degree at different regions of the protein. In addition, we observed that the highly conserved functional motif of Aquaporin family namely NPA/NPAR motif shows the greatest degree of decrease in dynamics. **Conclusion:** Our findings suggest that NPAR motif may play a key role in regulation of water permeability under environmental perturbation. This finding highlights the importance of understanding the protein-environment interactions and has significant implication to the fabrication of aquaporin based biomimetic membrane which requires the incorporation of aquaporins in non-native environment.

Keywords: aquaporin, structural dynamics, high osmolarity, amide hydrogen deuterium/exchange mass spectrometry

P10.06 In Vivo Measurement of Mammalian Ribosome Maintenance

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Introduction and Objectives: The eukaryotic ribosome is a huge molecular machine composed of 5 RNA strands and ~80 unique proteins. It translates mRNA into proteins, and is therefore critical in the maintenance of protein homeostasis. The number of ribosomes in the cell is carefully regulated by control of both synthesis (assembly) and degradation (ribophagy). Control of ribosome quality is an important part of the regulation. Poor ribosomal quality leads to poor control of protein homeostasis and is connected to cancer, anemia, and aging. Ribosome quality is verified in part by the rate of ribosomal protein synthesis. The Ribosome Quality control Complex (RQC) tests the 60S subunit of stalled ribosomes for its capability to produce protein. The RQC controls whether the stalled 60S remains in the pool of active ribosomes. What happens to the ribosomes that fail the test is less well understood. Our objective is to identify a repair or maintenance strategy which allows ribosomes to be refurbished. **Methods:** We used previously described methods for in vivo metabolic labeling with deuterium to monitor synthesis of ribosomal RNA, 70+ proteins within the assembled ribosome, and 1000+ free proteins within the cell. Ref: Price, et al. Mol.Cell.Prot. 2012, 11 (12), 1801-1814 **Results and Discussion:** We find that exchange of ribosomal proteins between the cytosol and the assembled structure is protein specific and in response to cellular needs changes independent of the ribophagy rate. **Conclusion:** We propose a mechanism for ribosomal maintenance whereby variation in the synthetic burden on the ribosome may increase ribosomal fidelity and hence the quality of newly synthesized proteins. We provide in vivo measurements showing stimulus dependent increases in exchange rates for proteins within the assembled ribosome without accompanying changes in ribophagy relative to control. This model provides a mechanistic explanation for the frequently observed link between reduced ribosome synthetic burden, improved protein homeostasis and increased longevity.

Keywords: Kinetic Proteomics, Protien Turnover, Metabolic Labeling, Aging and Longevity

P10.07 Crosslinking Study of IAPP Oligomers

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Introduction and Objectives: Islet amyloid polypeptide (IAPP) appears to be an intrinsically disordered protein that is normally expressed in the beta-cells of pancreatic islets. Amyloid deposits containing IAPP are often observed in the islets of individuals with type-2 diabetes, and are associated with beta cell death. Currently in protein misfolding disease research, it is believed that misfolded aggregated forms of proteins execute their toxic actions through interaction with specific cellular targets. The goal of the project is to gain insights into the IAPP-related diabetes pathogenesis by elucidating the structure of the oligomeric aggregated species of IAPP. We prepared IAPP oligomers in-vitro and studied their structure through crosslinking with multiple crosslinking reagents of various reactivities. Crosslinks will provide structural constraints to construct a model. **Methods:** Stocks of IAPP suspended in DMSO were diluted with PBS pH 7.4 and allowed to form oligomers for 1 hour at room temperature. IAPP was then crosslinked, the crosslinking reactions were quenched, and the crosslinked protein was digested using proteinase K. When possible, crosslinked peptides were enriched using avidin beads before being analyzed by LC-ESI-MS/MS using an Orbitrap Velos Pro mass spectrometer. DXMSMS Match of ICC-CLASS (Creative Molecules Inc.) was used for the identification of crosslinks. **Results and Discussion:** We have applied a panel of crosslinking reagents to IAPP oligomers (CBDPS, ABAS, and TATA), yielding crosslinks of both intra- and inter-chain origin. The longer crosslinking reagents (CBDPS) provide structural constraints that are used to determine the arrangement of monomers in the oligomer complex, while shorter reagents (ABAS, and TATA) are used to determine detailed features of the complex subunits. These constraints were then combined to construct a molecular model of human IAPP oligomers. As more crosslinking constraints are identified, a more precise model can be refined. **Conclusion:** Crosslinking analysis has yielded protein interaction structural details of the oligomers of human IAPP

Keywords: Islet Amyloid Polypeptide, structural proteomics, Mass spectrometry, Peptide Crosslinking

P11: POSTER SESSION - OTHER

P11.01 Permanent Proteins in the Urine of Healthy Humans during the Mars-500

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Introduction and Objectives: The adaptive plasticity of the human proteome is significant problem in current biomedical investigations. The urinary proteins serve as indicators of various conditions in human normal physiology and disease pathology. Comparison of the normal urinary proteome with the urinary proteome from patients with a defined disease can detect proteins expressed differently from one another. But diagnostic biomarkers of the various diseases usually have been searched in group of patients without a defined investigated disease, which cannot be referring to the healthy category. **Methods:** Using mass spectrometry proteome analysis, the permanent constituent of the urine was examined in the experiment Mars-500 (520 days isolation of healthy volunteers in a terrestrial complex with an autonomous life support system). **Results and Discussion:** Seven permanent proteins with predominant distribution in the liver and blood plasma as well as extracellular localization were identified. Analysis of the overrepresentation of the molecular functions and biological processes based on Gene Ontology revealed that the functional association among these proteins was low. Previously we analyzed urine protein composition samples of ten Russian cosmonauts performed long flight missions on the International Space Station. It was identified more than 200 proteins, among which there were the proteins specifically associated with space flight, as well as permanent proteins, independent from the flight, presented on all stages of observations during the experiment. All proteins analyzed in this study were represented among these permanent proteins. **Conclusion:** The results showed that the identified proteins may be independent markers of the various conditions and processes in healthy humans and that they can be used as standards in determination of the concentration of other proteins in the urine.

Keyword: proteome urinary Mars-500 isolation

P11.02 Dynamics of Proteome Urine of Healthy Volunteers Exposed 105-Day Isolation

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Introduction and Objectives: Using mass spectrometry proteome analysis, the permanent constituent of the urine was examined in the experiment Mars-500 (520 days isolation of healthy volunteers in a terrestrial complex with an autonomous life support system). **Methods:** All samples were analyzed using mass ion cyclotron resonance mass spectrometer with Fourier transformation LTQ FT MS (Thermo) on the basis of the AMT-tags (accurate mass and time tags) approach. **Results and Discussion:** Among more than 20 000 trypsin' peptides we found out 690 proteotypic ones and identified about 600 urine proteins. For physiological interpretation of the proteomic data it was used bioinformatic tool ANDSystem. Application of ANDSystem revealed proteins that are most closely associated with regime of sodium intake, as well as build the network of their interactions. Using cluster analysis (Self Organizing Maps) method it has been identified clusters of proteins with similar dynamics of appearance and disappearance in the urine of volunteers in the experiment. It was revealed that there were two experimental period in which the protein composition of the urine significantly altered - 7 and 13 weeks, which may be associated with the transition of volunteers to reduced sodium consumption; in the first case - from high to medium and the second - from the middle to low (from 12 to 9 and from 9 to 6 g/day, respectively). Performed clustering k-means with the assessment of the reliability of the events that proteins of the same processes that provide the same function with the same cellular localization, according to Gene Ontology, going inside these clusters, with filtering of false-positive results. **Conclusion:** Thus, this bioinformatic approach allowed for a comprehensive analysis of proteomic data with access to the physiological processes of the human body with the assessment of their dynamics during the experiment.

Keyword: proteome urine isolation

P11.03 Proteomic Changes Associated with Reproductive Periods in Male Polychaetous Neanthes Arenaceodentata

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Introduction and Objectives: The polychaetous annelid Neanthes acuminata complex is cosmopolitan in distribution and being used in many types of research including longevity and toxicology. The reproductive pattern in this species of Neanthes is unique because female reproduces once and the male can reproduce up to nine times. Females die after laying eggs and males, after fertilization, incubate the embryos until the larvae leave the male's tube 21-28 days later. The purpose of this study is to measure the proteomic changes in the male after each reproductive period. **Methods:** The percent success of male reproduction was measured up to nine successive reproductive periods by the larvae present at the end of the incubation period. iTRAQ based quantitative proteomics was used to measure the dynamics of protein expression patterns. **Results and Discussion:** The percent success of male in producing juveniles increased through the first three reproductive periods then decreased, but the number of juveniles produced was similar through all nine reproductive periods. Several proteins were identified which altered the expression pattern. The abundant expression of muscular and contractile proteins may have influenced the body weight and successful reproduction. **Conclusion:** Proteins associated with fertilization and sperm maturation may provide a clue on molecular mechanisms of a male capable of reproducing many times.

Keyword: Polychaetes, Neanthes, reproduction, proteomics

P11.04 Redox Regulation of SAT1 Activity

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Introduction and Objectives: Oxidative stress refers to elevated levels of intracellular reactive oxygen species (ROS) such as superoxide anion (O2), hydrogen peroxide (H2O2), and hydroxyl radicals (OH-). ROS generated in the cell are normally cleared by glutathione peroxidase, thioredoxin, and other oxidation-reduction (Redox) related molecules. Redox signalling refers to free radicals, ROS, and other electronically-activated species that act as messengers in cells. There is a growing list of redox-active enzymes using intrinsic redox changes to control their biological activity. **Methods:**
Results and Discussion: Here, we found that spermidine/spermine-N1-acetyltransferase 1 (SAT1) contains redox sensitive reactive cysteine residues. Importantly, we discovered that the extremely inducible SAT1 enzyme activity under oxidative stress is governed by a redox regulated SAT1 protein conformation change mechanism. The oxidative stress induced SAT1 activity requires its redox sensitive reactive cysteine residues. We also showed that polyamine analogues induced SAT1 activation via a different mechanism. We identified a potential redox motif or redox switch on SAT1. All drugs targeting SAT1 are polyamine analogues or antagonist-based and are known for their cytotoxic side-effects. Our SAT1 redox regulatory mechanism opened a new avenue for designing high efficacy drugs that do not target SAT1 polyamine substrate binding sites but target SAT1 redox motif instead. **Conclusion:**

P11.05 Effects of Different Glaucoma Drugs on Proteomic Expression Levels in Corneal and Conjunctival Cells

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Introduction and Objectives: Benzalkonium chloride (BAC) is the most common preservative used world-wide in cosmetics and eye drops; however, it must be used carefully as it is toxic to epithelial cells and the pathways in this toxicity are largely unknown. In this study, the effects of common ophthalmic drugs for glaucoma were uncovered by exposing human corneal and conjunctival epithelial cells (HCE and IOBA-NHC) to treatments with and without BAC. It was hypothesized that these drugs may be linked to inflammatory mechanisms and cell death. **Methods:** HCE and IOBA-NHC cells were exposed to a preservative-free ophthalmic medication tafluprost or a similar drug latanoprost which contains BAC or BAC by itself for 24 hours. A dilution of 1:300 was applied to tafluprost

and latanoprost. BAC concentration of 0.000067% was used, which equals to the BAC concentration in 1:300 diluted latanoprost. The proteomic profiles of treated and untreated cells were analyzed with NanoLC-TripleTOFMS using SWATH technique. Mixed-effects ANOVA model was implemented to data and Benjamini-Hochberg procedure was used for multiple testing correction. **Results and Discussion:** The SWATH library for >2700 proteins was created from the samples and 2299 and 1972 proteins were relatively quantified for IOBA-NHC and HCE cells respectively. Statistical analysis identified 29 differentially expressed proteins for IOBA-NHC cells (fold change>1.25 or <0.8, q-value<0.25) and 28 for HCE cells (fold change>1.5 or <0.67, q-value<0.25). For the IOBA-NHC cell line, INHA(1.491.07, p=0.0005) and PSMB8(2.521.32, p=0.008), which are both related to apoptosis, were overexpressed in cells receiving BAC. For the HCE cell line, HSPD1(1.641.13, p=0.008), OAS3(1.821.17, p=0.01) and LAMP1(7.851.81, p=0.01) were overexpressed in BAC-treated samples and these are connected to immune system activation. **Conclusion:** Proteomic profiling revealed the BAC induced changes with the induction of apoptotic pathways and inflammation in both HCE and IOBA-NHC cells. These potential novel proteomic biomarkers will be further analyzed in ongoing clinical studies of glaucoma patients.

Keywords: Cell line, Preservative toxicity, Mass spectrometry, SWATH

P11.06 A Highly Sensitive Strategy for Quantitative Analysis of Cancer Related Protease in Living Cells

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Introduction and Objectives: Protease plays essential roles in a wide range of biological processes and human diseases, such as cancer. One of the fundamental ways to carry out cancer therapy is to control apoptosis. Caspase family proteases have been identified as a central mediator for the initiation and propagation of apoptosis. To better understand their key roles in cell apoptosis and cancer development, it would be crucial to develop probes to sensitively quantify the dynamic changes of caspases activity in different conditions in living cells, which may provide great assistance for clinical diagnosis and cancer therapy. **Methods:** ICP-MS is a highly sensitive analytical technique used for element quantification that can determine most of elements below ppb level with excellent mass resolution and dynamic range. Herein, we developed a simple and effective method for sensitive quantification of caspases activity in apoptosis cells by synthesizing new nanoprobe with element labeled substrate peptide of caspases and gold nanoparticle conjugates. **Results and Discussion:** As a proof of concept, caspase-3 was chosen as a model system. After caspase-3 cleaves the substrate peptide of the nanoprobe in living cells, the element tagged on the cleaved peptide was collected and detected by ICP-MS for quantitative determination of the activity of caspase-3 in the range from 5 ng/mL to 70 ng/mL. **Conclusion:** To the best of our knowledge, this is the first study to reveal the activity of caspase-3 produced in STS-induced apoptosis and may provide deeper insights for the understanding of cell apoptosis process. Furthermore, this simple designed method could be expanded as a general strategy to quantitatively determine the activity of other proteases in living cell and explore their functions and biological roles.

Keywords: cancer cells, ICPMS, quantitative analysis, protease

P11.07 A Strategy for Large-Scale Analysis of Asymmetric Inheritance of Old-Age Proteins at Cell Division

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Introduction and Objectives: Budding yeast cells undergo asymmetric cell division through which daughter cells arise from mother cells. While mother cells have a limit of the number (from 20 to 30 times) of producing daughter cells before their death due to ageing of mother cells, daughter cells which emerge via budding process are rejuvenated to refresh ageing stage. During asymmetric cell division, some groups of older proteins originally localized in mother cells are known to be also asymmetrically inherited into mother and daughter cells. In order to explore a hypothesis that old-age proteins specifically retained in mother cells are associated with ageing process, we carried out systematic analysis of asymmetric inheritance of old-age proteins. **Methods:** We conducted an experimental strategy as follows. Cell wall of mother cells synchronized at G1 stage are labeled with biotin, followed by incorporation of stable isotope-containing amino acid into newly synthesized proteins during the progress of single cell cycle. Then mother and daughter cells are separated with streptavidin-immobilized magnetic beads. Fractions of newly synthesized and old-age proteins were compared between mother and daughter cells by quantitative mass spectrometric analysis in a proteome-wide scale. **Results and Discussion:** In consistency with previous report, proteins that were newly synthesized in daughter cells during cell division were involved in cell wall biogenesis and ion transport through plasma membrane, demonstrating proof-of-principle of our strategy. On the other hand, old-age proteins related to aerobic respiration in mitochondria and proteolysis in vacuole were dominantly retained in mother cells. Given that functions of mitochondria and vacuole are known to decrease in the ageing process, our finding could reflect accumulation of old-age and likely damaged proteins causing dysfunction of these organelles. **Conclusion:** Our experimental frame work would provide global view of asymmetric protein inheritance and lead to understanding of how old-age proteins affect cellular ageing process.

Keywords: Asymmetric cell division, Ageing, Asymmetric protein inheritance, Budding yeast

P11.08 Proteomic Profiling of Six Snake Venoms in Taiwan for Discovering Snakebite Biomarkers

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Introduction and Objectives: Taiwan is located in South Pacific, a subtropical region that has more than forty snake species, and six of them are responsible for most of the clinically significant snakebites. Four of these venomous snakes belong to the Viperidae family that include Deinagkistrodon acutus(DA), Viridivipera stejnegeri(VS), Protobothrops mucrosquamatus(PM) and Daboia russelii siamensis(DRS), while the other two belong to the Elapidae family, Bungarus multicinctus(BM) and Naja atra(NA). Although, there have been four types of antivenoms available for treating snakebites caused by the six snakes, there have no any method or assay developed for rapid identification of the envenoming species. Therefore, this study aimed to discover snakebite biomarkers of the six clinical significant species in Taiwan for further development of multi-target rapid test. **Methods:** We used shotgun proteomics technology (LC-MS/MS and GeLC-MS/MS) to comprehensively profile the six snake venom proteomes and find some proteins may be specific for each venom. **Results and Discussion:** The venom proteome of DA, PM and VS share higher

similarity with each other. Bungarotoxin and cardiotoxin family proteins were mainly observed in BM and NA, respectively. Protein species detected in DRS were more complex than those in the other five snakes' venoms. Comparison of the six venom components revealed that some proteins may be specific for each venom. Using NA as an example, we discovered several venom proteins as snakebite biomarker candidates, and the major candidates belong to two protein families. We generated specific antibodies against one candidate and confirmed this candidate is specifically existed in the venom of NA, which could be applied to discriminate NA bite from other snakebites in Taiwan. **Conclusion:** This finding confirmed the feasibility of our strategy to find snakebite biomarker(s) with high potential for clinical application. Similar strategy is currently applied to find specific snakebite biomarker(s) for the other five venomous snakes in Taiwan.

Keywords: snakebite detection, biomarker, snake venom, venom proteome

P11.09 Resolving Discrepancies in Nephelometric Total IgG and Subclass Measurements with Mass Spectrometry

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Introduction and Objectives: Introduction: IgG subclasses are measured clinically to diagnose immunodeficiency and IgG4 related disease. In some patient samples, the sum of the immunonephelometric quantification of the individual subclasses (sum (IgGs)) is much greater than the immunonephelometric total IgG test. The mechanism behind the discrepant results is unknown. Objective: To compare sum (IgGs) and total IgG immunonephelometric measurements with those as measured by tryptic digest LC-MS/MS and by protein electrophoresis (PE). **Methods:** Serum samples from patients with and without IgG4 related disease (IG4RD) were measured for sum (IgGs) and total IgG by immunonephelometry, LC-MS/MS and electrophoresis, respectively. **Results and Discussion:** 40 samples with a range of IgG4 values were retrieved and tested. In the IG4RD cohort, the sum (IgGs), when measured by immunonephelometry, was greater than total IgG as measured by any of three methods (immunonephelometry, PE or TDTMS). This bias between sum (IgGs) and total IgG was predicted by the IgG4 level and was absent when IgG4 was at normal levels. This bias [sum (IgGs) – total IgG] was not evident when sum (IgGs) was measured with LC-MS/MS. In serum samples, with the largest bias [sum (IgGs), immunonephelometry – total IgG, any method], there were marked discordances in IgG2 levels: IgG2 by immunonephelometry was grossly elevated relative to IgG2 by LC-MS/MS. Discussion: Some commercial immunonephelometric methods measure IgG subclasses in a manner that is grossly discordant with that measured by other methods (LC-MS/MS, PE). Comparison of the individual LC-MS/MS subclass measurements with that of the corresponding immunonephelometric measurements highlights an apparent interference in the immunonephelometric IgG2 subclass measurement that is mediated by IgG4 levels in patients with IgG4 related disease. **Conclusion:** Mass spectrometric measurement of IgG subclasses can highlight interferences in immunonephelometric measurements.

Keywords: Immunonephelometry, LC-MS/MS, IgG subclasses

P11.10 Proteomic Profiling of the [PSI+] Yeast Prion Strain by Quantitative Mass Spectrometry

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Introduction and Objectives: Prions proteins can adopt a second conformation that induces the formation of amyloid fibrils that are responsible for the transmissible spongiform encephalopathy in mammals. In contrast to mammalian prion, yeast prion proteins are controversially considered as advantageous for natural survival rather than being toxic. [PSI+] describes the yeast prion state in which the translational termination factor, Sup35, forms amyloid fibrils, resulting in an increase of non-sense suppression and giving a distinct phenotype. Due to non-sense suppression in [PSI+], part of its proteome might have an increased amount of C-terminal extended sequences, which could potentially affect the stability of the proteins positively or negatively. By differentiating the proteomes between [psi-] and [PSI+] states and identifying the C-terminal extended proteins, it would provide us some insights for what biological pathways are affected and how they provide survival advantages for being in prion state. **Methods:** To profile the proteomes of [psi-] and [PSI+] yeast strains, cells were metabolically labelled using SILAC approach and three biological replicates were analyzed. Tryptic digested peptides were fractionated by offline basic reversed-phase chromatography using HPLC and were run on a high-resolution Bruker Impact II Q-TOF mass spectrometer. The data was processed using MaxQuant (v.1.5.2.8) and analyzed with Perseus (v.1.5.1.6). **Results and Discussion:** We successfully identified 3827 proteins (≥ 2 peptides, FDR < 1%), in which 3770 quantified in total cell lysates. These numbers cover >80% of actively expressed proteome in yeast. Among these IDs, proteins with C-terminal extension were identified and quantified. **Conclusion:** The current data shows that the proteomes between [psi-] and [PSI+] are surprisingly very similar. Future work will explore whether the proteome may be affected in different growth conditions or in different prion strains. Together we hope this data will contribute to our understanding of how the biological pathways are affected in [PSI+] prion state.

Keywords: Quantitative spectrometry, CTAILS, Stop condon read-through, Yeast prion

P11.11 Colims: An Open Source Lims System to Automate Proteomics Data Management, Processing and Analysis

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Introduction and Objectives: 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 (notably the HUPO PSI's mzML, mzIdentML, mzQuantML) to upload into public repositories such as PRIDE is sometimes missing. Here, we present colims, 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. This way, the storage of large quantities of data can happen in a controlled manner on a preferably 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 failures. Colims currently supports import from MaxQuant and PeptideShaker, two commonly used software packages for analyzing mass spectrometric datasets. The latter supports various search engines input such as X!Tandem, MS-GF+, MS Amanda and Mascot. The imposed use of controlled vocabulary terms for the metadata facilitates the export into public repositories through for example ProteomeXchange. This makes colims a key component in any proteomics research group.
Methods: not applicable
Results and Discussion: not applicable
Conclusion: not applicable

Keywords: lims system, Bioinformatics, mass spectrometry-based proteomics

P11.1 Gas Phase Interactions of Nucleosides with Organomercuric Compounds

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Introduction and Objectives: The knowledge of the modes of bonding of metal ions with biomolecules, including DNA, is crucial for a definitive understanding of the mechanisms of the physiological functions of metal ions. Many organometallic compounds are considerably more toxic than the corresponding free metals. As far as mercury compounds are concerned, attention is mainly focused on highly toxic methylmercury ($MeHg^+$) and inorganic mercury (Hg^{2+}), since the latter one can be transformed into $MeHg^+$ via abiotic and biotic processes. In vitro interactions of model nucleobases nucleosides and nucleotides with heavy metals, such as Hg (II), can potentially provide a molecular basis for the genotoxicity and environmental impacts of mercury compounds.Gas-phase studies may provide useful insights about the mechanism occurring at the molecular level between Hg (II) species and DNA building block. In this respect, we started a combined experimental and theoretical study on the reactivity of nucleobases (NB) and nucleosides (NC) towards organomercuric compounds (R = CH3, n-Bu and t-Bu).
Methods: MS and MS/MS experiments were performed with a QqTOF instrument. Tandem MS experiments combined to infrared multiple photon dissociation IRMPD) were performed in order to probe the structure of the generated complexes. Theoretical calculations were carried out in the framework of Density Functional Theory.
Results and Discussion: MS experiments have notably shown that interaction of RHg^+ ions with nucleosides (NC = adenosine, guanosine, uridine, thymidine and cytidine) gives rise to abundant $[R(Hg)NC]^+$ complex through electrospray ionization (ESI).Upon activation, these complexes mainly lose sugar. They dissociate according to an alkyl transfer reaction associated with elimination of Hg^0, to form $[R(NB)]^+$ species. This process is particularly overwhelming with methyl mercury chloride (R =CH3).Tandem MS experiments combined IRMPD probed the structure of the generated complexes. Remarkably, methylation of uracil results in an ion isobaric to protonated thymine, and with data on a series of methylated uracils.
Conclusion: not applicable

Keywords: ab initi calculations, nucleoside, organomercuric compounds, Mass spectrometry

P11.13 Crowdsourcing Cardiac Mitochondrial Proteomics Knowledge in Gene Wiki

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Introduction and Objectives: Human protein data is now widely available in published literature and gene/protein knowledgebases, it remains largely inaccessible to the general public. Wikipedia, specifically Gene Wiki, is an existing platform with an established user base and interface that enables collective curation efforts. The Cardiac Gene Wiki project strives to improve the accessibility of protein knowledge by means of a structured crowdsourcing approach and novel curation tools.
Methods: The Cardiac Gene Wiki team established standardized criteria to score 556 human heart mitochondrial proteome pages for completeness and a protocol for populating incomplete pages with content. A software program was developed to analyze these pages automatically, indicating the level of curation of the human heart mitochondrial proteome. Another program was developed to perform simultaneous literature searches for genes and relevant clinical search terms on PubMed. This tool may be tailored to mine existing and upcoming data repositories such as WikiData and the bioCADDIE data discovery index (DDI). Ongoing updates of these efforts, as well as the annotation and curation tools, will be available on the HeartBD2K web portal (http://www.heartbd2k.org).
Results and Discussion: To date, 57 of the 556 selected proteins have been updated by the Cardiac Gene Wiki team, increasing the completion status from <1% to 10%. The analysis tool achieved an accuracy of 93% compared to manual scoring, thus validating its ability to assess Gene Wiki articles. The curation tool minimizes time-intensive tasks associated with academic writing. This initial effort provides a workflow for effectively curating publicly accessible content.
Conclusion: The Cardiac Gene Wiki team employs these tools and crowdsourcing mechanisms to aggregate unstructured knowledge in biomedical literature and organize the information into a structured, user-friendly format for a broad community of users. Access to integrated protein knowledge will expedite large-scale proteomics and biomedical research.

Keywords: cardiac, Mitochondria, crowdsourcing, curation

P11.14 Novel Serum Biomarkers Differentiate Psoriatic Arthritis from Psoriasis without Psoriatic Arthritis

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Introduction and Objectives: There is a high prevalence of undiagnosed psoriatic arthritis (PsA) in psoriasis patients. Therefore identifying soluble biomarkers for PsA will help in screening psoriasis patients for appropriate referral to a rheumatologist. Our purpose was to investigate whether serum levels of novel markers discovered by quantitative mass spectrometry (MS) of synovial fluid and skin biopsies,

differentiate PsA patients from those with psoriasis without PsA (PsC). **Methods:** Serum samples were obtained from 100 patients with PsA, 100 with PsC, and 100 healthy controls. Subjects were group matched for age and sex. No patient was undergoing treatment with biologics at the time of serum collection. Using enzyme-linked immunosorbent assays, four high-priority markers, previously discovered by quantitative mass spectrometry of synovial fluid and skin biopsies, were analyzed in the serum: Mac-2-binding protein (M2BP), CD5-like protein (CD5L), Myeloperoxidase (MPO), and Integrin-β 5 (ITGB5), as well as previously established markers Matrix metalloproteinase-3 (MMP3) and C-reactive protein (CRP). Data were analyzed using logistic regression, and receiver operating characteristic (ROC) curves were plotted. **Results and Discussion:** Polychotomous logistic regression showed that ITGB5 (p=1.18E-05), CRP (p=1.40E-06) and to a lesser extent M2BP (p=1.97E-03) are markers that are significantly different between the three groups (PsA, PsC and Controls). Compared to controls, CD5L, ITGB5, M2BP, MPO, MMP3 and CRP were independently associated with PsA, while only CD5L, M2BP and MPO were independently associated with PsC alone. Compared to PsC, ITGB5, M2BP, and CRP were independently associated with PsA. ROC analysis of this model shows an AUC of 0.85 with a 95% CI of (0.80, 0.90). Although there are significant correlations between markers, their magnitudes are not large. The largest correlation is between ITGB5 and M2BP (r= 0.24; p=3.51E-05). **Conclusion:** Thus, CD5L, ITGB5, M2BP, MPO, MMP3 and CRP are markers for PsA. However, only ITGB5, M2BP and CRP, differentiate PsA from PsC.

Keywords: psoriasis, arthritis, screening, Immunoassay

P11.15 STD Pathogens Determined in Semen Using PCR And "FLOW-Through" Hybridization Technology

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Introduction and Objectives: Background: The prevalence of sexually transmitted Disease (STDs) among hotel-based sex workers (HBSWs) in Karachi, Pakistan, was studied. These hotel workers are considered as high risk group because of their age, economic independence, low education and residence in a place away from their family Aim: The aim of this study was to access in health care facilities for diagnosis and common pathogens of STDs, those causing infertility and Chlamydia trachomatis, Neisseria gonorrhoeae and Mycoplasma hominis. Genital wart is a highly contagious sexually transmitted disease caused by some sub-types of human papillomavirus (HPV). **Methods:** Semen samples were obtained by masturbation into sterile containers after sexual abstinence of 48 to 72 hours. Samples were subjected to semen analysis within one hour of collection and processed for freezing within two hours of collection. The concentrations of sperm as well as sperm motility were also determined. DNA extraction was extracted of all the samples and the PCR assay was performed. The amplicons are subsequently hybridized to pathogen-specific capturing probes via "Flow-through" hybridization. **Results and Discussion:** During our study we came across with the STI pathogens present in our population and the reason for infertility was the main cause. When Chlamydia trachomatis and Neisseria gonorrhoeae were detected in their wife's were also screened and these STI pathogens were identified. Screening for bacterial STI pathogens, Mycoplasma hominis, Chlamydia trachomatis and Neisseria gonorrhoeae are strongly recommended because these pathogens can cause serious reproductive complications such as pelvic inflammatory disease, ectopic pregnancy. **Conclusion:** The main route for the transfer of STI pathogens were the men special those who visited commercial sex workers or hotel-based sex workers as they were working in other cities and the complained for infertility

Keywords: STD, Infertility , Semen analysis, PCR

P11.16 L-FABP Up-Regulates Cellular Angiogenesis and Migration in Hepatocellular Carcinoma

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Introduction and Objectives: HCC is notoriously resistant to systemic therapies, and often recurs even after aggressive therapies. Accordingly, identifying HCC biomarkers can improve diagnostics and therapeutics. Liver fatty acid-binding protein (L-FABP), an oncogenic protein, expresses abundantly in hepatocytes, and is known to be involved in the lipid metabolism. In this study, we investigated the role of L-FABP in HCC tumorigenesis. **Methods:** We used the expression correlation analysis to study the association between L-FABP levels and tumor progression in HCC patients. In vitro and in vivo studies were performed to get insight into the mechanisms underlying. **Results and Discussion:** We found that the expression of L-FABP was highly correlated with VEGF-A in HCC patients. L-FABP in vivo promoted tumor growth and metastasis significantly by the xenograft model. The mechanism investigation of L-FABP in the tumorigenesis showed that L-FABP associated with VEGFR2 on membrane rafts, and then activated Akt/ mTOR/P70S6K/4EBP1 and Src/FAK/cdc42 pathways, resulting in the up-regulated VEGF-A expression level accompanied with an increased angiogenic potential, and a higher migration activity. **Conclusion:** These findings indicate that L-FABP plays a role in the tumorigenesis could be a potential target for HCC chemotherapy.

Keyword: Hepatocellular carcinoma, L-FABP, VEGF-A, angiogenesis

P11.17 Genipin Inhibites Hepatocellular Carcinoma by Modulating the AKT/mTOR Pathway

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Introduction and Objectives: Hepatocellular carcinoma (HCC) is notorious for poor prognosis due to widespread metastasis and late recurrence. The development of effective therapy is therefore in an urgent need. In this study, we investigated the anti-HCC effects of an active component, genipin, form the Chinese herbal medicine Gardenia jasminoides. **Methods:** We used in vitro model to assess the effects of genipin on HCC cell migration/invasion and study the mechanisms underlying. The molecular docking was used to examine the interaction between geninpin and mTOR. Tumor xenograft analysis was performed to evaluate the efficacy of genipin in vivo. **Results and Discussion:** We found that genipin had an inhibitory effect on the migration and invasion of HCC Huh7 cells. The studies of mechanism underlying revealed that the inhibition of cell migration/invasion by genipin is related to the inhibition of VEGFR2 activation and F-actin reorganization. In addition, genipin inhibited the AKT/mTOR pathway and the results of molecular docking indicated that genipin fit into the ATP binding site of mTOR. In vivo validation using a mouse xenograft model showed that genipin had inhibitory effects on tumor growth and metastasis. **Conclusion:** The results show that genipin inhibited HCC Huh7 cell migration by targeting AKT/Mtor pathway. Genipin could be potentially developed as a chemotherapeutic agent for HCC.

Keyword: hepatocellular carcinoma, genipin, migration, mTOR

P11.18 Automatic Fast Standard Curve Generation Using Qualis-SIS for Quantifying Hundreds of Peptides

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Introduction and Objectives: One challenge in multiplexed targeted quantitative proteomics is the large amount of data that needs to be reviewed, analyzed, and interpreted. Qualis-SIS is a tool to generate standard curves from multiplexed MRM experiments and determine analyte concentrations in biological samples. The data processing is instantaneous, but following the stepwise calculations in the results is still possible. **Methods:** We keep the matrix unchanged and generate standard curves by spiking-in heavy-labeled peptides at 6 to 8 concentration levels, each of which has a minimum of three to five replicates. To qualify for curve generation, the analytical replicates within a given level must be both precise and accurate. We allow different weighting and concentration level removal methods including the FDA criteria for precision and accuracy. **Results and Discussion:** After acquiring the data on a triple-quadrupole mass spectrometer and exporting it from the vendor software, QualisSIS can be used interactively for quantification. We used a CSF dataset with 375 peptides measured in quintuplicate at 7 SIS-concentration levels, spanning a 10,000-fold concentration range. The analysis took 6.5 seconds for the results to appear on the screen, ready for navigation. This is for fixed set of parameters, for which manual reanalysis (if parameters needed adjustment) would be cumbersome; this can now be accomplished in seconds using our software. We tested how our algorithm handled incorrect peak picking and interferences in the input data. We used a dataset for 101 peptides each measured in quintuplicate at 6 concentration levels. Out of the 3030 data points we were able to use QualisSIS to identify 16 peptides for which incorrect peak picking/integration had been performed at one concentration level. **Conclusion:** QualisSIS is an online tool for fast MRM data analysis and allows interactive adjustment of parameters and automated quantification of hundreds of peptides in seconds.

Keywords: Automated Calculation, Limits of Quantification, Multiplexed MRM, standard curve

P11.19 Multiplexed Quantification of Preterm Infant Plasma Proteins by MS-Based Immunoassays

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Introduction and Objectives: Newborn health screening has been implemented in the clinics for the diagnosis of metabolic disorders by detecting low molecular weight molecules. Methods for monitoring multiple plasma protein biomarkers in preterm infants or newborns have not yet been established. Due to the low blood amount (ca. 125 mL) of preterm infants are multiplex methods essential for such biomarker analysis. Routine analysis of plasma proteins would allow monitoring e.g. organ development or inflammation and could prevent severe health damage, consequently improving preterm infants' overall health. **Methods:** Advanced MS instrumentation allows targeted detection and quantification of multiple analytes in low sample volumes (<5 µl). However, the complexity of plasma as sample matrix requires targeted analyte enrichment to enable the routine analysis of hundreds of clinical samples per day. We employ specific antibodies, which allow the selective enrichment of tryptic peptide groups comprising the same C-terminal amino acid sequence (TXP antibodies). Enriched peptides are then quantified by high-resolution parallel reaction monitoring. 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 quantitative results due to incomplete proteolytic sample digestion. Adding Stable Isotope Labeled recombinant Protein standards (SILP), prior to sample processing, can eliminate potential sample processing errors. **Results and Discussion:** Here we developed TXP-MS based immunoassay for the identification and accurate quantification of 10 plasma proteins in 1 µL plasma. This low volume meets the assay requirements to monitor plasma proteins in preterm infants. We combined an optimized tryptic digestion protocol with a single immunoaffinity enrichment step followed by high resolution Parallel Reaction Monitoring (PRM) MS analysis. We quantified 10 proteins in 100 plasma samples from newborns and preterm infants. **Conclusion:** This is the very first quantitative analysis of protein biomarkers in preterm infants that is relevant to organ development and disease status.

Keywords: MS-based Immunoassay, Plasma Protein Biomarker, Preterm Infants, newborn

P11.20 A Library Containing MRM-Suitable Peptide Surrogates for All Human Proteins

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Introduction and Objectives: Among the different approaches for selecting surrogate peptides representing a target protein in an MRM experiment, our software PeptidePicker with its v-score is distinctive in its integrative approach. It integrates information about the protein, its tryptic peptides, and the suitability of these peptides for MRM which is available online in UniProtKB, NCBI's dbSNP, ExPASy, PeptideAtlas, PRIDE, and GPMDB. The v-score is a simple scoring that reflects our "best knowledge" for selecting candidate peptides for MRM. **Methods:** Here we present an updated approach that we used to pre-compile a list of all possible surrogate peptides for all proteins in the human proteome. The new approach extends our previous approach with advanced error handling and fault tolerance logic. We also updated different module to make the software faster and more robust when working in the "proteomics ecosystem" of data repositories. **Results and Discussion:** Using our stringent selection criteria, we attempted to compile a list of surrogate peptides of all human proteins represented in UniProtKP/SwissProt. The final list includes 165k suitable MRM peptides covering 17k proteins out of the 20k human reviewed proteins. Compared to average of 2-4 minutes per protein for retrieving and integrating the information, the precompiled list includes all peptides available instantly. Our results shows also that more than 3k of the proteins provided with lower level of existence in UniProtKB have a mass spectrometry evidence on the protein level in at least one of the used databases. **Conclusion:** The pre-compiled list of surrogate MRM-suitable peptides allows a more cohesive and faster design of multiplexed MRM experiments. Around 3k proteins did not pass our stringent selection criteria, for which relaxing the later would be a suitable alternative. Our intention is to keep this list up-to-date as proteomics data repositories continue to grow.

Keywords: multiple reaction monitoring (MRM), Online Data Repositories, data integration, Protein Evidence Level

P11.21 Nanoparticle Ferric Pyrophosphate in Iron Deficiency, Its Impact on Plasma Proteins in Vivo

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Introduction and Objectives: Iron deficiency anemia (IDA) is considered to be a global health issue affecting a significant population worldwide. Nanosized iron salts due to their increased solubility with decreasing particle size finds potential application in food fortification to combat iron deficiency anemia. Further, nanoparticles on exposure to a biological system can get adsorbed by plasma proteins which may in-turn influence the biological activities of many proteins. However, limited knowledge exists about the effect of nanoparticles in a biological system. The present study addresses the efficacy of synthesized ferric pyrophosphate in its nano form (10-30 nm) as a potential food fortificant in iron deficiency anemia and measures its toxicity in a rat model. Additionally, the effect of the nanoparticle on protein molecules in vivo has been explored. **Methods:** Nanoparticle ferric pyrophosphate [NP-Fe4(P2O7)3] was synthesized and characterized using various standard techniques. The relative bioavailability of NP-Fe4(P2O7)3 with respect to a gold standard, ferrous sulphate (FeSO4) was calculated using hemoglobin regeneration efficiency method in anemic rats. Two-Dimension Nano-LC/MS based label free quantification was performed for quantifying the plasma proteins in both NP-Fe4(P2O7)3treated and FeSO4 treated groups. **Results and Discussion:** Relative bioavailability of NP-Fe4(P2O7)3 salt was found to be 103.02% with respect to FeSO4. Histopathological examinations of different organs did not show any significant toxicity attributable to NP-Fe4(P2O7)3. Although the findings from the histopathology did not show any change in the cell morphology, plasma proteomics analysis showed a decreasing and increasing trends for proteins Fetuin-B and Haptoglobin in their concentrations with increasing dose levels of NP-Fe4(P2O7)3. **Conclusion:** The nanoparticle ferric pyrophosphate could be a promising food fortificant in combating iron deficiency anemia, while Fetuin-B, a negative acute phase protein, might be a potential candidate for detecting biological responses to the nanoparticle exposure in vivo.

Keywords: proteomics, nanoparticles, 2D Nano LC/MS

P11.22 LFQProfiler - A Free Plugin for Label-Free Quantification in Proteome Discoverer

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Introduction and Objectives: Label-free quantification (LFQ) of peptides and proteins has become a very popular analytical technique in particular in clinical proteomics. Large-scale studies comprising hundreds or even thousands of LC-MS experiments require efficient computational processing tools. Here, we present the integration of an OpenMS-based LFQ workflow into the Proteome Discoverer platform. **Methods:** Proteome Discoverer (PD, Thermo Fisher Scientific) is an integrated software solution for analyzing LC-MS proteomics data. It includes a workflow engine facilitating the creation of custom data analysis pipelines for peptide/protein identification and quantification. While PD contains workflow nodes for a variety of tasks, including quantification strategies such as SILAC, iTRAQ, or TMT, it does not yet provide a solution for LFQ. OpenMS is an established and well-tested open-source software project providing a plethora of tools for various tasks in LC-MS-based omics data analysis, including LFQ. **Results and Discussion:** We have implemented an OpenMS-based LFQ workflow (LFQProfiler) for Proteome Discoverer featuring two novel community nodes: (i) the OpenMS FeatureFinder node, which can be used in the processing step of a PD workflow, and (ii) the OpenMS LFQ node, complementing the workflow in the consensus step. The former performs run-wise MS1 feature detection and can be combined with arbitrary search engine adapters and validation nodes. The latter performs all remaining steps, including retention time alignment, mapping of identifications to quantitative features, intensity normalization, and protein quantification. The results can be inspected conveniently within the PD environment and may in addition be exported to a tabular file format for downstream statistical analyses. **Conclusion:** We present the implementation of an OpenMS-based LFQ workflow within the PD platform. Our community nodes can be combined with existing nodes for peptide identification and validation. Results can be inspected within PD and exported for more sophisticated downstream analyses. LFQProfiler is available free of charge from the OpenMS website (OpenMS.de).

Keywords: quantification, OpenMS, Proteome Discoverer, label-free

P11.23 Mining of Proteins Related to Prevention of Adipogenic Differentiation by Vitamin A in Cattle

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Introduction and Objectives: In beef steers fed diets low in vitamin A, adipocyte differentiation and intramuscular fat deposition were increased without affecting subcutaneous adipocytes. This study was conducted with the aim of identifying proteins in the blood that have responses to the vitamin A restriction through proteomics study. **Methods:** Sixty-three Korean native steers were randomly divided into three groups and given different levels of vitamin A supplementation: High, Medium and Low. Blood samples were collected individually via the external jugular veins and analyzed by 2-DE. **Results and Discussion:** The results of the blood analyses showed that vitamin A concentrations in the Low group were significantly lower than those in the High or Medium groups ($P < 0.05$), which corresponded to the levels of vitamin A added to the different diets. In addition, two up-regulated and five down-regulated proteins were identified in a proteomics analysis. To confirm the tendency of these candidate proteins, we used the PPARgamma2-transduced BEFS cell, which is derived from spontaneously immortalized bovine embryonic fibroblasts (BEFS). After adipogenic differentiation of these cells, these cells were cultured with 10 microM 9-cis retinoic acid (RA) for 16 d. The number of lipid-laden cells and PPARgamma2 expression were decreased by the addition of RA and the mRNA expression of the candidate proteins increased or decreased significantly ($P<0.05$). **Conclusion:** These results showed that the RA inhibited adipocyte differentiation induced by the PPARgamma2 in culture of BEFS-PPARγ2 cell. In addition, these up or down-regulated proteins identified in a proteomics analysis may play an important role in adipocyte differentiation and interfere with the action of PPARgamma2 in beef cattle.

Keyword: Vitamin A, Beef steers, Proteomics, Adipogenic differentiation

P11.24 Affinity Proteomics on Glycemic Deterioration - A DIRECT Study

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Introduction and Objectives: The WHO predicts diabetes to become the 7th leading cause of death by year 2030 and 90% of the patients be diagnosed with type-2 diabetes. To tackle this disease, an EU project denoted DIRECT (DIabetes REsearCh on patient sTratification, www.direct-diabetes.org) was initiated to gather research platforms all over Europe to gain a deeper molecular knowledge in type-2 diabetes. The consortium is organized in an integrative framework including genomic, metabolomics and proteomic analysis. **Methods:** Sample material was collected from numerous sites in Europe within the two main studies, Glycaemic deterioration and Treatment response in type-2 diabetes. To select proteins of interest, a biomarker task force was formed. For proteomics, the Biobank Profiling group from KTH at Science for Life Laboratory, conducted affinity-based plasma analysis using bead arrays with antibodies from the Human Protein Atlas (www.proteinatlas.org). The generated data will become an element for subsequent multi-omics integration in DIRECT. **Results and Discussion:** Bead arrays were constructed with 640 antibodies based on the candidates proposed by the task force. To narrow down the number of binders, a set of 1,000 samples from the two studies was analyzed and used to score antibodies based on assay data and bioinformatics evaluation. With a set of 384 antibodies, >3100 samples were subsequently analyzed to explore proteins in patient plasma collected at baseline. The poster will demonstrate how we built this targeted array, designed and performed large-scale affinity proteomics, and provide initial insights considering sample related aspects. **Conclusion:** Affinity arrays have enabled to perform plasma profiling in > 4000 samples using a selection of protein candidates. Interesting initial findings will be evaluated using other immune- or mass spectrometric assays, to provide the DIRECT consortium with validated targets. The aim of this study will be to develop assays for the molecular markers discovered in relation to progression of diabetes and treatment response.

Keywords: DIRECT, type-2 diabetes, biomarker, Affinity assays

P11.25 Assessment of Awareness about Immunization among Parents in Population of Karachi, Pakistan

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Introduction and Objectives: Introduction: Immunization is safe and effective measure against avertable-diseases in all over the world that prevents children from life threatening infectious diseases. Awareness and attitude regarding importance of vaccines among parents play significant role to vaccinate their children. However, Pakistan has some of high rates of deaths among children due to lack of awareness and poor health management, mainly; improper-vaccination. Objectives: To evaluate the status of awareness and attitude about importance of immunization in parents of different status (Upper, middle, lower class) in population of Karachi. **Methods:** A survey-based Cross-sectional-study was conducted in different towns of Karachi. Total 600 Parents were included who have at least one child under-the-age of 10. Out of 600-Questionnaire, 200 were filled by parents in different areas by visiting them door to door, 200 were filled by parents in various schools while the remaining 200-Questionnaire were filled by the parents visiting hospitals. Informed-consent with regards to confidentiality was provided. Epi-info and SPSS software were used for data management. **Results and Discussion:** Data collected from all three status. Majority of High-class respondents 79% were aware of children immunization, Middle-class 64%, Whereas, Lower-class 49% respectively. 68% respondents of all three classes agreed that child immunization is important for children's healthy future. 70% of the respondents were of opinion that immunization is more beneficial than harmful, While only 42% respondents answered correctly about vaccination schedule. Generally, Parents have good knowledge about importance of immunization with average of 70.5% responding correctly. Although, Lower class needs to have more awareness about Importance of Immunization and knowledge for proper scheduling to vaccinate their children. **Conclusion:** Data shows that Parents have positive attitude and awareness about importance of immunization in general but lacking proper knowledge and guidance. therefore, The study results reinforce recommendations for use of educational programmes to improve the immunization knowledge and practice.

Keywords: Childhood immunization, Vaccination, infectious diseases

P11.26 Host Defense-Related Proteins in Bovine Milk during Subclinical Staphylococcus Aureus Mastitis

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Introduction and Objectives: Staphylococcus aureus is the most common contagious pathogen associated with bovine subclinical mastitis. Presently, a sensitive and specific procedure to identify animals with S.aureus subclinical mastitis is not yet available. Current diagnosis of S.aureus infectionsrelies on time-consuming procedures such as bacteriological culture of milk samples along with non-pathogen specific screening tests (e.g. somatic cell count SCC). Milk contains a range of proteins of moderate or low abundance that contribute to host defense. Identification of these proteins and their variable responses to pathogenic stimuli would enable the characterization of candidate biomarkers of subclinical mastitis. The objective of this study was to profile proteomic changes in bovine milk during subclinical mastitis by comparing whey samples collected from healthy uninfected cows (n=7) with low SCC with cows that are S. aureus-positive (n=7). **Methods:** Two different proteomic approaches were used to identify differentially expressed milk proteins between the two groups; In-solution digestion using trypsin enzyme followed by separation and identification of the peptides through direct liquid chromatography tandem mass spectrometer (LC-MS/MS) and two-dimensional difference gel electrophoresis (2D-DIGE) followed with in-gel digestion and identification through (LC-MS/MS) **Results and Discussion:** In total, 250 milk proteins were identified in both infected milk whey and control using direct LC-MS/MS with FDR of 0.1%. Over 60% of the proteins were common to both groups, and 64 proteins were significantly up/down regulated in S.aureus infected milk. Notably, 50 proteins were identified in mastitic whey uniquely and associated with host defense. Furthermore, Significant differences in protein expression detected in mastitic samples by 2D-DIGE-MS approach including serotransferrin, polymeric immunoglobulin receptor, complement C3 and other host defense-related proteins.To our knowledge, this is the first study that utilizes 2D-DIGE, to characterize protein expression in milk whey from cows with mastitis **Conclusion:** Comparison of two proteomic approaches can aid in the identification of biomarkers of subclinical mastitisandprovidenewinsightsintothe host-pathogenrelationshipduringS aureusmammaryinfectionindairy cows.

Keywords: Difference Gel Electrophoresis, Milk proteins, subclinical mastitis, host defense

P11.27 Pulmonary Fibrosis: TAILS N-Terminomics Unravels the Role of MMP12

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Introduction and Objectives: Pulmonary fibrosis is a progressive chronic disease characterized by excessive accumulation of extracellular matrix in interstitial and alveolar spaces and consequent disruption of basement membrane. Even though it was shown matrix metalloproteases (MMPs) are involved in the pathogenesis, the exact mechanism remains elusive. Macrophages are a major source of pro-fibrotic TGFß and anti-inflammatory MMP12. Utilizing MMP deficient mice we aim to analyze the roles of MMP12 in early events of fibrosis using the initial development of bleomycin-induced lung fibrosis as a model. To do so we will characterize changes in pulmonary proteome and N-terminome in presence and genetic absence of MMP12. **Methods:** Pulmonary fibrosis was induced by intra-tracheal instillation of 1.5U/kg bleomycin in B10.RIII Mmp12$^{+/+}$ and Mmp12$^{-/-}$ mice. Animals were sacrificed at day 7 and 14. Lung proteome extracts were analyzed by Terminal Amine Isotopic Labelling of Substrates (TAILS) to identify proteins and their N-termini. Simultaneous labelling and blocking of primary amines at protein level was achieved using TMT™. After digestion with trypsin, internal tryptic peptides possessing free N-termini were covalently bound to a commercially available polyaldehyde polymer (www.flintbox.com). Remaining unbound peptides-representing mature and processed protein neo-N-termini, were recovered by ultra-filtration and analysed by LC-MS/MS. **Results and Conclusion:** We identified in total more than 5,000 unique peptides and more than 2,000 unique proteins by combined shotgun and terminomics approaches. In fibrotic lungs, we found changes in proteins known to be involved in inflammatory process, including components of complement and coagulation cascades. However, many of these were cleaved in a MMP12 dependent or independent process potentially altering their bioactivity and hence proposing roles in connective tissue homeostasis and perturbations including fibrosis. **Conclusion:** Our ongoing studies highlight the power of TAILS in characterizing and quantifying N-terminome changes of proteins involved in pathology and casts new light on new proteolytic connections in regulating the initiation of fibrosis.

Keywords: matrix metalloprotease (MMP), fibrosis, inflammatory disease, TAILS N-terminomics

P11.28 A Cross-Sectional Study on Dietary Factors among Teenagers in Karachi

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Introduction and Objectives: Introduction: The increasing burden of non-communicable diseases in Pakistan like HTN, DM as-well-as anemia, growth retardation etc. secondary to obesity and malnutrition respectively, possesses a major public-health concern and can be prevented by a healthy diet and life-style. Objectives: To assess the nutritional dietary habits/life-style among the School-going teenagers of Karachi and their co-relation with the current Asian-standard guidelines of BMI. **Methods:** A survey-based cross-sectional study among teenagers from Grade-8th to Grade-10th in 5 government and 5 private schools of Karachi. Data was collected using pre-tested questionnaire from 500-teenagers after taking informed consent. Height and weight of the students were measured and BMI was calculated on calibrated-scale. SPSS-software was used for data-evaluation. **Results and Discussion:** 43.4% of the teenagers were underweight, 34.4% had normal weight-and-height, Overweight and obese were 12.2%. And 10% respectively. 52% of underweight teenagers' belonged to lower SES (socio-economic-status), 39% from middle-class while 9% from high SES. Improper nutrition, skipping meals mainly breakfast and dieting were main factors respectively. Out of all overweight respondents, 34% frequently consumed junk-food, 22% had high caloric-beverage intake, 18% were due to eating several times a day, 14% were lacking physical-activity, 10% due to unknown-reason or may be genetically and 2% due to lack of sleep and stress. Most students did not meet the recommended dietary habits. Under nutrition is comparatively higher but obesity and under-nutrition both co-exist in teenagers and are directly related to high and low socio-economic status respectively. Balanced-diet and regular physical activity would be an effective recommendation for both. **Conclusion:** Dietary habits of teenagers were found to be unhealthy and significantly associated with BMI. Study revealed that both under and over nutrition co-exist among teenagers because of Socio-economic factors and unhealthy life style. We need to create awareness and interest regarding healthy diet, proper body-weight-management and regular exercise among this future generation.

Keywords: Dietary habits, Teenagers, Socioeconomic status, BMI, Overweight, Malnutrition

P11.29 Antidiabetic Natural Flavonol

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Introduction and Objectives: not applicable

Methods: not applicable

Results and Discussion: Investigation of the EtOAc fraction of Tagetes minuta L. (Asteraceae) aerial parts has afforded a new flavonol glucoside, minutaside A (quercetagetin 6-O-(6-O-hexanoyl)-b-D-glucopyranoside) (1), together with four known flavonoids: axillarin 7-O-β-D-glucopyranoside (2), quercetagetin 3,7-dimethoxy-6-O-β-D-glucopyranoside (3), quercetagetin 7-methoxy-6-O-β-D-glucopyranoside (4), and quercetagetin 6-O-β-D-glucopyranoside (5). Their structures were established by multiple spectroscopic methods in addition to HRESIMS and comparison with literature data. The antioxidant and anti-diabetic activities of the isolated flavonoids were evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and α-amylase inhibition assays. Compounds 1 and 5 showed significant antioxidant activity (84.1 and 83.0 % at a 20 μM dose, respectively). Compounds 1, 4, and 5 exhibited strong a-amylase inhibitory activity compared with acarbose (a reference a-amylase inhibitor). However, 2 and 3 showed moderate activity. Molecular modeling studies of 1-5 that included docking, flexible alignment, and surface mapping were performed to evaluate their recognition profile toward a-amylase receptor. In docking simulations, 5 displayed a binding mode similar to that of acarbose in the active site of α-amylase enzyme. **Conclusion:** not applicable

Keywords: Natural products, Antidiabetics, Flavonol

P11.30 Glygosylation Sites and Oligomerization of MRJP1 – Application of Bottom up, Top down and HDX-MS

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Introduction and Objectives: A large family of major royal jelly proteins (MRJPs) is present in the honey bee (Apis melifera) royal jelly as well as in various tissues. In the honey bee brain, MRJP1 concentration decreases during the ontogenetic and behavioral differentiation from nurse to forager worker subcastes as shown in quantitative proteomic analyses. MRJP1 is a 57 kDa glycoprotein that drives queen development through an Egfr-mediated signaling pathway. MRJP1 purified from royal jelly comes with an interacting peptide (apisimin, 5.4 kDa). Self-association of MRJP1 was observed in solution apparently dependent on apisimin. Also, MRJP1 binds to over 50 proteins as we showed by affinity purification followed by bottom up and top down MS analyses. Herein, we focus on characterizing the glycosylation pattern and on understanding the oligomerization process of MRJP1. **Methods:** native PAGE, bottom up MS, top down MS and HDX-MS **Results and Discussion:** Using native PAGE in combination with bottom-up mass spectrometry identification, we detected carbohydrate-dependent oligomer formation. Two glycosylation sites were mapped on MRJP1-apisimin complex. Native mass spectrometry allowed us to identify the MRJP1-apsimin tetramer (231 kDa) in MS1. In MS2, upon increasing of source CID energy, two intense groups of peaks showed up - one around 20.1 kDa, which seems to be products of fragmentation with glycopeptide species and another around 51.8 kDa, corresponding to the MRJP1 monomer without apisimin. Finally, Hydrogen Deuterium Exchange Mass Spectrometry (HDX-MS) is being used to understand the influence of the glycan moiety on the tertiary structure of the protein as well as on the interaction between MRJP1 and apisimin. **Conclusion:** MRJP1 is glycosylated on two sites at least. The oligomerization of MRJP1, that forms a tetramer, is carbohydrate-dependent.

Keywords: HDX-MS, Top down, Apisimin, MRJP1

P11.31 Proteomic Analysis of Rhodopsudomonas Palustris CGA009 Grown under Simulated Microgravity

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Introduction and Objectives: Rhodopseudomonas palustris is a gram-negative bacterium and found ubiquitously in soil and freshwater. In this study, we profiled the changes in Rhodopseudomonas palustris proteome caused by simulated microgravity created with RWV. **Methods:** Rhodopseudomonas palustris CGA009 was obtained from American Type Culture Collection and maintained on Tryptic Soy Broth media. Fresh colonies were picked from Tryptic Soy Agar media in 55ml RWVs. The vessels were installed on laboratory-made bioreactor and rotated at the rate of 5rpm vertically (simulated microgravity) or horizontally (ground 1G control) during the incubation. After four days of incubation at 30˚C, the bacterial growth was determined by measuring optical density at 600nm. Rhodopseudomonas palustris was harvested by centrifugation at 6,500 rpm for 20min with following two washes with ice-cold 1 x PBS. **Results and Discussion:** Subunit of succinyl-CoA synthetase showed the highest increase under simulated microgravity. The expression of 70 kDa bacterial chaperon protein DnaK was also increased under simulated microgravity. DnaK is a homolog of the well-known eukaryotic chaperon HSP70. Previous studies showed that the expression level of HSP70 was altered by microgravity in various systems. Another stress response protein, late embryogenesis abundant protein (LEA) was found to be increased. LEA proteins has been associated with cellular tolerance to dehydration, which may be induced by freezing, saline conditions, or drying. Rhodopseudomonas palustris might sense simulated gravity as a stress factor and respond to it by overexpressing stress response proteins. **Conclusion:** It is reported that microgravity may increase the pathogenicity and cause the cellular stress in some bacteria. Therefore it is important to understand changes in Rhodopseudomonas palustris under microgravity at molecular level for the better use of this bacterium in space. Employing 2D-PAGE based protemics analysis, we reported the alterations in the proteome of Rhodopseudomonas palustris caused by simulated microgravity.

Keywords: Rhodopseudomonas Palustris CGA009, Simulated Microgravity, Rotating-Wall Vessel (RWV), 2 Dimensional-Polyacrylamide Gel Electrophoresis (2D-PAGE)

P11.32 Integrated Systems Biology Lung Analysis of OMICS Endpoints for Product Assessment

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Introduction and Objectives: Cigarette smoking causes severe diseases such as chronic obstructive pulmonary disease (COPD) and cardiovascular disease (CVD). ApoE-deficient mice are prone to developing premature atherosclerosis and emphysema making them an ideal model to investigate the effects of cigarette smoke and the development of the disease. We evaluated the effects of combustible cigarette smoke (CS) from a reference conventional cigarette (3R4F) and aerosol from candidate modified risk tobacco product (THS 2.2). **Methods:** ApoE$^{-/-}$ mice were exposed for up to 8 months to smoke from 3R4F or aerosol from THS2.2 for 3 hours/day, 5 days/week to a target nicotine concentration of 30 μg/l. After 2 months exposure to CS, cessation and switching groups were further exposed for up to 6 months to fresh air, or THS 2.2 aerosol, respectively. The changes in expression levels of the lung were detected using 'Omics' approaches, such as transcriptomics, proteomics and lipidomics, to quantitate and identify biomarkers and networks perturbed. **Results and Discussion:** Significant increase in the expression levels were detected in the mice in response to 3R4F-exposure in all of the "Omics" endpoints compared to the levels of sham-exposed mice, while smoking cessation and switching to THS2.2 resulted in lower expression effects compared to 3R4F. The broad biological impact of CS exposure including effects on immune-system, xenobiotic and oxidative stress, metabolism, and ER stress protein clusters. **Conclusion:** Our work demonstrates the advantage of using integrated systems biology/toxicology Omics approach on the ApoE$^{-/-}$ mouse model to study the diseases associated with cigarette smoking and investigate the mechanisms underlying the benefits of smoking cessation or switching to THS2.2.

Keywords: Systems biology, proteomics, transcriptomics, Lipidomics

P12: POSTER SESSION - EPIGENETICS AND HISTONE LANDSCAPE

P12.01 Towards Understanding Cellular Signaling into Chromatin

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Introduction and Objectives: The cellular response to external stimuli such as growth factors, hormones, viruses and drug treatments are often times measured by using microarray or RNA-Seq profiling to detect changes in gene expression. This suggests that the cellular response to these stimuli require mechanisms to signal for the reprogramming of chromatin and transcriptional networks to ultimately alter gene expression patterns. At the heart of this signaling into chromatin, are kinase mediated phosphorylation signaling pathways that relay the cellular activation information from the outer cell membrane into the nucleus. While phosphorylation signaling pathways have been studied in the past, both at the classical biochemical individual protein level and more recent in more large-scale phosphoproteomic experiments, several outstanding questions still remain. How long does it take for global dynamic signaling pathways to reach the nucleus? What is the mechanism for signaling pathways to activate gene expression patterns (e.g. modification or transcription factors or machinery, chromatin remodelers or histones)? Can direct signaling pathway target genes be robustly isolated and characterized? Towards answering these questions, we have been developing novel approaches to understand both the temporal dynamic aspects of cellular signaling and to characterize the target genes of such signaling into chromatin. **Methods:** Here we use chemical biology approaches to introduce labeled ATP analogs into cells and nuclei to specifically label newly phosphorylated proteins to mark these new PTM sites, and also affinity isolate these species. Quantitative proteomics using high resolution mass spectrometry was then performed to identify the modified species. Custom-software to quantify the ATP analogs was also developed. **Results and Discussion:** The approaches created to monitor signaling into chromatin are based on combining innovative in vivo metabolic or chemical labeling of specific protein PTMs with mass spectrometry and genomic readouts for a quantitative examination of cellular signaling linked to transcriptional or epigenetic activation. **Conclusion:** Signaling pathways affect chromatin response.

Keywords: histone, epigenetic, signaling, phosphoproteomic

P12.02 Characterization of Histone PTM Crosstalk by Middle-Down Mass Spectrometry and Data Integration

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Introduction and Objectives: Combinatorial post-translational modifications (PTMs) of histone proteins play an important role in modeling chromatin structure, which modulates gene expression, DNA repair, chromosome condensation and leads to propagation of epigenetic traits. One of the largest challenges in current chromatin biology is to characterize the relationships between co-existing histone PTMs, the order and hierarchy of their deposition and their distinct biological functions. Commonly used methods mostly rely on indirect measurement of co-occuring PTMs on separate peptides, thus complicating quantification of their mutual relationship. **Methods:** We developed a workflow to analyze the co-existing marks as revealed by "middle-down" MS experiments of histone proteins. The implemented new method quantifies positive and negative interplay between pairs of methylation and acetylation marks in proteins based on measurements on the same peptides. We further developed a public web repository (http://crosstalkdb.bmb.sdu.dk) allowing to inspect and statistical assess the results. The detection of crosstalk patterns is followed by data integration with epigenomics (ChIP-seq) and transcriptomics data. **Results and Discussion:** The observed features are not only in accordance with previously reported examples of crosstalk but also revealed novel types of interplay. Comparison between different cell lines shows that the observed interplays are conserved and independent from PTM abundance. Integration with ChIP-seq data allowed mapping of the chromatin regions where these combinatorial marks reside. Further investigation of the involved pathways reveals a well-defined hierarchy of biological functions within single and binary histone marks. **Conclusion:** The results show that we gather deeper insight into chromatin function by consideration of multiple marks measured with mass spectrometry approaches. The workflow provides an unprecedented tool for in-depth analysis of PTM crosstalk.

Keywords: histone, post-translational modification, crosstalk, middle-down

P13: POSTER SESSION - PROTEIN NETWORKS AND COMPUTATIONAL BIOLOGY

P13.01 Characterization of the Protein Complex Landscape of Murine Tissues

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Introduction and Objectives: In recent years, the elucidation of protein identities and abundances within tissues has provided an important resource to biologists. However, few studies have provided tissue specific protein-protein interaction (PPIs) information. As traditional high-throughput PPIs approaches are untenable for tissues, we have applied an alterative means to generate this resource: size exclusion chromatography protein correlation profiling (SEC-PCP). **Methods:** To assess the interacome across tissues we utilized size exclusion chromatography coupled to protein correlation profiling SILAC (SEC-PCP-SILAC). Both SILAC (Lys-6) and normal mice were generated and seven tissues (Brain, Lung, Liver, Heart, Skeletal Muscle, Thymus and Kidney) isolated, subjected to complex extraction and SEC under non-denaturing condition. SILAC labeled sample were then used to generate a global reference mixture that was added to all non-labeled samples to provide a means to compare between and across SEC fractions from different tissues. The resulting samples were analyzed on a Q Exactive and processed with MaxQuant. Using Matlab, protein chromatograms across the SEC gradients were assessed, based on correlation and co-enrichment, to determine PPIs. **Results and Discussion:** Using our PCP-SILAC approach, 9063 protein groups were identified across the seven tissues with 8231 protein groups leading to the generation of unique Gaussian fitted profiles. Using this data, the interactome of each tissue was determined; quality characteristics of these data, e.g., precision, FDR and FNR, were equal to or better then typical tagged based approaches. Interestingly a large proportion of proteins were only observed within a limited range of tissues, suggesting the interactome of each tissue is highly specialized. From the detected Guassians fitted profiles, 31518 protein interactions could be determined with a precision of ~65% based on comparison to the CORUM database. **Conclusion:** We have generated the first quantitative interactome map of

murine tissues, representing one of the largest interactomes to data.

Keywords: Bioinformatics, Interactomics, Tissues, SILAC

P13.02 Global Survey of Protein Complexes in Nematode Species (WormMap)

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Introduction and Objectives: Protein complexes are macromolecular assemblies responsible for many cellular biochemical activities. Systematic experimental characterization of protein complexes can provide rich insights while facilitating annotation of uncharacterized proteins, but to date has largely been restricted to simple model organisms. Whereas many large-scale genetic screens have been reported for C. elegans, proteomic research on worm has lagged. To address this gap, we have applied a comprehensive biochemical methodology to systematically isolate, identify protein complexes present in mixed C. elegans populations. **Methods:** We performed sixteen co-fractionation experiments on soluble protein lysates extracted from mixed stage C. elegans populations. Proteins present in each of the many thousands of fractions collected were identified and quantified by nano-flow liquid chromatography coupled to Orbitrap tandem mass spectrometry (LC-MS/MS). Pair-wise protein elution profile similarities were calculated using three different similarity metrics. To predict worm protein-protein interactions, these features were then integrated with external functional evidence using a support vector machine (SVM). **Results and Discussion:** Physically associated proteins co-elute during non-denaturing chromatography, which was exploited by us to define human protein complexes. Similarly, we fractionated soluble C. elegans protein complexes, and subsequently identified their components based on their correlated retention times. Pairwise similarities between the elution profiles of individual worm proteins were combined with functional evidence as features using a data-mining algorithm to predict co-complex associations. We benchmarked our results against annotated co-complex interactions, which revealed good prediction performance. In total, we predict 10,077 putative high confidence protein-protein interactions for C. elegans, involving 2208 proteins. **Conclusion:** We have generated the first global map of chromatographically resolved protein complexes for C. elegans, which encompasses the largest collection of worm protein-protein interactions supported by both biochemical and functional evidence to date. In addition to benefiting worm biologists, this network should serve as a resource for the broader biological community, providing novel information about the molecular associations.

Keywords: C. elegans, portein complexes, protein-protein interactions, machine learning

P13.03 Targeted Proteomics-Driven Computational Modeling of the Signaling Pathways in the Immune System

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Introduction and Objectives: Osteoclasts are monocyte-derived multinuclear cells that directly attach to and resorb bone. Sphingosine-1-phosphate (S1P) regulates bone resorption in mice by functioning as both a chemoattractant and chemorepellent of osteoclast precursors through two G-protein coupled receptors that antagonize each other in an S1P-concentration dependent manner. To quantitatively explore the behavior of this chemosensing pathway, we applied targeted proteomics, transcriptomics, and detailed pathway modeling with the Simmune toolset. **Methods:** RAW 264.7 cells were used as model osteoclast precursors, RNA-seq was used to identify expressed target proteins, and selected reaction monitoring (SRM) mass spectrometry using internal peptide standards was used to perform absolute abundance measurements of pathway proteins. For those proteins that could not be accurately quantified, the corresponding transcript values were used to estimate protein abundances. **Results and Discussion:** By using these abundance values as simulation input parameters, we were able to generate in-silico results consistent with in-vitro microscopy measurements and to predict signaling behaviors not used to parameterize the model. These findings demonstrate the feasibility and value of combining mass spectrometry-based measurements with detailed computational pathway modeling for advancing biological insight. We expanded this methodology to the Toll-like receptor signaling, our main area of interest, where we model the changes in the TLR signaling pathway upon LPS stimulation using the protein molecule abundance numbers obtained at 5 time points during 12 hours of LPS treatment of RAW264.7 cells. **Conclusion:** Combining these quantitative measurements of pathway component abundance with Simmune's powerful toolset, we were able to not only reproduce many experimentally observed features of the modeled pathway but also point out aspects of the simulated cells responses that emerged from the model structure as opposed to having been put in as desirable outcomes a priori. This research was supported by the Intramural Research Program of the National Institute of Allergy and Infectious Diseases, National Institutes of Health.

Keywords: quantification, protein networks, proteomics, modeling

P13.04 Identifying Cell Cycle Feedback Loops via Single Cell Proteomics in Xenopus Laevis Eggs

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Introduction and Objectives: Positive and negative feedback loops in biological signaling systems play a critical role in eliciting and controlling specific cell behaviors. Significant research is currently focused on trying to identify where feedback loops are in cellular signaling networks. Because their activity often results in phenotypes that are different from cell to cell, feedback loops must be studied at the single cell level using approaches such as fluorescence microscopy or flow cytometry. A main challenge in uncovering feedback loops is that current approaches are limited to measuring just a few proteins in each cell whereas cellular networks often consist of hundreds of proteins that should be measured since they could be feedback connected under different conditions and times. **Methods:** In this study 34 proteins are quantified with SRM-MS using Xenopus laevis eggs as a single cell model for the cell cycle. A statically powerful number of individual eggs are collected at time points across the first cell cycle and cytoplasmic proteins are quantified. In order to form statistical relationship models between proteins across different stages of the cell cycle we use regression and correlation analyses. The non-symmetric nature of regression analysis allows us to differentiate between protein relationships that are unidirectional (open loop) and bidirectional (feedback loop). **Results and Discussion:** Protein dynamics measured across the cell cycle agreed with expected behavior from previous publications. This includes Cyclin-A, Cyclin-B, Emi1, and DNA replication protein expression

dynamics. A linear regression analysis of the data confirmed known protein relationships and feedback loops, as well as previously undescribed relationships. This analysis is also being used to show which proteins are key drivers of the cell cycle and which play a more passive role. **Conclusion:** Here we present results on this method to uncover the topology of feedback-connected regulatory networks based on statistical analysis of protein expression in single-cells.

Keywords: Targeted proteomics, Quantitative, Single-Cell, Cell Cycle

P13.05 The Protease Web: A Pervasive and Complex Network Generating a Multitude of Protein Isoforms

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Introduction and Objectives: Proteases mold the functional state of proteomes by generating stable, shortened protein species. Despite the importance of these processes, human proteases are not well understood on a systematic level. In particular, our understanding of the interactions of proteases and their inhibitors through cleavage and inhibition that play a crucial role in the regulation of biological systems is lacking. Here, we provide strong evidence that these interactions largely expand beyond the classically described protein cascades, e.g. the complement system and coagulation, thus forming a pervasive proteolytic network, the protease web. **Methods:** We compiled large-scale proteolytic data to investigate the regulatory potential of the protease web. Using graph modeling of annotated cleavage and inhibition information, we investigated connectivity in the protease web. **Results and Discussion:** We identified a pervasive human protease web, where interactions between protease classes and cascades are common with protease inhibitors often forming network hubs. The model describes how proteases can potentially influence the cleavage of many more proteins than their direct substrates through protease activation pathways or inactivation of protease inhibitors. We successfully predicted a perplexing proteolytic pathway in vivo, where activities of matrix metalloprotease (MMP)-8 and neutrophil elastase are linked by an inactivating cleavage of serpin A1 by MMP8. This was validated biochemically, in cell culture and in vivo. To enable researchers to globally investigate the protease web, we created a web-based software termed PathFINDer that performs queries of the protease web and returns proteolytic paths from the query protease to a set of substrates, each path representing a biochemical mechanism that can be validated. **Conclusion:** Our findings supply systematically derived and validated evidence for the existence of the protease web. PathFINDer helps to explore this network and its impact on the proteome, thus facilitating mechanistic interpretation of proteolytic events in vivo and prediction of protease drug target off target effects.

Keywords: Protease network, Protein modifications, Protease inhibitors, Protein cleavage

P13.06 Identification of Disease Related Pathways by Integration of Phosphor and Global Proteome Profiling

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Introduction and Objectives: By integrating multi-types of omics data using network analysis, more comprehensive model which explains the molecular basis of cellular changes can be obtained. However, the approach for the identification of targetable pathways from the network model, which have high regulation power to the remaining network and can be confirmed by small scale experiment is still challenging. **Methods:** Here, we proposed a framework for effectively identifying targetable signaling pathways from disease system through the integration of global proteome profiles and phosphoproteome profiles. The framework combines kinase-substrate analysis (KSEA), TF-target analysis and network analysis so that targetable signaling pathways composed of deregulated kinases and TFs can be identified. **Results and Discussion:** We applied the framework for the identification of targetable pathways from the AD-related model system affected by mitochondrial specific Aβ accumulation. Firstly, we performed global and phosphor proteome profiling through iTRAQ analysis from the mouse hippocampal tissues with mitochondrial specific Aβ accumulation and control tissues without treatment. From the profiling, we identified 468 differentially modified proteins (DMPs) and 330 differentially expressed proteins (DEPs). We also identified 30 upstream kinases of 468 DMPs and 25 upstream TFs of 330 DEPs using KSEA and TF-target analysis. Through integration of DMPs, DEPs, kinases and TFs based on network analysis, we suggested targetable signaling pathways including key kinases and TFs for the modulation of perturbed network caused by mito- Aβ accumulation. **Conclusion:** In this study, we proposed frameworks for combining phosphoproteomic and global proteomic profiling through network based integration methodology for the effective identification of disease-related signaling pathways. These approaches can be applied to other disease systems, as well as the systems we introduced, for the exploration of targetable pathways for clinical treatment.

Keywords: Signaling pathway, Neurodegeneration, Network analysis, Itegrated OMICS

P13.07 Highest Connected Isoforms and Their Protein-Level Expression for the Human

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Introduction and Objectives: A single gene, especially in higher organisms, can generate multiple splice isoforms that carry different or even opposing biological functions. The identification of "canonical" or "dominant" or "principal" isoforms is important for understanding gene regulation and evolution. For example, a recent study showed that most highly expressed genes have a single dominant isoform based on proteomic evidence. Using RNA-seq data, major isoforms with the highest expression values were also identified. In contrast to these dominant or major isoforms defined by transcript- or protein-level expression, we have proposed Highest Connected Isoforms (HCIs) based on a functional relationship network in the mouse. **Methods:** HCIs showed differential expression signals at the transcript and protein level compared to non-highest connected isoforms (NCIs). Using the established method in our previous work, here we report the identified HCIs in the human. **Results and Discussion:** For each of the 6,157 multi-isoform genes based on RefSeq annotation, we calculated the average functional relationship (AFR) for each isoform and obtained 6,157 HCIs and 11,805 NCIs in total. For 36% of the multi-isoform genes, their fold change values, the ratio of the maximal to minimal AFR score, exceeds 1.5, showing the large-scale existence of differential functional relationships. Next, we are going to look at the expression signals of HCIs and NCIs at both transcript and protein level. Also, the HCIs will be compared to

such as the APPRIS principal isoform to investigate their relationship. **Conclusion:** We expect that the human isoform network will contribute to more precise understanding of gene functions and the HCIs will expand our knowledge of "canonical" isoforms from a functional perspective.

Keywords: Network, Major Isoform, Expression

P13.08 An Algorithm for a Complex Filtering Query System of Proteomics Data

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Introduction and Objectives: Protein and peptide features such as confidence scores or spectral counts are often used to perform different types of proteomics data filtering. Often, multiple filters are used to limit the data, but the end results of the sequential application of such filters are heavily dependent on the order in which they are performed. To our knowledge, there are currently no computational tools that allow these filters to be applied simultaneously. Here, we present and compare two strategies to apply complex logical combinations of filters over protein and peptide features. **Methods:** We present a first approach where F is the set of all filters and in which each filter fi∊F is independently and sequentially applied to the whole dataset. The results Rfi from each filter fi are then combined by taking the unions or intersections of these sets. We propose a second approach based on the modeling of the relationships between protein and peptides as a bipartite graph G=(V1,V2,E), where V1 is the set of proteins, V2 is the set of peptides, and ev1,v2∊E is the set of all edges connecting a protein v1∊V1 to a peptide v2∊V2. For this approach the entire logical combination of all filters in F is applied individually to the different elements of G instead of applying each filter to the whole dataset independently and sequentially. **Results and Discussion:** Using a theoretical dataset and applying different queries with various levels of logical complexity, we comprehensively show the advantages and limitations of each approach. **Conclusion:** We finally propose a single iterative algorithm based on a bipartite graph model to filter proteomics data using a complex logical combination of filters. Our algorithm can be easily incorporated in any proteomics data analysis software package and has been implemented in the Proteomics INTegrator (PINT) tool, providing a powerful web-based filtering query system for proteomics datasets.

Keywords: Proteomics data querying and filtering, Algorithm comparison, Bipartite graph

P13.09 Efficient Identification of Cross-Linked Peptides with StavroX and MeroX in Structural Proteomics

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Introduction and Objectives: Cross-linking in combination with mass spectrometry (MS) presents a powerful technique to study protein-protein interactions and to determine three-dimensional protein structures. However, the bottleneck of this technique is the reliable identification of cross-links from the highly complex datasets obtained with modern mass spectrometers. As the number of cross-linked products increases quadratically, novel software is urgently needed allowing a fast and reliable analysis. These requirements are met by the software tools StavroX [1] and MeroX [2]. **Methods:** StavroX and MeroX are freely available software tools (www.StavroX.com) programmed in Java. Both tools exhibit a high flexibility and an easy-to-use, self-explanatory graphical user interface. StavroX can handle different kinds of cross-linked products (e.g. disulfides as naturally occurring cross-links, cross-links originating from amine-reactive cross-linkers, such as BS^3 or DSS and photo-activatable amino acids). MeroX is designed for analyzing data from MS/MS-cleavable cross-linkers, such as the urea-linker [3] or DSSO [4]. **Results and Discussion:** We investigated several protein systems cross-linked by different reagents (amine-reactive, photo-reactive, MS/MS-cleavable) with StavroX and MeroX. MeroX identified cross-link specific fragments in MS/MS spectra and efficiently scored cross-links at a low FDR. With the advancement of the chemical cross-linking/MS approach for structural proteomics, there is an urgent need for developing novel MS/MS-cleavable cross-linkers that facilitate the assignment of cross-links. Both programs can handle isotope-labeled cross-linkers (deuterated, ^{13}C, ^{15}N) as well as isotope-labeled proteins (^{15}N). StavroX and MeroX are constantly updated to keep pace with instrumental development. **Conclusion:** With StavroX and MeroX, two software tools are available that allow analyzing cross-linking datasets in a highly automated, robust, and reliable fashion. References: [1] Götze, M., et al., J. Am. Soc. Mass. Spectr. 23 (2012): 76-87. [2] Götze, M., et al., J. Am. Soc. Mass. Spectr. 26 (2015): 83-97. [3] Müller, MQ., et al. Anal. Chem. 82 (2010): 6958-6968. [4] Kao, A., et al., Mol. Cell. Prot. (2010): mcp-M110.

Keywords: MS/MS-cleavable cross-linker, cross-linking mass spectrometry, bioinformatic

P13.10 ProHits 2.0: A Bioinformatics Management and Analysis System Optimized for Interaction Studies

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Introduction and Objectives: Quantitative proteomics data can be a challenge to handle, appropriately and efficiently analyze, and then subsequently present to an audience in simple and intuitive ways. Data needs to be archived, searched, scored, compared and visualized, often requiring a variety of tools. These challenges are compounded by the growing use of data-independent acquisition (DIA) methods that require novel tools for the searching and scoring of data. Our ProHits system was specifically designed to handle many of these challenges, but we sought to update it to handle the unique requirements of DIA data, as well as incorporate new means for data visualization. **Methods:** The ProHits Data Management interface was redesigned to accommodate DIA workflows. New tools incorporated into ProHits include SAINTexpress and DIA-Umpire, as well as a suite of visualization tools that use novel as well as preexisting R libraries. New DIA solutions are also being implemented, including the spectral searching tool MSPLIT-DIA and the statistical quantification tool MAP-DIA. **Results and Discussion:** ProHits (prohitsms.com) is a laboratory information management system that allows for the storage and analysis of MS data. Recent additions to ProHits include the incorporation of tools for the handling of DIA data, including DIA-Umpire. A module dedicated to interaction proteomics enables interrogation and scoring of DDA or DIA data via Significance Analysis

of INTeractome (SAINT) software. We have recently coupled data scoring with visualization tools that are capable of displaying multiple parameters from quantitative protein–protein interaction data sets, including absolute and relative abundance measures (from either spectral counts or intensity measurements), fold change between samples and confidence metrics. **Conclusion:** The open source system ProHits now handles and/or facilitates the storage, searching, interrogation, analysis and visualization of MS data acquired either through data-dependent or –independent means, functioning as a single easy to use post-acquisition pipeline.

Keywords: ProHits, Data-Independent Acquisition, Data Visualization

P13.11 Visualizing and Analyzing Protein Interactome Data

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Introduction and Objectives: Quantitative interaction proteomics data can be a challenge to efficiently analyze and subsequently present to an audience in a simple and easy to understand format that still conveys sufficient levels of information. Since pre-existing tools, such as Matlab and R, have a difficult learning curve and solutions generated with them can be problematic to port between individuals, we sought to develop tools that could utilize simple input formats and generate a variety of graphical outputs depending upon the experiment and needs of the user. **Methods:** To create these tools we developed novel as well as used preexisting functions from R and Perl, with inputs supplied through an HTML interface. **Results and Discussion:** The web-tools we have developed are capable of displaying multiple parameters from quantitative protein–protein interaction data sets supplied in simple formats. Given a set of "bait" proteins with detected "prey" interactions, dot plots can be generated to display absolute spectral counts for the preys, relative spectral counts between baits, and confidence levels for the interactions. Fold change results between numerous baits with their associated confidence level can be displayed as heat maps or dot plots. Pairwise bait analyses can be performed displaying spectral counts, confidence score and fold change differences in a scatter plot format. Lastly, heatmaps displaying the correlation between preys can be generated that facilitates the assignment of proteins to potential complexes. **Conclusion:** The tools we have developed make it easy for the user to visualize and interpret their data, identify important interaction changes and present this information to others in an intuitive way. They are freely accessible to the community at http://prohitstools.mshri.on.ca.

P13.12 A High Throughput Software Solution for Treating Data Independent Acquisition Results

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Introduction and Objectives: Data Independent Acquisition (DIA) represents a powerful screening technique for the comprehensive and accurate quantitation of biological samples, particularly their protein component. As the method sequentially collects MS/MS fragmentation spectra on all ions within a given m/z range, it affords the opportunity for retrospective analysis of unknowns and new targets of interest. Obtaining peak performance demands not only on finding an optimum balance of instrument speed, sensitivity, and selectivity, but also a robust and comprehensive search tool to extract and validate quantitative results from a DIA analysis. Herein we present a complete and high throughput DIA pipeline from method to quantitative results on a Q-OT-IT Tribrid mass spectrometer. **Methods:** Two non-small cell lung cancer lines, one showing marked resistance to the tyrosine kinase inhibitor Erlotinib, were established and used to assess the quantitative performance of a Q-OT-IT Tribrid mass spectrometer. A series of data dependent acquisition (DDA) discovery experiments were performed to build a spectral library. Several DIA experiments were undertaken including classicDIA and wide-isolation selected ion monitoring (WiSIM). **Results and Discussion:** The results obtained showed a general reduction in expression of proteins belonging to the canonical MAPK pathway and an increase in abundance of downstream proteins of the mTOR pathway for the drug resistant cell line, consistent with previous results. However, a substantially higher number of peptide targets were identified at 1% FDR and quantified than in previous analysis. Finally, in spite of successive reduction of the load on column, a comparable number of quantified peptides were obtained. **Conclusion:** The large numbers of identifications obtained provide an inclusive list from which to select significantly regulated proteins of interest for future targeted analysis.

Keywords: Spectronaut, DIA, Fusion, Orbitrap

P13.13 ProteomeXchange: Enabling Proteomics Data Sharing in the Public Domain

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Introduction and Objectives: The overall aim of the ProteomeXchange (PX) Consortium (http://www.proteomexchange.org) is to enable data sharing in the field by providing a common framework and infrastructure for the cooperation of mass spectrometry proteomics resources (PMID: 24727771). PX defines and implements consistent, harmonised, user-friendly data deposition and exchange procedures among the members. **Methods:** In the first stable implementation of the data workflow, the PRIDE database (EMBL-EBI, Cambridge, UK) is the point of submission for tandem MS/MS experiments, while PeptideAtlas (Institute for Systems Biology, Seattle) provides a repository for Selected Reaction Monitoring experiments called PASSEL. Having imported all public data from Tranche and having accepted new MS/MS dataset submissions since early 2013, the MassIVE repository (University of California San Diego) has since joined PX, thus demonstrating PX's unifying role in the proteomics community by inclusion of members that were not part of the initial Consortium. **Results and Discussion:** The implementation of PX has resulted in a fast increase of publicly available proteomics datasets (by April 2015 almost 2,000 datasets had been submitted to any of the three PX partners since mid 2012). The main common access point is ProteomeCentral (http://proteomecentral.proteomexchange.org), which provides the ability to search datasets in all participating PX resources. It is possible for everyone to get subscribed to the announcements of new datasets as they become publicly available, via e-mail, RSS or using Twitter (@proteomexchange). **Conclusion:** PX is actively changing the "culture" in the proteomics field, together with some funding agencies and scientific journals, by promoting and enabling sharing of proteomics data in the public domain.

Keywords: Mass spectrometry, data repositories, sharing data, databases

P13.14 Oxidative Stress Dependent Regulation of DJ-1: An Interatomic Point of View

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Introduction and Objectives: DJ-1 protein, a Parkinson's disease (PD) associated protein, has already proved to be involved in neuroprotection against oxidative stress. Moreover, evidences suggest that DJ-1 is also regulated by oxidative stress, and in fact its oxidized state is considered its active form. Therefore, by providing a comprehensive characterization of DJ-1 dynamic interactome under oxidative stress conditions, this work elucidates the mechanisms through which DJ-1 exerts its neuroprotective role in response to oxidative challenges. **Methods:** Using affinity purification combined with SWATH-MS (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. The main mechanisms of DJ-1 action were predicted by monitoring the interactions changes and highlight groups of proteins with similar behavior. To establish the importance of DJ-1 stress regulation in the newly interactions/mechanisms identified, pull-down assays of DJ-1 recombinant forms - WT and mutants that mimics different DJ-1 oxidative states - were also performed. Finally, the stress dependent regulation of DJ-1 interactions was further evaluated at the molecular level by native mass spectrometry analysis of isolated protein complexes. **Results and Discussion:** Novel DJ-1 binding partners were identified, pointing to new mechanisms for DJ-1-mediated neuroprotection, and also the dynamics and the regulation of these interactions was characterized. **Conclusion:** This work largely contributed to the elucidation of the DJ-1 neuroprotective mechanisms. Furthermore, many of the proteins identified are well established in distinct cellular functions implicated in PD ultimately contributing also to a better understanding of the disease. Work supported by Fundação para a Ciência e Tecnologia (FCT), Portugal, projects reference PTDC/NEU-NMC/0205/2012 and PEst-C/SAU/LA0001/2013-2014, and co-financed by "COMPETE Programa Operacional Factores de Competitividade, QREN, the European Union (FEDER – Fundo Europeu de Desenvolvimento Regional) and by The National Mass Spectrometry Network (RNEM) under the contract REDE/1506/REM/2005. SA is supported by PhD fellowship reference SFRH/BD/81495/2011.

Keywords: Dynamic Interactome, oxidative stress, AP-SWATH-MS, DJ-1 and Parkinson's Disease

P13.15 Large Scale Quantitation of SILAC Proteomes Using Retention and Drift Time Profiling

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Introduction and Objectives: SILAC is routinely applied in LC-MS based quantitation. Comparison of multiple samples requires high degree multiplexing, needing higher separation power LC-systems, advanced MS, and informatics tools that can utilise high-resolution/mobility separation data. Principle and application of novel informatics is described to enable SILAC quantitation using commercial software originally developed for large scale quantitation of label-free LC-MS data, incorporating novel retention and ion mobility drift time profiling based scoring algorithms. **Methods:** High-resolution precursor/product ion LC-MS data were acquired in DDA/DIA/IM-DIA mode. Data was peak detected with ProgenesisQI using default noise-filtering and a zero-intensity thresholds. A search approach was utilised whereby multiple fixed modification searches were conducted. Isotopic profiles with identifications were exported to ProteoLabels. Modifications were determined automatically by ProteoLabels and tolerances set using observed distributions. Pairs were detected, allowing identification of only one of light/heavy, and scores calculated using mass shift, chromatographic and drift profile. Protein grouping was conducted and ratios reported. **Results and Discussion:** Five SILAC and one dimethyl dataset, were used to evaluate ProgenesisQI/ProteoLabels quantition. A number of samples were created with expected ratios for validation. Informatic analysis of technical LC-MS replicates was conducted to determine reproducibility. ProgenesisQI affords co-detection across samples/datasets, including both technical/biological replicates, and time-course samples, which increased on average the number of detected isotopic clusters by 2.1 fold compared to single run data. The number of quantifiable pairs was found to be significantly increased by a factor of 1.8, requiring a minimum of two peptides for quantitation, compared to commercially available software for the analysis of DDA/DIA/IM-DIA data, with subsequent protein amino acid coverage increase. **Conclusion:** Median weighted average protein and peptide pair ratios determined by ProgenesisQI/ProteoLabels analysis were found to be in good agreement with previous results for the dataset. Combined retention and drift time similarity profiling improved the pair scoring, reducing quantitation FDR and improving quantitation precision.

Keyword: silac, quantitation, proteolabels, dimethyl, labeled, stable isotope

P13.16 PSEA-Quant: A Protein Set Enrichment Analysis on Quantitative Proteomics Datasets

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Introduction and Objectives: Most quantitative mass spectrometry (MS)-based proteomics methods tend to produce large lists of quantified proteins with varying levels of reproducibility. The size and complexity of such datasets make their biological interpretation only possible through the use of sophisticated computational algorithms. We previously proposed a statistical approach (PSEA-Quant) that computationally identifies protein sets, derived from the Gene Ontology (GO) and Molecular Signature databases, that are significantly enriched with reproducible quantification measurements of abundant proteins across a set of replicates. Originally, PSEA-Quant only supported the analysis of datasets that were quantified using either spectral counting or stable isotope labeling. We now present an extension of PSEA-Quant that allows the analysis of a greater variety of quantitative proteomics techniques to cater to a greater pool of proteomics users. **Methods:** PSEA-Quant is a web-based protein set enrichment analysis algorithm for quantitative proteomics datasets. We extended the existing approach to allow the analysis of quantitative proteomics datasets produced using label-free intensity-based quantification from extracted ion chromatograms, selected reaction monitoring, and multiple reaction monitoring. The statistical analysis of quantitative proteomics datasets generated by the vast majority of popular MS-based quantification techniques is now facilitated by PSEA-Quant's user-friendly input format. **Results and Discussion:** We show that PSEA-Quant can process quantitative proteomics datasets produced by most popular MS-based quantification techniques and pipelines. We also demonstrate that PSEA-Quant provides valuable insights about the underlying biological mechanisms involved in cystic fibrosis using a label-free protein quantification analysis of a cell line expressing a CFTR mutant.

Conclusion: This extended version of PSEA-Quant, which is available online, will thus facilitate the analysis of most quantitative proteomics datasets. When applied to such datasets, PSEA-Quant can provide a better understanding of biological mechanisms and diseases processes.

Keywords: quantitative proteomics, Gene Ontology, Bioinformatics, Functional Enrichment Analysis

P13.17 PRIDE Proteomes: A Protein Centric View of PRIDE Archive Data

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Introduction and Objectives: The PRIDE database (http://www.ebi.ac.uk/pride) at the EMBL-European Bioinformatics Institute (Cambridge, UK) is one of the main public repositories for mass spectrometry based proteomics data and the initial submission point for MS/MS data in the ProteomeXchange consortium (http://www.proteomexchange.org). The amount of data in PRIDE is constantly growing. Together with the data management component, the other main challenge in proteomics resources such as PRIDE is to provide an aggregated and quality-scored version of the peptide/protein identifications found across all the submitted projects, in order to decide which information is more reliable. **Methods:** PRIDE Proteomes uses the publicly available data submitted to PRIDE as the base. At present, it utilizes a spectrum clustering approach (the new PRIDE-Cluster-H algorithm) to quality-score the originally submitted peptide-spectrum matches. Those peptide sequences are mapped to protein sequences using the UniProtKB/Swiss-Prot "complete proteome" databases. **Results and Discussion:** PRIDE Proteomes is a new resource providing an integrated, quality-filtered, protein centric view of the data in PRIDE (http://wwwdev.ebi.ac.uk/pride/proteomes). The resource is now available for four species: human, mouse, rat and Arabidopsis. In this first release, we have used a conservative quality scoring for the PSMs, to ensure data reliability. In the near future we plan to use results coming from reprocessed datasets to complement the information provided by the spectrum clustering approach. **Conclusion:** This new resource provides an interactive representation of the data across datasets deposited in PRIDE, organised by species.

Keywords: database, data reliability, data aggregation, proteomics resource

P13.18 Systems Biology Analysis of Common and Disease-Specific Pathways in Neurodegeneration

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Introduction and Objectives: Parkinson's disease (PD) and Amyotrophic Lateral Sclerosis (ALS) are complex neurodegenerative disorders, whose etiology and pathogenesis have not been completely characterized. Many biochemical, environmental and genetic mechanisms have been proposed to play a role in the neuronal damage and loss for both diseases. Thus, we aim at the identification of common and distinct pathways of these neurodegenerative diseases by a systems biology meta-analysis of proteomics data available in the literature. **Methods:** We performed a meta-analysis of the literature of all the proteomic investigations of neuronal alterations in PD, ALS and Alzheimer's disease (AD), used as control (non-motor neurodegenerative disease). We combined these data with genes linked to PD and ALS found in a curated disease-gene database (DisGeNET). By using GO Consortium, we identified biological processes altered in PD, ALS and both. **Results and Discussion:** 1088 proteins were associated to PD, 457 to ALS and 142 to both diseases. Biological processes mostly involved in PD turned out to be chromatin organization (GO:0006325; p-value $3.78\ 10^{-19}$), mitochondrion organization (GO:0007005; p-value $2.22\ 10^{-08}$) and protein folding (GO:0006457; p-value $9.31\ 10^{-06}$), whereas in ALS RNA and mRNA processing (GO:0006396; p-value $3.44\ 10^{-15}$, GO:0006397, p-value $1.03\ 10^{-09}$) were the most over-represented categories. In addition, we found common deregulated pathways, i.e., translation (GO:0006412; p-value $6.24\ 10^{-27}$), SRP-dependent co-translational protein targeting to membrane (GO:0006614; p-value $1.41\ 10^{-19}$) and protein transport (GO:0015031; p-value $1.90\ 10^{-09}$). **Conclusion:** PD and ALS pathogenesis have some common mechanisms, because they are both neurodegenerative diseases. Moreover, common biochemical altered pathways may explain a high prevalence of comorbidity. The meta-analysis allowed us also to highlight the disease-specific pathways, which may justify the degeneration of different neuron populations (dopaminergic neurons of the midbrain in the case of PD and motor neurons in the case of ALS).

Keywords: Parkinson's disease, Amyotrophic lateral sclerosis, Over-representation analysis

P13.19 Reactome: Pathway Analysis of Proteomics Data

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Introduction and Objectives: The Reactome pathway database (http://reactome.org) [1,2] is a collaboration between Ontario Institute of Cancer Research, New York University, and the EMBL-European Bioinformatics Institute (Cambridge, UK). Reactome provides open, high quality, manually curated pathways for human and orthology-based projections for model species. We are currently developing Reactome into a comprehensive resource for the pathway-centric analysis of proteomics data, supporting the transformation of data into knowledge. **Methods:** We have developed a highly optimised Reactome pathway analysis module, allowing analysis of proteome scale qualitative of quantitative datasets within seconds. Additional external data like molecular interactions, protein structures, or gene and protein expression data can be visualised in a context sensitive manner through integrated JavaScript widgets and web services. **Results and Discussion:** Reactome pathways are arranged in a hierarchical structure. After submitting a qualitative or quantitative protein expression dataset, users are offered a high level, genome-wide view of pathway analysis results projected onto the Reactome hierarchical structure. From there, users can zoom in to detailed maps for pathways of interest, and view a projection of their own data, as well as external data like molecular interactions. Through the use of web services and JavaScript widgets provided by the external data resources, we minimise data update problems, maximise software code re-use, and provide users a "look and feel" they might already be familiar with for that external data type. **Conclusion:** Reactome offers a freely available, fast and stable interface for the analysis of proteomics data in a pathway context, accessible both interactively and through web services. References: 1: Jupe S, Fabregat A, Hermjakob H. Expression data analysis with Reactome. Curr Protoc Bioinformatics. 2015 Mar 9;49:8.20.1-9. 2: Croft D, et al. The Reactome pathway knowledgebase. Nucleic Acids Res. 2014 Jan;42(Database issue):D472-7

Keywords: pathway analysis, Reactome

P13.20 Sequence-Based Identification of Cis-Regulatory Elements in Intrinsically Disordered Protein Regions

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Introduction and Objectives: Protein interactions in cis that can activate or (auto)inhibit protein function play an important role in the fine-tuning of regulatory and signaling processes in the cell. So far, there exist no methods that predict the location of cis-regulatory elements (CREs) in proteins. Here, we introduce a computational approach that identifies intrinsically disordered protein segments that contribute to protein function regulation via interactions in cis with a per-protein accuracy of 76.3% and true discovery rate of 0.58. With its help, we map the usage of cis-regulation in the MAP kinase pathway and demonstrate that about one fourth of all proteins in this pathway have CREs. We further reveal that disease-causing mutations are highly enriched in predicted CREs, specifically mutations that are associated with lymphoid neoplasms, osteosarcomas and carcinomas. The introduced computations tool opens up new avenues in the discovery of cis-regulatory elements in proteins and facilitates the identification of disease-causing mutations that disrupt autoinhibition.
Methods: 'not applicable'
Results and Discussion: 'not applicable'
Conclusion: 'not applicable'

Keywords: cancer, Protein interactions in cis, Interaction prediction, signaling

P13.21 Assessment of Network Systems Using Background Information to Reveal Relevance of Proteomic Data

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Introduction and Objectives: A useful strategy to interpret proteomic results is based on the analysis as a protein interaction network. However, when proteomic results are limited, data could be disconnected and/or inconclusive. The introduction of background information by network analysis systems is an alternative to build conclusive networks from limited data, but consensus in the reliability of its application is lacking. Our aim was to assess several network analysis systems against small data sets of comparative proteomic studies using as control random protein sets, and compare the networks obtained from proteomic and random data, initially and during network growth by adding background nodes.
Methods: Protein data came from 3 different comparative proteomic experiments with 10, 12 or 40 proteins. As controls 3 random protein sets were generated by R from Swiss-Prot (Organism Human), to 6 total random sets. We attracted in open source and free network systems, as STRING, PCViz, visANT, UniHI and iRefWEB.
Results and Discussion: The growth and enrichment of networks from proteomic sets is unmistakable, compared with that of random sets. Initial connection among nodes of proteomic sets is higher than that of random sets and nodes are more interconnected, as the network grows. Obtained proteomics networks, unlike random networks, reveal a higher number of linked seed nodes with few connections and faster connection of seed nodes in function of added nodes. Despite nodes of random sets can connect to background nodes, the connection among them remains low and a main network is not generated as do the proteomic sets.
Conclusion: The enriched of proteomic networks using background nodes provides highly interconnected networks and brings meaning in a specific way, unlike the random networks. We propose a pipeline for bioinformatic analysis of small comparative proteomic date and suggest recommendations of operation and criteria for adding background nodes.

Keywords: protein interaction network, network analysis systems, background data, proteomic

P13.22 jPOST: Development of Japan ProteOme STandard Repository/Database

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Introduction and Objectives: Sharing experimental data is important for the scientific community, for example, to validate and reuse the data. In Japan, the Database Integration Coordination Program has been promoted to share the life science data. We have joined this program and developed a new repository for MS raw and processed data to share the proteomics data. However, the processed data by submitters are generally in a wide variety of qualities because each experiment has been analyzed under the different criteria. To ensure the quality of processed data, we have developed our own workflow for reanalysis of the deposited raw data, and the results are stored in the curated database. Here, we introduce the current status of our developing system "jPOST," Japan ProteOme STandard repository/database.
Methods: We have been developing jPOST, which consists of three parts: MS raw and processed data repository, reanalysis workflow, and curated database. The repository and curated database store users' deposited data and our reanalysis data, respectively. The reanalysis workflow includes the peak picking, peptides/proteins/PTMs identifications, and the quantification.
Results and Discussion: The data repository accepts MS raw and processed data from all over the world. The function for the assignment of the global accession number to deposited data will be added in jPOST to join the ProteomeXchange consortium. The deposited raw data are entered into our reanalysis workflow, and only the results from carefully-screened high-quality data that meet our criteria are stored in the curated database. We also store detailed metadata such as samples, instruments, tools, and their settings and parameters into the database, we will thus provide a detailed search interface like the faceted search for the curated data.
Conclusion: We have developed jPOST consisting of the repository for deposited data, the reanalysis workflow for ensuring the quality of processed data, and the curated database for storing reanalysis results.

Keywords: workflow, database, jPOST, repository

P13.23 Real-Time Peptide Sequencing

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Introduction and Objectives: The dramatic improvement in mass spectrometers' throughput during the past few years has made the data analysis increasingly difficult and time consuming. This article presents new peptide sequencing software, Novor that is capable of performing both de novo sequencing and database search on an MS/MS spectrum. Compared to the state-of-the-art, Novor significantly improves both the speed and accuracy of these two functions. Novor is free for academic research.
Methods: A decision tree is used to combine 169 scoring features to calculate the algorithm's confidence on each amino acid in the sequencing

result. The de novo sequencing algorithm in Novor tries to maximize the sum of amino acid confidence in the de novo sequence. Then, the de novo sequence tag is used to find approximate sequence matches in the sequence database efficiently. These database matches are scored with another scoring function, which uses logistic regression to combine the de novo score with several other scoring features. **Results and Discussion:** On a MacBook Pro laptop (quad core), Novor can perform the combined de novo sequencing and database search analyses at the speed of 100 spectra per second. Its accuracies for de novo sequencing and database search are significantly better than the PEAKS and Mascot software, respectively. The UniProt human database (90411 proteins) is used for testing the search speed. Novor opens the possibility to sequence the peptides at the same time as the spectrometer is acquiring the data, and therefore eliminates the waiting time for the data analysis after the data is produced. **Conclusion:** Novor can perform both de novo sequencing and database search analyses on an MS/MS spectrum. Its accuracy is better than the most popularly used tools for these two functions, respectively. Its speed exceeds today's fastest mass spectrometer, making it the first and only software for real-time peptide sequencing analysis.

Keywords: peptide sequencing, real-time, de novo, database search

P13.24 An Automated Alignment Strategy to Obtain Comprehensive Data Matrices in Targeted Proteomics Data

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Introduction and Objectives: Targeted proteomic techniques such as SWATH-MS and SRM comprise a set of sensitive and accurate methods to quantify protein analytes across many experimental conditions. Such high-quality proteomic data matrices are essential for systems biology approaches or for the analysis of clinical cohorts, however, ensuring consistent quantification of a high number of peptide analytes across multiple LC-MS/MS runs is currently challenging. **Methods:** Here we present TRIC, a software which performs automated, consistent peak-picking across multiple targeted LC-MS/MS runs to produce quantitative data matrices with very few missing values. TRIC is specifically designed for targeted proteomics data (such as high-throughput SWATH-MS data) and written in the Python language. It uses a graph-based alignment strategy based on non-linear retention time correction to integrate information from all available runs. Through alignment and transfer of identification confidence, TRIC can resolve ambiguous identifications and boost identification confidence for low-confidence identifications. **Results and Discussion:** When applied to SWATH-MS data, the algorithm was able to reduce the identification error by more than 3-fold at constant recall, while correcting for non-linear chromatographic effects. On a pulsed-SILAC experiment performed on human iPS cells, TRIC was able to identify quantify 1834 proteins across all time points and substantially increased the quantitative completeness and biological information in the data. The number of proteins identified in five or more samples increased by 59.8 % after applying TRIC, providing direct insights into protein dynamics of iPS cells. Our analysis demonstrates the importance of consistent peak picking in targeted proteomics datasets where manual analysis is infeasible. The proposed TRIC algorithm automates cross-run peak picking and constitutes the last missing piece of an analysis pipeline able to automatically analyze massively parallel targeted proteomics datasets with high throughput and confidence.

Keywords: proteomics software, SWATH-MS, computational proteomics, Targeted proteomics

P13.25 ProteoSuite – An Open Source Framework for Quantitative Proteomics Based on PSI Data Standards

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Introduction and Objectives: 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. **Methods:** ProteoSuite is a Java-based software package, integrating external libraries or packages, such as ProteoAnnotator (http://www.proteoannotator.org/), searchGUI (https://code.google.com/p/searchgui/), mzqLib (https://code.google.com/p/mzq-lib/), x-Tracker (http://www.x-tracker.info), openMS (http://open-ms.sourceforge.net/) and application programming interfaces, such as jmzML, jmzIdentML and jmzQuantML. ProteoSuite is released under permissive licence, free for academic or commercial users. **Results and Discussion:** In the version 1.0 release, we include a quantitation routine for label-free analysis, a genome annotation routine using MS/MS data, and generic peptide/protein identification search capability. 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. Future releases will incorporate support for tag-based protein quantification.

Keywords: software, quantitation, java, label-free

P13.26 A Deeper Understanding of mRNA Processing through Proximity Proteomics

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Introduction and Objectives: Regulation of gene expression is a complex process: control of the proper processing, transport, translation and degradation of messenger RNAs (mRNAs) is tightly regulated. For example, in the cytosol, mRNA cycles between active sites of translation (polysomes) and other macroscopic structures where mRNA stalled in the process of translation initiation are held (stress granules; SGs) or degraded (p-bodies; PBs) as a regulatory mechanism. Understanding the structural organization of SGs and PBs and other RNA-containing structures is of clinical relevance, however, due to challenges in their biochemical purification, the precise composition of these structures remains unclear. **Methods:** We have used a recently-introduced in vivo biotinylation approach called BioID to identify novel protein components of SGs and PBs and proximity partners of various mRNA processing factors. In BioID, a protein tagged with a mutated bacterial biotin ligase (BirA*) biotinylates nearby proteins in vivo; these partners are then recovered on streptavidin and identified by mass spectrometry. **Results and Discussion:** By generating BioID profiles of approximately 70 proteins involved in mRNA regulation (60% of which with strong SG or PB signature), we identified multiple new components p-bodies and stress granules, which were further validated by immunofluorescence microscopy. The consequences of the depletion of each new component on the dynamics of formation and dissolution of the stress granules was determined. Importantly, generating a high-resolution BioID map of the RNA processing and degradation machinery enabled us to uncover the architecture of these structures. We also identified new links between the mRNA processing machinery and other cellular processes, namely membrane trafficking. **Conclusion:** BioID enabled the discovery of the composition of p-bodies, and stress granules. Interestingly, stress granules components could be identified without applying stress, implying a constant cycling of the SG components between soluble and bound forms. Our data greatly expand the biological knowledge regarding the regulation of mRNA processing.

Keywords: interaction proteomics, Systems biology, Subcellular Proteomics, Regulation of gene expression

P14: POSTER SESSION - MEMBRANE PROTEOMICS

P14.01 Expression & Association of CDK10 with ETS2 during Human Corneal Wound Healing

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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 and Discussion:** 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.

Keyword: cornea, CDK10, migration, trancription factor

P14.02 An Effective Plasma Membrane Proteomics Approach for Small Tissue Samples

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Introduction and Objectives: Despite their high biological importance, plasma membrane proteins (PMPs) often remain underrepresented when applying currently available proteomics strategies. Traditional isolation methods mostly rely on the removal of the non-plasma membrane fraction from the sample by ultracentrifugation. This approach requires high sample loads and delivers low yields that are still obscured by high levels of contamination from other cellular compartments. Advancing the quest for new drug targets therefore demands the development of innovative research strategies with straightforward PMP enrichment methodologies, especially for tissues that are only available in very low amounts. **Methods:** In this study, we performed an 'acute slice biotinylation assay' (ASBA) on mouse coronal brain slices followed by streptavidin pull-down to separate the PMPs in the so-called PMP enriched fraction from the rest of the proteome in the wash-through fraction. Liquid chromatography mass spectrometric analysis was performed on a nanoLC connected to a Thermo Scientific LTQ Velos Orbitrap mass spectrometer. Ingenuity Pathway Analysis (IPA; Qiagen Ingenuity systems, Redwood City, CA, USA) was used for cellular component assignment. The enrichment of proteins in the PMP enriched fraction was investigated by analysing the identifications within this fraction relative to a background composed of all proteins identified in the wash-through and PMP enriched fraction Similarly, information about the enrichment of proteins in the wash-through fraction was retrieved. **Results and Discussion:** Here we report an effective technical workflow where biotinylation of acute ex vivo tissue slices and streptavidin pull-down is followed by shotgun proteomics. It allowed the selective extraction and identification of more than 800 proteins of which 60% are associated

to the plasma membrane based on IPA, including (G-protein coupled) receptors, ion channels and transporters, and this from mm3-scale tissue. **Conclusion:** The workflow has the potential to solve both the problem of poor extraction efficiency and high sample consumption of the common PMP extraction protocols used today.

Keywords: membrane, Plasma membrane, TMT

P15: POSTER SESSION - PHOSPHOPROTEOMICS AND CELL SIGNALING

P15.01 Identification of Adaptor Proteins Included in the TNFR2 Signaling Complex

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Introduction and Objectives: TNFR1 signaling mechanisms, which involve the functions of adaptor molecules, have been widely researched. However, the mechanism by which TNFR2 activates downstream signals is poorly defined. In this study, a TNFR2-specific agonist (R2-7), which has a binding selectivity to TNFR2 and retains a bioactivity, was generated from a mutated TNF library using phage-display technology to activate TNFR2 specifically. TNFR2- expressing cell line was used to analyze the molecular assemblies associated with TNFR2 signaling. **Methods:** TNFR2 adaptor molecules were analyzed using a proteomic approach. Binding of the TNF homotrimer to TNFR2 induces aggregation of the receptors and subsequent recruitment of cytosolic signaling proteins, resulting in the formation of a ligand–TNFR2 complex that initiates downstream signal transduction. R2-7–FLAG promoted formation of the TNFR2 complex specifically, and subsequent affinity purification by anti- FLAG immunoprecipitation enabled us to concentrate the target proteins. These precipitates were analyzed by liquid chromatography mass spectrometry (LC-MS) with shotgun analysis. **Results and Discussion:** A MASCOT database search indicated that, 600 and 570 proteins were identified with and without R2-7–FLAG stimulation, respectively. Comparative analysis of results obtained for stimulated versus unstimulated cells indicated that 142 proteins were specifically detected in the stimulated cells. These proteins were considered to be potential TNFR2-signaling-related proteins and as candidates as adaptor molecules. Among identified proteins we found that APP3 showed the highest protein score and coverage after TNFR2 and TRAF2. **Conclusion:** We found that APP3 was considered as a new adaptor protein of TNFR2. These results indicate that APP3 binds to TNFR2 specifically. We focused on the potential role of APP3 in TNFR2 signaling, and proceeded to analyze more detail functions of this molecule in the future.

Keywords: TNF mutant protein, TNFR2 signaling, aminopeptidase P3

P15.02 The NCI60 Phosphoproteome

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Introduction and Objectives: The NCI-60 panel is collection of cell lines which originate from different tissue types. It is extensively characterized on a molecular level and is widely applied to study the in vitro cytotoxicity of therapeutic compounds. In this study we set out to measure the phosphoproteome of the NCI-60 cell line panel and correlate it to available drug response data in order to answer if the basal phosphopeptide abundance is a suited predictor of drug response. **Methods:** A Fe-IMAC column connected to an Aekta HPLC system was used for comprehensive enrichment of phosphopeptides from 60 different cell lines. Enriched eluates were directly measured on an Orbitrap Q-Exactive HF. For full proteomes, Fe-IMAC column flow throughs of the corresponding phosphopeptide enrichments were additionally fractionated. MaxQuant was used for data analysis and quantification (label-free). **Results and Discussion:** Here we present a quantitative phosphoproteome profile of the NCI-60 panel. Phosphopeptide abundance changes were used for clustering and were integrated and compared to already available large scale “omics” datasets. Moreover, we re-measured the corresponding 60 full proteomes to unprecedented depth and used both datasets to model drug response profiles for a large collection of therapeutic compounds. **Conclusion:** We provide a comprehensive resource of NCI-60 wide phosphorylation, which can be broadly utilized in basic cancer treatment research.

Keyword: Phosphorylation, NCI-60 panel, cell lines, drugs

P15.03 Target Recognition Properties of Pin1 Revealed by Quantitative Peptide Microarray

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Introduction and Objectives: Pin1 is a unique peptidyl prolyl isomerase (PPIase) which specifically recognizes the substrates with the phosphorylated Ser/Thr-Pro motifs, followed by catalyzing the cis-trans conformational transition of Proline. So far, there is no systematic view of the association of Pin1 with its diverse targets. In order to understand the rules that govern the target recognition of Pin1, herein we report a peptide microarray for high-throughput measuring the equilibrium dissociation constant of Pin1-peptide interaction. **Methods:** We synthesized 87 peptides derived from known or putative binding motifs of Pin1, where every peptide is 11 mer in length and contains a centrally located Ser/Thr-Pro. With the equilibrium dissociation constants of Pin1 for each peptide representing the binding motifs of Pin1 targets measured by peptide microarray, the target recognition properties of Pin1 were analyzed by sequence logo and binding scores. The binding affinity of Pin1 to its substrate peptides was validated by surface plasmon resonance (SPR) and the cis/trans conformational change was investigated by nuclear magnetic resonance (NMR) spectroscopy. **Results and Discussion:** We found Pin1 prefers binding the motifs with centralized pSer/Thr-Pro flanked by small and non-charged amino acids. Interestingly, distinctive amino acid preferences were found in peptides with either high or low binding affinity for Pin1. Surprisingly, there is no significant difference in binding selectivity or binding affinity between the PPIase domain and full length Pin1, which underlines the significance of the PPIase domain in Pin1 target recognition. In addition, Pin1 catalyzes the cis/trans isomerization regardless the binding affinity.

Conclusion: Our study is the first comprehensive study on the binding specificity and affinity of Pin1 with its substrates and provides a global biophysical picture on Pin1 target recognition properties.

Keywords: Substrate specificity, Pin1, Peptide microarray

P15.04 Proteomics of ALK Signaling in Neuroblastoma

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Introduction and Objectives: Neuroblastoma is a tumor of the sympathetic nervous system, accounting for around 10% of pediatric cancer-related mortality. Oncogenic anaplastic lymphoma kinase (ALK) functions as a driver of neuroblastoma and several other cancers, including non-small cell lung cancer and lymphoma. Currently, ALK tyrosine kinase inhibitors (TKIs) are used and tested in the clinic to treat ALK-positive cancers. We applied a quantitative mass spectrometry (MS)-based proteomics approach to delineate druggable ALK signaling in neuroblastoma cells. **Methods:** In our set-up we treated an ALK-amplified neuroblastoma cell line NB1, displaying constitutive ALK activity, with three different ALK TKIs (crizotinib, TAE684 and LDK378). SILAC was used to quantitatively assess how drug-treatment affected the ALK interactome and phosphoproteome. For the interactome we immunoprecipitated ALK and for the phoshoproteome we enriched phosphopeptides by both TiO2 and anti-phosphotyrosine antibodies. Samples were analyzed by nano-flow LC-MS/MS on a Q-Exactive mass spectrometer and data were processed using MaxQuant. **Results and Discussion:** From the interactome dataset we identified both known and novel ALK interactors, including 57 proteins displaying significantly decreased association with ALK upon TKI treatment. Selected candidates, including the novel full-length ALK interactor SHP2, were validated by co-immunoprecipitation and western blotting. In addition, peptide pull-down MS-data was generated to validate and map binding-sites of interactors binding to ALK phosphotyrosines. In the phosphoproteomics analysis we identified and quantified 11,122 sites including 506 phosphotyrosines. Our analysis supported the role of the PI3K/Akt signaling axis as a major player in mediating ALK oncogenic activity and identified the transcription factor FoxO3a as a down-stream target of full-length ALK. In addition, we identified a new ALK signaling adaptor linking ALK to PI3K/Akt signaling. Currently, the functional impact of these ALK effectors are being studied using siRNA-mediated knock-down and inhibitors. **Conclusion:** This comprehensive analysis of ALK signaling using quantitative proteomics has revealed novel and important aspects of ALK function in neuroblastoma.

Keywords: ALK, neuroblastoma, Mass spectrometry, phosphoproteomics

P15.05 Phosphoproteomic Analysis of Signaling Networks during Egg Activation in Sea Urchin

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Introduction and Objectives: After Upon fertilization, activated eggs undergo a dynamic symphony of molecular transformations induced by a wave of intracellular calcium. Most prominent are surface alterations, metabolic activation, cytoskeletal reorganization and cell cycle reentry. While the activation process appears to be broadly evolutionarily conserved, the signaling networks mediate the response to calcium release are not fully known. To address this gap, we applied large-scale phopshopeptide profiling affinity enrichment together with unbiased high-throughput 2D LC-MS/MS screening over a time course analysis of egg activation using the sea urchin Strongylocentrotus purpuratus as a model system. **Methods:** we applied large-scale phopshopeptide profiling affinity enrichment together with unbiased high-throughput 2D LC-MS/MS screening over a time course analysis of egg activation using the sea urchin Strongylocentrotus purpuratus as a model system. **Results and Discussion:** We identified striking phospho-signatures at both 2 and 5 minutes post fertilization as compared to unfertilized eggs and the 2 cell zygote stage, which revealed critical pathways and downstream effector proteins. Overall, we mapped 8301 distinct phosphosite modifications on 3599 phosphoproteins, of which 354(10%) were differentially regulated. Enrichment analysis revealed signaling cascades conserved in human while revealed exhibiting both well-known kinase substrate relationships and many unexpected functional connections. **Conclusion:** This study represents the most comprehensive study of the signaling systems responsible for egg activation to date, providing unprecedented mechanistic insights and valuable novel avenues for future investigations.

Keywords: phosphoproteomics, sea urchin, egg activation

P15.06 Quantitative Proteomics Analysis Unravels Functional Roles of Englerin A in Renal Cancer

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Introduction and Objectives: Englerin A (EA) is a small molecule natural product with selective activity against renal cancer cells. Although EA has been suggested to exert its cancer inhibitory roles through various mechanisms, its renal cancer-specific mode-of-action (MOA) remains enigmatic. Our aim is to elucidate EA's biological MOA through identification of its renal cancer-specific direct as well as downstream protein targets using SILAC Mass Spectrometry and Network Biology. **Methods:** SILAC-labeled A498 renal cancer cells treated with 100 nM EA for 24 hours were used for proteome and phosphoproteome profiling. Fractionated trypsin digested peptide samples were subjected to liquid-chromatography mass spectrometry analysis. Phosphopeptide enrichment was performed using TiO2 approach for phosphoproteome profiling. The acquired MS data was processed using MaxQuant (v. 1.3). Kinase-substrate enrichment was implemented using predictions from NetworKIN (v. 3.0), and integrative network analysis was carried out by MetaCore, STRING and Reactome FI, and rendered using Cytoscape. **Results and Discussion:** The whole proteome analysis identified over 4000 proteins, but with no significant EA-responsive protein changes. Phosphoproteomics analysis identified 10940 phosphorylation sites of which 671 sites exhibited EA-dependent phosphorylation changes. Integrative analysis of motifs and interaction networks suggested that kinases implicated in apoptosis such as p38, PKD1, and JNK were potentially activated upon EA treatment. It also revealed that EA may restore aberrant chromosome segregation and spindle checkpoint functions in renal cancer cells via inactivation of TTK and PLK1. Western blot analysis confirmed renal cancer specific-p38 activation, suggesting EA's effects in stress mediated cytotoxicity. **Conclusion:** This study highlights that renal cancer-specific anti-tumor effects of EA are a consequence of altered phosphorylation landscape associated kinase signaling events. Network analysis identified EA-induced

specific downstream signaling pathways that potentiate cytostasis and cytotoxicity, uncovering plausible MOA of EA in renal carcinoma.

Keywords: Renal cell carcinoma, Englerin A, phosphoproteomics, Network biology

P15.07 Elucidating Dynamic Receptor Tyrosine Kinase Signaling with Deep Phospho-Seq

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Introduction and Objectives: Regulatory protein phosphorylation serves as a fast and efficient way for eukaryotic cells to control cellular responses to external stimuli. Receptor tyrosine kinases (RTK) in particular, are of great interest due to their role in being key regulators of cell fate, differentiation along with disease progression and development via extensive signaling cascades. Here we develop and apply novel technical workflows to study phospho-signaling dynamics initiated by RTKs in very great depth using offline high resolution phosphopeptide chromatography in combination with orbitrap tandem mass spectrometry (MS). We developed a fast and efficient sample preparation protocols to reduce biological variability along with incorporation of automated high resolution peptide level fractionation and enrichment of phosphorylated events for in depth coverage. Using this technology we are able to obtain a comprehensive map of the dynamic phosphoproteome as a result of the activation of different RTKs. The large dataset arising from our high resolution MS analysis allowed us to perform large scale quantitative and discovery based analysis of RTK signaling. **Methods:** NIH/3T3 cell line were stimulated with ligands specific for various receptor tyrosine kinase (RTK). Cells were lysed using boiling Guanidinium Chloride and compared with standard RIPA buffer. Proteins were digested into peptides using sequential addition of Lys-C followed by Trypsin. Peptide mixtures were fractionated using basic reversed phase chromatography. Fractions were automatically concatenated into 10 fractions. Peptides were enriched directly from concatenated fractions using Titanium Dioxide and washed on hydrophilic filter plate and eluted into 96 well plate using vacuum. Samples were loaded into C18 STAGE-Tip until analyzed by LC/MS. All samples were analyzed on Q-Exactive HF Orbitrap system operating high resolution (120k resolution MS and 60k resolution for MS/MS). One hour LC gradients were used for all samples. Subsequent raw files analyzed using Maxquant software package with Andromeda search engine.
Results and Discussion: not applicable
Conclusion: not applicable

Keywords: phosphoproteomics, signaling, Receptor tyrosine kinase, Phospho-seq

P15.08 Targeted Quantitation Evolved: HD-PRM

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Introduction and Objectives: Parallel reaction monitoring (PRM) yields very sensitive quantitation regardless of MS1 features, but requires knowledge a priori of which analytes must be targeted by the mass spectrometer for MS/MS. Scheduling targets according their retention times is often done to increase duty cycle, but can cause missed quantitation due to chromatographic variations. A new instrument method, High Density Parallel Reaction Monitoring (HD-PRM) actively screens for targeted endogenous peptides using their spiked-in isotopically labeled analogues, monitors MS/MS data to validate against reference spectra, and switches to high resolution/increased sensitivity mode to collect MS^2 data precisely when the peptide is eluting, overcoming the issue of missing data, increasing the number of targets, and dramatically enhancing sensitivity[1,2]. **Methods:** Stable isotope labeled tryptic peptides for many critical proteins of the human MAPK, WnT, and mTOR signaling pathways were synthesized and used to generate spectral libraries. These peptides were spiked in fixed concentrations into samples consisting of lysates of two non-small cell lung cancer cell lines, one which had developed resistance to a tyrosine kinase inhibitor. An HD-PRM method was run on the Thermo Scientific™ Q Exactive™ HF using 60k resolution MS^2. **Results and Discussion:** HD-PRM enabled more complete quantitation of the proteins of the target signaling pathways in a routine and reproducible fashion while being insensitive to minor chromatographic fluctuations. Protein expression data was consistent with previously msxDIA analysis, but showed a noticeable increase in sensitivity, identifying many additional peptides below the detection limit of data independent acquisition. **Conclusion:** The use of HD-PRM enabled the quantification of more targets than would be possible by conventional PRM methods and also yielded absolute quantitation information, something not possible by conventional DIA methods. Finally, the spectral library generated for this experiment is applicable to other assays, where similar protein targets are of interest and similar gradients are employed.

Keywords: signaling, PRM, pathway, DIA

P15.09 Multi-Layered Proteomics Unveils Molecular Switches of Ligand-Dependent EGFR Outputs

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Introduction and Objectives: Receptor tyrosine kinase (RTK) endocytosis has been proposed as a key conductor of the cell signaling orchestra as it can coordinate the core variables of signal transduction: duration, intensity and spatial distribution. Concomitant with signal propagation, activated ligand-RTK pairs undergo intracellular endocytic trafficking and the balance between the numbers of receptors that undergo degradation versus those that are recycled to the cell membrane after internalization is critical to determine the dynamics of downstream signaling networks, which ultimately affects cellular outcome. Perturbations of this delicate balance often lead to pathogenesis, such as cancer and diabetes. The concept of functional selectivity, where the amplitude and duration of signaling cascades mediated through the same receptor are ligand-dependent, is well-known for GPCRs. As recently demonstrated in our laboratory for FGF receptor (Francavilla, et al. 2013), this concept is now emerging in the RTK signaling field. **Methods:** Here, we performed mass spectrometry-based quantitative proteomics to elucidate the core signaling pathways activated by Epidermal Growth Factor (EGF) Receptor in response to the recycling ligand Tumor Growth Factor alpha (TGF-α) or to EGF, the canonical ligand that induces receptor degradation. **Results and Discussion:** The integration of several datasets (phosphoproteome, ubiquitylome, interactome, proteome) collected in a time-resolved manner revealed that the two ligands shared a common core of signaling proteins, although their dynamics were often different. While EGF induced transient signaling, TGF-α promoted sustained signaling leading to increased cell proliferation and migration. In particular, proteins

involved in the endocytic machinery were differentially regulated and from these we identified and functionally validated 'cellular switches' that control the endocytic EGFR trafficking and ultimately decide the cellular fate. These results, based on a multidisciplinary approach, which combines multi-layered proteomics and functional assays, identify ligand-dependent mechanisms for the control of EGFR trafficking and long-term responses. **Conclusion:** Not applicabile

Keyword: EGFR, phosphoproteomics, functional selectivity, endocytosis

P15.10 Fast and Accurate Site Localisation of Phosphopeptides via Spectral Library Matching

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Introduction and Objectives: Nowadays tens of thousands of phosphopeptides are reported as identified, but there are no computational tools to validate such reports. In the presentation we will show a new automated method to validate such results by comprehensively and unambiguously localising phosphorylation sites on proteins. At the heart of the method lies a novel computational simulation of mass spectra that are 'virtual' phosphopeptide spectra. We will present how we localise phosphorylation sites on proteins at high sensitivity and how well it scores compared to existing methods. **Methods:** To prevent false localization reporting, a spectral library of phosphopeptides needs to contain all possible isoforms, which can be readily accomplished by the simulation algorithm. Computational simulation of phosphorylated peptides is based on actual dephosphorylated or non-phosphorylated peptides. We show that SpectraST accurately matches virtual phosphopeptide spectra to localise the phosphorylation sites on actual phosphopeptides. The simulated spectral library was evaluated based on results from datasets consisting of 1) synthetic 'test case' phosphopeptides, 2) a large-scale HeLa phosphopeptide study and 3) the Marx et al. synthesized data of >100,000 singly phosphorylated peptides. Results were compared to other existing methods including sequence database searching followed by phosphorylation site localisation. **Results and Discussion:** The results obtained from the HeLa sample and the synthetic peptides indicate that SimSpectraST is a sensitive method for the site-specific identification of phosphopeptides. Furthermore the new method developed outperforms all other methods. The simulation was developed based on HCD spectra and should also work on other beam-type MS instruments such as Q-TOFs, and is probably applicable to ETD fragmentation. **Conclusion:** Spectral library searching of simulated MS/MS phosphorylated peptides successfully leads to accurate phosphorylation localization outperforming other available and tested programs. The method serves as an effective orthogonal method for supplementing and/or validating the phosphorylation sites obtained by existing database searching and localisation tools.

Keyword: phosphopeptide, signalling, phosphorylation, spectral library

P15.11 Enrichment of Multiphosphorylated Peptides Using Novel Magnetic TiO2-Based Nanomaterial

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Introduction and Objectives: One of the challenges in phosphoproteomics is the enrichment and analysis of multiphosphorylated peptides. A widely used enrichment techniques for phosphopeptides utilize either metal ions e.g. Fe^{3+} or metal oxides, e.g. TiO2. Materials made from TiO2 are able to bind multiphosphorylated peptides as well as monophosphorylated peptides but elution of multiphosphorylated peptides is difficult due to their extremely high binding to TiO2 material (1). The magnetic nanomaterial based on TiO2 introduced in this work with different structural state significantly increases the number of eluted and analyzed multiphosphorylated peptides, as compared to common TiO2 materials available today. **Methods:** A peptide mixture consisted of tryptically digested phosphorylated and nonphosphorylated proteins was used as sample. The phosphopeptide enrichment was performed either with TiO2 microparticles (Titansphere, GL Sciences) or with the novel magnetic nanomaterials. Binding of phosphopeptides was performed in 80% acetonitrile/5% trifluoroacetic acid/1M lactic acid and eluted with diluted ammonia solution. All samples were analyzed by MALDI LTQ Orbitrap XL mass spectrometer. **Results and Discussion:** Number of identified monophosphorylated peptides was the same for all tested materials. However, elution fractions obtained from the new magnetic nanomaterial contained significantly higher portion of multiphosphorylated peptides, as compared to commonly used commercial TiO2 beads. These results indicate an improved performance in the analysis of multiphosphorylated peptides. In addition, enriched samples were obtained in a single step using only one type of carrier in comparison to SIMAC protocol (1). **Conclusion:** The presented material showed the high enrichment efficiency especially for multiphosphorylated peptides that are difficult to elute from TiO2 in general. Another advantage is its magnetic property making the material handling easier during enrichment steps. All these results and properties predetermine this material as ideal tool for phosphoproteomics studies in various organisms. 1.Thingholm, T. E., et al. Molecular&Cellular Proteomics 7, 661–671(2007). Acknowledgements: This work was supported by EU project NADINE (No. 246513).

P15.12 Protein Kinase Substrate Specificity Determination by Prediction Algorithms and Peptide Microarrays

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Introduction and Objectives: Over a million phosphorylation sites (P-sites) in the human proteome are targeted by at least 568 protein kinases. To ensure cell signalling fidelity, protein kinases must exert high specificity for their substrates. Recognition by a particular kinase is strongly influenced by the amino acid sequence surrounding a substrate P-site. The substrate specificities for most of the human protein kinases have not been explored. **Methods:** We developed a protein kinase substrate prediction algorithm (KSP v 1.0) that was trained with empirical data from ~10,000 known protein kinase-protein substrate P-site pairs. This algorithm permitted protein kinase consensus sequence prediction matrices based on the primary amino acid sequences of 492 catalytic domains of human protein kinases. Optimal consensus peptides were produced by SPOT synthesis, and 445 of these were printed in triplicate on glass slides to allow individual testing of their

phosphorylation in the presence of ATP with over 200 different recombinant human kinases. Detection of phosphorylated peptides was achieved with ProQ Diamond stain, and the signals were quantified with a microarray scanner, and analyzed using ImaGene® 8.0 image analysis software. **Results and Discussion:** Over 90,000 protein kinase-peptide substrate combinations were tested and the top 8,000 kinase-peptide sequence pairs were used to define optimal consensus P-site sequences for 200 protein kinases. These sequences correlated well with P-site consensus sequences that were derived for a subset of ~80 protein kinases for which there was sufficient published empirical data. They also matched well with predictions generated from the KSP v 1.0 algorithm. The findings were then used to further train a more advanced KSP v 2.0 algorthm, and the results have been posted in the PhosphoNET website at www.phosphonet.ca. **Conclusion:** Knowledge of protein kinase substrate specificities is useful for the identification of new P-sites in the human phosphoproteome, and the development of high-resolution maps of kinase-dependent phosphorylation signalling networks.

Keywords: Peptide microarray, Substrate specificity, Protein phosphorylation, Protein kinase

P15.13 Protein Kinase Substrate Specificity Determination by Prediction Algorithms and Peptide Microarrays

Steven Pelech[1], Shenshen Lai[2], Dominik Sommerfeld[2], Javad Safaei[3], Dirk Winkler[1]
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Keywords: Protein kinase, Protein phosphorylation, Substrate specificity, Peptide microarray

P15.14 Metal-Immobilized Magnetic Nanoparticles for Enrichment of Phosphopeptides by Mass Spectrometry

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Introduction and Objectives: Protein phosphorylation is one of the most important post-translational modifications that mediates many cellular events. Developing high selective enrichment methods for phosphopeptides has being of great significance for sensitive and in-depth phosphoproteome analysis. **Methods:** A set of different metal ions-immobilized magnetic nanoparticles was developed. In brief, the modified process included the covalent linkage of DOTA and TCPP via ethylenediamine, in which aminefunctionalized magnetic nanoparticles were used as the precursor. Then, different metal ions were immobilized on the surface of the magnetic nanoparticles by the chelation reaction of DOTA. **Results and Discussion:** A new set of different metal ions–immobilized (Ti^{4+}, Zr^{4+}, Fe^{3+}, Tb^{3+}, Tm^{3+}, Ho^{3+}) magnetic nanoparticles, Fe_3O_4@TCPP-DOTA-M, was prepared. A model protein, α- casein and a protein mixture of α- casein and BSA (1: 50) were used to test the enrichment efficiency of the phosphopeptides respectively. For the model protein α-casein, 15 phosphopeptides were identified with Fe_3O_4@TCPP-DOTA-Tb and Fe_3O_4@TCPP-DOTA-Ti respectively. And 14, 11, 11, 13 phosphopeptides were identified by Fe_3O_4@TCPP-DOTA-Zr, Fe, Tm, Ho, indicating the enrichment efficiency of metal iones Tb^{3+} and Ti^{4+} immobilized materials was better than other metal ions immobilized materials. Even in the tryptic digest of α-casein and BSA (1: 50), 14 phosphopeptides were easily detected with Fe_3O_4@TCPP-DOTA-Tb and Fe_3O_4@TCPP-DOTA-Ti, suggesting the novel materials possess higher selectivity in phosphopeptide enrichment. To further demonstrate the enrichment applicability of Fe_3O_4@TCPP-DOTA-Tb and Fe_3O_4@TCPP-DOTA-Ti in a real biological sample, the materials were utilized to isolate phosphopeptides from the tryptic digest of HeLa cells. In total, 9048 phosphopeptides corresponding to 2103 phosphoproteins were identified in a single mass spectrometric analysis. **Conclusion:** In summary, our newly developed materials have high selectivity and sensitivity for the enrichment of phosphopeptides and potential application in deep phosphoproteome analysis.In summary, our newly developed materials have high selectivity and sensitivity for the enrichment of phosphopeptides and potential application in deep phosphoproteome analysis.

Keywords: Enrichment, Mass spectrometry, Magnetic nanoparticles, Phosphopeptides

P15.15 Regulatory Roles of Conserved Phosphorylation Sites in the Activation T-Loop of the MAP Kinase ERK1

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Introduction and Objectives: The catalytic domains of most eukaryotic protein kinases are highly conserved. Phosphorylation of the activation T-loop, a variable region between kinase catalytic subdomains VII and VIII, is a common mechanism for stimulation of phosphotransferase activity. The MAP kinase Extracellular signal-regulated kinase 1 (ERK1) serves as a paradigm for regulation of protein kinases in signaling modules. We investigated the possible roles of three conserved phosphosites in the activation loop of ERK1 flanking the well-documented T202 and Y204 (TEY) activating site. **Methods:** Mutants of ERK1 were generated for the flanking phosphosites T198, T207 and Y210. These mutants were explored for their phosphorylation at these residues and the pTEpY site with phosphosite-specific antibodies, and for their ability to phosphorylate the ERK1 substrate myelin basic protein (MBP).

Results and Discussion: In vitro kinase assays with MBP using the purified ERK1 phosphosite mutants supported the functional importance of T207 and Y210, but not T198 in negatively regulating ERK1 phosphotransferase activity. A T207E mutation abolished the phosphotransferase activity of ERK1 without affecting the phosphorylation at the TEY site by the upstream kinase MEK1. The Y210 site could be important for proper folding of ERK1 in this regulatory region, since the mutation of this residue caused decreases in protein solubility, and the Y210F mutant was not recognized by MEK1 for phosphorylation in vitro. The phosphorylation of Y210 was enhanced in presence of MEK1. We propose the Y210 phosphosite of ERK1 becomes hyperphosphorylated after the phosphorylation of the TEY site by MEK1, which inhibits its catalytic activity. Our data also indicated that the T207 site appears to be inhibitory, and arises from autophosphorylation. **Conclusion:** Our findings contribute to an improved understanding of the activation of MAPKs and many protein-serine/threonine kinases in general. Hyper-phosphorylation within the kinase activation T-loop following the initial activation by upstream kinases may serve as a general mechanism for protein kinase inhibition to prevent prolonged cell signalling.

Keywords: Enzyme regulation, MAP kinase, Protein phosphorylation, Protein kinase

P15.16 Preservation of Phosphorylation Signaling States with Heat Stabilization for Bottom-Up Proteomics

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Introduction and Objectives: Protein phosphorylation states are affected by sampling causing analytical results to differ from in vivo phosphorylation levels. Rapid heat induced enzyme inactivation is an efficient way to minimize such post-sampling changes and preserve in vivo like levels of phosphorylation states for analysis. The ability to analyze in vivo like levels are vital when investigating signal transduction and searching for disease related biomarkers. Two studies comparing heat stabilized and just snap frozen brain tissue and liver using affinity phosphorylation enrichment followed by HPLC-MS based bottom-up proteomic has been conducted to investigate the effect of preservation of phosphorylation levels. **Methods:** In the first study phosphorylated tryptic peptides from mouse brain was affinity isolated using a pTyr specific antibody and analyzed using HPLC-MS in a bottom-up proteomic approach. In the second study human liver biopsies with 30 min post-sampling time on ice was digested with trypsin and TIO2 enrichment was used to isolate phosphorylated peptides for analysis using HPLC-MS in a bottom-up proteomic approach. **Results and Discussion:** Study 1: 1/3 of identified phosphorylated peptides showed at least 50% higher phosphorylation levels in heat stabilized samples compared with a handful showing comparatively higher level in snap frozen samples. 60 phosphorylations were only detected in heat stabilized samples including phosphorylations on clinically relevant proteins such as Tau and BCAR1. Study 2: 33% more phosphorylated peptides were detected in heat stabilized clinical liver biopsies compared to snap frozen biopsies even when which 30 min post-operation time on ice prior to treatment. Identified peptides from both treatments were distributed over all functional groups indicating no bias due to heat treatment. **Conclusion:** Heat inactivation of enzymes directly post-sampling preserves phosphorylation states closer to in vivo levels enabling more accurate study of cell signaling and detection of disease relevant biomarkers.

Keywords: phosphorylation, preservation,, Sample Preparation, heat inactivation

P15.17 Production and Characterization of Polyclonal Generic Phosphotyrosine-Specific Antibodies

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Introduction and Objectives: Reversible protein-tyrosine phosphorylation plays a critical role in cell regulation under normal and pathological conditions, which has made generic phosphotyrosine (pY) antibodies valuable tools for biomedical research. A recent study that compared the specificities of three widely-used monoclonal pY antibodies has raised concerns about strong sequence motif selectivity and low overall coverage rates. These issues can potentially introduce significant bias in pY site identification and quantification. In this study we describe a novel strategy of generating a pool of polyclonal antibodies from a large set of physiological pY-site sequences. **Methods:** Peptides with 7 to 20 amino acids, and 1 to 7 phosphorylation sites found in 210 human protein kinases, were used to immunize 28 rabbits. The sera from these rabbits were subjected to ammonium sulphate fractionation, pooled, and then affinity-purified on pY-agarose columns to produce PYK. The specificity of the purified PYK antibody, was assessed with phosphopeptide microarrays, including the Jerini Peptide Technologies Phosphatase Peptide Microarray, which features 6,099 peptides representing diverse human pY-sites. **Results and Discussion:** The rabbit polyclonal PYK pY antibody was compared to the well known 4G10, PY20 and PY100 mouse monoclonal antibodies. PYK was extremely stable to repeated freeze thaw, and proved to be more sensitive for detection of phosphopeptides on arrays and for proteins following Western blotting of EGF-treated A431 cell lysates than the monoclonal antibodies. PYK detected a larger number of diverse pY-containing peptides than 4G10, PY20 and PY100 on phosphopeptide microarrays, and unlike these other antibodies, acidic amino acid residues surrounding the pY-sites were not negative determinants for antibody recognition. **Conclusion:** Since acidic amino acids are positive determinants for substrate recognition for most protein-tyrosine kinases and flank most protein-tyrosine phosphosites, our findings indicate that the PYK antibody may be more useful for enrichment and analysis of physiological pY-sites than the commonly used mouse monoclonal antibodies for such purposes.

Keywords: Phosphotyrosine antibody, Phosphotyrosine, Protein phosphorylation, Phosphopeptide microarray

P15.18 Evaluation of Protein Kinase Inhibitors Using Motif-Targeting Phosphoproteomics

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Introduction and Objectives: Protein kinase inhibitors are clinically used as molecular targeted agents for cancer therapy. It is essential to evaluate these kinase inhibitors for drug development since there is a possibility that the drugs demonstrate unexpected behaviors caused by their off-target activities. Phosphoproteomics enabled us to monitor cellular phosphorylation events in a large scale, however current technology is inadequate to profile whole effects on signaling pathways by treatment with protein kinase inhibitor, especially on low-abundant phosphotyrosines. In this study, we developed a high-throughput method for profiling of kinase inhibitors with motif-targeting phosphoproteomics using in vitro kinase reaction. **Methods:** Human cancer cells were stimulated with each protein kinase inhibitor in the presence of sodium pervanadate. HeLa lysate proteins were subjected to reductive alkylation followed by in-solution digestion with trypsin. After desalting, phosphopeptides were enriched with hydroxy acid-modified metal oxide chromatography (HAMMOC) using a titania

tip. The enriched phosphopeptides were dephosphorylated with alkaline phosphatase and the target phosphopeptides were re-phosphorylated by in vitro kinase reaction using a mixture of recombinant protein kinases. Finally the phosphopeptides were enriched again with HAMMOC, and analyzed with data-dependent nanoLC-MS/MS using Q Exactive (Thermo fisher). **Results and Discussion:** Previously, we profiled in vitro substrates of 93 recombinant human tyrosine kinases using in vitro kinase assay. Based on the substrate data we decided the optimum combination of the tyrosine kinases to profile physiologically phosphorylated tyrosines in depth. Using a mixture of the tyrosine kinases, about 1,000 phosphotyrosines were identified in single LC-MS/MS run. The approach was applied to evaluation of kinase inhibitors, and we successfully quantify their influence on signaling molecules with the target motifs. **Conclusion:** The motif-targeting phosphoproteomic approach is useful for deep characterization of molecular-targeted drugs.

Keywords: Protein kinase, phosphorylation motif, molecular-targeted agent, signal transduction

P15.19 Proteomic and Phosphoproteomic Study of Human Macrophage Kinome after Interaction with Candida Cells

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Introduction and Objectives: Candida albicans is a dimorphic fungus that in a setting of congenital, induced or disease-related immune dysfunction can cause cutaneous, mucocutaneous and life-threatening systemic diseases. In the host side, macrophages are key cells in the antifungal innate immune response through phagocytosis and direct pathogen killing. Here, we develop and optimize a proteomic and a phosphoproteomic approaches for the study of the human macrophage kinome after interaction with C. albicans cells. **Methods:** For macrophage labelling, we used the SILAC method with switched labelling. After incubation of macrophages from the THP1 cell line with C. albicans cells (MOI 1:1) during 3 hours, the protein lysate was enriched in ATP-binding proteins using the ActivX desthiobiotin ATP probes (Thermo Scientific) kit. Then, proteins were reduced, alkylated and in-gel digested using trypsin. For phosphopeptide enrichment, sequential elution from IMAC and TiO2 chromatography was performed. Then, samples were analysed by LC-MS/MS using an LTQ-Orbitrap and the fragment ions extracted for a protein database using Mascot. **Results and Discussion:** Preliminary results showed that we were able to identify 987 proteins and quantify 899 of them. The functional analysis of the proteins showed that 86 of them were kinases, including several ones that are known to be important in the immune response as Mekk4, Src, Rsk1, Mapk3, Mek2, Syk, Pkr, Lyn, Tao3, Irak4, Csk and Pak2. Regarding the phosphorylation results, we were able to quantify more than 100 phosphorylated peptides from proteins involved in the regulation of the immune response and signal transduction such as Mapk1, Mekk2, Cdk, and Pkm among several others. **Conclusion:** With this work we successfully quantify the abundance and study the phosphorylation of ATP binding proteins enriched from human macrophages after interaction with Candida albicans which will shed a light on the signaling pathways that are differentially regulated during interaction. This work is supported by FP7-PEOPLE-2013-ITN, IMRESFUN Project.

Keyword: Candida albicans, macrophages, kinases, cell signalling

P15.20 Identifying Novel Signaling Mechanisms Underlying Insulin Release from Glucose Stimulated Beta Cells

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Introduction and Objectives: Glucose stimulated insulin release from pancreatic beta cells (PBCs) takes place via calcium-dependent exocytosis in a biphasic manner. Defects in exocytosis or other signaling mechanisms can lead to a decrease in the release of insulin from beta cells and subsequently diabetes. Therefore, a thorough investigation of the signaling mechanisms behind the glucose-mediated release of insulin will not only provide new knowledge about the way beta cells secrete insulin, but may also offer new targets for therapeutic intervention. Here we aimed to identify novel signaling pathways involved in the initial release of insulin from PBCs using quantitative strategies for phosphoproteomics on low amounts of material. **Methods:** PBCs were isolated from rats and stimulated with low and high glucose concentration for 5 min (500 Islets of Langerhans per condition). Phosphorylated peptides derived from proteins purified from the two conditions were then enriched by the TiSH procedure (combining TiO2, SIMAC and HILIC) and examined using quantitative phosphoproteomics. **Results and Discussion:** We identified >3000 phosphosites in 1400 proteins and found 452 phosphosites, which exhibited a significant change after 5 min of glucose stimulation. Among the proteins showing a significant increase in phosphorylation, we found a number of kinases, including PKC Camk2g, Map2k2, PK3C3, Prkaca, Rps6ka3, MI and JAK2. Pathway analysis of the proteins with changed phosphorylation, revealed an over-representation of the proteins involved in the phosphorylation (kinases and phosphatases), proliferation, protein translation, gene expression and cell development, as well as signaling pathways, such as insulin, leptin, AMPK and Ca^{2+}-signaling. Moreover, data on changes in the phosphoproteome of glucose stimulated PBCs at several time-points (5-20 min) will be presented at HUPO14. **Conclusion:** The majority of the identified proteins showing changed phosphorylation have not previously been associated with glucose stimulation, which illustrate the potential of using global phosphoproteomics as an unbiased technique to identify novel signaling mechanisms.

Keywords: glucose-stimulated insulin secretion, pancreatic beta cells, phosphoproteomics, exocytosis

P15.21 Phosphoproteomic Analysis Using the WW and FHA Domains as Biological Filters

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Introduction and Objectives: Protein phosphorylation plays a key role in regulating various biological events. Cellular protein phosphorylation data have been obtained using phosphoproteomic approaches, but the detection of low-abundance or fast-cycling phosphorylation sites remains a challenge. Enrichment of phosphoproteins greatly enhances the spectrum of low-abundance but biologically important phosphoproteins. Previously, we used 14-3-3ζ to selectively enrich for HeLa cell lysate phosphoproteins. However, because 14-3-3 does not isolate phosphoproteins lacking the 14-3-3-binding motif, we looked for other domains that could complementarily enrich for phosphoproteins. We here assessed and characterized the phosphoprotein binding domains Pin1-WW, CHEK2-FHA, and DLG1-GK. **Methods:** Using a strategy based on affinity chromatography, phosphoproteins were collected using phosphoprotein-binding domains from the lysates

of HeLa cells treated with phosphatase inhibitor (calyculin A or okadaic acid) or cAMP activator forskolin. The molecules and phosphorylation sites were identified by liquid chromatography tandem mass spectrometry (LC-MS/MS) following tryptic digestion and phosphopeptide enrichment. **Results and Discussion:** We identified different subsets of phosphoproteins associated with WW or FHA in addition to 14-3-3ζ, whereas only a limited number of phosphoproteins were obtained by GK. WW- and FHA-bound phosphoproteins were nuclear and membrane enclosed lumen mainly, different from that of 14-3-3ζ, cytoplasmic and membrane protein mainly. KEGG pathway analysis revealed that both WW and FHA are capable of precipitating phosphoproteins involved in the cell cycle and cancer development, with additional signaling substrates precipitated by FHA. To capture PKA signaling molecules, we found 26, 46, and 36 PKA candidate substrates from HeLa cell lysate stimulated with forskolin, using the WW domain, FHA domain, and 14-3-3ζ proteins, respectively. **Conclusion:** Our Kinase-Oriented Substrate Screening (KiOSS) method with the use of phosphoprotein-binding domains are applicable and useful for the identification of novel phospho-substrates for kinases and can therefore be used as biological filters for comprehensive phosphoproteome analysis.

Keywords: WW domain, FHA domain, phosphorylation, phosphoproteomics

P15.22 MS-Based Analysis of Thiol-Redox and Phosphorylation Cross Talk in Human Bronchial Epithelial Cells

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Introduction and Objectives: Hydrogen peroxide (H2O2) serves as a second messenger in signal transduction. It causes oxidation of protein cysteinyl thiols e.g. in signaling proteins, thereby altering protein activity and function. However, it is largely unknown which proteins act as thiol-based redox switches, to which proteinogenous targets they do cross talk to, or which parts of the cellular signaling networks are activated in response to cellular redox signaling. **Methods:** Protein thiol-oxidation and phosphorylation were studied in human bronchial epithelial cells after exogenous pulse of H2O2 in a time course format. Identification and quantitation of protein cysteinyl thiol oxidation was achived by labeling with cysteine-reactive tandem mass tags or sulfenic-acid-selective probes and high-resolution mass spectrometry after enrichment of labeled peptides. Protein phosphorylation was identified and quantified in SILAC-treated cells after TiO2-based enrichment of phosphopeptides or after immunoprecipitation of tyrosine-phosphorylated peptides and high-resolution mass spectrometry. Bioinformatics was applied for the separated datasets to extract affected signaling networks and physiological pathways. **Results and Discussion:** Reversible thiol oxidation was observed immediately after addition of H2O2 and the quantity of affected proteins decreased in the course of the treatment period. Identified targets include redox proteins (e.g. TXN) and signaling proteins (e.g. ACP1). In contrast, the number of proteines with changes at the level of phosphorylation appeared as increased during the treamtent period. Proteins with increased phosphorylation included ERK1/2. Our results indicate that oxidation of specific cysteinyl thiols in distinct protein targets triggers the activation of phospho relay cascades and the integration of redox signaling in the cellular signaling networks. **Conclusion:** Analyzing the cross talk between thiol redox-modifications and protein phosphorylation gives new insights into the mechanisms and networks in response to H2O2-mediated redox signaling. The identification of thiol switches and targets may have implications for the understanding of the cellular communication stratetgies and thus for manipulation of cellular function e.g. during therapeutic interventions.

Keywords: redox proteomics, phospho proteomics, signal transduction, PTM analysis

P15.23 Comparison of Label-Free and TMT Quantification for Phosphoproteome Analysis of Apoptotic Cells

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Introduction and Objectives: Protein phosphorylation is one of the most important and well-studied post-translational modifications (PTM) participating in most cellular and development processes, including apoptosis. Major efforts in recent years have lead to the development of a multitude of LC-MS workflows that allow monitoring thousand of phosphorylation sites within one LC-MS experiment. With their multiplexing capabilities, isobaric labeling techniques (e.g. TMT) are becoming increasingly popular, also for phosphopeptide quantification, since they considerably reduce instrument time when analyzing fractioned samples. Despite numerous successful applications, a thorough evaluation of possible implications of TMT labeling on phosphopeptide identification and phosphorylation site localization for global phosphoproteomic studies is still missing. **Methods:** Proteins were extracted from either normally grown or apoptotic human cells and subjected to phosphopeptide enrichment using TiO2 beads. Peptide quantification was either carried out by label-free quantification of unfractionated samples or by TMT labeling. Here, an aliquot of each sample was labeled with TMT 10-plex reagents, combined and subsequently fractionated using off-line High-pH reversed phase liquid chromatography. Phosphopeptides were analyzed on a fast scanning, high-resolution Q-Exactive HF mass spectrometer. The power of the two different quantification methods including number of quantified phosphopeptides, pathway coverage, quantification precision and phosphorylation site localization confidence were compared. **Results and Discussion:** We found that while TMT labeling significantly reduced the number of identified phosphopeptides compared to unlabeled samples, in total, more phosphopeptides could be quantified from fractionated TMT labeled samples with similar MS instrument time. Along these lines, the higher precision of TMT quantitation additionally increased the number of detected significant phosphorylation site changes and the achieved coverage of affected pathways. Interestingly, TMT labeling slightly enhanced the fraction of peptides of which the phosphorylation site could be localized with at least 95% confidence. **Conclusion:** To conclude, TMT labeling in combination with high-pH fractionation is well suited for global phosphoproteome analysis.

Keywords: TMT, Phosphopeptides, High pH fractionation, label free quantification

P15.24 Comparison of SWATH, MS1 Intensity and Spectral Counting for Quantitation of Interaction Partners

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Introduction and Objectives: The combination of affinity purification and mass spectrometry is successfully used to identify protein interaction networks in cells. However, the quantitative measurement of variations in these interactions across different conditions remains a challenge. We utilized the non-catalytic region of tyrosine kinase (NCK) adaptor protein as a model to compare the performance of 3 label-free techniques, i.e. MS1 peak integration, spectral counting using data-dependent analysis, and

data-independent acquisition with SWATH to quantify variations in the interaction of several of its binding partners and their phosphorylation status. **Methods:** Wild-type or mutant NCK-expressing 293T cells were lyzed and subjected to affinity purification. Proteins were digested with trypsin and analysed by LC/MS/MS. Peptides were quantified by SWATH on a Triple-TOF 5600+ mass spectrometer and by MS1 peak integration on an Orbitrap Fusion mass spectrometer. Both instruments were used for spectral counting. For SWATH, a library was created using data-dependent analysis. Mascot and Scaffold software were used for spectral counting quantification; Peak View and Skyline software were used for SWATH quantification analysis; MaxQuant was used for label-free analysis. Variable window sizes were tested with SWATH acquisition in order to use the best method for the SWATH quantification experiment. **Results and Discussion:** Over 300 proteins were commonly identified at 1% FDR with the different methods and 30 phosphorylation sites were found. Comparison of quantification results between the 3 methods (spectral counting, MS1 peak integration and SWATH) is presented and the common interactors are further analyzed. For each technique, the sensitivity and the quantification accuracy will be discussed. **Conclusion:** Comparable results were obtained with the three methods; however MS1 peak integration and SWATH gave results with higher accuracy than spectral counting as expected. SWATH and MS1 peak integration allowed reliable quantification of phosphopeptides. In addition, these results provide further knowledge on the NCK interactors and their role in signal transduction

Keywords: quantitative proteomics, DIA, label free, phosphorylation

P15.25 Low-Bias Phosphopeptide Enrichment from Scarce Samples Using Plastic Antibodies

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Introduction and Objectives: Phosphospecific enrichment techniques and mass spectrometry (MS) are essential tools for comprehending the cellular phosphoproteome. Here, we report a fast and simple approach for low sequence-bias phosphoserine (pS) peptide capture and enrichment that is compatible with low biological or clinical sample input. **Methods:** The approach exploits molecularly imprinted polymers (MIPs, “plastic antibodies”) featuring tight neutral binding sites for pS or pY that are capable of cross-reacting with phosphopeptides of protein proteolytic digests. The versatility of the resulting method was demonstrated with small samples of whole-cell lysate from human embryonic kidney (HEK) 293T cells, human neuroblastoma SH-SY5Y cells, mouse brain or human cerebrospinal fluid (CSF). **Results and Discussion:** The approach exploits molecularly imprinted polymers (MIPs, “plastic antibodies”) featuring tight neutral binding sites for pS or pY that are capable of cross-reacting with phosphopeptides of protein proteolytic digests. The versatility of the resulting method was demonstrated with small samples of whole-cell lysate from human embryonic kidney (HEK) 293T cells, human neuroblastoma SH-SY5Y cells, mouse brain or human cerebrospinal fluid (CSF). **Conclusion:** We have developed a new and highly versatile phosphopeptide enrichment technique that can be easily applied to scarce clinical samples. The SCX/pS-MIP method is unique with respect to its compatibility with low sample inputs, its programmable selectivity and its low sequence bias in combination with its robustness, speed and simplicity.

Keywords: phosphoproteomics, Molecularly imprinted Polymers

P16: POSTER SESSION - NEUROLOGICAL DISORDERS

P16.01 DBP Associated with the Aspirin in the Prevention of Cerebral Thrombosis

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Introduction and Objectives: To investigate changes in expression of proteins and protein isotypes before and after aspirin therapy in cerebral thrombosis patients and analyze how Vitamin D-binding protein (DBP) interacts with aspirin during the secondary prevention of atherothrombotic disease. **Methods:** The plasma samples were obtained from 18 clinical cerebral thrombosis patients before and after aspirin treatment respectively. To find more potential protein biomarkers, two-dimensional electrophoresis (2DE) and mass spectrometry were taken to measure differential proteins between the two groups. Furthermore, an interaction between DBP and actin was confirmed with coimmunoprecipitation and western blot. **Results and Discussion:** There were 11 proteins, which were more than 1.5-fold difference in expression levels between the experiment and control groups, selected and identified by mass spectrometry analysis. Level of DBP significantly increased and actin had the opposite trend after aspirin therapy, which was confirmed by western blot. Protein–protein interaction network of DBP and related proteins was shown in IPA software. **Conclusion:** These results indicate roles of DBP in the actin scavenge system and consequently, a correlation between the ascended DBP and aspirin therapy in cerebral thrombosis patients has been demonstrated. DBP is probably involved in a new regulator in the therapy of atherothrombotic disease.

Keywords: Vitamin D-binding protein, aspirin, cerebral thrombosis patients, proteome analysis

P16.02 Proteomic Analysis of Cerebrospinal Fluid from Acutely Injured Spinal Cord Patients

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Introduction and Objectives: Spinal cord injuries (SCI) are categorized in the clinical setting, but the categories poorly reflect the underlying heterogeneity of these injuries, making translational medicine and trials of new therapies time consuming, expensive and error prone. Our earlier experiments identified six inflammatory cytokines and structural proteins as potential biomarkers for human SCI, which when combined were better predictors of neurologic recovery than the currently used ASIA clinical scale of injury severity (AIS). To expand on these findings, here we used targeted quantitative proteomics

on cerebrospinal fluid (CSF) to identify molecular biomarkers which can differentiate between different injury types and predict clinical outcomes. **Methods:** 30 acute spinal cord injury patients were classified with injury severity AIS A, B or C (high, medium, low severity respectively; 10 patients each) with CSF collected at 24, 48, and 72hr post injury. The CSF was depleted using a MARS antibody column, removing the 14 most abundant serum proteins. MRM transitions were optimized against 201 proteins, and compiled into 2 LC-dMRM methods. Protein abundances were measured for the three times points for each patient and compared to positive pooled (24hr post injury) and negative (uninjured) controls. **Results and Discussion:** Preliminary inspection shows 33 CSF proteins upregulated in SCI patients 24hrs post injury compared to uninjured controls ($p < 0.5$). Many of these proteins show significant differences between AIS A injuries (most severe) and AIS B (middle severity) injuries, as well as between AIS B and AIS C (least severe). Differences in these latter two injury classifications are more difficult to diagnose clinically, and are where much of the clinically undetectable heterogeneity in human SCI lies. **Conclusion:** Grouping these proteins' expression levels over time and comparing them to known patient histories and outcomes will create a biomarker panel that has strong predictive power for not only injury severity, but also future clinical outcomes.

Keywords: biomarkers, CSF, MRM, Spinal cord injury

P16.03 Chitinase 3-Like Proteins as Candidate Cerebrospinal Fluid Biomarkers for Multiple Sclerosis

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Introduction and Objectives: Multiple sclerosis (MS) is an inflammatory disease characterized by an initial demyelinating event (CIS), followed by remission periods and relapses occurring at irregular intervals. Clinical symptoms and brain imaging allow diagnosis with a good sensitivity. However, there is still a need for prognostic biomarkers of conversion to MS and disease progression after a CIS. This study aims at identifying such biomarkers by comparing the cerebrospinal fluid (CSF) proteome from patients with MS and symptomatic controls and from patients with rapid (<1 year) and slow (>2 years) conversion to MS after the first demyelinating event. **Methods:** Quantitative proteomic analysis of human CSF samples (n=78) using high-resolution mass spectrometry and isobaric mass tag labeling or label-free quantification procedures, followed by verification by ELISA of candidate biomarkers in CSF and serum from a different cohort comprising control, CIS and MS patients at different disease stages (n=123). **Results and Discussion:** We identified 30 proteins exhibiting different abundances in CSF of control and MS patients and 6 proteins differentially expressed in CSF from CIS patients with rapid and slow conversion to MS. Proteins up-regulated in CSF from MS patients include two chitinase-3-like proteins, CHI3L1 and CHI3L2. Higher levels of CHI3L1 and CHI3L2 were also found in serum from remitting relapsing MS patients, compared with controls. CSF and serum CHI3L1 levels increased with the disease stage, and CIS patients with high CSF (>189 ng/mL) and serum (>33 ng/mL) CHI3L1 converted more rapidly to MS ($p<0.05$ and $p<0.001$ respectively). **Conclusion:** This study identifies CHI3L1 and CHI3L2 as candidate biomarkers of MS associated with the disease stage and highlights the potential of serum CHI3L1 in clinical practice using a less invasive procedure than a lumbar puncture. Additional candidate prognostic biomarkers of MS identified in this study are currently verified by parallel reaction monitoring.

Keywords: multiple sclerosis, cerebrospinal fluid, quantitative proteomics, prognostic biomarker

P16.04 Quantitative Analysis of the Hippocampal Postsynaptic Density Using DIA LC-MS

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Introduction and Objectives: The postsynaptic density (PSD), a highly specialized protein complex located at neuronal terminals, is responsible for the transduction and modulation of glutamatergic signaling between neurons in excitatory synapses. Defects in major PSD scaffolding proteins (i.e. Shank family members) are associated with neuropsychiatric disorders such as autism and schizophrenia. **Methods:** We established an ion-mobility separation (IMS) enhanced data-independent, label-free LC-MS workflow for the in-depth evaluation of crude synaptosomes, synaptic junctions and PSDs isolated from mouse hippocampus. The validated workflow was applied for the analysis of hippocampal PSDs from wildtype and Shank3 mutant mice. Mouse hippocampal PSD was isolated using two ultracentrifugation steps and two Triton X-100 extractions. Crude synaptosomes, synaptic junctions and isolated PSD fractions were tryptically digested and peptides analyzed by LC-MS using a Synapt G2-S HDMS mass spectrometer (Waters) coupled to a nanoAcquity UPLC system. MS analysis was performed in DIA mode with IMS using optimized collision energies ($UDMS^E$). Raw data processing and database search was performed in PLGS3.02. Post-processing and label-free quantification analyses were done using the in-house developed software tool ISOQuant. **Results and Discussion:** Using a novel DIA LC-MS approach, we established a reference proteome dataset of crude synaptosomes, synaptic junctions, and PSD derived from mouse hippocampus, which across all fractions comprised 49,491 peptides corresponding to 4,558 protein groups. Of these, 2,102 protein groups were identified in highly purified PSD in at least two biological replicates. Analysis of wildtype and Shank3 mutant mice revealed differential expression of proteins associated with key PSD functions as well as neuropsychiatric disorders. Thus, our combined datasets provide a deeper insight into the molecular processes and functions of the hippocampal PSD. **Conclusion:** A comprehensive PSD proteome analysis obtained by a DIA MS workflow without any pre-fractionation revealed differential expression of key PSD proteins between wildtype and Shank3 mutant mice.

Keyword: Postsynaptic density, Hippocampus, Data-independent acquisition, label-free quantification

P16.05 PC12 Cells Expressing a Dp71∆78-79 Dystrophin Mutant That Stimulates Neurite Outgrowth

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Introduction and Objectives: Dp71 plays an important role in nervous system, disrupt of Dp71 expression has been correlated with the severity of the cognitive impairment of DMD patients. PC12 cells are used as a model to study the neuronal function of Dp71 because it can acquire a neuronal phenotype in response to Nerve Growth Factor (NGF). This phenotype based on morphometric analysis of cell differentiation has

been more efficiently achieved when the dystrophin mutant Dp71Δ78-79 is stably expressed (PC12-C11 cells), suggesting that the absence of the last two exons of Dp71 have an important role in stimulating neuronal differentiation in this cellular model. The goal of this work was to perform a proteomic analysis to investigate the protein profile of PC12-C11 cells. **Methods:** By using 2DE we compared the expression profile between undifferentiated and NGF-differentiated PC12 and PC12-C11 cells. **Results and Discussion:** In undifferentiated cultures: one protein was downregulated and five were upregulated. Moreover in differentiated cultures: ten proteins were downregulated and seven upregulated. Interestingly, the higher upregulated protein was HspB1, a protein implicated in neurite outgrowth through the cytoskeleton restructuration, with an increase of 5.2 fold. A5 was the second upregulated protein with an increase of 2.2 fold. In addition, changes in HspB1 and A5 expression were observed and validate by western blot and immunofluorescence assays. In differentiated PC12-C11 cells, HspB1 was observed into the cytoplasm and along the neurites, A5 was also localized into the cytoplasm, but it was minimal along the length of neurites. Surprisingly, expression of Dp71Δ78-79 decreases neuronal differentiation relevant proteins as: secretogranin-2, Neurosecretory protein VGF and neurofilament light polypeptide. **Conclusion:** These results show that the Dp71Δ78-79 affects the expression profile of PC12-C11 cells, enhancing HspB1 expression that has an essential role in the neurite outgrowth.

Keywords: Dp71Δ78-79, Differentiation, Neurite outgrowth, PC12 cells

P16.06 Semi-Quantification of Cerebrospinal Fluid Biomarkers for Autism Spectrum Disorder and ADHD

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Introduction and Objectives: We aim to explore the pathophysiological mechanisms for neurodevelopmental disorders as Autism and Attention-Deficit/Hyperactivity disorder (ADHD). The goal is to identify new biological markers for disease diagnosis and prediction of treatment responses. A valuable source for biomarkers for brain disorders is cerebrospinal fluid (CSF), as it contains large numbers of endogenous proteins with great potential value as biomarkers. **Methods:** Analysis by nano-flow liquid chromatography-mass spectrometry (Q-Exactive instrument) in combination with Tandem Mass Tag (TMT) labeling, enables multiplexed semi-quantification of proteins from a small amount of CSF (100 ml). In this study, six different TMT reagents (TMT 6-plex), each with a unique reporter mass, were used for labeling of the tryptic digested proteins. This methodology was employed on a unique CSF patient material from discordant and concordant twins with ASD and/or ADHD and healthy twin pairs (N=44, RATSS cohort, http://ki.se/en/kind/the-project-ratss). **Results and Discussion:** In this proteome-wide pilot study, more than 700 proteins could be identified and semi-quantified. Many neurospecific proteins showed significantly ($p<0.05$) altered CSF protein levels in the ASD/ADHD patients as compared to healthy controls. Several of these proteins are involved in processes as synaptic plasticity and transmission, cell-adhesion, CNS development and complement activity (innate immunity). A few examples of altered proteins are Cadherin 13, Neurocan, Neuroserpin, Neurexin 2, Superoxide dismutase and Monocyte differentiation antigen CD14. The results also show that the genetic factor is strong regarding CSF protein levels, as monozygotic twins have more similar protein profiles than dizygotic twins. The findings in this study will be followed up and validated in larger patient cohorts and with targeted methods. **Conclusion:** This study clearly demonstrates a promising approach for finding relevant biomarkers for complex brain disorders. A future panel of biological markers would be very helpful in the diagnostic assessment of patients and to explore treatment responses.

Keywords: Autism/ADHD, Semi-quantitation (TMT), biomarkers, cerebrospinal fluid

P16.07 Structure Associated Proteins as Key Players in the Hippocampal Progression of Alzheimer's Disease

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Introduction and Objectives: Alzheimer's disease (AD) is the most common form of neurodegenerative dementia and shows a specific progression in the human hippocampus. Braak et al. (2000) observed that neuronal damage of different hippocampal regions (CA1, CA2, CA3, fascia dentata) occurs in a time dependent manner. The CA1 region is affected first by developing a early neurofibrillary tangle formation. Unfortunately it is still unknown why AD moves through the human hippocampus. **Methods:** We decided to analyze the content of the human hippocampal regions of interest (CA1, CA2, CA3, fascia dentata) by performing a differential proteomic study (label free LC-MS/MS approach, spectral counting) combined with a couple of functional analyses. We used the latest laser-microdissection technique to separate the human hippocampal regions and quantified them by using 6 biological post mortem replicates (healthy controls). For our validation experiments we used immunohistochemistry with hippocampal tissue from healthy controls and hippocampal AD tissue. **Results and Discussion:** By using human hippocampal post mortem tissue we was able to identify specific protein concentration gradients of prominent structure associated proteins. These candidate proteins might be the reason for the specific AD progression in the human hippocampus. **Conclusion:** Here we show new insights and prospects for the hippocampal progression of AD by presenting new structure associated candidate proteins.

Keywords: Hippocampus, Neurodegeneration, label free, Alzheimer's disease

P16.08 Anoctamin 2 as an Autoimmune Target in Multiple Sclerosis

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Introduction and Objectives: The increasing availability of recombinant components of the human proteome and multiplex array platforms provide unique opportunities for both targeted and discovery-driven analyses of autoantibody repertoires. We previously identified enriched affinity for 51 out of 11,520 human protein fragments by plasma IgG of individuals with multiple sclerosis (MS). Almost all of these antigens were novel autoantibody targets not previously described in the context of MS. Ayoglu et al (2013)

Autoantibody profiling in multiple sclerosis using arrays of human protein fragments. Molecular & Cellular Proteomics 12: 9. 2657-2672 Sep. **Methods:** Here, we present an in-depth analysis and further characterization of these previously identified targets together with targets suggested in literaure (e.g. KIR4.1), using an independent cohort of 2,169 plasma samples from MS cases and population-based controls on bead-based antigen arrays. **Results and Discussion:** We confirmed and strengthened the presence of autoantibodies against one of our previously proposed targets, a calcium-activated chloride channel protein called anoctamin 2 (ANO2), in ~15% of MS cases. Here, autoantibodies against ANO2 revealed the most prominent difference within the IgG repertoire between MS cases and controls. These results were reproduced for a subset of samples in independent assays performed at a different laboratory. Using peptide arrays, ANO2 autoantibody epitopes were mapped with higher amino acid resolution. Additionally, we found that the conspicuous HLA complex MS-associated risk genes interacted strongly with the presence of ANO2 autoantibodies, reinforcing a potential role of the ANO2 auto-reactivity in MS ethiopathogenesis. Further immunofluorescence analysis on human MS brain tissue revealed a clear increase in ANO2 staining as small cellular aggregates near and inside MS lesions. **Conclusion:** These findings demonstrate the potential for the existence of an ANO2 autoimmune sub-phenotype in MS. They lay the ground for further studies focusing on this particular target with regard to its pathogenic role in MS, either directly or as an epiphenomenon

Keywords: Autoimmunity profiling, multiple sclerosis, affinity proteomics

P16.09 Neuroproteomic Profiling of 277 Brain-Enriched Proteins in CSF and Plasma

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Introduction and Objectives: Advancements in transcriptomic technologies have enabled comparative studies allowing identification of genes with housekeeping as well as tissue-enriched expression. Based on RNAseq analysis of 32 different human tissues, a set of genes has been identified with enriched expression in the brain [1]. The corresponding protein products of these genes are of special interest for protein profiling of cerebrospinal fluid (CSF) and plasma from patients with neurological disorders. [1] Uhlén (2015) Tissue-based map of the human proteome, Science **Methods:** We have developed a workflow for multiplex protein profiling of body fluids using the antibody suspension bead array technology and antibodies from the Human Protein Atlas. Through direct labeling of proteins, the current setup allows profiling of 380 samples and 380 analytes in a single assay. **Results and Discussion:** As a pilot study, we have profiled 1500 CSF and plasma samples from MS and ALS patients for a subset of the brain enriched proteins. We found disease associated profiles of two proteins, neuromodulin (GAP43) related to MS disease progression and neurofilament medium polypeptide (NEFM) that was found elevated in plasma of ALS patients [2,3]. The levels of GAP43 and NEFM are currently further evaluated through analysis of 380 CSF and 350 plasma samples from patients with Parkinson's disease, Alzheimer's disease and Lewy body dementia. In addition, analysis of all 277 brain-enriched proteins is ongoing for investigation of their potential association to neurological diseases. [2] Häggmark (2013) Antibody-based profiling of cerebrospinal fluid within multiple sclerosis. Proteomics [3] Häggmark (2014) Plasma profiling reveals three proteins associated to amyotrophic lateral sclerosis, Ann Clin Transl Neurol **Conclusion:** This study presents a novel approach for protein profiling of brain-enriched proteins and the exploration of their association to neurological disorders.

Keywords: Neuroproteomics, cerebrospinal fluid, plasma, Antibody microarray

P16.10 HIV-1 Clade B Induces a Pro-Apoptotic While Clade C an Anti-Apoptotic Mechanism in Human Astrocytes

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Introduction and Objectives: One of the consequences of HIV-1 infection among patients is HIV-associated neurocognitive disorders (HAND). It is suggested that the degree of Neuro-AIDS vary according to HIV-1 clades. HIV-1 B and C clades account for the majority of HIV-1 infections, clade B being the most neuropathogenic. There are no established mechanisms of HIV-mediated neuropathogenesis, and therefore, it remains the subject of active research. We hypothesized that HIV-1 clade B induces deleterious pathways in human astrocytes vs. HIV-1 clade C. **Methods:** We used a proteomic approach on human astrocytes treated with Tat and Gp120 proteins from HIV-1 clade B and C to identify protein expression differences. Cytokine and chemokines analysis were performed using cytometric bead assay. **Results and Discussion:** Among the significantly upregulated proteins by HIV-1 Tat and Gp120 are pro-apoptotic, endoplasmic reticulum and oxidative stress markers and numerous metabolic factors. Also, HIV-1 clade B and C proteins induced a pro-inflammatory response with the expression of key inflammatory cytokines including IL-1β, IL-6 and IL-8. These data suggest that HIV-1 Tat B/C and Gp120 B/C induce differential protein profile in human astrocytes. **Conclusion:** Our findings demonstrate that HIV-1 clade B appears to induce an inflammatory, pro-apoptotic, ER and oxidative stress response while HIV-1 clade C seems to be associated with anti-apoptotic mechanisms in human astrocytes. The project described was supported in part by NIH/NIMHD/RCMI grant # G12MD007583 and Title V PPOHA grant number PO31M105050 from the U.S. Department of Education to UCC.

Keywords: HAND, Astrocytes, ER Stress, HIV

P16.11 Identification of Synaptic Tau Hyperphosphorylation Sites Induced by β Amyloid in APP/PS1 Mice

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Introduction and Objectives: Alzheimer's disease is an amyloid-induced tauopathy that leads to neurodegenerative dementia. Increasing evidence suggests that tauopathy transmits across neurons through synaptic network connections. It is plausible that the transmission of tauopathy is induced by β amyloid interacting with tau at neuronal synapses. Our goal is to investigate the effect of amyloid pathology on tau proteins located 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, including phosphosites of tau. **Results and Discussion:** We observed that amyloid-induced phosphorylation of synaptic tau protein affected mainly 6 sites in the flanking regions of the

microtubule binding domain. All six sites (181, 199, 202, 396, 400, 404) were apparently targeted by proline-directed kinases, and they showed correlated increase. Increased phosphorylation at these sites could have promoted the dissociation of tau from microtubules due to electrostatic repulsion. **Conclusion:** Tau hyperphosphorylation in APP/PS1 mice is relatively modest compared to human AD, but it may reflect early cross-talks between amyloid and tau pathology. Tau hyperphosphorylation in APP/PS1 mice is relatively modest compared to human AD, but it may reflect early cross-talks between amyloid and tau pathology. Our data suggest that amyloid release at synapses may trigger synaptic tauopathy and its progression.

Keywords: phosphoproteomics, Alzheimer's disease, tau, synapse

P16.12 Reveal the Pathophysiology of the Chronic Fatigue Syndrome with a Quantitative Proteomic Approach

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Introduction and Objectives: Myalgic encephalomyelitis (ME), also known as Chronic fatigue syndrome (CFS) is a neurological disorder with currently unknown etiology. The typical symptoms of ME/CFS include pain, persistent fatigue (exhaustion) and sleep difficulty, etc. However the complete pathophysiology of the disease is still unclear. In this study, we applied a MS-based quantitative proteomics approach to investigate the pathophysiology of ME/CFS. **Methods:** Cerebrospinal fluid from 20 ME/CFS patients and 10 healthy matched volunteers were individually analyzed by nLC-Orbitrap MS platform with a label-free approach. The protein expression quantitation was extracted from Maxquant 1.5.1.8, and followed by bioinformatics analysis. A comprehensive pathway analysis was performed on significantly altered proteins by Ingenuity IPA. **Results and Discussion:** A total of 336 proteins were identified and quantified by mass spectrometry. Whereas 22 proteins presented significant difference between disease and control subjects,10 proteins were reported to relate to ME/CFS for the first time. These significantly changed proteins are involved in several pathways involved in neurological, metabolism and immunological disease, eg glycoprotein biogenesis. In pathway analysis, novel upstream regulators were predicted in ME/CFS patients, including IL6 Receptor and IGF1. Furthermore, the downstream analysis of the proteome profiling indicated that ME/CFS patients have an elevated level of glucose metabolism and suppressed lipid metabolism and chemotaxis of myeloid cells. **Conclusion:** In this study, we successfully identified a list of altered proteins in ME/CFS patients as compared to healthy controls, including both known and novel potential biomarkers for ME/CFS. These biomarkers could act as potential diagnostic, prognositic or therapeutic markers. Several novel pathways were reported altered within the ME/CFS disease. Moreover, pathway analysis results partially explained the causal relationship of disease syndrome, and indicates that ME/CFS is also a metabolic disorder.

Keywords: Myalgic encephalomyelitis, Chronic fatigue syndrome, Label free proteomics, pathway analysis

P16.13 Utilizing Single-Shot Proteomics to Monitor Synaptic Health in Alzheimer's Disease

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Introduction and Objectives: Alzheimer's disease (AD) is a complex progressive brain disorder that currently affects over 35 million people worldwide. Current clinical approaches allow AD diagnosis at a late stage where most therapies fail to achieve significant results because of the massive neuronal death that has already occurred. A robust platform capable of reliably identifying alterations that may correlate with the onset and progression of AD would be of high value. Synaptic loss is the best correlate of cognition across the spectrum of AD. Here we present an optimized workflow to identify novel protein signatures capable of monitoring altered synaptic neuronal health of AD-patients. **Methods:** The presented method combines rapid and unbiased single-tube sample preparation with single-shot label-free high-resolution mass spectrometric (MS) analysis to analyze protein expression changes within very small (2-5ml) volumes of cerebrospinal fluid (CSF). Following nano-Ultra-High Performance Liquid Chromatography, samples were subjected to 4 hr single-shot analysis on a Q-Exactive MS. Data files from technical triplicates were analyzed using Proteome Discoverer 1.4. Proteins satisfying ≤1% false discovery rate were subjected to label-free quantitation using Scaffold 4.4.1. **Results and Discussion:** CSF samples from 15 normal and 15 early stage AD subjects were analyzed. Our robust workflow allowed effective use of the precious clinical samples to discover altered protein expression patterns in AD patients. The single-shot analysis identified more than 700 CSF proteins including a majority of published AD-associated biomarkers, plus additional discriminatory signatures not previously linked to AD. Novel discriminatory signatures await further validation in big AD cohorts. **Conclusion:** Our method allows assessment of disease modifications with unprecedented sensitivity and minimal sample requirements. Discovery of novel discriminatory signatures of progressive neural degeneration in AD will facilitate monitoring of disease progression, response to drug treatment, or lead to new strategies for disease interception during early stages of neurodegeneration.

Keywords: Alzheimer, Synaptic health, biomarker, Mass spectrometry

P16.14 Quantitative Analysis of APPswe/PS1-dE9 Brains: An Amyloid Plaques Mouse Model of Alzheimer Disease

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Introduction and Objectives: Alzheimers disease is the leading disease causing dementia and has become a global health care concern. The disease causes neurodegeneration and inflammation in the brain, and is marked by two hallmarks; the amyloid plaques and neurofibrillary tangles. Mutations in the genes for APP and PS1 are known contributors to early onset of Alzheimers disease given heavy plaques loads in the brain. The amyloid plaques are hypothesized to cause changes in the cells by unknown mechanisms leading to the observed cell death. We investigated the quantitative changes in the brain tissue of a double transgenic mouse model by a comprehensive large scale proteomic study providing information about changes in proteins, phosphorylation and glycosylation states. **Methods:** We utilized brain tissue from the double transgenic mouse model

APPswe/PS1-dE9, compared to healthy litter mates at 3, 12 and 21 months of age in 3 biological replicates. Brain tissue samples were homogenized and ultra-centrifuged to separate membranes from soluble proteins. All samples were labeled with iTRAQ and underwent the TiSH protocol, a comprehensive workflow to recover phosphorylated as well as glycosylated peptides. **Results and Discussion:** Preliminary results indicate substantial early stage protein regulations associated to the membrane. Significant up-regulation of apolipoprotein E and clusterin were observed in response to the increasing amyloid plaques loads in the brain tissue. These proteins are known to be involved in removing the toxic components of plaques from the brain, and the results are therefore consistent with disease development. All data are not fully analyzed and up-following biological validation of results will also be performed. **Conclusion:** The study will provide crucial data regarding the proteome related changes in response to increasing amyloid plaques loads. The comprehensive TiSH workflow enables analysis of both phosphorylation and glycosylation changes and will help to understand the pathological effects of amyloid plaques in the brain.

Keywords: Alzheimers disease, quantitative proteomics, phosphoproteomics, Glycosylation

P16.15 Quantitative Proteomics Reveals New Insights into Chaperone Malfunction Linked Neurodegeneration

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Introduction and Objectives: Malfunction of the endoplasmic reticulum (ER) resident SIL1/BiP chaperone complex leads to accumulation of misfolded proteins, which is regarded as a major cause of neurodegeneration. Mutations in SIL1/Sil1 are responsible for the multi-systemic disorder called Marinesco-Sjögren Syndrome (MSS) and mutant "woozy" mouse phenotype. One of the clinical hallmarks of SIL1/Sil1 deficiency is Purkinje-cell degeneration leading to ataxia in human and mouse. However, other cerebellar cell types are obviously protected suggesting the presence of compensatory factors. Here we used quantitative proteomics to identify these factors in order to improve our knowledge about the role of SIL1/BiP chaperone complex in neuronal function **Methods:** Three 26-week old Sil1 deficient mice and three control mice were sacrificed and their cerebella isolated. After tissue lysis, protein extraction and digestion, peptides were labeled with iTRAQ 8plex and samples were pooled. After fractionation using a RP HPLC at pH 6.0, fractions were analyzed by nano-LC-MS/MS on a LTQ Velos Orbitrap mass spectrometer. Data were processed using Proteome Discoverer 1.4. In addition, label free quantification was done with single shot LC-MS/MS measurements and using Progenesis and PeptideShaker. Immunohistochemistry (IHC) studies and transmission electron microscopic (TEM) studies were performed for data validation. **Results and Discussion:** We could quantify 2,660 proteins (≥ 2 unique peptides, 1% FDR), ~6% of which were differentially regulated (t test p-value ≤ 0.05). Notably, several down-regulated proteins (Calb1, Cacna1a, and Pcp2) are indicative of Purkinje-cell loss, whereas increase of Gfap reflects the known proliferation of glia cells, confirming the sensitivity of our analysis. Other regulated proteins are connected to crucial processes like brain development (ApoD), cerebellar differentiation (Grid2) and apoptosis (Erp29). These results were confirmed by IHC and TEM studies, respectively. **Conclusion:** Our findings show particular role of the Sil1/BiP system in Purkinje-cell survival and suggest that other cerebellar cell types are protected from cell death by expressing compensating factors.

Keywords: Protein folding dysfunction, Neurodegenaration, quantitative proteomics, Purkinje-cell loss

P16.16 H2S Role in Amyotrophic Lateral Sclerosis: Unravelling New Cellular and Molecular Mechanisms

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Introduction and Objectives: Amyotrophic lateral sclerosis (ALS) is a lethal disease characterized by a progressive motor neuron degeneration. Many etiologic factors are implicated, however it is accepted that a severe mitochondrial dysfunction leads to an unavoidable neuronal death. Hydrogen sulphide (H2S) is an essential body product, mainly produced in the brain by astrocytes and microglia through the cystathionine-β-synthase (CBS), a cytoplasmatic enzyme that accumulates in mitochondria under oxygen sensitive conditions. Our recent data show poisonous levels of H2S in ALS patients and in the familial ALS mouse model $SOD1^{G93A}$. Therefore, the aim of this study is to further unravel the complexity of H2S metabolism and the molecular mechanisms through which H2S could contribute to the ALS-related neurodegeneration **Methods:** Deeper proteomics and metabonomics analysis was performed on neuronal tissues derived from $SOD1^{G93A}$ mice, male and female, treated and untreated with hydroxylamine hemihydrochloride (AOAA), a CBS inhibitor, at different developmental/disease stages. Differential protein expression of total protein extracts and mitochondrial enriched fractions were evaluated with a shotgun proteomics analysis based on nLC-MSE. Moreover, mice metabonomic profiling was investigated through a comprehensive gas chromatography approach. **Results and Discussion:** we looked putative dysregulated biological processes linked to H2S metabolism between the cyosolic and mitochondrial compartments. We focused on key proteins, and their post translational modification, that are affected directly by H2S, and that could be relevant for ALS, such as GADPH and actin. In parallel we focused on ALS proteins with reactive cysteine that may be regulated by H2S such as mitochondrial complexI subunits and SOD1. Our data show that the increased H2S amount in ALS could further distress an already compromised mitochondrial function. **Conclusion:** H2S toxic effects seem to associate with phenotype development in ALS. Our study introduces H2S as a new player to the cohort of pro-inflammatory/degenerative factors that could be involved in the aetiology of ALS.

Keywords: proteomics, Amyotrophic lateral sclerosis, Hydrogen sulphide

P16.17 Implementation of Mass Spectrometry for Detection Amyloid-β Peptides in Plasma

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Introduction and Objectives: Early diagnostics of Alzheimer's disease (AD) is necessary for treatment patients at the earliest stage. Amyloid precursor protein (APP) is the precursor protein to amyloid-β (Aβ), the main constituent of senile plaques in AD and are likely to be relevant to AD pathogenesis. A decrease of the APP672-713(Aβ1-42) or in the [APP672-713(Aβ1-42)/APP672-711(Aβ1-40)] ratio in cerebrospinal fluid (CSF) has shown potential as an AD biomarker. A serious problem during diagnostics is CSF collection due to its invasiveness and sampling duration. Hence, a blood marker is suitable

for screening for early AD because it is more practical for routine use than collection of CSF or imaging approaches. The aim of this research was to develop an approach for isolating Aβ from plasma via immunoprecipitation for early and routine diagnostics of AD followed by mass spectrometry. **Methods:** Aβ was immunoprecipitated from plasma samples collected from AD sufferers and analyzed via mass spectrometry by adding isotopically labeled internal standards. **Results and Discussion:** The use of MS to detect Aβ-related peptides from human plasma from AD sufferers was shown. **Conclusion:** A preliminary approach for quantitative analysis of amyloid-β peptides in plasma by immunoprecipitation followed by mass spectrometry was developed.

Keywords: Alzheimer's disease, Mass spectrometry, immunoprecipitation, amyloid-β peptide

P16.18 Functional Analysis of Protein Complexes in Alzheimer's Disease

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Introduction and Objectives: Alzheimer's disease, characterized by progressive memory deterioration and other cognitive functions is associated with aberration of multiple interacting pathways and molecular mechanisms . These alterations appears in the form of early prodromal symptoms with episodic memory followed by further decline and loss of general cognitive functioning during the final syndromal dementia stage. The present study aims to decipher the structure and dynamics of complex network of protein-protein interactions which is important for understanding many aspects of living systems in depth ranging from molecular organization to cellular pathways. **Methods:** Multiprotein complexes were isolated and separated from human autopsied brain tissue from normal controls and AD patients and subjected to BN-PAGE followed by determination of complex components by SDS-PAGE and identification by Mass Spectrometry. The interaction of identified proteins was analyzed by STRING databases and some were also validated by co-immunoprecipitation **Results and Discussion:** An interesting observation of this study is a complex resolved and comprised of novel interacting partners including glyceraldehyde-3-PO4 dehydrogenase, actin cytoplasmic, microtubule associated protein 1B and glial fibrillary acidic protein and proteolipid protein. The interaction of GAPDH and actin was also confirmed by coimmunoprecipitation. In total thirteen complexes comprising of more than thirty proteins mainly membrane proteins, cytoskeletal proteins and metabolic enzymes were resolved from AD brain tissue. Our results are in accordance with the previous findings on normal control subjects where the metabolic enzymes are in close association with cytosekletal proteins taking the advantage of transport from one site to another and inturn providing energy to the cytoskeleton. Earlier studies from our lab demonstrated an altered expression and aberrant post translational modification of proteins identified in this study. **Conclusion:** The present study concluded that multiple proteins function in a complex in AD as in the normal subjects. However alteration in their expression level in AD suggests changes in their function.

Keyword: Interactomics, multiprotein complexes, coimmunoprecipitation, Mass Spectrometry, Alzheimer's Disease

P16.19 Identification of Brain-Related Cerebrospinal Fluid Proteins in Neurodegenerative Diseases

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Introduction and Objectives: The Human Protein Atlas (HPA) provides various subgroups of human proteome including the secreted and membrane, as well as tissue-specific proteome, which can serve as a valuable tool in proteomic research. Specifically, brain-enriched and brain-inclusive group-enriched proteins may have a specific neurodegenerative disease-related role and potentially serve as signature-biomarkers in cerebrospinal fluid (CSF). To define the brain-related signature proteins, HPA database is used to create a list of proteins of interest and compared with "deep" CSF proteome generated experimentally. The resulting proteins are the basis for SRM assay development, followed by validation of final protein candidates in different groups of neurodegenerative disorders. **Methods:** Six non-pathological, CSF samples from three female and three male individuals (age range: 32-72 years) were selected for "deep" CSF analysis. Samples were normalized by total protein and subjected to strong cation exchange chromatography, followed by in-solution trypsin digestion and LC-MS/MS analysis. Brain-enriched and group-enriched proteins of secreted and/or membrane origin were retrieved from HPA and compared against CSF proteome. **Results and Discussion:** Mass spectrometric analysis of fractionated CSF identified 2615 protein groups in all samples combined. The number of protein groups identified per individual sample ranged from 1109 to 1421. HPA-retrieved brain-enriched, secreted/membrane proteins (n=196) as well as group-enriched, secreted/membrane proteins (n=138) were compared with CSF proteome. The final list of 33 brain-enriched and 24 group-enriched proteins, reproducibly detected in all individual CSF samples, was prepared for SRM assay development. **Conclusion:** A combination of HPA database and experimental search of proteins in specific body fluid followed by SRM method development can be applied as an initial step in search for disease biomarkers specific for a particular tissue. In this study, a list of brain-related CSF proteins with SRM assay is generated and will be further investigated in different neurodegenerative disorders, such as Alzheimer's and Parkinson's diseases, Frontotemporal and Vascular dementias.

Keywords: biomarkers, cerebrospinal fluid, selected reaction monitoring, Neurodegenerative diseases

P16.20 Advancing Alzheimer Disease Diagnostics: A Clinical Assay to Quantitate Amyloid-Beta Peptides in CSF

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Introduction and Objectives: While Alzheimer disease has a defined pathology on autopsy, in vivo diagnosis is challenging—particularly in early stages of disease when treatment opportunities are greatest. Toward the development of an in vivo diagnostic model, cerebrospinal fluid biomarkers amyloid-β (Aβ) and tau proteins have been extensively studied and are now included in research diagnostic criteria. Until recently, quantitative analysis of Aβ peptides in CSF had relied almost exclusively on the use of immunometric assays. In order to side step known immunoassay reagent issues, matrix effects, heterophile antibody interference, therapeutic antibody

interference, and major adsorption losses, we endeavored to develop an antibody-free quantitative LC-MS/MS assay for Aβ40 and Aβ42 peptides. **Methods:** The assay was developed using equipment common to clinical laboratories, i.e. high performance liquid chromatography coupled to a triple quadrupole mass spectrometer (LC-MS/MS). A full clinical analytical validation was performed including assessments of sensitivity, selectivity, linearity, precision, interferences and stability. Due to the propensity of Aβ peptides to aggregate and adhere to surfaces, we also systematically characterized conditions and techniques that resulted in minimal adsorption and aggregation events. **Results and Discussion:** We identified an appropriate surrogate matrix for the calibrators that minimized unintentional aggregation and adsorption events. We achieved a coefficient of variation of less than 15% at the lower limit of quantitation. Using clinical samples, a method comparison with the Innogenetics ELISA revealed Aβ42 concentrations from LC-MS/MS were greater than those reported by the ELISA (similar to the relationship previously observed between UPLC-MS/MS and Luminex methods), whereby only the LC-MS/MS method was traceable to gravimetric targets. **Conclusion:** We developed an antibody-free LC-MS/MS workflow to quantitate amyloid-β peptides in cerebrospinal fluid that is suitable for clinical use. A study to evaluate diagnostic performance of our mass spectrometric method is currently underway using specimens from the biobank at the Clinic for Alzheimer's Disease and Related Disorders.

Keywords: Alzheimer's disease, amyloid-beta, clinical assay, dementia

P16.21 Cell-Type and Brain-Region Resolved Mouse Brain Proteome

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Introduction and Objectives: The complex structural and functional organization of the mammalian central nervous system (CNS) with its enormous diversity of cell types of different morphology, connectivity and function warrants the application of global and systematic approaches. However, a concerted effort to resolve the brain proteome is missing. The development of more sensitive and powerful high resolution MS technologies now allows in-depth coverage of nearly complete proteomes of mammalian cells.To resolve the mouse brain proteome and to determine the basis for cellular specialization in the CNS, we have performed a global analysis of protein levels in the adult mouse brain with its major brain regions and cell types. **Methods:** We analyzed whole mouse brain in biological quadruplicate after tryptic digestion of lysates and fractionation of resulting peptides using strong anion exchange chromatography. We performed LC-MS/MS analysis with 4-hr runs per fraction and higher energy collisional dissociation in a quadrupole Orbitrap mass spectrometer equipped with a high-field analyzer. In addition, we analyzed ten brain regions and cell types with our 'single-shot analysis' approach using 4 hr gradients. Spectra were searched with the Andromeda search engine integrated into MaxQuant. **Results and Discussion:** Here, we further optimized our 'single-shot analysis', which resulted in the detection of more than 11,500 different proteins in only four hours replicate analysis. Such a deep proteome coverage in a single run was made possible by matching to the 'brain proteome library'. Comparisons of the 12,934 identified proteins in CNS cell types with deep sequencing data of the transcriptome indicated deep coverage of the proteome. Cell-type specific proteins represent about a tenth of the total proteome and show an overrepresentation of integral membrane proteins. **Conclusion:** Our data and the rapidity with which it can now be generated, suggest that MS-based proteomics be implemented into neuroscience to resolve the full molecular complexity of the brain with cell resolution.

Keywords: match between runs, Mouse brain proteome, brain regions, Neuronal and glial cells proteome

P16.22 Pathophysiology of Human Parkinson's Disease Involves Multiple Proteostatic Systems

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Introduction and Objectives: Parkinson's disease is a devastating neurologic disorder affecting dopaminergic neurons of the nigral- striatal pathway in the aging human brain. Pathophysiology of this disease is attributed, but not limited to protein aggregation, mitochondrial dysfunction, oxidative stress, inflammation, and loss of proteostasis. Emerging proteomics methodologies provide a platform to integrate pathophysiologic mechanisms to advance knowledge concerning the etiology and bring forward potential therapies for the disease. We aimed to use existing data from public databases as well as our own data to integrate knowledge at gene expression and protein levels to form a clearer and deeper view on proteostatic changes in Parkinson's disease. **Methods:** Data used for integration included public data sets and our own generated data that included quantitative proteomics, mass spectrometry, bioinformatics, and associated functional genomics data including next-generation sequencing and RNA interference studies. **Results and Discussion:** Using computational approaches we link human α-synuclein, the main component of Lewy-bodies, a pathological hallmark of Parkinson's disease, with genes and their protein products that constitute the proteasome, a major proteolysis unit of eukaryotic cells. In chemical models of Parkinson's disease, we observe the up regulation of heat shock proteins and down regulation of activated in blocked unfolded protein response genes suggesting further perturbations in maintaining protein homeostasis. Finally we integrate data from human disease tissues with model organism data to find common factors in pathogenesis of the disease. **Conclusion:** Taken together our results show a widespread pathology of multiple stress conditions that affect folding, integrity, and degradation of proteins that contribute to pathology of human Parkinson's Disease. These findings highlight the importance of protein hoemostasis in maintaining the function and integrity of human neural systems.

Keyword: protein aggregation, protein folding, data integration, synuclein

P16.23 Quantitative Analysis of N-Linked Glycoprotein in the Brain of Alzheimer's Disease Model

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Introduction and Objectives: 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 and site-specific glycan-peptide analysis for determination of N-gycoproteome heterogeneity **Methods:** Initially, comparative proteomics by iTRAQ was performed to identify the differentially expressed proteins among AD mouse and age-matched wild-type (WT). Next, the glycopattern of brain proteins was profiled

by lectin bloting and then the N-glycoprotein profiles were quantitatively compared by 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). At last, comprehensive structure ananlysis and quantification of intact glycopepetides were conducted to find difference of site-specific glycopeptedes in the disease models. **Results and Discussion:** In total, 3389 proteins were quantified in both samples, of these, 78 proteins exhibited significant modulation between AD and control($p< 0.1$) that mapped to pathways associated with energy production, lipid metalism, small molecule biochemistry, celluar movement etc. The staining intensity of WGA, SNA, MAL-I and Jaclin were found to be significantly lower in the Alzheimer's disease compared with WT controls, indicating that the level of protein sialylation and galactosyl (b–1,3) N-acety was significantly lower in the brain of AD. we further quantified 46 glycopeptides corresponding to 41 glycoproteins with altered abundance in AD compared with WT controls. The structure analysis and quantification of site-specific glycopeptides are going on in our laboratory. **Conclusion:** Protein glycosylation altered in the brain AD mouse models indicating its important roles in the development of Alzheimer's Disease. The findings of aberrant glycosylations in AD may help understand the mechanisms of neurodegenerative diseases.

Keywords: Mass spectrometry, Alzheimer's disease, quantitative glycoproteome, iTRAQ

P16.24 Proteomic Analysis of Human Microglial Cells Activated by Amyloid β Peptide

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Introduction and Objectives: Microglial activation in the central nervous system is a key event in the neuroinflammation that accompanies neurodegenerative diseases such as Alzheimer's disease (AD). Among cytokines involved in microglial activation, amyloid β (Aβ) peptide is known to be a key molecule in the induction of diverse inflammatory products, which may lead to chronic inflammation in AD. However, proteomic studies of microglia in AD are limited due to lack of proper cell or animal model systems. **Methods:** In this study, we performed a proteomic analysis of Aβ-stimulated human microglial cells using SILAC (stable isotope labeling with amino acids in cell culture) combined with LC-MS/MS. **Results and Discussion:** Results showed that expression of 60 proteins was up- or down-regulated by 1.5 fold or greater. Among these, ER-resident proteins such as SERPINH1, PDIA6, PDIA3, and PPIB were revealed to be key molecular biomarkers of human microglial activation, by validation of the proteomic results by immunostaining, PCR, ELISA, and western blot. **Conclusion:** Our data suggests that ER proteins play an essential role in human microglial activation by Aβ and may be important molecular therapeutic targets for treatment of AD.

Keywords: amyloid β, human microglia, tandem mass tags, LC-MS/MS, amyloid beta, human microglia, tandem mass tags, LC-MS/MS, amyloid β, human microglia, microglia, activation, amyloid

P17: POSTER SESSION - TRANSLATIONAL PROTEOMICS

P17.01 Plasma Kininogen and Diabetes Related Kidney Disease

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Introduction and Objectives: Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia and it has become a global epidemic in the past years. The heterogeneity in the development of diabetes complications represents a big challenge in the advance of new strategies for prevention. The ability of microalbuminuria to predict early progressive renal function decline diabetic patients has been questioned and previous studies have described plasma kininogen as a potential biomarker of early progressive renal function decline. **Methods:** In the present study we propose the investigation of plasma kininogen as a potential biomarker of early stages of diabetic nephropathy in plasma of type 2 diabetic patients using mass spectrometry. Plasma samples from diabetic (n=7), hypertensive diabetic (n=5), as well as hypertensive diabetic with chronic kidney disease patients (n=6), were collected and processed. Individual protein samples were analyzed by NanoUPLC tandem nanoESI-LC MS^E **Results and Discussion:** Using previous data from our study in spontaneously diabetic mice we have selected 4 peptides as standards to develop a monitoring method for plasma kininogen fragments in our samples. Our study groups had different levels of kidney damage and we have found that the monitoring of plasma kininogen using MS showed a strong correlation with microalbuminuria. **Conclusion:** Our data supports the use of plasma kininogen as a protein biomarker of early kidney damage. We believe that a large prospective study is now necessary to monitor this protein in a big population to assess its predictive value of detecting kidney damage. Besides, it is imperative that new studies also correlate this protein with other potential kidney damaging diseases.

Keywords: diabetes, kininogen, NOD mice, kidney disease

P17.02 Proteins of the Human Eye. From Bottom-Up Proteomics to Top-Down Peptidomics

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Introduction and Objectives: Quantitative bottom-up analyses of human eye proteins provide useful information with respect to ophthalmological practice . Yet a top-down approach is superior in the context of clinically relevant biomarker discovery. However, top-down proteomics (particularly peptidomics) is many times more challenging than conventional trypsin-based bottom-up strategies. **Methods:** Indeed many biologically important endogenous peptides are small and extremely low abundant. Hence they are difficult to detect, especially in a background of abundant proteins. In contrast to tryptic protein fragments, native peptides do not obligatorily contain a basic (carboxyterminal) residue, which typically makes them 'fly well' in MS. Additionally, Lys and Arg lacking peptides are lost in typical bottom-up analyses, which, by convention, require, at least, the presence of 2 or more representative peptides for unequivocal protein/proteoform identification.

Results and Discussion: Therefore, LC MS/MS based peptidomics often requires so-called de novo sequencing efforts, requiring the highest possible data quality (excellent mass measurement accuracy) to facilitate laborious and time-consuming 'manual' interpretation of tandem mass spectra. In this context, we have evaluated the optimized quadrupole-orbitrap combination (ThermoFisher Q Exactive Plus) in combination with the latest version of the de novo software Peaks (v7.5) for their ability to detect and identify non-tryptic peptides in ocular fluids and conditioned corneal tissue culture media. Various endogenous peptides could be idenified, which were missed from typical bottom-up analysis of the same human eye fluid samples. **Conclusion:** Our data illustrate that both the enhanced instrument performance and ever increasing power of the bioinformatics solutions for peptide tandem MS data interpretation help to filter biologically relevant information from the large datasets generated by high throughput experiments. Literature cited [1] Raus et al. (2015) EuPA Open Proteomics 9: 8-13. [2] Kumar et al. (2015) EuPA Open Proteomics in press We gratefully acknowledge the scientific support by Drs. T. Arrey, M. Kellmann and T. Moehring (ThermoFisher; Bremen, Germany).

Keywords: peptidomics, human eye, Top-down

P17.03 Characterizing the Urinary Peptidome to Infer Protease Activity in the Diabetic Kidney

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Introduction and Objectives: Proteolytic activity may be responsible for early structural changes in kidneys of patients with type 1 diabetes mellitus (T1D). Evidence suggests that this activity may be specific to some proteases and their substrates and that resulting peptides generated within kidney may be excreted into urine. We postulated that urine peptidome of healthy controls and subjects with early T1D may be informative in regards to proteins cleaved in the kidney and proteases causing the cleavage. **Methods:** We developed methods for urine peptidomics using two urine samples from a healthy volunteer on different days. Peptides (<10 kDa) were reduced and alkylated, then subjected to solid-phase extraction, lyophilization, and fractionation using strong cation exchange (SCX) chromatography. Fractions were desalted and analyzed by LC-MS/MS on Q-Exactive mass spectrometer. We also examined StageTip and ProteoSpin methods in lieu of SCX. Raw files were analyzed by MaxQuant software. Peptide Extractor and Peptidomic Enzyme Tabulator were used to infer proteases, which generated the identified peptides. We are now collecting urine samples from T1D adolescents and healthy age/sex-matched controls. **Results and Discussion:** Peptide fractionation using SCX yielded the greatest number of peptide/protein identifications. We identified 2067 peptides from 422 proteins in two healthy samples. As measures of intra-individual biologic variability, 48% and 77% of peptides and proteins respectively were shared between both samples. Uromodulin, collagen and clusterin fragments were the most abundant kidney-derived peptides. Proteolysis occurred near the C-terminus of proteins. Many collagen fragments were found along the entire sequence lengths, likely due to sequence repeats inherent in collagen proteins. The top predicted candidates responsible for the proteolysis of kidney-derived proteins included plasmin, trypsin and Arg-C proteinase. **Conclusion:** Our methods enabled deep sequencing of urine peptidome and imputation of active kidney proteases. Understanding early changes in the kidney protease activity caused by hyperglycemia may lead to novel insights into diabetic kidney disease.

Keywords: proteases, diabetes mellitus, peptidomics

P17.04 Extended Boiling of Peanuts Reduces IgE Reactivity While Retaining Allergen Peptides

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Introduction and Objectives: Peanut allergy affects 1-3% of children in developed Western countries, and is the leading cause of food-induced anaphylaxis and anaphylaxis-related fatalities in children. Peanut allergy tends to be persistent with a 10-year remission rate of only 20%. Avoidance of peanuts cannot be guaranteed and accidental ingestion may be dangerous and even fatal. A safe and effective treatment is therefore urgently needed. Peanuts contain 12 water-soluble allergens termed Ara h1 to Ara h13. Oral desensitization of allergic patients using peanut flour shows promise for the treatment of peanut allergy, but the very high rate of severe adverse events during treatment has prevented routine use of this therapy. We have investigated the effect of extended boiling of peanuts to determine if boiled peanuts are sufficiently hypoallergenic to allow safe desensitisation of peanut-allergic children in the first step of a biphasic protocol which uses roasted peanuts as a second step. **Methods:** The effect of boiling raw peanuts for up to 12 hours on peanut allergens was investigated by 1D/2D electrophoresis. Reduced allergenicity was determined by western blot, inhibition ELISA and by patient skin prick test. The presence of fragmented allergen peptides was monitored by qTOF mass spectrometry. **Results and Discussion:** Extended periods of boiling induced hydrolysis of peanut proteins with leaching of intact peanut allergens into cooking water. Two hour boiling induced an 8-fold reduction of peanut IgE reactivity as determined by inhibition ELISA. Mass spectrometry revealed the presence of small (7-22 amino acid) peptides from Ara h1, 2, 3, 6, 10 and 11 that were retained within boiled nuts. **Conclusion:** Extended boiling of peanuts markedly reduces IgE reactivity and allergenicity whilst progressively accumulating small peptides derived from hydrolysis of allergenic proteins. These properties suggest the potential application of hypoallergenic boiled peanuts prefacing graded-dose introduction of roasted peanuts in a biphasic oral immunotherapy approach to treat peanut-allergic children.

Keywords: Peanut, Allergy

P17.05 Proteome Profiling in Rat Tears by High Resolution Mass Spectrometry

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Introduction and Objectives: New biomarkers play an important role for the drug development process including prediction of therapeutic response and early diagnosis. Tear is a readily available biological fluid and can be useful sources for molecular biomarkers in translational research. However, rat tear is not fully characterized at the proteome level. Here we present a comprehensive proteomic profiling of

rat tear using shotgun based high resolution mass spectrometry. **Methods:** Tear samples were collected from Sprague-Dawley (SD), Wister, and spontaneously diabetic Torii (SDT) rats using Schirmer tear production measuring strips. Schirmer's strips were treated with 80% methanol to extract metabolites followed by tryptic digestion. The resulting peptides were separated by nanoLC with a length of 50 cm and the MS/MS data were acquired on an LTQ Orbitrap Elite or a Q Exactive high resolution mass spectrometer. Acquired data were analyzed with Skyline and Progenesis QI softwares. **Results and Discussion:** In the step of tryptic digestion, we tried in-strip digestion of proteins (i.e. tryptic digestion without protein extraction from the strip) to avoid the loss of proteins. In our procedure, endogenous metabolites and tryptic peptides were successfully profiled using the sequential extraction. More than 500 proteins were identified in the single NanoLC-MS/MS run with an amount of rat tear fluid less than 1 μL. The method allowed profiling of totally over 1000 proteins present in the rat tear fluid. Current results show that approximately 65% of the total protein in the rat tear fluid have in common with the proteomes of human eyes and tears. We also tried a shotgun approach based on label free quantification with high resolution mass spectrometry. Longitudinal characterization of the rat tear proteomes will be presented and discussed. **Conclusion:** The analysis revealed the identification of more than 1000 proteins in rat tear fluid.

Keywords: tear proteome, label free quantification, biomarker, high resolution mass spectrometry

P17.06 Multiple Sclerosis Lesion Stage Markers from Quantitative Brain Proteomics of Mouse Models

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Introduction and Objectives: Multiple sclerosis is a diverse chronic inflammatory demyelinating disease of the central nervous system, and is one of the leading causes of neurological disability among young people. Current therapies are most effective if diagnosed in the very early phase, and discovery of novel disease-associated proteins might contribute to earlier diagnoses and better supervision of disease progression and treatment response. **Methods:** We investigated two multiple sclerosis mouse models, the "immune-mediated" experimental autoimmune encephalomyelitis and the "re- and demyelinating" cuprizone, using TMT-labeling and label-free quantitative brain proteomics. Three regulated proteins quantified with both methods were investigated in brain sections using immunohistochemistry of tissue from mouse models, and human homologues were investigated in human multiple sclerosis tissue. **Results and Discussion:** By quantification of 4375 proteins using proteomics we revealed differences in protein regulation between the multiple sclerosis models and control mice, and noticed that there were few similarities in protein regulations between the models in the brain section investigated. Decreased levels of ERMIN in cuprizone were validated in mouse brain sections during the disease course showing decreased immunopositivity at the disease peak. ERMIN positive oligodendrocytes reappeared at the remyelination stage which was confirmed for the human homologue in white matter chronic inactive multiple sclerosis lesions. Increased levels of LGMN and LAT1 in cuprizone and decreased levels of the LAT1 in experimental autoimmune encephalomyelitis were observed. Cellular morphologies indicate that human homologues were expressed mostly in astrocytes in white matter active lesions, and that LAT1 was also present in several macrophages. **Conclusion:** The translational potential of proteomics analyses of multiple sclerosis mouse models was confirmed in human multiple sclerosis brain tissue increasing our understanding of early processes of lesion development.

Keyword: Quantitative proteomics, Multiple sclerosis, Mouse models, IHC

P17.07 Microfluidics Coupled Mass Spectrometry for Multi-omics/ Targeted Assays in Translational Research

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Introduction and Objectives: Translation medicine is an interdisciplinary science that aims at combining the information taken from bench to bedside. In this process molecules are isolated and identified in discovery and then utilized in the clinical setting as biomarkers of health and disease to better develop therapies. Due to the complexity of deriving meaningful information from omic studies, the development of new analytical technologies is critical. Here we present the utilization of a microfluidic LC coupled with mass spectrometry for multi-omic discovery and targeted studies. **Methods:** Chromatography was performed on a nanoscale LC system integrated microfluidic device (150μm x 10cm) packed with 1.7μm particles. Analytes from plasma and urine were eluted under liner gradient conditions. Mass spectrometry was performed on QToF and tandem quadrupole instruments operating in positive and negative ESI mode. **Results and Discussion:** Proteomics, lipidomics, metabonomics, and glycomics studies using plasma and urine were performed over the course of one week. Test compounds and known endogenous molecules in matrix were monitored for system performance. Sensitivity increase using the microfluidic device ranged from 5 to 40-fold compared with 2.1 mm id UHPLC. Peptides showed the greatest increases in sensitivity versus small molecules. Chromatographic peak capacity ranged from 450-480 compared with 600 for a 30 minute separation on the analytical system with no significant performance degradation observed over 1000 injections. The inherent increase in sensitivity is further demonstrated with the ability to perform multiple injections from limited sample volumes with rodent samples from an acute model toxin study using 2-bromophenol. The results used to verify effected biochemical pathways. **Conclusion:** System utilization , robustness, throughput, sensitivity, and handling limited sample volumes for multi-omic studies is illustrated for an integrated microfluidic device when compared with 2.1 mm id UHPLC.

Keywords: Translational Research, Multi-Omics, Microfluidics

P17.08 Gel Based and Shotgun Approaches to Identify Salt Stress Responsive Proteins of Panax Ginseng

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Introduction and Objectives: Salt stress is one of the major abiotic stresses affecting the yield of ginseng (Panax ginseng C. A. Meyer). The objective of this study was to identify bio-marker(s), which is responsive in salt stress in ginseng using proteomics approaches. **Methods:** Detached ginseng plants of different growth stages (3, 4

and 5 years) were exposed to the hydroponic solution with 5 ds/m salt concentration for 0, 24, 72 and 120 hours. Total proteins were extracted from the leaves using MgNP40 extraction buffer and secreted proteins were collected from hydroponic solution, following phenol extraction for isolation of secreted proteins. Totals and secreted proteins were used for further proteome analysis using 2-DE and shotgun approaches, respectively. **Results and Discussion:** Spots detected in colloidal CBB stained 2-DE and secreted proteins were identified by searching in Ginseng database and an in-house developed Ginseng RNA-seq database. Differentially expressed proteins were identified by using MALDI-TOF/TOF MS and LC-MS/MS. The identified proteins were mostly involved in diverse functional categories including metabolic process, binding, oxidation-reduction process, and transport etc. Interestingly, Glycosyl hydrolases family, Thaumatin family, C2 domain, Hsp70 protein, and Metalloenzyme superfamily were up-regulated at the 72 and 120 hour, while Ribulose bisphosphate carboxylase, Fructose-bisphosphate aldolase class-1, and Ribosomal protein L44 were down-regulated. **Conclusion:** Thus, we suggest that these proteins might participate in the early response to salt stress in ginseng leaves.

Keywords: Salt stress, Secreted proteins, RNAseq, Ginseng

P17.09 Comparative Proteomic Analyses of Soybean Seed Cultivars Differing in Protein and Oil Content

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Introduction and Objectives: This study aimed to develop the differential protein profiles of soybean (Glycine max) seeds (cv. Saedanbaek and Daewon) differing in protein (47.9% and 39.2%) and oil (16.3% and 19.7%) contents. **Methods:** For proteome analysis, the total seed proteins were subjected to protamine sulfate (PS) precipitation to deplete high-abundance seed storage proteins (SSPs) in the pellet (PSP) fraction and to enrich low-abundance proteins in the supernatant (PSS) fraction. **Results and Discussion:** Two-dimensional gel electrophoresis (2-DGE) analysis of proteins from the PSS fractions resulted in the detection of 54 differential spots between Daewon and Saedanbaek, where protein identity for 48 spots was successfully assigned by MALDI-TOF/TOF. Gene ontology analysis of the identified proteins revealed that increased protein abundance in Saedanbaek was largely associated with the nutrient reservoir activity (42.6%), which mainly included SSPs (subunits of glycinin and β-conglycinin). To validate this observation, the same approach was used to investigate seeds of wild soybean (G. soja) cv. WS22 and WS15 differing in their protein content; 46.9% and 56.5%, respectively. A similar result was found showing increased SSPs abundance in WS15. Furthermore, Western blot results confirmed the 2-DGE-based proteomics results. **Conclusion:** Findings presented and discussed in this study suggest that increased accumulation of SSPs is one possible cause for higher protein content in soybean seeds.

Keywords: protamin sulfate, 2-DGE, soybean, seed

P17.10 Selected Reaction Monitoring (SRM) for Quantification of Angiotensin-II Signature Proteins in Urine

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Introduction and Objectives: Angiotensin-II (AngII), the main effector of the renin-angiotensin system, mediates kidney disease progression. However, there are no specific markers of kidney AngII activity. We previously defined 83 AngII-regulated proteins in vitro, which reflected kidney AngII activity in vivo. We aim to quantify these AngII-regulated proteins in urine, and establish their role as markers of kidney AngII activity in patients with chronic kidney disease (CKD). **Methods:** SRM assays were developed for 37 peptides corresponding to 18 previously identified AngII-regulated proteins. We first spiked in crude unlabeled peptides into normal urine. To assess reproducibility and recovery, we spiked in bovine serum albumin (BSA), and the corresponding 7 heavy-labeled proteotypic peptides. Precipitation with acetonitrile, and digestion with lys-C/trypsin was optimal for detection of AngII-regulated peptides. Heavy peptides corresponding to 13 identified AngII-regulated peptides were purchased and spiked in, prior to digestion. After digestion, 20mg of total protein/sample was subjected to C18 microextraction and analyzed on triple-quadrupole mass spectrometer (TSQ-Quantiva). We quantified AngII-regulated peptides in urine samples of 9 patients with CKD. **Results and Discussion:** Biological replicate CVs were <6% for BSA peptides in normal urine, and recovery was ~100%. Calibration curves demonstrated linearity (R^2>0.99) and CVs<20% in the concentration range of 8/13 peptides in normal and CKD urine samples. Deamidated glutamine and arginine fragments constituted 20-50% of peptide peak areas. Most peptides were quantified in all urine samples, with two peptides above the LOQ in few urine samples. We are now exploring the relationship between urine excretion of AngII-regulated peptides successfully quantified, and clinical parameters linked to CKD progression. **Conclusion:** We have developed and optimized a protocol for SRM measurement of protein biomarkers in urine. Eight AngII-regulated peptides were successfully quantified in urine samples. Future studies will examine whether urine excretion rate of AngII signature proteins in large cohorts of patients with CKD predict disease progression and outcome.

Keywords: selected reaction monitoring, kidney disease, angiotensin II, Urine

P17.11 Biomarker Combination to Predict Infection in Aneurysmal Subarachnoid Haemorrhage

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Introduction and Objectives: Aneurysmal subarachnoid haemorrhage (aSAH) is a medical emergency associated with high rates of mortality/morbidity. Infections such as pneumonia or urinary tract infection occurring during the hospital stay are one of the main causes of outcome worsening and death. A personalized preventive antibiotic therapy, using biomarkers is needed. The aim of this study was to use omics-based strategies to find biomarkers of infection prediction in aSAH patients. **Methods:** Plasma samples of aSAH patients were collected daily from the admission to the hospital to ten days after. 'Omics techniques were used to compare the samples of infected (n=3) and non-infected (n=2) patients, in order to identify differentially expressed markers. The most promising ones were selected for further WB and ELISA verification in 54 infected and 27 non-infected patients. The predictive performances were established using Mann-Whitney U tests and ROC curves. The molecule and clinical parameters combination was established using PanelomiX. **Results and Discussion:** Omics strategies identified 17 differentially expressed proteins and a metabolite between infected and non-infected patients. SAA

and NP, the two most promising ones, correlated with infection development from four days after hospitalisation to ten days after. Furthermore, we observed that the concentrations of SAA were significantly higher in patients that will develop an infection during hospitalisation already at the admission to the hospital (p=0.002, 80% SP - 71.8% SE). The combination of SAA with NP and two clinical parameters (white blood cells and age) improved importantly the individual performance of SAA to differentiate between infected and non-infected patients, reaching an AUC of 94.4% (100%SP, 83%SE). **Conclusion:** Our data suggested that the combination of SAA and NP with the white blood cells and the age of the patients, could be an efficient tool to tailored the anti-biotherapy and thereby potentially to improve the long-term outcome of aSAH patient.

Keywords: biomarker, Subarachnoid Haemorrhage, Infection

P17.12 Identification of TEX101 Protein Interactome in Testicular Germ Cells by Immunoprecipitation-MS

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Introduction and Objectives: Protein-protein interactions and their functional role in spermatogenesis and fertilization remain unexplored in the field of reproductive biology. In our previous studies, a testis-specific protein TEX101 emerged as a biomarker for various types of male infertility. Functional interactions of human TEX101 during spermatogenesis still remain unknown. Studies in mice suggested that TEX101 function was exerted through its interactions with other testicular germ cell surface proteins, and such interactions were crucial for the production of competent spermatozoa. Since TEX101 may be a ubiquitous cell-surface chaperone which regulates the maturation of several sperm membrane-bound proteins, we aimed at identifying the interactome of human TEX101 protein in testicular germ cells. **Methods:** Human recombinant TEX101 was used as an immunogen to generate mouse monoclonal antibodies. Screening by immunocapture-SRM facilitated the selection of antibodies which bound to the native form of TEX101 on the surface of testicular germ cells and seminal plasma, as demonstrated by flow cytometry and our in-house ELISA. **Results and Discussion:** Immunocapture-SRM revealed 18 antibodies which recognized the native form of TEX101. Top 5 antibodies targeting at least two different TEX101 epitopes were confirmed by flow cytometry of human testicular germ cells and used to develop an immunoprecipitation-mass spectrometry (IP-MS) platform for identification of TEX101 interactome. Isolation of germ cells from normal testicular tissue and cell lysis were optimized to suit IP-MS and to preserve native protein complexes. IP-MS provided a list of candidate proteins interacting with TEX101. Immunoprecipitation-SRM assays were developed to confirm TEX101 interactome in testicular germ cells and seminal plasma. **Conclusion:** Elucidation of protein-protein interactions in human testicular germ cells is important for understanding the biology of spermatogenesis and fertilization. Identification of disrupted interactions of germ cell-expressed proteins may shed light on molecular mechanisms leading to idiopathic male infertility.

Keywords: Immunoprecipitation-mass spectrometry, Interactome, testicular germ cells, male infertility

P17.13 Discovery of Novel Subtype-Specific Ovarian Cancer Biomarkers via Integrated Tissue Proteomics

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Introduction and Objectives: It is evident that ovarian cancer (OvCa) is not a single disease but is made up of several distinct subtypes, including serous, endometrioid, clear cell and mucinous. The gold-standard biomarker CA125 performs well in serous but not in the other histotypes. We hypothesize that a more focused discovery effort (on non-serous OvCa biomarkers and/or markers that can complement serum CA125) may bring about a sensitivity that is acceptable for all histotypes and be suitable for early diagnosis of OvCa. **Methods:** Tissues from patients diagnosed with endometrioid (EC), clear cell (CC), and mucinous ovarian carcinoma (MC), as well as from their appropriate controls (endometriosis and healthy endometrium for EC and CC; mucinous cystadenoma for MC) were subjected to proteomic analysis using a label-free, offline 2D-LC-MS/MS based approach. Discovery candidates were then filtered using an in-house developed algorithm combining publicly-available resources with our own warehouse of transcriptomic and proteomic sets. **Results and Discussion:** Over 5000 unique proteins were identified in this proteomic exercise; specifically, approximately 1500 protein unique to MC, 1100 unique to CC, and 3000 unique to EC were identified when comparing the appropriate cases and controls. A curated list of 60 high-potential candidates was generated after using a range of bioinformatics tools to ensure criteria based on factors including (but not limited to) tissue specificity, cellular localization and transcriptional upregulation were met. These 60 candidates represent putative subtype-specific markers which will be further analyzed and validated in serum cohorts. **Conclusion:** The identification and validation of markers specific to the non-serous subtypes of OvCa remains an unmet clinical need. With our list of putative subtype-specific markers, we aim to develop a novel biomarker panel able to detect all OvCa histotypes with greater sensitivity and specificity than any existing clinical tools.

Keywords: Early diagnosis, Ovarian cancer, biomarker

P17.14 Protein Biomarkers Link Olfactory Signal Transduction to Social Immunity

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Introduction and Objectives: The honey bee (Apis mellifera) plays a critical role in agriculture through pollination– their estimated contribution to North American agriculture exceeds $10 billion. Honey bee colony losses of the last decade can be attributed to a combination of factors including pathogens and parasites. Bees, however, have evolved individual and colony level social immune responses. We identified protein markers and explored molecular mechanisms of social immunity; in particular defense behaviors associated with the rapid detection and removal of dead or infected larvae.

Methods: We performed quantitative shot gun proteomics of antennal samples in parallel with field assays for colony level social immune behaviors. To explore relevant molecular mechanisms, we expressed recombinant odorant binding proteins (OBPs) and characterised their binding to different ligands. **Results and Discussion:** We identified biomarkers of immune defense behaviors associated with the rapid removal of dead or infected larval bees from the colony. Intriguingly, these proteins were all involved in semiochemical sensing (OBPs), nerve signal transmission or signal decay, which indicates the series of events required to respond to an olfactory signal from dead or diseased bees. We also characterized the classes of ligands that these proteins might be helping bees detect. **Conclusion:** Our data suggest that neurosensory detection of odors emitted by dead or diseased larvae is a likely mechanism behind complex and important colony-level defenses of honey bees against pathogens and parasites.

Keyword: quantitative, Apis, immunity, mechanism

P17.15 Multiplexed SRM Assay for Measurement of Tissue Kallikrein-Related Peptidases in Biological Fluids

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Introduction and Objectives: Tissue kallikrein-related peptidases (KLKs) are a group of 15 serine proteases, expressed in multiple tissues and responsible for the coordination of various physiological functions such in cancer, semen liquefaction and skin desquamation. ELISA immunoassays were previously developed for all KLKs. In this work, we focused on development of a multiplex SRM assay for simultaneous quantification of all 15 KLKs and analytical comparison of ELISA and SRM assays. **Methods:** Trypsin digests of recombinant KLKs were used to identify 5 proteotypic peptides per each KLK. The peptide with the most intense and reproducible signal was selected for protein quantification. Proteotypic peptides were multiplexed in a single SRM assay (15 peptides and 45 transitions) and analyzed using nanoflow liquid chromatography coupled to quadrupole-ion trap mass spectrometer. SRM assay was validated in biological fluids including seminal plasma (SP), cervico-vaginal fluid (CVF) and sweat. We tested the performance of the assay on three mass spectrometry platforms including a triple quadrupole mass spectrometer, a quadrupole-ion trap mass spectrometer and a quadrupole-orbitrap mass spectrometer. **Results and Discussion:** Heavy isotope-labeled synthetic peptides with a quantifying tag were used for absolute quantification of KLKs in biological fluids, such as SP, synovial fluid, sweat, CVF, ovarian cancer ascites, and cerebrospinal fluid. LODs ranged from 10ng/mL to 1000ng/mL. We have successfully quantified proteins in individual clinical samples and we evaluated the analytical performance of the assay. We also investigated the correlation between the clinical ELISA tests and the mass spectrometry based assay. **Conclusion:** The present work reports a multiplex SRM method for absolute quantification of all 15 KLKs in various biological fluids. The assay was proven to be an accurate, reproducible, sensitive and highly specific alternative to the existing antibody-based assays and it allows the detection of several analytes simultaneously without compromising sensitivity.

Keywords: Mass spectrometry, selected reaction monitoring, chromosome 19, human tissue kallikrein-related peptidases

17.16 Stress Chaperone GRP78 Confers Protection against Neurocognitive Impairment in HIV+ Polydrug Users

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Introduction and Objectives: Due to the high prevalence of neurocognitive impairment (NCI) in HIV infected and drug abuse patients worldwide, there is an urgent need to study the synergism between these disorders and their impact on the immune response. We hypothesize that HIV infected polydrug users (PDU) have a greater impact on NCI with alterations in T-lymphocyte Endoplasmic Reticulum (ER) stress protein and pro-inflammatory cytokine expression from HIV+/HIV-PDUs. To test this hypothesis, we will 1) identify NCI in HIV+ participants with and without polydrug addiction, 2) analyze the cytokine and ER stress protein profiling from HIV+/HIV-PDUs. **Methods:** NCI was measured by psychological and homocysteine analysis in HIV-/HIV+ PDUs. Alterations on protein expression were detected through a proteomic approach and cytokine profiling was achieved by means of flow cytometry. The sample population distribution was: 10 HIV+PDU+ and 10 HIV-PDU+. HIV-PDU+ participants had higher NCI and expression of proinflammatory cytokines as compared to HIV+PDU+. **Results and Discussion:** Proteomic data showed that polydrug use contributes negatively to NCI in HIV infected participants and that ER stress marker GRP78 was downregulated in NCI participants as compared to non NCI ones. Moreover, GRP78 expression diminished with HIV infection. This was correlated with the expression of IL-8, IL-6 and IL-12p70 proinflammatory cytokines in HIV+ participants. **Conclusion:** Our findings showed that downregulation of GRP78 may be a pontential biomarker to identify NCI in HIV positive PDU participants. This study will contribute to a deeper understanding of the cellular and molecular mechanisms linking drug addiction and HIV infection. This project was funded by NIMHD/NIH grant # G12MD007583 and PO31M105050 from the U.S. Department of Education to UCC.

Keywords: HIV, Drug Abuse, Endoplasmic Reticulum Stress, Neurocognitive Impairment

P17.17 Proteome Analysis of Skeletal Muscle from a Tailored Pig Model of Duchenne Muscular Dystrophy

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Introduction and Objectives: Duchenne muscular dystrophy (DMD) is a severe human disease caused by mutations in the dystrophin gene (DMD gene), leading to progressive muscle degeneration and a dramatically reduced life span. Recently, a tailored pig model of DMD (deficient of DMD gene exon 52) was generated (Klymiuk et al. Hum Mol Genet 2013) which better reflects the human pathology than the widely used mdx mouse. The aim of our study was to detect progressive proteome disturbances in skeletal muscle of this large animal showing important biochemical, histological and functional hallmarks of the human disease. **Methods:** We performed a label free quantification (LFQ) analysis of biceps femoris muscle samples from 2-day-old and 3-month-old DMD and WT pigs (n=3 per group). Separation was performed using ultra high pressure chromatography with long separation columns (50 cm). MS-analysis and quantification was performed on an Orbitrap XL instrument in combination with MaxQuant. A 2-way ANOVA with age and genotype as explanatory variables was performed using the R package. **Results and Discussion:** In total we identified 1429 proteins (FDR < 1%). In the 2-day old DMD group, 22 proteins with higher and 43 with lower abundance

compared to age-matched WT pigs, were detected. In the 3-month old group, the number of differently abundant proteins between the genotypes increased markedly to 372, of which 269 were significantly increased and 103 decreased in abundance in DMD vs. WT pigs. Beside other findings, ontology analysis of these proteins indicates serious aberrations of the respiratory chain pathway during growth and development of DMD pigs. **Conclusion:** Our proteome study of the first tailored large animal model of DMD revealed a large number of stage specific proteome changes, which provide new insights into the sequence of molecular derangements of dystrophic muscle. Furthermore, early alterations of the muscle proteome may be used for evaluation of new therapies.

Keywords: Large animal models, proteome analysis, LC-MS/MS, Muscular dystrophy

P17.18 Early Embryo Development in Mammals: mSRM Based Quantification of 27 Key Proteins in 9 Stages

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Introduction and Objectives: Early embryogenesis is a highly critical period of mammalian development. Morphology of pre-implantation development has been well studied, however molecular processes, particularly at the level of proteins, are still poorly understood. To identify molecular key players during early development, we had recently performed holistic iTRAQ proteome analyses using the bovine model (Deutsch et al., 2014), which shows crucial similarities to the human reproduction system. Here we present a multiplexed SRM assay for 27 developmentally relevant proteins. **Methods:** Oocyte maturation, in vitro fertilization and in vitro production of embryos was performed as described previously (Deutsch et al., 2014). SRM based quantifications using AQUA peptides were performed using batches of 10 oocytes, zygotes or embryos (n=6), corresponding to 1 μg total protein. LC-MS/MS was performed on a Q-Trap 5500 instrument. GraphPad Prism was used for statistical analysis (multiple T-tests). **Results and Discussion:** A sensitive multiplexed SRM assay was developed covering 27 proteins relevant for early embryonic development. Proteins were reliably quantified in nine developmental stages (MII oocytes up to expanded blastocyst). Individual proteins follow characteristic expression profiles. In a Principal Component Analysis based on SRM quantifications of five selected proteins, MII-oocytes, zygotes, 2-cell and 4-cell embryos were clearly separated. The absolute protein content per embryo was so far quantified for nine proteins covering a variety of biological functions, revealing protein amounts per embryo from low femtomole down to the attomole range. **Conclusion:** Despite silence of transcription and translation, the proteome in oocytes, zygotes and early embryonic stages is highly dynamic. Protein expression during embryo development was precisely quantified for nine characteristic stages from the oocyte to the expanded blastocyst. Absolute quantification of nine developmentally relevant proteins provides an essential data basis for systems biology oriented pathway modelling and prediction algorithms.

Keywords: zygote, mammalian development, embryo, SRM

P17.19 A Multi-Proteomics Approach to Biological Fluids: A Key to Translate a Discovery into Utility

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Introduction and Objectives: Deep knowledge of body fluid proteomes in health and diseases and development of robust assays is an ambitious target which would impact tremendously clinical studies. Here we present an integrated proteomic approach for the investigation of plasma proteins from biomarker discovery to validation. Featuring the common link of antibody capture and strategy of enrichment, the platform integrates four modules 1) SBA: sample profiling by suspension beads arrays; 2) immunocapture-MS (ic-MS): analysis of proteins captured and antibodies selectivity; 3) ic-LMX: MS generated hypothesis verification by SBA; 4) ic-SRM: development of a quantitative assay for cross-platform biomarker verification. Applying the same affinity reagent across assay systems adds a higher layer of characterization and certainty about assay specificity. **Methods:** Proteins were affinity-enriched from un-depleted plasma and analyzed with Thermo Q Exactive Orbitrap HF,Thermo (ic-MS) or with Agilent-6490 QQQ LC/MS (ic-SRM). For ic-LMX assays, protein captured were probed with an SBA for higher abundant plasma proteins, putative targets and their co-interactors. The process was automated using a Thermo KingFisher™ Flex magnetic bead handler. Data analysis and representation was performed, using Proteome Discoverer, MassHunter, Skyline and R. **Results and Discussion:** A larger set of antibodies previously applied in plasma profiling studies using SBAs were analyzed by automated ic-MS. We searched the data for PTMs, protein isoforms and common contaminants through a population statistic approach to ultimately investigate antibody selectivity and to identify novel protein-protein interaction. Information collected in the ic-MS exploratory experiments were verified and supported by ic-LMX, while an ic-SRM assay was developed for selected targets. The rate success of the workflow was correlated to protein concentration, antibody selectivity in plasma, and peptides detectability. **Conclusion:** This multi-proteomic platform supports (1) the discovery, verification and cross-assay validation of biomarkers; (2) sandwich assay development through antibody selectivity investigation, and (3) the characterization of the plasma proteome and investigation of its interactome.

Keywords: mass-spectrometry, Affinity-Proteomics, biomarker, plasma

P17.20 The Oxidized Proteome of Peripheral Blood Mononuclear Cells

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Introduction and Objectives: Peripheral blood mononuclear cells (PBMCs) are a popular model system to study the physiological and metabolic activity of cells within the body. PBMCs have enabled a very broad collection of biomedical applications. Monitoring gene expression and posttranslational modifications are very promising areas in biomarker discovery and translational research. In this study, we aimed to have the most extensive proteome map of PBMCs and monitor the in vitro effect of reactive peroxide at low concentration. **Methods:** PBMCs from a healthy male individual were purchased from AllCells. 1mill cells aliquots were in vitro treated with 5 mM H_2O_2 for 0, 2, 10, 30 and 80

min. Sample preparation was performed using the Mass Spec Sample Prep Kit for Cultured Cells (Pierce, Rockford IL). Peptide digests were then analyzed by LC-MS/MS analysis on a Thermo Scientific™ Q Exactive™ HF mass spectrometer . Database search and oxidation site localization were performed using Thermo Scientific™ Protein Discoverer 2.0. Inferno was then used for further statistical analysis and ProteinCenter was used to extract biological context. **Results and Discussion:** Access to the complete atlas of gene expressions and posttranslational modifications in PBMCs will permit more sophisticated studies such us the selection of potential biomarkers that could be used for many purposes ranging from diagnostic, prognosis or even help selecting the appropriate therapy for a patient. The bioinformatic analysis of the results yielded the identification of over 8000 proteins. In addition, over 5000 proteins were accurately quantified and over 7000 oxidation events were identified. **Conclusion:** Overall, this study not only adds significant value in the mechanistic understanding of redox signalling, but it also creates a valuable protein repository that could lead to the development of new therapeutic strategies. This work constitutes the largest proteomics dataset for PBMCs to date and one of the most comprehensive proteomics warehouse in the clinical proteomics field.

Keywords: Redox, proteomics, Orbitrap, Mass Spec Sample Prep Kit for Cultured Cells

P17.21 Pre-Cardiac Surgery Urinary Biomarkers to Identify Risk for Acute Kidney Injury

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Introduction and Objectives: Hospital acquired acute kidney injury (AKI) is associated with a mortality from 10% in uncomplicated patients outside the ICU to 70% in the ICU setting. AKI remains a significant complication of cardiac surgery requiring cardiopulmonary bypass with up to 16% of patients developing AKI post-operatively. One contributing factor toward the failure to alter the course of AKI is the absence of adequate markers to predict AKI and patient prognosis. We hypothesized the risk of the development of post-cardiac surgery AKI could be categorically differentiated with surrogate biomarkers identified in pre-surgery urinary proteomes. **Methods:** Urine was obtained prior to cardiac surgery. AKI was defined as a 0.5 mg/dl increase in serum creatinine for patients with a baseline creatinine of <1.9 mg/dl and a 1.0 mg/dl increase in serum creatinine if baseline creatinine was 2.0 mg/dl or greater. Urine proteins were isolated and analyzed by 2D-LCMS. Spectral count data were compared using t-test examining the direction and magnitude differences. ELISA tests were used to confirm proteomic data in an expanded patient sample set. Functional grouping of regulated proteins was assessed using pathways analysis tools. **Results and Discussion:** 15 proteins were increased in the AKI(neg) group and 24 were more abundant in AKI(pos) group. These proteins sub-grouped into markers of (1) tubular injury (complement components), (2) previously described AKI biomarkers (angiotensinogen, alpha1-microglobulin) and (3) novel markers (histidine rich glycoprotein-HRG, afamin, corticosteroid-binding globulin). Pathway analysis suggested alternative complement pathway as strongly regulated in patients that later developed AKI. ELISA values for HRG and pre-operative serum creatinine achieved receiver-operating characteristic score of 0.900 for predicting post-operative AKI. **Conclusion:** Alternative complement pathway components are highly expressed in the urine of patients that developed AKI following surgery. The data indicate that urinary proteomes of patients awaiting cardiac surgery can be used to define those at risk of developing post-surgery AKI.

Keywords: biomarker, acute kidney injury, Urine

P17.22 Characterization of Chronic Venous Ulcers through Its Inflamatory Exudate: A Translational Approach

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Introduction and Objectives: Chronic venous ulcers (VUs) are defined as wounds located below the knee that do not heal within a 6-week period and they represent a significant impact on patient quality of life and a worldwide public health problem. This project aimed to identify potential molecular biomarkers in the inflammatory exudates of easy and difficult healing VUs in order to assist their prognosis/diagnosis. **Methods:** Inflammatory exudate from 38 ulcers of 28 patients with VUs from outpatient of Dermatology from School of Medicine of Botucatu were collected and the ulcers areas were calculated by comparing T=0 and T=90 days. The samples were submitted to in solution protein digestion and ESI-Q-ToF-MS/MS were performed. Mascot, Scaffold Q+ and String software were used for protein identification, quantification of protein expression, function and protein-protein interaction classification. **Results and Discussion:** The patient were predominantly composed of female sex (64.3%), mean age of 70.3 years, 83% of whites, 57.1% of hypertension, 25% with Diabetes mellitus, 10.7% heart failure and 14.3% of smoking. It was observed that 63.2% had a lower reduction to 40% of your initial area, no regression or increase in lesion area (ulcers of healing difficult) while 36.8% had greater reduction than 40% of your initial area (ulcers of healing easy). Proteomic analysis was performed comparing these two clinical groups and 122 proteins were identified. Twenty-three proteins presented differently expressed and the majority of proteins are related with hemostasis, regulation of body fluid levels, negative regulation of protein metabolic process, regulation of biological quality, protein binding and regulation of cell death and apoptosis.Our findings have showed a higher incidence of ulcers of healing difficult, however, the differentially expressed proteins can assist to better comprehension about healing of VUs. **Conclusion:** The present study confirms the importance of translational medicine for the prognosis/diagnosis in the screening routine in a dermatology outpatient.

Keywords: Chronic venous ulcers, Inflammatory exudate, Proteomic Analysis, Healing

P17.23 Bubalus Bubalis Buffaloes Blood Plasma Proteome Applied Translational Medicine

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Introduction and Objectives: Significant scientific breakthroughs have occurred in recent years involving basic experimental research, which has affected the biotechnology sector, regarding the development of new bioactive compounds from raw materials of animal origin. Accordingly, domestic buffaloes, Bubalus bubalis, have gained considerably ground in the world economy as raw material providers. CEVAP has been developing a new fibrin sealant, which is used as a scaffold for stem cells, a biological glue in surgical procedures and as an aid for treating chronic venous ulcers in humans. This sealant is composed by a serine protease from Crotalus durissus terrificus snake venom and a fibrinogen-rich cryoprecipitate from blood plasma of

domestic buffaloes. Therefore, the objective of this work was characterizing the abundant proteins present in the blood plasma of healthy B. bubalis. **Methods:** The samples were submitted to 2D-electrophoresis and the proteins were subject to SDS-PAGE 10% (m/v). The protein spots were cut out, in-gel protein digestion was performed and the peptides were submitted to ESI-Q-ToF-MS/MS. Mascot, Scaffold Q+ and String softwares were used for protein identification, function and protein-protein interaction classification. **Results and Discussion:** Afterwards, it was evidenced the presence of 129 proteins with molecular masses between 100 and 20 kDa and isoelectric point 4-7. Of these, 112 were identified including albumin, fibrinogen-α, fibrinogen-β, fibrinogen-γ, immunoglobulin, α-1-antiproteinase, α-1B-glycoprotein, α-2-HS-glycoprotein, α-2-macroglobulin, apoliprotein A-1, antithrombin-III, endopin-2B, fetuin-B, retinol binding protein, transferrin, transthyretin and vitamin-D binding protein. Most of these proteins were presented in ruminant bloods and are related to the signaling via of the complement system and coagulation cascade. **Conclusion:** The present study has promoted a better understanding of the composition of the most abundant proteins in blood plasma of healthy buffaloes. These findings will support future studies on the development of a specific diagnostic platform of sanitary conditions of donor buffaloes that provide raw material for the fibrin sealant at CEVAP.

Keywords: Proteomic Analysis, Blood Plasma, Bubalus bubalis, Fibrin Sealant

P18: POSTER SESSION - CARDIOVASCULAR AND HAEMATOLOGICAL PROTEOMICS

P18.01 Peptide MRM Comparison Using Integrated Microfluidics/ Nanoscale LC with High/Low Resolution MS

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Introduction and Objectives: Biomarker validation is technologically challenged requiring a large number of samples with high-throughput, whilst maintaining high sensitivity, high resolution, large dynamic range and excellent selectivity. Miniaturized LC systems offer improved mass-sensitivity but often lack throughput, robustness and reproducibility. Comparing an integrated microfluidic device with nanoscale LC, using different MS platforms, for the quantification of marker peptides and proteins is presented. **Methods:** Stable Isotope Labeled peptides whose light analogues are putative biomarkers for cardiovascular disease were spiked at various levels into digested human serum. A reversed phase gradient was employed on various LC-MS systems using tandem quadrupole and hybrid Q-Tof instruments. **Results and Discussion:** MRM chromatograms using high and low resolution mass spectrometry, showed peptides to be resolved from background over the entire dynamic range for candidate biomarkers. For example, <100 amol TAENFR on-column was readily detected with no background matrix interference using the integrated microfluidic device in combination with a low resolution mass spectrometer under high flow and throughput conditions. Comparatively, 30 amol ESDTSYVSLK on-column was detected using nanoscale LC in combination with a high resolution platform. Ultimate sensitivity levels equaled sub 10 amol on-column using nanoscale LC in combination with either low or high resolution mass spectrometry. MRM transitions were inspected ensuring a minimum of two transitions per peptide. Typical retention time reproducibility for the monitored peptides at microfluidic and nanoscale levels equaled 0.02 min standard deviation. Levels of putative peptide biomarkers in matrix were determined, ITLYGR at 650 amol/200 ng of plasma using the microfluic/ tandem quadrupole, whilst GYSIFSYATK (45 amol/200 ng of matrix) using nanoscale/Q-Tof setup operating with and without ion mobility. **Conclusion:** Comprehensive comparison of various LC-MS platforms for the quantitative analysis of potential peptide biomarkers, including ion mobility based MRM technology. Ultimate sensitivity levels equaled sub 10 amol on-column with either low or high resolution mass spectrometry.

Keywords: Integrated Microfluidics, MRM, Ion Mobility, Cardiovascular biomarkers

P18.02 Proteomic Analysis of Human Cardiac Endothelial, Smooth Muscle, Myocytes, and Fibroblasts

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Introduction and Objectives: The heart is composed of multiple constituent cell types; the primary cells being fibroblasts (FB), cardiomyocytes (CM), endothelial (EC) and smooth muscle (SMC) cells. Cardiac proteomic studies often compare healthy and diseased tissues, but these studies are limited by lack of knowledge of the protein complement of the individual cell types, and thus are hindered to identify cellular origin of any disease-associated protein expression changes. **Methods:** We employed membrane sub-fractionation to attain membrane-enriched and -depleted fractions from 2-3 week cultured human cardiac FB, cardiac muscle-derived CM, coronary artery EC, and coronary artery SMC. Trypsinized peptides from each fraction were then subjected to 9-cycle MuDPIT analysis on the LTQ-Orbitrap. Protein abundance was determined using adjusted spectral counting. Protein expression was assessed using human cardiac tissue staining in the Human Protein Atlas (HPA). **Results and Discussion:** Shotgun proteomics identified a total of 2320 FB, 2310 CM, 2247 EC, and 2209 SMC proteins and 2853 proteins overall. Statistical analysis identified 367 FB-enriched, 97 CM-enriched, 340 EC-enriched, and 52 SMC-enriched proteins. We investigated the functional annotations of our enriched datasets via Gene Set Enrichment Analysis (GSEA). GSEA revealed functional roles of each cell type by protein clusters associated with “response to hypoxia” for FB, “junctions” for CM, “endothelium development” for EC, and “regulation of signal transduction” for SMC. Rank-ordering of cell type-specific proteins was determined with bioinformatic integration with transmembrane helix predictions, Phenotype Ontology, and publically available microarray datasets. Select examples were confirmed for subcellular localization with high resolution confocal microscopy, immunogold electron microscopy, and sucrose density gradient biochemistry. For several of the highly ranked membrane proteins, we assessed HPA to identify supportive and non-supportive staining of our protein candidates. **Conclusion:** Our study has provided the most complete proteomic analysis of specific human cardiac cell types. Potential markers of each cell type have been validated by HPA.

Keyword: membrane, MudPIT, cell-specific, cardiac

P18.03 Smyd1 Interactome in Skeletal and Cardiac Myocytes and Its Involvement in Novel Cellular Functions

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Introduction and Objectives: Previous studies show that the myocyte-specific methyltransferase Smyd1 plays a crucial role in cardiac development; however its function in adult heart is unknown. We have shown that cardiac-specific deletion of Smyd1 leads to pathologic hypertrophy and gene expression changes, ultimately progressing to heart failure. Overexpression of Smyd1 inhibits these hypertrophic and transcriptional changes thus highlighting a novel role for Smyd1 in heart disease. Two Smyd1 variants are expressed in the cytosol and nucleus of cardiac and skeletal myocytes where they methylate proteins; however, the difference between these variants' functions and interactomes has never been investigated. To elucidate the mechanisms governing Smyd1 function and identify novel methylation targets, we performed the first global, unbiased analysis of Smyd1 binding proteins using co-immunoprecipitation and mass spectrometry. **Methods:** Flag-tagged Smyd1a and Smyd1b were individually expressed in skeletal myoblasts or primary cardiac myocytes via adenoviral-mediated expression. Co-immunoprecipitation was performed for each of these variants and immunoprecipitates were trypsin digested overnight and analyzed on an Orbitrap Velos Pro mass spectrometer. Peptide identification was perfomed using Proteome Discoverer 1.4 software and bioinformatic analysis was performed using the DAVID Bioinformatics Resource. **Results and Discussion:** We identified 65 and 66 Smyd1 binding proteins in skeletal and cardiac myocytes, respectively. While there was significant overlap between these two proteomes, we also identified 19 unique Smyd1b binding partners. Additionaly, co-immunoprecipitation experiments in cells treated with hypertrophic agonist uncovered 45 unique binding partners. Gene ontology analysis revealed an enrichment of proteins involved in cytoskeletal organization and cardiac tissue development, as well as previously unrecognized Smyd1 processes including cellular metabolism. **Conclusion:** Our results highlight key differences in the molecular function and regulation of the Smyd1 variants and implicates their involvement in novel cellular functions. Together this knowledge enhances our understanding of the role of Smyd1 in regulating cardiac morphology and physiology with specific implications for heart failure progression.

Keywords: binding partners, Smyd1, heart disease, hypertrophy

P18.04 Identification of Altered Cell Signaling Pathways in B-Lymphocytic Chronic Leukemia

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Introduction and Objectives: 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. 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,... **Methods:** ,... 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. **Results and Discussion:** We will present differential protein profiles (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 **Conclusion:** This study could provide information for the diagnosis, prognosis and treatment as well as improvement in the knowledge of B-CLL.

Keyword: Biomarkers, protein microarrays, high-density analysis, functional proteomics

P18.05 Quantification of 90 Proteins by MRM in Biobank Samples from Patients with 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. The aim of the study is to quantify 90 target proteins with putative links to ischemic heart disease and myocardial infarction in large patient cohort from LUNDHEARTGENE Biobank and link the quantitative proteomics data with genomics and patient registry data. **Methods:** Human plasma samples together with QC samples (pooled plasma samples) were handled and digested according to our previously optimized protocol in 96-well plate format. The protein 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). Data evaluation were done with the aid of Skyline v2.6 (MacCoss Lab) and Anubis v1.4.1 (http://proteomics.bmc.lu.se/anubis/) softwares, further statistical analysis was done using Microsoft Excel, R and Matlab v7.11. **Results and Discussion:** 380 individual plasma samples were analyzed from subjects with ST-segment elevation myocardial infarction (STEMI), non-ST elevation myocardial infarction (NSTEMI), stable and unstable angina, as well as chest pain of non-cardiac origin. 90 proteins were quantified in one assay by monitoring at least one unique tryptic peptide per protein. Evaluation of the sample preparation, optimal heavy internal standard amounts, and calculation of the reproducibility and LOQ were done. **Conclusion:** NanoLC-MRM-MS analysis was performed together with stable isotope dilution strategy for highly reproducible quantification of the target proteins. The assay is able to accurately quantify proteins in 5 orders of magnitude dynamic range (~5 mg/mL to 50 ng/mL) in whole plasma digest.

Keywords: MRM, myocardial infarction, human plasma, biobank

P18.06 Cardiac Proteome Changes Induced by Radiation Exposure in a Mouse Model

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Introduction and Objectives: There is renewed interest in space exploration. A major risk to astronauts is prolonged exposure to Galactic Cosmic Radiation which results in increased free radicals at the cellular level which can cause DNA and protein damage, metabolic oxidative stress and oxidative PTMs on proteins. Exposure heightens the risk of cardiovascular disease (CVD). We used a mouse model of radiation exposure to identify protein and PTM changes associated with exposure to radiation, altered redox biology, and their impact on cell injury and CVD. **Methods:** Mice were exposed to 1 GeV Fe ions at 0.5 Gy/min at doses of 0 Gy, 0.5 Gy and 5 Gy. Heart tissue was subjected to proteomics analyses. LC-MS/MS: Q Exactive MS with Waters NanoAcquity HPLC. Label-free quantification: Scaffold (Proteome Software) and Progenesis LCMS (Nonlinear Dynamics). MS/MS data processing: Proteome Discoverer (Thermo-Fisher) and Mascot (Matrix Science). Meta-analysis: Trans Proteomic Pipeline (ISB), Scaffold and STRAP PTM (in-house) software. Ingenuity Pathway Analysis (IPA, QIAGEN) was used to identify dose-dependent biological pathways. **Results and Discussion:** Label-free analysis identified more than 1,900 features changing as a function of dose (p=0.05 ANOVA)/ MS/MS (p=0.05 FDR). 50% of the peptides had PTMs. PCA/ hierarchical clustering showed changes which were dose-dependent. IPA indicated pathway-specific changes including CVD related pathways: Post-Translational Modification, Energy Production, Lipid Metabolism, Free Radical Scavenging, Cardiovascular Development and Function and Metabolic Disease. Known markers of CVD were observed to change with dose including Troponin I (changes are related with cardiomyopathy, ventricle hypertrophy). We observed PTM changes on proteins related to cardiac function and metabolism. that were both site- and region-specific. **Conclusion:** Discovery of potential biomarkers: peptide, proteins, PTMs will allow us to gain insight into the etiology of CVD, understand host physiological response to pathogenesis and afford earlier detection, improve diagnosis and treatment. Funded by NHLBI Contract HHSN268201000031C, NSBRI Pilot CA00002 and NIH grant P41 GM104603.

Keywords: biomarkers, radiation, oxidative stress, cardiovascular disease

P18.07 Proteomics and Metabolomics of Starvation Response in Heart Tissue under Normal and MetS Conditions

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Introduction and Objectives: Metabolic Syndrome (MetS) is associated with obesity, dyslipidemia, elevated fasting blood glucose levels, insulin resistance and a reduced capacity to achieve cardioprotection from Ischemic Pre-Conditioning (IPC). In the heart MetS is associated with a decreased ability to respond to stressors. A possibly related response is that of autophagy, which is known to be induced by nutrient depletion. Autophagy is known to be impaired in the livers of animals with insulin resistance and hyperinsulinemia. Whether MetS impairs autophagy is unknown. **Methods:** Groups of aged-matched male FVBN mice (Jackson labs) were maintained, and fed ad libitum with normal chow (Teklad 2014, 13% kcal% fat) or a lard-based high fat diet (D12492, Research Diets) for 20 weeks. Fasting animals underwent a 24hr fasting period before harvest. Whole hearts were harvested, and for proteomic analysis, cytoplasmic preparations were made by IN-Sequence cytoplasmic preparation (Kane LA et al. 2007). Protein preparations were derived from 16 animals with four in each category (chow, fasting, Diet Induced Obesity, DIO fasting). Digestion utilized the FASP procedure, and MS analysis was by an Orbitrap Elite. Separate whole heart portions (12 animals, 3 in each category) were sent to metabolomics core facilities at Johns Hopkins University. **Results and Discussion:** There was an increase in proteins related to autophagy, as well as amino acid, nucleotide, and fatty acid metabolism in the DIO mice. These processes were linked via the GSK3B and MIST kinase signaling pathways. Finally, as well, there were changes in major autophagy proteins. Interestingly, many metabolites, including nitric oxide were highly abundant in DIO mice. Many showed a decreasing fasting abundance relative to non-fasting DIO mice which was a less pronounced pattern in the proteomics data. **Conclusion:** Under MetS conditions autophagic signalling pathways in the heart are affected in a fashion that may compromise the starvation stress response through autophagy.

Keywords: autophagy, fasting, metabolites

P18.08 Human Macrophage Redox and Phosphorylation Signaling in Cardiomyopathy from T. Cruzi Infection

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Introduction and Objectives: Chagasic cardiomyopathy (CCM), caused by Trypanosoma cruzi,, represents the third greatest tropical disease burden globally. Increased population mobility, among other factors, has increased the US human incidence to >300,000 patients, with >10 million patients globally. We pioneered the hypothesis that heart tissue damage occurs via two mechanisms: parasite factors that alter immune capacity to clear pathogens, and parasite and host cell factors that activate phagocytosis and the cardiopathology associated with chronic disease. Both redox and phosphorylation signaling pathways are implicated in the host response to infection. The objective of our work focused on developing a combined global proteomic approach to monitor the changes in abundance, oxidation (cysteinyl-S-nitrosylation—SNO), and phosphorylation (PO) of cellular proteins with the goal of characterizing the important proteins regulating the host response to infection. **Methods:** The literature supports the hypothesis that PO and SNO impact these signal transduction pathways in an inverse manner; i.e., PO and SNO are opposite sides of the "same coin." Although individual examples are reported, global demonstrations are difficult to perform. To accomplish this, we combined our SNOFlo method with a PO-specific method thereby allowing quantitative characterization of both PTMs globally and specifically across uninfected and infected human macrophages **Results and Discussion:** We will present global and specific changes in abundance, SNO, and PO, emphasizing those proteins that demonstrate increased SNO concomitant with decreased PO, and visa versa confirming the inverse correlation between these two PTMs. In addition we will present the signal transduction pathways that facilitate parasite persistence and lead to the cardiomyopathy in Chagas disease **Conclusion:** Chagas pathology derives from T. cruzi's resistance to host defenses, and the long-term futile host response that leads to cardiac injury. Understanding the redox and phosphorylation signal transduction mechanisms that underlie these effects may lead to more effective therapies

that clear the parasite and/or protect cardiac tissue

Keywords: cardiomyopathy, phosphorylation, cysteinyl-S-nitrosylation, Chagas disease

P18.09 Moving from Inventory to Personalized Medicine: Reducing Cardiovascular Mortality

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Introduction and Objectives: More than 130 years ago, it was recognized that platelets are key mediators of hemostasis. Nowadays, it is established that platelets participate in additional physiological processes and contribute to the genesis and progression of cardiovascular diseases. Recent data indicate that the platelet proteome comprises >5000 proteins. By implication, in patients experiencing platelet disorders, platelet (dys) function is almost completely attributable to alterations in protein expression and dynamic differences in post-translational modifications. Consequently, platelet proteomics will represent an invaluable tool for characterizing the fundamental processes that affect platelet homeostasis and thus determine the roles of platelets in health and disease. **Methods:** Quantitative mass spectrometry, analysis of postranslational modification, data analysis and integration. **Results and Discussion:** In principle, quantitative proteomics provides a powerful, highthroughput technology to reveal multiple changes in platelet structure and functions, both in health and disease. However, only little is known to date about which types of changes in protein expression levels predict for abnormal platelet functions in defined genetic or clinical settings. Our earlier work indicated that, for platelets from healthy subjects, the intersubject variance was small for both low and high copy numbers, with 85% of the quantified proteins showing (almost) no variation between healthy donors.From this, one can hypothesize that only a subset of platelet proteins determines variation in platelet functions. **Conclusion:** In the past few years, it has become increasingly clear that platelet proteomics can provide novel insights into basic research questions and thus improve our understanding about the fundamental processes that regulate platelets and can also contribute to the diagnosis of platelet disorders. The protein composition and the phosphorylation patterns of platelets will be useful to understand certain disease states and therapeutic interventions. In particular, quantitative phosphoproteomic studies will pave the way for a refined understanding of platelet properties.

Keywords: Quantitative mass spectrometry, multi omics, postranslational modification

P18.10 Establishing a High Throughput Proteomic Assay for Cardiac Troponin I in Heart Disease Diagnostics

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Introduction and Objectives: Cardiac Troponin I (cTnI) is the preferred diagnostic biomarker for various myocardial diseases. Phosphorylation of specific Ser/Thr residues on cTnI are relevant to several pathological conditions. For example, women have a lower diagnostic threshold for cTnI then men, and current techniques fail to diagnose adverse cardiovascular events. We hypothesize that the disease-induced forms of cTnI are increased in women and will facilitate risk stratification. This study focuses on establishing a highly sensitive, high throughput quantitative screening for cTnI and its disease-induced posttranslational modifications (14 phosphorylation sites and 2 proteolytic products) in tissue and blood samples by mass spectrometric analysis and ultra-sensitive Single molecule arrays (Simoa) to improve heart disease diagnostics in patients which failed to be diagnosed by current methods. **Methods:** An automated robotic high throughput intact protein immunoprecipitation assay was developed for identification and quantification of cTnI phosphorylation in tissue optimizing the sensitivity by comparing on-bead digestion with in-solution elutate digestion. Recombinant cTnI spiked with serum was analyzed using multiple reaction monitoring mass spectrometry (MRM-MS). Furthermore, the Simoa cTnI kit (Quanterix) was used to quantitate human TnI in serum. **Results and Discussion:** Successfully, an automated antibody-based cTnI enrichment from plasma was established and optimized . Only minor differences among the different IP methods were detected, although the on bead digestion proved most efficient. Overall approximately 80% of protein was recovered during enrichment compared to unenriched digests. The ultra-sensitive Simoa assay was capable of detecting cTnI in serum samples with an LLOD of 0.04 pg/ml as defined by recovery +/- 20% and CV<20. **Conclusion:** A quantitative assay was developed to enable the detectection of as little as 0.04 pg/ml cTnI in human serum and a novel method using an automated IP to quantify cTnI and its posttranslational modifications by MRM-MS in heart tissue was developed to improve the diagnosis of heart disease.

Keywords: cardiac Troponin I, biomarker, heart disease, proteomics

P18.11 Alterations of the Proteome and Transcriptome in Induced Sputum Samples in Early-Stage COPD Subjects

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Introduction and Objectives: Chronic obstructive pulmonary disease (COPD) is one of the most prevalent lung diseases, characterized by poorly reversible airflow limitation and incorporating both emphysema and chronic bronchitis. Cigarette smoking has been identified as the main risk factor for disease development and progression. In a basic model of COPD, the disease is initiated when the physiologic response mechanisms to cigarette smoke exposure are compromised, e.g. because of long-term exposure effects combined with aging-related changes. We conducted a case-controlled clinical study to investigate to which extent the biological effects in this chronic-exposure-to-disease model are reflected in the proteome and cellular transcriptome of induced sputum samples. **Methods:** In the design of the study, 60 age- and gender-matched individuals for each of four study groups were selected: current asymptomatic smokers, current-smoker COPD patients, former smokers, and never smokers (total of 240 subjects). Induced sputum was collected, the cell-free supernatant was analyzed by quantitative proteomics (isobaric-tag based), and the cellular mRNA fraction was analyzed by microarray-based expression profiling. **Results and Discussion:** The sputum proteome of current smokers (asymptomatic or COPD patients) clearly reflected the common physiological responses to smoke exposure, including alterations in mucin/trefoil proteins, peptidase regulators, and a prominent xenobiotic/oxidative stress response. The latter also induced the sputum transcriptome perturbations, which additionally revealed an immune-related polarization change. The (long-term) former smoker group showed minor observable effects when compared to never smokers.

Conclusion: In summary, our study demonstrates that sputum proteomics/transcriptomics analyses can capture the complex physiological response to cigarette smoke exposure, which appears to be only slightly modulated in early-stage COPD patients.

Keyword: COPD, Transciptomics, Quantitative proteomics, Systems biology

P18.12 Prioritizing Proteomics Translation Using Literature Data

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Introduction and Objectives: A major goal of the HUPO Human Proteome Project (HPP), and the field of proteomics at large, is to identify effective ways to broadly disseminate quantitative proteomics technologies. To attain maximal impact, the development of quantitative assays should be judiciously prioritized based on the importance of the proteins to research communities. This may be achieved by identifying biologically significant proteins such as using network analysis. An alternative approach is to identify which proteins are most "popular", i.e., most studied by researchers and thus for which quantitative assays are likely to be high in demand. **Methods:** We developed a data science method to identify the most studied proteins using publicly available literature data. We queried PubMed using specific search terms (e.g., "heart [MeSH term/All fields] or cardiac [All fields]" for cardiac-related articles). Using a custom software BD2KPubMed, we then tallied the occurrences of each protein being referenced to the retrieved articles, and further calculated the semantic distance between a protein with a given search term. A list of top "popular proteins" in each field is then retrieved. **Results and Discussion:** We retrieved highly investigated proteins in up to 14 major human and mouse tissues, including heart, brain, liver, kidney, lungs, and gut. Our analysis revealed that most of biomedical research is narrowly focused on few distinct, organ-specific proteins. The lists of top-50 proteins in each of the above six major organs illuminate a number of interesting observations on the biology of these systems. Besides aiding in the development and optimization of high-impact quantitative assays by the proteomics community, we used this approach to visualize the trend and focal points of biomedical research in each subject. **Conclusion:** We present a list of high-impact proteins and describe a generalizable method to monitor the focal points and trends of biomedical research.

Keywords: Translational proteomics, Quantitative assays, Data science

P18.13 Autoantigen Identification from Mitral Valve Proteomic Profiles of Rheumatic Heart Disease Patients

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Introduction and Objectives: Introduction: Rheumatic Heart Disease (RHD) is an infection induced autoimmune sequelae of Group A Streptococcal pharyngitis, affecting mitral valve in 70% cases. The burden of RHD continues to be a prominent cause of cardiovascular morbidity and mortality in developing countries like India. Involvements of a few cross reactive antigens have been identified yet the pathogenic mechanism is not clear. In here, we tried to identify such antigens from mitral valve tissues to provide insight into unexplained mechanism of the disease. Objective: Analysis of mitral valve proteins by proteomic approach from Rheumatic Heart Disease patients **Methods:** Mitral valve tissues were collected from RHD patients (n=25) and controls (n=10) along with serum samples from pharyngitis, ARF (Acute Rheumatic Fever) and RHD after Institute's ethical approval. Proteomic profile of tissue samples were carried by different methods like Western blot, LC-MS and validated by 2D-Gel Electrophoresis (2D-GE) followed by MALDI-TOF analysis. **Results and Discussion:** Results: Autoantigens were identified from mitral valve tissues of RHD patients by comparing their protein profile with controls. Western blot analysis of mitral valve proteins against ARF and RHD sera identified three distinct cross reactive proteins of ~79 kDa, ~55 kDa and ~38 kDa. These fragments were further confirmed by LC-MS analysis and MASCOT search and identified as serotransferrin, lactate dehydrogenase and vimentin. These proteins were validated by 2D-GE followed by MALDI-TOF analysis. Discussion: The study unveiled new autoantigens, serotransferrin and lactate dehydrogenase, from mitral valve of RHD patients whose role is yet to be decrypted. **Conclusion:** Protein expression profile of mitral valve, from RHD patients, highlighted some known as well as unknown proteins. Further elucidation of their associated pathway and gene interaction may also provide information for new therapeutic targets for prevention of the disease.

Keywords: Mitral valve, protein profile, autoantigens, Rheumatic heart disease

P18.14 High Definition Lipoproteomics Identifies Novel Proteins Predictive of Coronary Artery Disease

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Introduction and Objectives: Lipoproteins have an integral role in the pathogenesis of CAD. Quantitative proteomics is evolving to become an indispensable tool in the era of precision medicine. Using an unbiased lipoproteomic discovery workflow we sought to investigate the lipoproteomic differences between stable CAD patients and controls subjects and to reveal novel pathways/mechanisms that underpin this common disease. **Methods:** Stable CAD patients (n=21, mean ± SD; age 71yrs ± 3.15) were compared with age, sex and ethnicity matched control subjects (n=7; mean age 76yrs ± 6.65). All patients and controls were on statins to negate the effect on the lipoproteins. Plasma was co-incubated with a lipophilic affinity resin. Isolated lipoproteins were reduced, alkylated, digested and analysed using label-free high definition ion mobility enabled mass spectrometry. Raw data was analysed using Progenesis Qi software with a stringent false discovery rate of 1%. **Results and Discussion:** 168 proteins showed significant differential expression between the CAD patients and the control subjects ($P<0.05$). CAD patients had selective depletion of antioxidants; glutathione peroxidase 3 (GPX3) ($p=2.04E-4$), isoform 2 of clusterin ($p=8.24E-4$) and serum paroxonase-1 (PON1) ($p=1.85E-5$) compared with controls. Furthermore, CAD patients had enrichment of proteins concerned with inflammation; serum amyloid A-1 ($p=1.43E-7$), mannan binding lectin serine protease 1 (MASP1) ($p=1.35E-7$) and galectin-3-binding protein ($p=2.08E-4$) compared with the control subjects. A CAD biomarker model comprising of alpha-1-anti-chymotrypsin, angiotensinogen, apolipoprotein CII, carboxypeptidase B2, complement C3, inter alpha trypsin inhibitor heavy chain H4 and isoform 5 of hereditary hemochromatosis protein, accurately discriminated between all the CAD patients and the controls subjects ($p<0.001$). **Conclusion:** Patients with CAD have a distinct pathognomonic lipoproteomic cargo. The measurement of certain lipoprotein associated proteins may

assist in the early identification of such patients before symptom onset.

Keywords: coronary artery disease, lipoproteomics, biomarkers

P19: POSTER SESSION - PROTEIN MODIFICATIONS (OTHER THAN PHOSPHOPROTEINS)

P19.01 Truncated Protein Isoforms and Their Genesis in the Human Proteome

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Introduction and Objectives: The human proteome continues to be difficult to characterize, partly due to the complex mix of the multitude of protein species with numerous post-translational modification. One key irreversible modification is protein truncation that results in shortened protein species, which often have drastically altered bioactivity. Such protein truncations are results of protease cleavage, alternative translation, or alternative splicing. We have created the database TopFIND (http://clipserve.clip.ubc.ca/topfind/), the major resource for N and C termini data in five species including human. We present an analysis of the extent and genesis of N-terminal truncation of proteins in human and the software tool TopFINDer that allows the high-throughput analysis of the terminome. **Methods:** Our web accessible terminomics database, TopFIND, contains ~9,000 N-termini observed by terminomics techniques, as well as N-termini inferred from cleavage sites (~20,000), alternative splicing (~20,000), and alternative translation (~450). For our analysis, we downloaded human N-termini from TopFIND and compared experimentally observed to inferred N-termini. **Results and Discussion:** Comparing terminomics derived and otherwise inferred N-termini, we found that a majority of experimentally observed termini lie internal in proteins, consistent with individual terminomics analyses of tissues and cells, which consistently report ~50% of terminal peptides mapping internally in proteins. Of these internal N-termini, 86% had no inferred N-termini in close proximity and thus our knowledge of N-termini generating processes fails to explain these neo N-termini. Filling such knowledge gaps requires software support. We present our tool TopFINDer that automatically puts experimentally termini lists into context with all data in TopFIND thus reporting known and inferred termini, protein domain information, and protease statistics for cleavages relevant to the list. **Conclusion:** We expect that TopFIND will greatly support proteomics research by providing an access point for all termini related data, a growing area of research.

Keywords: Protein truncation, Protein modifications, Protease cleavage, Protein termini

P19.02 Quantifying N-Glycosylation Distribution in Therapeutic Antibodies

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Introduction and Objectives: Glycosylation patterns determine the stability and bio-disposition of therapeutic antibodies in vivo, as well as the efficacy, folding, binding affinity, specificity and pharmacokinetic properties; therefore a complete characterization of the biotherpeutic IgG glycosylation is required. IgG has a known set of glycan isotypes on a known glycosylation site. Therefore it is possible to predict and target specific glycopeptides for characterization. Here, we describe a robust MS strategy to profile and confirm IgG –derived glycopeptides and their subclasses. **Methods:** Microflow LC/MS (Eksigent) using C18 RP column was performed on a QTRAP® 6500 system (SCIEX) using multiple scan types. Using in silico prediction of all possible glycan structures, a comprehensive list of MRM transitions to all possible glycopeptide species was created. Confirmation of glycopeptides was done using both co-elution of structurally specific MRMs as well as fragment matching of MS/MS spectra using SimGlycan software. **Results and Discussion:** We tested this targeted workflow using therapeutically relevant antibodies such as Anti-Herceptin. The resulting specific MRM data for sugar marker ions as well as differentiating core and bisecting glycopeptide ions on the digested IgG sample provided more specificity because of multiple co-eluting transitions. In addition, MRM triggered MS/MS further confirmed the identity of glycopeptide structures. MRM confirmed 24 glycoforms for peptide 1, 17 of these were further confirmed with MS/MS on Anti-Herceptin. In the two IgG1 isotype antibodies analyzed, similar glycopeptides were detected, however the abundance of each glycoform varied between the different IgG1 proteins. **Conclusion:** The MS workflow has been well optimized; however there is an opportunity to further optimize the LC separation. Future work will test the approach with a range of separation techniques. Combining the optimized separation with this MS workflow will provide a powerful strategy for the confirmation and quantitation of glycopeptides and enables studies like antibody clearance in biological samples.

Keyword: Quantitative glycopeptide profiling group triggered MRM

P19.03 Quantitative Site-Specific ADP-Ribosylation Profiling of DNA-Dependent PARPs

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Introduction and Objectives: Poly(ADP-ribosyl)ation has long been recognized as a surveillance mechanism that contributes to preserve genomic stability. Upon sensing DNA damage, poly(ADP-ribose) polymerases (PARPs) are activated and catalyze the formation of poly(ADP-ribose) polymers (pADPr) that act as a molecular scaffold promoting the accumulation of repair factors at DNA lesions. Current efforts were focused on the identification of ADP-ribosylation sites and the determination of the extent of poly(ADP-ribosyl)ated residues within DNA-dependent PARPs by mass spectrometry. **Methods:** In vitro automodified PARP-1, PARP-2 and PARP-3 were treated with hydroxylamine to convert the pADPr attached to glutamate and aspartate residues into a hydroxamic acid conjugate that generates a spectral signature easily interpretable by common peptide annotation algorithms. The rationale behind this approach was that the cumulative spectral count would serve as an index of ADP-ribosylation activity on targeted residues. High abundance poly(ADP-ribosyl)ated peptides were repeatedly identified from multiple iterations, providing a simple method to estimate preferred modification sites.

Results and Discussion: Thousands of hydroxamic acid-conjugated peptides were identified with high confidence and ranked based on their spectral count. This semi-quantitative approach allowed us to locate the preferentially targeted residues in DNA-dependent PARPs. In contrast to what has been reported in the litterature, automodification of PARP-1 is not predominantly targeted toward its BRCT domain. Our results show that interdomain linker regions that connect the BRCT to the WGR module and the WGR to the PRD domain undergo prominent ADP-ribosylation during PARP-1 automodification. We also found that PARP-1 efficiently automodifies the D-loop structure within its own catalytic fold. Interestingly, additional major ADP-ribosylation sites were identified in functional domains of PARP-1, including all three zinc fingers. Similar to PARP-1, specific residues located within the catalytic sites of PARP-2 and PARP-3 are also major targets of automodification. **Conclusion:** Together our results suggest that poly(ADP-ribosyl)ation hot spots make a dominant contribution to the overall automodification process.

Keywords: PARP, DNA damage response, poly(ADP-ribose), post-translational modification

P19.04 The Quantitative Multiple PTMomics Study in INS-1E Cells in Response to Cytokines Stimulation

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Introduction and Objectives: The fine-tuned crosstalk of diverse protein post-translational modifications (PTMs) can coordinate the protein states, and synergistically regulate protein function in specific cellular conditions. Inflammatory cytokines in beta cell can affect the PTMs, cause cell apoptosis, and impair insulin signaling. Characterization of PTMs in INS-1E cells stimulated by cytokines can shed light on the involvement of inflammation in diabetes mellitus. **Methods:** We synthesized a novel cysteine-specific phosphonate adaptable tag, and developed a TiO2-based multiple PTMomics strategy for co-enrichment of Cys peptides, phosphopeptides and glycopeptides. Here we employed this strategy combined with the iTRAQ-based quantitative proteomic strategy to characterize the Cys modification, phosphorylation and glycosylation, and their cross-talk in INS-1E -cells stimulated by cytokines (IFN$\gamma$and TNFα) for 12h and 24h. **Results and Discussion:** The co-enrichment efficiency for Cys peptides and phosphopeptides was 87.32%. In total, 7378 unique Cys sites (3928 proteins) and 11102 unique phosphorylation sites(2450 proteins) and 1130 N-linked glycosylated sites (868 proteins) were identified. PTMs were identified in 5240 proteins, and 359 proteins contain all three PTMs. Significant change ($\geq$1.5 fold) was observed in 2711 phosphopeptides (1158 phosphoproteins) and 3148 Cys peptides (1511 proteins). The TNF/IFN stimulation induces ROS production, regulates the PTMs of proteins involved in cell apoptosis, metabolism and STAT1/IRF1 signaling pathway. The phosphorylation and Cys oxidation levels of STAT1, STAT2, STAT3 are increased, and involved in apoptosis and insulin signaling, indicating the involvement of multiple PTMs in cytokines mediated beta cell apoptosis. The glycosylation patterns and potential crosstalk of the three PTMs will be further analyzed. **Conclusion:** The high efficiency and specificity of the multiPTMs strategy enable it a promising tool for simultaneous characterization of multiple PTMs and the possible crosstalk. The cytokines stimulation in INS-1E cell can increase the ROS production, induce multiple PTMs change, trigger the STAT1/IRF1 pathway, and finally leads to cell apoptosis and impaired insulin signaling.

P19.05 Large-Scale Identifications of Post-Translational Methylation by LC-MS/MS Are Subject to High FDRs

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Introduction and Objectives: A growing number of broad-scale LC-MS/MS-based post-translational methylation site discovery experiments have indicated that methylation is widespread in the proteome. In interpreting any LC-MS/MS-derived data for the purposes of methylation site discovery, a common requirement must be met: methylpeptide sequence matches must be identified at acceptably low false discovery rates (FDRs) following sequence database searching. The standard method of removing probable false positive peptide identifications involves performing searches against reversed databases to estimate FDRs (target-decoy approach); peptide sequence matches are then filtered to meet an estimated FDR threshold. **Methods:** It is, however, forseeable that when dealing solely with methylpeptide sequence matches, the target-decoy approach may not correctly estimate FDRs. One proposed reason for this lies in the fact that the mass differences between numerous amino acids are identical to those observed for methylation. Another potential reason relates to the fact that glutamic and aspartic acid residues have been shown to undergo esterification reactions in sample preparation protocols that feature methanol or ethanol. To account for these potential issues, orthogonal methylpeptide validation techniques – that is, independent forms of methylpeptide validation applied in conjunction to sequence database searches – can be of value. However the use of orthogonal methylpeptide validation in broad-scale methylation site discovery studies remains sporadic; this is a reflection of the fact that in-depth studies into methylpeptide FDRs have yet to be performed. **Results and Discussion:** Here we aim to provide the first such systematic investigation into methylpeptide FDRs across a range of sample preparation workflows and mass spectrometric instrument platforms. Our data indicate that methylpeptide FDRs are consistently high (>80%) across a range of sample preparation techniques and instrument platforms, and that esterification reactions are only a minor source of false positive methylpeptide sequence matches. **Conclusion:** These data provide insights into the necessity of employing orthogonal methylpeptide validation in broad-scale LC-MS/MS analyses.

Keywords: heavy-methyl SILAC, methylation, False discovery rates, LC-MS/MS

P19.06 Proteome-Wide Prediction of the Human Protein Ubiquitination Sites

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Introduction and Objectives: Protein ubiquitination regulates multiple eukaryotic cellular processes including cell cycle progression, transcriptional regulation, DNA damage repair and immune response. Identification of ubiquitinated proteins sites is a basic step to the understanding of their molecular mechanism in biological systems. However, it is often time consuming and expensive to screen the protein ubiqutination sites by web-lab experiments. The computational approaches are promising to map the proteome-wide ubiquitination profiles, yet none of the existing computational models offer satisfying performance. **Methods:** UbiSiteXplorer was constructed based on two categories of

biological features: peptide sequence similarity and peptide sequence properties. All these informative protein sequence features and protein sequence similarity were separately integrated into 6 SVM prediction models according to feature category, each SVM model outputs a score for each sample indicating its probability to be ubiquitinated under this certain proof, then all 6 scores are further processed by Logistic Regression (LR) method and finally an LR score is given representing the probability of this sample to be ubiquitinated with all features combined together. **Results and Discussion:** We developed a new prediction system for ubiquitinated site prediction with improved accuracy and efficacy. By analyzing various biological properties we found that ubiquitination sites tend to be located at more structured regions, with bigger size and average accessible surface area, with higher hydrophobicity, average flexibility indices. To facilitate this strategy used by the community, we developed this strategy into a web service, namely UbiSiteXplorer. UbiSiteXplorer is designed to address the requirements not only for ubiquitinated site prediction but also for the proteome-wide presentation of human unbiquitome. **Conclusion:** UbiSiteXplorer shows high sensitivity and specificity against both cross validation and independent test. Though the online server, users may submit their query proteins, and the detailed information about the supporting evidence for the prediction will be returned. The web interface of UbiSiteXplorer is available at http://prodigy.bprc.ac.cn/ubisitexplorer.

Keywords: Ubiquitination site, Prediction, Online service, post-translational modification

P19.07 A Universal Chemical Enrichment Method for Mapping Protein N-glycosylation

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Introduction and Objectives: Glycosylation is one of the most common and important protein modifications in biological systems. Many glycoproteins naturally occur at low abundances, which makes comprehensive analysis extremely difficult. It is well known that the interaction between boronic acids and diols is one of the strongest reversible covalent interactions in an aqueous environment. This covalent interaction provides a great opportunity to catch glycopeptides by boronic acid, while the reversible property allows their release without side effects. **Methods:** One common feature of all glycoproteins and glycopeptides is that they contain multiple hydroxyl groups in their glycans. From chemistry point of view, the reversible convalent interaction between boronic acid and cis-diols can be used to achieve universal glycopeptide/protein enrichment. We applied this chemical enrichment method on large-scale analysis of N-glycosylation sites. In our experiments, we systematically optimized the binding conditions, including buffer pH, and washing times. By combining the optimized enrichment method and MS we comprehensively mapped protein N-glycosylation in HEK293T whole cell lysates. **Results and Discussion:** First, yeast whole cell lysates were chose to opimize several experimental parameters. The greatest number of glycopeptides was identified when the enrichment was run at pH 10. Next, the optimal number of washes after binding was investigated. Finally, four washes resulted in the largest number of unique glycopeptides identified. After the enrichment samples were thoroughly dried and treated with PNGase F in heavy-oxygen water, and fractionated by high-pH reversed HPLC, and subsequently analyzed with an on-line LC-MS system. In the biological duplicate samples, 665 and 687 N-glycosylation sites were identified in 1274 and 1289 unique glycopeptides, respectively. A total of 816 glycosylation sites were identified. After the optimization, we move on to study the HEK293T glycoproteome. **Conclusion:** This boronic acid-based chemical enrichment method can be extensively applied to many complex biological samples for the comprehensive analysis of protein glycosylation.

Keywords: Protein N-glycosylation, Boronic acid, Chemical enrichment, Post translational modification

P19.08 Proteomic-Based Discovery of the Human Arginine Methylome

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Introduction and Objectives: Arginine methylation is an abundant protein post-translational modification with known roles in transcriptional regulation, RNA metabolism and DNA damage repair. Arginine methylation is catalyzed by a family of at least 9 enzymes called protein arginine methyl-transferases (PRMTs). PRMTs collectively promote three distinct methylation variations: mono-methyl arginine (MMA), asymmetric di-methyl arginine (ADMA) and symmetric di-methyl arginine (SDMA). All PRMTs are known to be over-expressed, amplified or mutated in at least one oncological indication, suggesting arginine methylation may play an important role in cancer progression. Despite the prevalence and disease relevance of arginine methylation, relatively little is known about substrate identity, modification site, or which enzymes are responsible. **Methods:** Here we describe the combination of multiple large-scale proteomics approaches aimed at in-depth discovery of the human arginine methylome. To identify PRMT substrates and putative regulatory proteins, we have performed co-immunoprecipitation experiments of all 9 PRMTs from HEK 293T cells using multiple different affinity tags, followed by protein identification by mass spectrometry. Next, to further identify arginine methylation substrates and map specific sites of modification, we performed immunoenrichment of methylated peptides followed by high-resolution mass spectrometry, using antibodies specific to MMA, ADMA and SDMA. Lysates from 293T cells over-expressing each of the 9 PRMTs were used for these peptide IPs, allowing for determination of enzyme-substrate specificity. **Results and Discussion:** This large dataset is currently being analyzed to gain both a global picture of all proteins modified by arginine methylation in the human proteome, and also to understand specific methylation substrates targeted by each PRMT. We will present data encompassing topics such as analysis of methyl-arginine substrate function, sequence motifs, site localization relative to protein domains and tertiary structure, and evolutionary conservation. These results will provide an invaluable scientific resource, enabling discovery of roles for arginine methylation in diverse cellular processes and disease mechanisms. **Conclusion:** not applicable

Keywords: post-translational modifications, arginine methylation, proteomics

P19.09 Characterization of Intact Glycoproteins by CESI-MS under Native and Denaturing Conditions

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Introduction and Objectives: Protein glycosylation is an important PTM that plays crucial roles in various biochemical processes. Over 40% approved biopharmaceuticals are glycoproteins. Comprehensive structural and quantitative characterization of glycoproteins is necessary to understand the functions of glycoproteins. The limited choice of separation methods under either native (neutral pH) or denaturing conditions coupled with mass spectrometry (MS) is among major obstacles towards complete characterization of intact glycoproteins and their complexes. Here, we demonstrate the power of combining high performance capillary electrophoresis (CE) and high-resolution MS-in a single dynamic process for the analysis of glycoproteins at both native and denaturing conditions. **Methods:** Intact prostate specific antigen (PSA, Lee Biosolutions) and Erythropoietin (EPO, gift from Prof. Albert Heck) were separated using a CESI 8000 High Performance Separation and ESI Module (SCIEX) and analyzed on either a LTQ Orbitrap Elite or Exactive™ Plus EMR mass spectrometer (Thermo Scientific™). A prototype neutral coated capillary was employed in all experiments. **Results and Discussion:** A neutral coated capillary was used to minimize protein adsorption onto the silica surface, eliminate electroosmotic flow, and enable efficient CE separation of intact glycoproteins under both native and acidic conditions. Both conditions resulted in detection of an identical number of nearly baseline resolved dominant peaks. Due to the improved signal/noise and efficient separation enabled by CESI-MS under both denaturing and native conditions, we were able to identify additional glycoforms compared to direct infusion experiments (at least 2.5 times more PSA glycoforms were detected by CESI-MS). Interestingly, CESI-MS experiments under both conditions enabled qualitative and quantitative analysis of 20-40% more hybrid glycoforms migrating later than the sialylated/complex glycan species. **Conclusion:** Integration of CE with HRAM mass spectrometry enabled thorough characterization of intact PSA and EPO under native and denaturing conditions.

Keyword: intact glycoprotein CESI-MS

P19.10 Assessment of RA-Specific Citrullinated Peptides in Synovial Fluid by PRM-MS Analysis

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Introduction and Objectives: Citrullinated protein/peptide epitope is recognized by anti-citrullinated protein/peptide antibody (ACPA), which is a major diagnostic or prognostic marker for rheumatoid arthritis (RA). Together with ACPA, specific citrullinated protein/peptide autoantigens in the major inflammatory lesions of RA may provide valuable information for the development of early diagnostic and therapeutic intervention. Hence, we performed global citrullinated peptide profiling of synovial fluids using mass spectrometry (MS) to identify RA specific citrullinated peptides and validate them in an independent cohort of RA patients by Parallel Reaction Monitoring (PRM)-MS analysis. **Methods:** Synovial fluids obtained from RA (n=4) and osteoarthritis (OA) (n=4) patients were pooled, separately. Peptides in each pooled synovial fluid were extracted using centrifugal filtration and solid phase extraction, and then analyzed by a Q-Exactive. ScaffoldPTM (v.2.1.3) was used to validate peptide identification and citrullination site mapping. Elevated levels of RA-specific citrullinated peptides and their unmodified peptides are validated by multiplexed PRM-MS analysis with synovial fluids from RA (n=30), OA (n=15), and ankylosing spondylitis (AS) (n=5). **Results and Discussion:** Form LC-MS profiling of synovial fluids (RA vs. OA), we identified total 1094 peptides originated from 120 proteins. Among them 121 peptides of 9 proteins (FGA, FGB, SAA1, HMGN2, PTMA, SRGN, COL5A3, PRG4, and APOBR) were found to be citrullinated. Several FGA peptides citullinated at R271, R308, R367, R425, R426, and R547 were uniquely elevated in RA synovial fluid. To verify RA-specific citrullinated peptides, multiplexed PRM-MS analysis of selected peptides was conducted. **Conclusion:** We have identified unique citrullinated peptides present in RA synovial fluid. To identify representing citrullinated peptides in RA, we have undertaken multiplexed PRM-MS analysis of selected citrullinated peptides using RA, OA, and AS synovial fluids. The eventual results from the independent cohort studies will confirm unique citullinated peptide markers present in RA synovial fluid, which may provide their diagnostic or therapeutic values for RA.

Keywords: parallel reaction monitoring, Citrullination, rheumatoid arthritis

P19.11 Ubiquitin and the Kiss of Death under Stress Conditions

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Introduction and Objectives: Protein quality control pathways monitor the proteome to avoid the accumulation of misfolded proteins and their aggregation, which has been associated to aging, as well as many neurodegenerative diseases. Protein quality control pathways can either assist refolding or target terminally misfolded proteins for degradation; a major challenge is to decipher how these triage decisions are made at the molecular level. We used a combination of genetics and proteomics to uncover and characterize several novel protein quality control pathways upon stress. Notably, we have characterized several pathways that rely on E3 ubiquitin ligases to target cytosolic misfolded proteins for proteasome degradation. Using mass spectrometry we first identified which proteins were ubiquitinated after stress and identified several features associated to these proteins [1, 2]. We then combined enrichment of ubiquitinated peptides with SILAC in order to identify substrates of the Rsp5/Nedd4 E3 ligase to decipher how the ligase recognized misfolded proteins [3]. More recently, we further characterized how the Rsp5 E3 ligase is reprogrammed under heat stress conditions to target its substrates to the proteasome with the help of two deubiquitinases. Our data provide a common framework to better understand the targeting of misfolded polypeptides by quality control pathways that play a major role in protein homeostasis. 1 - Fang NN et al., Nat Cell Biol 2011 13(11):1344-52. 2 - Ng AH et al., Mol Cell Proteomics 2013 12(9):2456-67 3 - Fang N.N. et al., Nat Cell Biol 2014 16(12):1227-37 **Methods:** not applicable

Results and Discussion: not applicable
Conclusion: not applicable

Keyword: ubiquitin, proteasome, misfolding, stress

P19.12 Targeted PTM Analysis by SWATH-MS: High Confidence Site Assignment and Quantification

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Introduction and Objectives: The detection and quantification

of posttranslational modifications (PTMs) on proteins using mass spectrometry-based proteomics has enabled their association with biological functions, signaling mechanisms or diseases. In discovery proteomics, PTM detection is commonly facilitated by database or open modification searching in combination with site-localization scoring algorithms. Targeted proteomics in contrast, enables quantification of modified peptides using specific assays whose modification types and site-localizations are often inferred from discovery proteomics data or synthetic versions of the query peptides. To differentiate between closely related peptide species, manual validation is often required for complex samples by acquisition or extraction of site-specific diagnostic fragment ions. Here we present a novel algorithm that enables automated confidence assessment of PTM detection and site assessment for targeted proteomics. **Methods:** Implemented using SWATH-MS and the OpenSWATH software, the algorithm generates and tests different hypotheses based on low- to high-confidence annotated spectral libraries from discovery proteomics. Using unique ion signature (UIS) or diagnostic transitions and a modified target-decoy approach, the confidence of the modification and site-localization for each candidate peptide signal can be estimated. The algorithm is validated and benchmarked using a new synthetic gold standard dataset. **Results and Discussion:** We demonstrate the application of the algorithm to modified human blood plasma proteins based on a longitudinal study of pairs of monozygotic and dizygotic twins. Using open modification searching for assay generation and our novel algorithm for targeted proteomics, we can quantify differentially modified proteins across hundreds of samples. This allowed us to assess the heritability of specific modifications in human blood plasma proteins and to differentiate between inherited and environmentally induced changes in PTMs. **Conclusion:** In comparison with existing methods, this enables targeted quantification of PTMs with high throughput and independent confirmation of modification type and site-localization. We demonstrate the ability of the system to accurately quantify modified peptides across large numbers of samples.

Keywords: posttranslational modifications (PTMs), Bioinformatics, data-independent acquisition (DIA), SWATH-MS

P19.13 Impact of Limited Proteolysis on Protein Function in Breast Cancer Mapped by Positional Proteomics

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Introduction and Objectives: The proteome is highly dynamic. Proteins, once translated, cannot be treated as static modules with a pre-defined function but instead are frequently converted by post-translational modification (PTM) into species with altered localization and function. Among the most fundamental and consequential protein modifications is limited proteolysis. In this study we investigate changes in the proteolytic conversion of proteoforms into a new proteoforms and the implications on protein function and localization on a proteome wide scale. **Methods:** We developed a unique set proteomic and bioinformatics of techniques to identify protein termini including their modifications and to derive the functional competence of the proteins from these identifications. The murine 4T1, 66cl4, 67NR breast cancer model is used. Protein termini are di-methylated or isotope labeled, terminal peptides enriched by TAILS and identified by LC-MS/MS. We use new computational models and network inference to predict changes in protein based on the TopFIND knowledgebase. Using position-weight-matrices built from data obtained by Proteomic Identification of Protease Substrate specificities (PICS) and TopFIND we identify the underlying proteolytic events. **Results and Discussion:** We present quantitative profiling of protein termini and their modifications in a mouse model for breast cancer metastasis. Comparative analysis reveals distinct proteolytic post-translational modification processes to be prevalent in murine breast tumors of increasing metastatic potential. Computational modeling reveals the underlying protease web and shows modulation of major cancer pathways by proteolysis. We then use over-expression and knock-down of select proteases detailed biochemical and cell biological validation and show protease specific effects on proliferation, tumor growth and metastasis. **Conclusion:** The proteomic data and show that proteolysis has strong impact on breast cancer progression, for example, by changing the function and location of multi-functional proteins.

Keywords: proteolysis, Protein termini, cancer, post-translational modification

P19.14 Identifying the Interactome of Human RNA-Binding E3-Ubiquitin Ligases

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Introduction and Objectives: Ubiquitin is a prevalent post-translational modification involved in regulation of protein function and stability. Recently, a group of Human RNA-binding E3-ubiquitin ligase (E3-RBPs) were identified and implicated as novel players in ubiquitin-mediated regulation of RNA processing. We sought to assess whether E3-RBPs are integrated into RNA-associated protein complexes. **Methods:** A target list of E3-RBPs was derived from a recently published census of Human RNA-binding proteins and a subset of E3-RBPs was subjected to affinity-purification and mass-spectrometry (AP-MS) analysis in HEK293T cells. **Results and Discussion:** We observed interactions between several E3-RBPs and RNA associated protein complexes. In particular, a previously uncharacterized E3-RBP, MKRN2 was observed to interact with a known complex implicated in nuclear mRNA export. In addition, we observed a series of novel interactions between the E3-RBP, RBBP6, and a cleavage-and-poly-adenylation subcomplex. **Conclusion:** These initial studies indicate that E3-RBPs may be candidate interactors and possible regulators of RNA-associated protein complexes.

Keywords: Ubiquitin Ligase, RNA Associated Protein Complexes, RNA Binding Proteins

P19.15 Analysis of N-Terminal Acetylation on RNA Polymerase II in Saccharomyces Cerevisiae

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Introduction and Objectives: N-terminal acetylation (Nt-Ac) is a common co-translational modification among eukaryotic proteins involving the transfer of an acetyl group to the α-amino group of the first residue by N-terminal acetyltransferases (NATs). NATs play an important role in the synthesis, stability and localization of proteins, yet little is known about their functional and pathophysiological implications at the molecular level. This study aims to identify the Nt-Ac state of the evolutionary conserved

RNA polymerase II (RNAP II) in Saccharomyces cerevisiae to better understand the roles of NATs and protein Nt-Ac in biology and disease. **Methods:** RNAP II was tandem affinity purified in S. cerevisiae using TAP-Rpb11. Peptide mixtures were prepared using five different combinations of endoproteinases. Each differentially-digested sample was independently analyzed by a 10-step Multidimensional Protein Identification Technology (MudPIT) using a Velos-Pro-Orbitrap-Elite hybrid mass spectrometer. **Results and Discussion:** Most RNAP II subunits with very small neutral amino acids such as Ser in the second position showed efficient iMet cleavage and subsequent acetylation of the first residue. Subunits with small hydrophilic amino acids such as Asp and Asn in the second position retained 85% to 100% of the iMet with the majority of them being acetylated. Subunits with large hydrophobic amino acids such as Phe and Ile in the second position retained >98% of the iMet with <2% being acetylated. Rpb1 with Val in the second position showed efficient iMet cleavage with <2% Nt-Ac-Val and Nt-Ac-Met. Lastly, Rpb9 with a tandem Thr in the second and third position showed 100% iMet cleavage and 70% Nt-Ac-Thr2 and 30% acetylation on Thr3 side chain. **Conclusion:** N-terminal acetylation was detected on all 12 RNA polymerase II subunits in S. cerevisiae. Further comparative analysis of NATs between yeast and higher eukaryotes in normal and mutated states might provide new insights into their functions and co-evolution of NATs and their substrates.

Keywords: N-terminal acetylation, RNA polymerase II, Multidimensional Protein Identification Technology

P19.16 SWATH-MS Technology for Citrullination: A Target for Neurodegenerative Diseases

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Introduction and Objectives: Citrullination, the post-translational (PTM) conversion of arginine to citrulline by the family of peptidylarginine deiminase (PADs), has been commonly implicated as abnormal pathological features in neurodegenerative diseases such as prion diseases, multiple sclerosis and Alzheimer's disease. This PTM could be a target for novel diagnostic or therapeutic agents. Therefore, an unambiguous and efficient method to identify citrullinated proteins and their modified residues is of extreme importance. **Methods:** The workflow combined human brain (n#5) protein solubilization, incubated in the presence and absence of PAD, in-solution LysC digestion, and acquisition of high-resolution full scan and fragmentation spectra. Spectral ion libraries of the maximally citrullinated proteomes were constructed by data dependent acquisition on an AB Sciex 5600 TripleTOF and the raw data was searched with ProteinPilot™ 5.0 to create a spectral ion library with emphasis on transitions that distinguish the citrullination site. SWATH-MS fragment ion maps were generated and individual SWATH-MS runs were matched against the spectral libraries created in the presence or absence of PAD. **Results and Discussion:** Using SWATH-MS method we were able to extract 88 citrullinated peptides from the PAD minus library and 161 citrullinated peptides from the PAD plus library. Interestingly, only 56 of these citrullinated peptides overlapped when the files were matched against each library. The majority of the citrullinated proteins were enzymes involved in the TCA chain. Within the cytosolic fraction, the enzymes of glycolysis, membrane binding and axon guidance and maintenance proteins were prominent. Nuclear targets included histones, elongation factor. Citrullinated proteins may become a useful marker for human neurodegenerative diseases. Therefore it is important to create a trustful method that can distinguish and quantities of citrullinated peptides in complex samples. **Conclusion:** This work overcomes the challenges of the identification and quantification of citrullinated peptides in complex mixtures.

Keywords: Neurodegeneration, SWATH MS, posttranslational modification,, Citrullination

P19.17 A Dynamic Picture of the Ubiquitinome upon Proteasome Inactivation

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Introduction and Objectives: The 26S proteasome is a 2.5 MDa protein complex, which degrades unneeded and damaged proteins in the cell. As such, it is critical in regulating proteostasis and controls key regulator abundance levels. Malfunctioning of the ubiquitin–proteasome system has been implicated in diseases such as cancer and neurodegenerative disorders. On the other hand, in cancer therapeutics the induction of apoptosis by proteasome inhibition using drugs is widely used. Current strategies are directed towards the development of more selective inhibitors that target the proteasome regulatory subcomplex and have less side-effects. We take a proteomics approach to dissect the molecular mechanisms of the proteasome regulatory subcomplex, which is essential for the development of better proteasome inhibitors. **Methods:** Targeted proteasome inactivation by selective RNAi knockdown or drugs is monitored at the proteome and ubiquitinome levels using a SILAC approach in Drosophila. **Results and Discussion:** Over 5,000 proteins and 10,000 diGly peptides were identified and quantified. After brief inactivation by drugs, proteins involved in stress response, cell cycle regulation, apoptosis and the UPS were upregulated (e.g., Hsp proteins) and accumulated. After prolonged inactivation, the abundances of several 100s of proteins were altered. Similar effects were observed after inactivation of the proteasome with RNAi knockdown of different subunits. Protein ubiquitination dramatically increased upon proteasome inactivation. Interestingly, many proteins showed dynamic ubiquitination changes in opposite directions on different target lysine residues within the same protein. Proteomic analysis of individual RNAi knockdown of three proteasome bound deubiquitinating enzymes indicated that each of them has a different and specific function. Finally, proteasome interactome profiling under different experimental conditions using LFQ based quantitation suggested that the proteasome itself is a dynamic complex that recruits different partners and/or (sub)complexes under specific conditions. **Conclusion:** Global analysis of the dynamic proteome and ubiquitinome after proteasome inactivation gives detailed insight into regulatory mechanisms of the proteasome.

Keywords: proteasome, ubiquitinome, SILAC, dynamics

P19.18 Comprehensive Proteomic Analysis of N-Terminal Acetylated Proteins in Caenorhabditis Elegans

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Introduction and Objectives: Protein N-terminal acetylation is one of the prominent post-translational modifications (PTM), which is broadly observed in eukaryotic proteins. Although the biochemical mechanisms of protein N-terminal acetylation have been extensively studied, its functional implication in organismal level remains largely elusive. To investigate any potential biological implication involved in N-terminal acetylation under metabolic

stress conditions, we utilized the free-living soil nematode, Caenorhabditis elegans,a well-established model organism in genetics and molecular biology. **Methods:** We performed nanoLC-MS/MS based analysis of protein N-terminal acetylation as well as quantitative comparisons between well-fed and starved animals by the label-free and the tandem mass tag (TMT) labeling analysis. **Results and Discussion:** Total 1926 N-terminal acetylated proteins corresponding to 3273 N-terminal peptides were identified by combining different proteases (Trypsin, LysC, GluC and chymotrypsin) followed by high resolution MS. The GO term analysis showed that majority of Nt-acetylated proteins is related to metabolic processes (32.9%). The quantitative analysis between the well fed vs. starved group revealed a group of proteins in which the Nt-acetylation is enriched in both conditions. We have selected two metabolic proteins, fructose 1,6-bisphosphatase/FBP-1 and transaldolase, which were overly Nt-acetylated when the animals are under starvation stress. The N-terminal amino acid sequence is very well conserved compared to that of humans. The transgenic animals, which express translation GFP fusion of FBP-1 and transaldolase were generated to examine protein stability. The transcripts level of the two genes remained unchanged upon starvation. By contrast, the GFP expression was markedly reduced when the animals were starved for 16 hrs, suggesting that the abundance of these proteins was modulated in translational or post-translational level. **Conclusion:** Here we demonstrate an analytical platform to analyze the N-terminal acetylated proteins in a model organism C. elegans. Our data show that the Nt-acetylation profile is dynamically modulated upon acute metabolic stresses.

Keywords: N-terminal acetylation, C. elegans, post-translational modification, metabolic stress

P19.19 Modulation of Surface Sialylation of HeLa Cells upon Different Growth Factor Stimulus

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Introduction and Objectives: Sialic acids are a family of more than 30 derivatives of neuraminic acid, typically found as terminal components of glycoproteins, proteoglycans and glycolipids in the surface of the cells. Due to their location in the cells, they have a key role during the first steps of the homeostasis acting in communication, interaction and defence. It has been observed a modulation in surface sialylation in different kind of cells upon stimuli. However, very few studies revealing the role of glycosylation in these processes have been conducted. The aim of this study was to investigate the alteration of sialylation on surface glycoproteins on HeLa cells after short stimulation using different growth factors. **Methods:** Membrane proteins from HeLa cells were enriched by Na2CO3 treatment and ultracentrifugation after EGF, TGF-α and HB-EGF stimulation for 5 min. The changes at the N-linked sialylated glycopeptides upon stimulation were assessed using iTRAQ 4-plex labelling. After digestion and dephosphorylation, iTRAQ labelled sialylated glycopeptides were enriched using TiO2, deglycosylated using PNGase F, fractionated using R3 at high pH and subsequently analysed by nLC-MS/MS using an Orbitrap Fusion. **Results and Discussion:** A preliminary experiment has shown that acute EGF stimulation of HeLa cells produces a significant desialylation of glycans on glycoproteins involved in cell adhesion, cell-cell communication and migration, and degradation or internalization of surface receptors. We also observed increased sialylation on glycoproteins on the surface of HeLa cells after short time EGF stimulation suggesting the presence of ecto-sialyltranferases or trans-sialydases on the surface of the cells. The present MS-based sialylation characterization strategy has allowed the study of the modulation of the sialylation pattern depending on the stimulation of different cellular pathways. The results suggest that changes in the sialylation pattern have a great influence on the cellular pathways and internal protein phosphorylation. **Conclusion:** Modulation of sialylation is dependent of different growth factors stimulus.

Keywords: Glycoproteomics, Sialylation

P19.20 Characterizing the Dynamic Change of Global and Site-Specific S-Nitrosoproteome and Glutathionylome

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Introduction and Objectives: S-nitrosylation, specifically targeting on cysteine residues by nitroso moiety, is a reversible post-translational modification that mediates important nitric oxide (NO)-based signaling in vivo. It can be reduced with glutathione to generate the glutathionylation that functions as a redox-sensitive switch and regulate redox signaling transduction. Due to the analytical challenges in their low abundance and labile nature, the dynamic interplay between S-nitrosylation and glutathionylation remains intangible. A quantitative approach for site-specific identification and quantitation of the S-nitrosylation and glutathionylation level on the same cysteine of proteins is needed to delineate their dynamic relationship. **Methods:** The Raw264.7 macrophage cells were stimulated with or without SNAP/L-cysteine for 10 to 60 min. Total cell lysate were harvested, and synthetic GSH@biotin was added into each sample for glutathionylation, following by S-alkylating biotin switch method and tryptic digestion. After affinity purification, the glutathionylated and nitrosylated peptides were analyzed by LC-MS/MS, searched by Mascot algorithm, and quantified by IDEAL-Q software. The candidate target of S-nitrosylation and glutathionylation was further validated by Western blotting. **Results and Discussion:** The results showed that S-nitrosylation signal was significantly increased in NO-stimulated cells and reduced by glutathionylation in time-dependent manner by Western blotting. Based on data-dependent MS/MS spectra, the S-nitrosylation and glutathionylation on cysteine residue can be site-specifically identified by mass shift of the characteristic marker ions from biotin or glutathione modification. On the proteome scale, 113 S-nitrosylated and glutathionylated peptides were concomitantly quantified and may be the common targets for inter-conversion of S-nitrosylation and glutathionylation, including Cys73 on thioredoxin. In addition, the structural diversity analysis showed that S-nitrosylation prefers to modify on positively charged and hydrophobic region, and glutathionylation prefers on acidic motifs. **Conclusion:** Our methodology may provide an effective proteomic approach to unravel the potential molecular targets and its dynamic change for S-nitrosylation and glutathionylation, which will allow the better understanding for redox switch regulation.

Keywords: S-nitrosylation, Glutathionylation, Redox switch, Dynamic change

P19.21 SanxotGhost: Toward the Global Analysis of the Posttranslational Modificome

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Introduction and Objectives: Given that posttranslational modifications (PTMs) of proteins play essential roles in most biological processes, the

systematic analysis of PTMs can reveal many insights into cellular function. As a complement to the weighted spectrum, peptide and protein (WSPP) model, the statistical approach we previously developed for the quantitative analysis of high-throughput proteomics experiments, here we present "SanxotGhost", a new statistical framework aimed at the quantitative analysis of wide variety of PTMs in deep proteomics experiments. In parallel, we are also developing a new approach to speed up the identification of PTMs in these experiments. **Methods:** The WSPP model was used to determine the technical variance at the spectrum, peptide, and protein level separately in proteomes. These variances were then used to calculate the standardized peptide ratio of the modified and non-modified forms using an algorithm that corrects for the local degrees of freedom at the protein level. When the protein ratio was missing (orphan peptides), standardized protein ratio, calculated using the grand mean of the entire population, was used instead. The rapid identification of PTMs was based on the probability distribution of peptide random match in decoy databases. **Results and Discussion:** PTM quantitation was validated with simulated random modifications in a null hypothesis experiment where peptide populations were checked for normal distribution. The method has been evaluated using massive experimental data from a wide variety of proteomes like infarcted pig myocardium, mitochondria from mouse ischemic heart and human plasma from pre-clinical subjects, where SanxotGhost has enabled the quantitation of numerous different PTMs. In combination with the PTM identification approach, which is being tested in different proteomes, SanxotGhost could pave the way for comprehensive analysis of the modificome. **Conclusion:** The multiplexed, quantitative analysis of PTMs on the large scale could be possible based on fast, reliable identification and quantitation of PTMs.

Keywords: PTM identification, PTM quantification, PTM FDR correction, Peptide Random Matching

P19.22 Automating Optimization of Compensation Voltages for Differential Mobility Separation Using Skyline

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Introduction and Objectives: When quantifying peptides in the complex matrices typical in proteomics research, one must to try to avoid interferences that could confound results quality. With QTRAP® system technology, one has added options for higher selectivity quantitation in addition to MRM analysis, the MRM3 workflow and the differential mobility separation (DMS) workflow. Use of DMS can improve quantitation of peptides by reducing both specific confounding interferences as well as reducing general background noise, simplifying peak integration. Each peptide of interest will have a unique compensation voltage (CoV) and therefore must be tuned like all compound dependent parameters on a mass spectrometer. In this poster, we will demonstrate a new workflow for the automated optimization of CoV values using Skyline Software, using an iterative tuning strategy. **Methods:** Separation of a trypsin digest of human plasma was performed on an nanoLC™ 425 System (SCIEX) operating in microflow mode. The MS analysis was performed on a QTRAP® 6500 system (SCIEX) equipped with SelexION® Technology (SCIEX). Skyline Daily version 3.1.1. was used to create an Analyst® Software acquisition method and optimize in an iterative fashion compensation voltages (CoV). MultiQuant™ software was used to export peak areas into a computational Excel sheet to show %CV versus % of peptides. **Results and Discussion:** We demonstrated the workflow using 88 peptides to 39 proteins in human plasma. CoV tuning can be used in combination with other Skyline optimization features.Using optimized CoV values, interferences for some of the peptides could be significantly reduced. **Conclusion:** DMS provides an orthogonal level of selectivity by separating components based on their chemical properties and mobility.The iterative approach to tuning the compound dependent CoV value has been implemented in Skyline software, allowing for the easy, rapid optimizing of many peptide CoVs in a small number of LCMS runs.

Keyword: Skyline, DMS, CoV optimization , peptide quant

P19.23 PAD4 Regulates Cellular Polyamine Production through Citrullinating the Negative Regulator Antizyme

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Introduction and Objectives: Peptidylarginine deiminase type 4 (PAD4) catalyzes the conversion of protein arginine to citrulline, which is called citrullination, a post-translational modification of proteins. PAD4 plays crucial roles in inflammation, differentiation, apoptosis, and histone-related gene regulation. In several human diseases, such as rheumatoid arthritis (RA) and cancers, PAD4 activity is dysregulated and the expression of PAD4 is highly found in disease-related tissues. Here, we demonstrate a novel substrate of PAD4, antizyme (AZ), the negative regulator of ornithine decarboxylase (ODC), which is the rate-limiting enzyme for polyamines biosynthesis pathway. Several studies have reported that polyamines play an important role in T cell activation and proliferation. In this study, we aim to clarify the possible mechanism that controls ODC activity through PAD4-catalyzed AZ citrullination. **Methods:** After in vitro citrullination, LC-MS/MS was used to identify the citrullinations sites of AZ. The functional assays including ODC inhibition assay, analytical ultracentrifugation, and in vitro degradation were performed to illustrate the functional difference between AZ and citrullinated AZ. The inducible expression system of PAD4 in Jurkat T cells was established and the cellular ODC activity and degradation were detected in the presence or absence of PAD4 expression and AZ citrullination. **Results and Discussion:** Citrullinated AZ showed the impaired ability in binding, inhibition with ODC, in addition to poor tendency of promoting ODC degradation. We applied LC-MS/MS analysis to study the site-specific information of AZ citrullinations after in vitro citrullination, and eight citrullinations sites were detected. According to our previous studies and MS results, we mutated these sites and found that C-terminal citrullination showed a significantly attenuation on inhibition of ODC. **Conclusion:** We demonstrate how citrullinations on AZ affect its inhibitory ability to ODC and provide the direct evidence to strengthen the link of PAD4 and polyamines biosynthesis pathway.

Keywords: T cell activation, Citrullination, Peptidylarginine deiminase, ornithine decarboxylase

P19.24 Protein-Adductome Analysis for Human Exposure-Monitoring with Environmental and Endogenous Mutagens

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Introduction and Objectives: DNA adducts have been used for an exposure marker of reactive chemicals in our environment but protein adducts can also be used for such purpose with higher sensitivity. Specific hemoglobin N-terminal adduct with glucose (A1C) or glycidol have already been reported. **Methods:** We took more comprehensive approach as "protein adductome" analysis using LC-MS/MS analysis (Advance nano-LC with Q-Exactive). A quantitative analysis was done by the Progenesis LC-MS software. **Results and Discussion:** We started with a detection of glycidol adducts of rat and human hemoglobin. We could not detect N-terminal adducts but could detect cysteine adducts only in rat. Then we targeted human serum albumin (HSA) because it has a reactive free cysteine residue (Cys34). In vitro treatment of HSA with various alkylating agents resulted in a formation of their adducts at Cys34 together with several basic amino acids. Reaction preference was different among those sites for each alkylating agents. We have extended these approaches to comprehensive detection of HSA Cys34 adducts. Using a similarity of MS/MS spectrum and retention time of un-modified Cys34-containing peptide, we have detected several adduct candidates with known and unknown structures. We will discuss on an efficient approach for the detection of unknown adducts and their confirmation. For endogenous protein adducts, we have analyzed glycation and 4-hydroxylnonenal adducts with HSA. It is interesting to analyze the relationship of these adducts against aging or obesity. **Conclusion:** The comprehensive detection of protein adducts enables human exposure monitoring for environmental and endgenous mutagens.

Keywords: adductome, alkylation, mutagen, exposure marker

P19.25 Identifying Protein Targets of Reactive Metabolite Covalent Binding by LC-MS/MS

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Introduction and Objectives: Drug-induced toxicity is a major obstacle in drug development, and is linked to the formation of reactive metabolites that can covalently bind to proteins. The liver plays a crucial role in the metabolism of xenobiotics, and hence the formation of reactive metabolites. Variations in the proteins targeted by different reactive metabolites could explain the range of toxicological effects and there is a need for improved methods for identifying these targets. Two strategies have been developed for the identification of protein targets of reactive metabolites. The first strategy is based on a 2D-LC-HR-MS/MS analysis of samples. The second strategy uses the popular click chemistry azide-alkyne Huisgen reaction for its ability to tag and purify targets of interest. **Methods:** Rat liver microsomes were incubated with acetaminophen and then digested. Resulting peptides were then cleaned up by solid-phase extraction and fractionated using strong cation exchange prior to a reverse-phase ultra-high pressure chromatography directly coupled to a high-resolution QqTOF mass spectrometer. APAP-treated samples were compared to the control samples using peptide spectral matching, statistical differential analysis, and a custom-built delta-mass filtering tool to pinpoint potential APAP-modified peptides, which were later rejected or confirmed based on their absence from the control samples. Alkyne-containing analogs of drugs known to form reactive metabolites were incubated in vitro resulting in the covalent binding to numerous protein targets. The alkyne moiety incorporated on the drug analogs acts as the handle for the clickbased purification. Purification via reaction with a custom azide-containing solid phase resin was used. Click-purified samples were analysed by UHPLC-HRMS/MS. **Results and Discussion:** not applicable
Conclusion: Two different strategies have been compared for the identification of protein targets of reactive drug metabolites. The click chemistry method shows much more promise in terms of rapidly identifying protein targets, however suffers from the fact that drug analogs need to be synthesized for this strategy.

Keywords: reactive metabolite, covalent binding, click chemistry, protein targets

P19.26 Mass Spectrometry-Based Analysis of Dynamic Protein Cysteine Modifications

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Introduction and Objectives: Ischemia/reperfusion (I/R) induces cardiomyocyte death and reactive oxygen species (ROS) generation. Reversible redox modifications of Cys thiols in the forms of protein sulfenylation (SOH), S-nitrosylation (SNO) and S-glutationylation (SSG) are widely implicated in myriad pathophysiological processes including cardiomyopathy and cardioprotection. Although the reversible cysteine modifications of cellular proteins has been reported as a key mechanism for nitric oxide (NO)-mediated cytoprotective effects in cardiovascular system against I/R injury, the underlying mechanism remains elusive. **Methods:** For in vitro experiments, embryonic rat cardiomyocytes (H9c2 cells) were exposed to hypoxia/reoxygenation (H/R) in control medium with or without S-nitrosoglutathione (GSNO). For in vivo study, ischemia was created in mice by ligating the left anterior descending coronary or ischemia followed by reperfusion for studying myocardial injury. Our previously established sequential iodoTMT-switch method was adapted to identify the reversible cysteine modification sites and to quantify the different extent of thiol modifications generated upon oxidative stress in the presence and absence of GSNO. The non-modified cysteines were first irreversibly alkylated with iodoacetamide (IAM), and the S-nitrosylated thiols were reduced with ascorbate prior to conjugation with iodoTMT1 tags, then other reversible modified thiols were reduced and labeled by TCEP and iodoTMT2 tag, thus allowing the selective enrichment of peptides containing formerly reversibly modified cysteines by anti-TMT resin. **Results and Discussion:** GSNO attenuates lactate dehydrogenase (LDH) leakage in cell model of H/R, and significantly reduced myocardial infarct size and cardiac troponin-I (cTnI) levels in mouse model of myocardial I/R injury. Collectively, we have quantitative dynamic change in various Cys-redox modifications from H9c2 cell undergoing H/R with and without adding GSNO, indicative of individual differences in susceptibility to S-nitrosylation or S-glutathionylation. **Conclusion:** We thus demonstrate how quantitative analysis of various Cys-redox modifications occurring in biological samples can be performed precisely and simultaneously at proteomic levels.

Keywords: S-nitrosylation, S-glutathionylation, redox-proteomics, Ischemia/Reperfusion

P19.27 Enhanced Purification of Ubiquitinated Proteins by Hybridized Ubiquitin-Binding Domains

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Introduction and Objectives: Posttranslational modification of proteins by ubiquitin (Ub) plays an important role in diverse cellular events, including cell division, differentiation, signal transduction and protein trafficking. Enrichment of ubiquitinated proteins is one of the key points for global analysis of ubiquitome. Previous reports showed that tandem repeated ubiquitin-binding domains (UBDs) is an attractive reagent for the purification of ubiquitinated proteins under native conditions, especially for ubiquitinated proteins with poly-Ub chains. However, different UBDs have varied features against specific types of poly-Ub linkages. Therefore, the screening and development of unbiased and highly efficient enrichment technology with UBDs is necessary for global ubiquitome study. **Methods:** In this study, we quantitatively analyzed the affinity features of a series of UBDs to all of the seven kinds of ubiquitin chains and ubiquitin monomer on protein substrates with the SILAC-Ub-AQUA approach. **Results and Discussion:** By applying these two optimized hybridized UBDs for affinity purification of UBCs, we successfully identified and confirmed 2790 and 3145 ubiquitinated proteins from yeast and mammalian cell, respectively. Among them, about 30% are newly identified substrates. The function of these newly identified UBCs has also been discussed. **Conclusion:** These results demonstrate that ThUBD is a promising reagent for the global ubiquitinated proteome research. This approach, bypassing the need of overexpressed tagged variants of ubiquitin and the use of antibodies to recognize ubiquitin remnants, is especially constructive to the analysis of tissues or clinical samples at protein level, further to identify novel biomarkers and targets for drug development.

Keyword: ubiquitination, ubiquitin binding domain (UBD), affinity purification, proteomics

P20: POSTER SESSION - IMAGING MASS SPECTROMETRY

P20.01 Matrix Coating Assisted by an Electric Field (MCAEF)-LDI Imaging of Prostate Cancer Tissue Biomarkers

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Introduction and Objectives: Prostate cancer pathogenesis needs to be fully deciphered, and the ongoing discovery of new and more reliable biomarkers to stratify the onset and progression of this disease is of great importance. In this study, we applied a newly-developed technique -- matrix coating assisted by an electric field (MCAEF) -- in combination with LDI-MS, to more comprehensively image endogenous compounds that allow the clear differentiation between the cancerous and non-cancerous regions of human prostate tissue. **Methods:** Prostate cancer (stage II) tissues were cryosectioned and thaw-mounted onto ITO-coated glass slides. Using matrix coating assisted by an electric field (MCAEF), the tissue sections were coated with various matrices, including quercetin, 9-aminoacridine (9-AA), and sinapinic acid (SA). 12-Telsa FTICR and MALDI-TOF/TOF instruments were used for metabolite and protein imaging, respectively. Accurate mass measurements, MALDI- or LC-MS/MS analysis, and metabolome or proteome database searches were performed for biomolecule identification. **Results and Discussion:** Using quercetin and 9-AA as two complementary MALDI matrices, (+)/(-)MCAEF-LDI/FTICR MS led to the imaging of 864 metabolites, of which 147 were uniquely detected in the non-cancerous cell region and 264 were uniquely detected in the cancerous cell region. 51 additional metabolites showed distinct distributions ($p<0.01$, t-test) between the two regions. Overall, 53% of the metabolites detected showed significantly different distributions in cancerous versus non-cancerous cell regions. Using SA as the matrix with MCAEF-LDI/TOF MS allowed the successful detection of 242 peptide and protein signals, with 64 species being uniquely detected in the cancerous region. Three potential cancer biomarkers were verified by immunohistochemical staining. In summary, the differential distribution of these biomolecules indicates significant molecular alterations in the prostate cancer cells. **Conclusion:** This study reports the largest group of biomolecules in prostate cancer imaged thus far by MALDI-MS and shows the potential of MCAEF-LDI MS imaging for enhanced in situ detection of biomarker candidates for prostate cancer.

Keywords: MCAEF, LDI-MS imaging, prostate cancer, biomarkers

P20.02 An Investigation into Multi-Model Tissue Imaging on a Single Section by DESI and MALDI MS

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Introduction and Objectives: Desorption electrospray ionization (DESI) is used in the field of mass spectrometry imaging (MSI). DESI results are of very high-quality, for lipids and small molecules similar to matrix assisted laser desorption ionization (MALDI) imaging technique. Here we present work showing the potential to analyze a single tissue section by DESI imaging with a subsequent analysis by MALDI imaging of the same tissue section. **Methods:** Experiments utilized a MALDI SYNAPT HDMS G2-Si mass spectrometer, incorporating ion mobility. MALDI experiments used a solid-state diode-pumped ND:YAG laser (1kHz repetition rate). DESI spray conditions were 1.5 μl/min (90:10 MeOH:water) with nebulizing gas pressure of 7bar. All data were processed and visualized using High Definition Imaging software. **Results and Discussion:** Two consecutive snap frozen tissues of human colorectal tumour were sectioned on a cryo-microtome to a 15 μm thickness and thaw mounted onto a conventional glass slide that was placed onto the DESI 2D stage directly from the freezer. DESI imaging experiment was conducted using a raster pattern that was defined over one of the tissue. After acquisition, matrix was evenly sprayed onto both tissue sections. MALDI Imaging experiments were carried out on both tissue sections: pristine and DESI altered tissue. DESI of the colorectal tumour section shows intense lipid peaks with specific species localized to defined regions of the tissue section. Both MALDI imaging datasets also show strong lipid signals. The overall MS spectra between the two MALDI experiments are very similar, in terms of peaks presents but also in terms of peak intensities and spatial localization, therefore indicating that the DESI spray passage did not chemically modify the tissue or delocalize the endogenous molecules. **Conclusion:** Some endogenous molecules were uniquely, or predominantly, present in one dataset versus the other, demonstrating a complementary aspect between MALDI and DESI, providing a more complete molecular picture of the tissue section.

Keywords: MALDI MS, Imaging, DESI

P20.03 Enhancing the Analytical Capabilities of DESI Imaging Using Ion Mobility Separation

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Introduction and Objectives: Matrix assisted laser desorption ionization (MALDI) mass spectrometry imaging (MSI) coupled with ion mobility separation has demonstrated significant utility over the last decade. Since its introduction ten years ago, desorption electrospray ionization (DESI) has been gathering momentum as a complementary MSI technique to the more traditional MSI approaches, proving especially beneficial for the analysis of metabolites/ lipids localization in tissue. In this study, we compare and contrast DESI imaging with MALDI imaging on the same ion mobility enabled mass spectrometer, with a variety of samples. We will demonstrate that additional classes of molecules are ionized by DESI which are clearly defined using ion mobility. **Methods:** Experiments were conducted on a MALDI SYNAPT HDMS G2-Si mass spectrometer, incorporating ion mobility. MALDI experiments used a solid-state diode-pumped ND:YAG laser (1kHz repetition rate). DESI spray conditions were 1.5µl/min (90:10 MeOH:water) with nebulizing gas pressure of 7bar. All data were processed and visualized using High Definition Imaging software. **Results and Discussion:** Different tissue samples including mouse brain sections and human tumor sections, have been analyzed using the same mass spectrometer by both MALDI and DESI. By keeping the parameters for the ion mobility and mass analyzer constant between the different ionization techniques, the ion distribution overlap could be studied in detail. One advantage of MALDI imaging using an ion mobility enabled MS is the ability to differentiate clustered matrix peaks from the tissue derived analytes (e.g. lipids) as two distinct nested trendlines are observed in the m/z vs drift time plot. **Conclusion:** A closer inspection of the ion mobility dimension obtained in the DESI imaging experiments revealed further trendlines in the 2D-plot , corresponding to either different classes of molecules or different charge states of ions, present at much lower abundance. Investigating the origin of these ions demonstrate differences between the fundamental mechanisms of these two imaging techniques.

Keywords: Imaging, DESI, Ion Mobility, MALDI

P20.04 Megapixel Tissue Imaging at High-Speed: Evaluation of a MALDI-TOF Prototype

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Introduction and Objectives: Currently major limitations of MALDI imaging experiments are the acquisition speed of and the spatial resolution of common instrumentation. In a prototype instrument we have addressed these limitations, enabling acquisition speed of up to 50 pixels/second at 10 µm resolution. Here we present first results using this prototype for most common application fields of Imaging, among them lipids, tryptic digests, and intact proteins. At throughput rates of several millions pixels per day, instrument robustness and maintenance become a serious issue. We demonstrate approaches that minimize instrument downtime. **Methods:** Fresh frozen and FFPE tissue sections were mounted onto conductively coated slides and dried in a desiccator. FFPE tissue was subjected to antigen retrieval and spatially resolved enzymatic digest according to established protocols. Matrices (HCCA, DHB and SA) were deposited using vibrational vaporization and sublimation. All imaging data was acquired on a prototype MALDI-TOF instrument. For pixel sizes of 10-50 µm, the laser was set to scan the complete area using a Gaussian beam of ~5 µm diameter at a 10 kHz repetition rate. Continuous sample stage movement, combined with dedicated software allowed acquisition of up to 50 adjacent, non-overlapping pixels/second. **Results and Discussion:** We evaluated the instrument performance using the following, common application examples:

Lipid distribution in rat testis

Tryptic peptides in FFPE tissue

Intact protein imaging Previous limits to spatial resolution was overcome by combining matrix deposition by sublimation, matrix recrystallization and improved capabilities of the prototype enabling analysing large tissue sections at high spatial resolution (30µm) routinely. Daily operation and maintenance is further improved by a novel ion source design. Finally, the aquisition software has been optimized and parallelized to exploit modern processor and OS advances.

Conclusion: In summary, we present a new platform that enables the acquisition of large sample cohorts in a reasonable time frame for the first time.

Keywords: MALDI, Tissue imaging, Protein imaging

P20.05 MALDI Imaging of a Novel and Orally Bioavailable Glucokinase Activator in Rat Kidney Tissue

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Introduction and Objectives: not applicable
Methods: not applicable
Results and Discussion: not applicable
Conclusion: Matrix-assisted laser desorption/ionization (MALDI) imaging mass spectrometry (IMS) is a remarkable new technology to assess the localization of proteins, lipids, small molecules and their metabolites present in tissue sections by direct ionization and detection. MALDI-IMS is a potential tool for the investigation of disease mechanisms and pathological analysis. Its advantages of sensitivity and, especially, radiolabel-free approach for imaging drugs and metabolites in tissues and whole body has led to increasing attention on application of MALDI-IMS to understand the distribution of both drug and its metabolites in drug discovery and development. In the present study, a method using MALDI-IMS was developed for the determination of Glucokinase activators (GKAs) Compound A and its metabolites in rat kidney tissue. Article-related renal changes of transitional epithelial hyperplasia, neutrophil infiltration, and clinical pathology were observed after oral administration of this compound to the female sprague dawley rats at a dose of 600 mg/kg for up to 4 consecutive days in toxicology study. The parent molecule, oxidation metabolites, and glutathione conjugate were detected in the kidney sample, and were found to be highly localized in pelvis which is one of the main branches of kidney. This data might provide insights to the renal toxicity observed after Compound Adosing.

Keywords: MALDI imaging, metabolite, Glucokinase, Mass spectrometry

P21: POSTER SESSION - GLYCOMICS IN BIOLOGY AND DISEASES

P21.01 Interplay between High Glucose Levels and Aspirin on Human Platelets

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Introduction and Objectives: The correlation between chronic hyperglycaemia and "aspirin resistance" in diabetes raised the issue of the potential influence of non-enzymatic glycation on the lowered platelet response to aspirin. The aim of this study was to evaluate the impact of aspirin-acetylation in presence of elevated glucose concentrations on platelet proteins and in particular on COX-1, through the characterization of the preferential acetylation and glycation sites. **Methods:** Platelets from healthy volunteers were lysed and protein extracts were incubated with 30 mM glucose for 24h followed by 500 μM aspirin for 30 min. A label-free tandem mass spectrometry approach was used to quantify the extent of acetylation and glycation at site level. The activity of COX-1 was also assessed in presence of glucose and aspirin in the same experimental conditions and its acetylation and glycation levels were specifically addressed as well. **Results and Discussion:** A significant decrease of protein aspirin-acetylation levels was observed in presence of glucose. Protein glycation was reduced as well after aspirin exposure for most of the identified proteins, including several specifically associated to platelets activation pathways. On the other hand, inhibition of COX-1 by aspirin increased with increasing glucose in a concentration-dependent manner (p for trend = 0.001). **Conclusion:** Overall, the mutual influence between aspirin-acetylation and glycation was assessed on platelets. While glucose did not hamper the aspirin effect on COX-1, we observed that the glycation of several platelet specific proteins was decreased in presence of aspirin. These findings highlight new insights on the interaction between glycation and aspirin-acetylation onto the platelets proteome.

Keywords: Platelets, Glycation, aspirin, Mass spectrometry

P21.02 Development of Bioinformatics Support for Quantitative Glycomics Using Tandem Mass Tags

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Introduction and Objectives: AminoxyTMT reagents enable relative quantitation of glycans in a MS/MS spectrum. However, there are no bioinformatics tools to automate the qualitative/quantitative analysis. We have implemented features within SimGlycan software that enables identification, quantitation of glycans and differential analysis across biological samples from complex mixtures. **Methods:** Glycans were released from standard glycoproteins, mAbs and labeled with aminoxyTMT reagents. MS/MS spectrum were acquired on Velos Pro or Orbitrap Fusion MS using HCD fragmentation. Data was subjected to SimGlycan for identification and quantitation. **Results and Discussion:** HCD fragmentation of high-mannose glycans at m/z 780, 861, 942, 1023, 1124 produced abundant TMT reporter, Y/B/A-type ions. SimGlycan identified N-glycan with residues (GlcNAc)2(Man)5 for the precursor at 780, N-glycan (GlcNAc)2(Man)6 for 861, N-glycan (GlcNAc)2(Man)7 for 942, N-glycan (GlcNAc)2(Man)8 for 1023 and N-glycan with (GlcNAc)2(Man)9 for 1124, which is a correct assignment for all high-mannose glycans present in the sample. Majority of the ions with intact TMT tag carry Na adduct and are the outcome of two glycosidic cleavages towards the reducing end. The program enabled quantitation by measuring reporter ion peak intensities. In order to nullify the interference of other nearby peaks onto TMT reporter ions custom correction factor was used. Statistics calculated as a measure of the relative amount/expression level of each glycan present in the samples constitute sum/average/median/standard deviation of the reporter ion intensities, ratio sum/average/median intensity of each TMT ion and the control TMT 130.13 ion, Log2 relative expressions of these ratios and number of MS/MS spectra which identifies each glycan. Differential analysis and data visualization were facilitated through charts. Heat map enables display of relative intensities of TMT reporter ions for each identified glycan. Cluster dot plot shows Log2 expression levels of a glycan at different TMT reporter ions. **Conclusion:** The performance of the software for other glycoproteins and mAbs were also examined in these studies.

Keywords: quantitation, Glycans, aminoxyTMT, Orbitrap

P21.03 mO-glycobase - An Integrated Database for O-glycosylation

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Introduction and Objectives: Glycosylation is one of the most important protein post-translational modifications and is involved in a variety of biological processes. However, unlike N-glycosylation, study on O-glycosylation is inadequate to a large extent, and still, the bioinformatics tools for O-glycosylation remains poor. Database concerning O-glycosylation remains incomplete and obsolete, which greatly hinders the development of O-glycosylation research. **Methods:** In this study, we create an improved database mO-GlycoDB for O-glycosylated proteins, sites and site-specific O-glycans, primarily based on some currently available databases such as O-glycobase and on retrospective manual extraction from literatures published since 1998. **Results and Discussion:** The database currently contains 884 experimentally confirmed O-glycosylated proteins and 4570 O-glycosites, which have been verified on the uniprot database at the same time, and related O-glycans. Our mO-glycobase is being updated and will soon be available on line. **Conclusion:** We believe that such a database will greatly facilitate research on O-glycosylation and O-glycoproteomics.

Keywords: database, glycan, O-glycosylation, Mass spectrometry

P21.04 Salivary Glycoprotein Characterisation Using Lectin Magnetic Bead Arrays

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Introduction and Objectives: Oral cancers present with non-specific symptoms meaning few patients are diagnosed early (30% of all cases

worldwide). Cancers have a profound effect on the glycosylation machinery and can carry structures markedly different to normal proteins. Human saliva provides numerous benefits for detecting putative glycoprotein biomarkers, and is in close proximity to tumours in the oral cavity. Lectin Magnetic Bead Array's (LeMBA) utilize a panel of different lectins to alterations in glycosylation of biological samples. **Methods:** We have optimised the use of LeMBA for the enrichment of glycoproteins in human saliva (saLeMBA). For saLeMBA, whole saliva from 1 healthy volunteer was used to test the conditions of the saLeMBA methodology (the amount of protein per pull down, Binding buffer volume, and the incubation time). Pooled whole saliva (n=4) from healthy volunteers was used for a triplicate reproducibility test using LC-MS/MS with PCA used for discriminate analysis. **Results and Discussion:** We have titrated the amount of protein binding to beads and optimized the buffers for denaturing to correspond to the overall low protein concentration in saliva compared to serum. We have shown the methodology is reproducible in a triplicate pull down of pooled saliva using high-performance liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS), suggesting that our methodology is robust and suitable for future clinical validation.Our saLeMBA methodology found proteins such as Extracellular Lacritin (AAL), Beta-glycoprotein 1 Extracellular glycoprotein lacritin (AAL), Beta-2-glycoprotein 1 (SNA), Beta-2-microglobulin (NPL) specific to their respective lectins, suggesting the lectins can recognise proteins specific to their glycan specificities. **Conclusion:** In summary we suggest that saliva optimized with LeMBA (saLeMBA) is a powerful tool for glycoprotein enrichment which could be used for biomarker discovery using human saliva in systemic diseases such as cancer.

Keywords: Lectins, LC-MS/MS, Saliva, Glycoproteins

P21.05 Development of Specificity-Enhanced PSA by Rapid Glycan Profiling

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Introduction and Objectives: Serum prostate specific antigen (PSA) is a powerful biomarker widely used for early detection of prostate cancer. However, unnecessary invasive biopsy tests and other follow-up cares have emerged as profound social issues worldwide, which are caused by high false positive rate of PSA test. In this study, we performed comprehensive and quantitative profiling of glycan structures on serum PSA using energy resolved oxonium ion monitoring (Erexim) technology (Anal Chem, 2012, 84:9655) in order to identify glycan alterations specific to prostate cancer development or progression. **Methods:** The Erexim parameters of 4000 Q TRAP LC/MS system were optimized for quantify multiple glycan structures on PSA in 25 minutes run. Glycopeptide enrichment efficiency was also maximized using 96-well aminopropyl silica plate. As a prostate cancer progression model, quantitative PSA glycan profiles were acquired using prostate cancer cell lines WPE1-NA22, NB14, NB11, NB26 (in order of increasing malignancy), and their original normal prostate epithelial cells WPMY-1. Finally, tumor progression-associated glycan signatures were validated using sera from prostate cancer patients or benign prostate hypertrophy patients. **Results and Discussion:** The Erexim technology allowed us to quantitatively monitor 57 glycan structures without enzymatic glycan release or chemical labeling. This enabled rapid and reproducible quantification of glycan varieties even from 0.1% content structures. The Erexim analysis of PSA from 5 cell lines revealed glycan structures showing gradual changes in association with increase of tumor malignancy, which could improve accuracy of Gleason grading-based histological malignancy diagnosis. These specific glycan structure alterations also had potential to distinguish tumor-derived PSA from benign disease-derived one. **Conclusion:** In-depth glycoform characterization of PSA by Erexim analysis could define not only malignancy states of prostate cancer but also origin of PSA.

Keywords: biomarker, prostate specific antigen, glycoform, N-glycosylation

P21.06 N-Glycosylation Analysis of Formalin Fixed Paraffin Embedded Samples by Capillary Electrophoresis

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Introduction and Objectives: Formalin-fixed paraffin-embedded (FFPE) samples are generally used in histology as well as to archive clinical and pathological samples. The most commonly used fixative is buffered formalin that cross-links the amino groups of proteins through the formation of -CH2- bridges preserving the structural integrity of the cells. FFPE tissue collections, with their accompanying clinical outcome information, are invaluable resources for translational studies of cancer and other diseases. However, this huge sample collection was mainly used so far for nucleic acid analysis. The objective of this work is to show that formalin fixed samples hold a great promise in glycomics studies. **Methods:** The carbohydrate moiety of intact and formalin treated standard glycoproteins and human serum as well as human carcinoma cell lines were removed by PNGaseF digestion. The released glycans were labeled with a charged fluorophore and analyzed by capillary electrophoresis with laser induced fluorescent detection (CE-LIF). Mouse tumor biopsy samples and their FFPE counterparts were homogenized and subject the same glycan removal, labeling and analysis process. **Results and Discussion:** Preliminary experiments with standard glycoproteins showed identical glycosylation patterns before and after formalin treatment. Extending this work to human carcinoma cell lines revealed some differences between the glycan patterns of untreated and formalin fixed cells, probably due to the fact that the latter one represented cell surface glycans only. In case of mouse tumor tissues, the whole cell glycome was investigated from formalin fixed and paraffin embedded samples. **Conclusion:** Our results suggested that the sugar moiety of glycoproteins remained intact after formalin treatment, thus, can be used to discover disease associated glycosylation changes at the molecular, cellular and tissue levels. The ability to effectively profile N-glycans form FFPE blocks offers new opportunities to understand disease associated glycan profile changes, even retrospectively from large hospital archives.

Keyword: glycomics, FFPE, capillary electrophoresis, N-glycans

P21.07 Investigation of QTOF Collision Energies to Allow Identification of Complete N- and O-Glycopeptide

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Introduction and Objectives: In most naturally occurring glycoproteins, pools of glycans are attached to one or more glycosylation sites. The analysis of each individual site is challenging and requires the analysis of glycopeptides. For this, quadrupole time of flight (QTOF) mass spectrometers (MS) with high mass accuracy, fast duty cycles, and high m/z range are highly suited due to the usability of multiple collision energies. The systematic investigation of optimum collision energies for the glycan and the peptide part of glycopeptides as well as the software supported data interpretation is presented in this approach. **Methods:** Synthetic N-glycopeptides were analysed on a QTOF MS instrument (impact II) with CaptiveSpray nanoBooster (Bruker Daltonics). Tryptically digested standard glycoproteins (fetuin, antibodies) were separated by nano LC before MS analysis. Collision energies were systematically varied. Glycopeptide spectra were detected and the peptide masses were determined automatically (ProteinScape 4.0). Glycan structures were identified using the integrated GlycoQuest search engine, and for peptide identification Mascot (Matrix Sciences) was used. **Results and Discussion:** The fragmentation parameters on QTOF instruments were systematically investigated using synthetic glycopeptides and glycopeptidemixtures.Thisallowedidentifyingconditionsresultinginmaximum sequence information on both, peptide and glycan parts of glycopeptides. The energies required for optimal glycan fragmentation were found to be clearly below the ones necessary for the peptide part. Nevertheless, the data showed a narrow energy range of +/- 5 eV produced spectra that resulted in the highest scores. The optimized parameters were successfully applied on digested monoclonal antibodies and complex glycoprotein mixtures, which allowed the identification of complete N- and O-glycopeptides. This improved methodology is particularly useful in the fields of glycoproteomics research as well as biopharmaceutical development and quality control. **Conclusion:** Applying different collision energies on a QTOF instrument, the peptide and the glycan part of glycopeptides were fragmented. This allowed the identification of complete glycopepides.

Keywords: QTOF, Collision energies, Data interpretation, Glycopeptides

P21.08 In-Depth Analysis of Site-Specific N-Glycosylation in Vitronectin from Human Plasma

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Introduction and Objectives: The characterization of site-specific micro-heterogeneity in glycoprotein is very important for understanding cell biology and disease processes. Vitronectin is well known to be a multi-functional glycoprotein in blood and the extracellular matrix, which is related to hepatocellular carcinoma (HCC). **Methods:** Here, we systematically analyzed the site-specific N-glycopeptides of vitronectin in human plasma by tandem mass spectrometry combined with immunoprecipitation and HILIC enrichment. Vitronectin was purified with immunoprecipitation by monoclonal antibody from plasma and digested to tryptic N-glycopeptides. Then, enrichment with HILIC materials was used, and followed by analysis with nano-LC/MS/MS. **Results and Discussion:** The sequences of N-glycopeptides were identified from the mass spectra by high-energy C-trap dissociation (HCD) and collision-induced dissociation (CID). In HCD mode, oxonium ions were used for recognizing glycopeptides and y ions for sequencing the peptide backbone. In CID mode, Y ions were used for characterizing their glycoforms. As a result, total 17 site-specific N-glycopeptides were completely identified at all of three N-glycosylation sites of vitronectin in human plasma, including 12 N-glycopeptides first reported. **Conclusion:** Finally, we specifically found that three hybrid and four complex glycopeptides of tri-antennary forms with outer-fucosylation increased in HCC human plasma.

Keywords: Glycoproteomics, immunoprecipitation (IP), Vitronectin, N-glycopeptides

P21.09 Millibeads-Based Lectin Array toward the Fully Automated Glycan Profiling

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Introduction and Objectives: The chip-based lectin microarray is known as a simple and sensitive glycan analysis technology, which is applied to the differential glycan profiling of a tissue protein mixture to find out disease-related N-/O-glycosylation alteration in the uppermost stream of glycobiomarker discovery. Focused differential glycan profiling assisted by a specific antibody against a target glycoprotein is ideal for the glycobiomarker verification. The usability of the technology has been increased gradually; however, simplicity is still debatable especially in the full automation for robust manipulation. Here we show a concept for the fundamental technology toward the "fully automated glycan profiling". **Methods:** We adopted a unique millibeads-based array called "Beads array In Single Tip (BIST®)", a lectin array in a nozzle-type tip form with beads (ø1 mm) coated with 10 different lectins vertically arranged at equal intervals in 30 spacer beads. The lectin-glycoprotein binding reaction, antibody-protein reaction, and washing processes were automatically manipulated by Purelumn® System (Precision System Science). The intense lectin signals were generated by a chemiluminescent scanner (BISTnner®, Precision System Science) after the addition of a chemiluminescent substrate. **Results and Discussion:** In a pilot experiment, two different biotherapeutic erythropoietin (EPO) reagents (epoetin beta [Eposin] and darbepoetin alfa [NESP]) were analyzed. The ten lectins used were selected based on the results of 45-lectin microarray. By the automated manipulation within 30 min for 100 ng of EPO, unique signal patterns of 10 lectins were obtained depending on the binding specificities of lectins. The profiles of Eposin and NESP with or without Sialidase A treatment were consistent with those analyzed by our previously developed system using a 45-lectin microarray chip. **Conclusion:** We confirmed the possible use of millibeads-based lectin microarray. The alternative array format is the best fit for the fully automated glycan profiling in exchange for reducing the numbers of lectins.

Keywords: glycan profiling, glycomics, lectin microarray, glycobiomarker

P22: POSTER SESSION - SUBCELLULAR PROTEOMICS

P22.01 Deep Proteomics of Nucleocytoplasmic Partitioning and the CRM1-Dependent Nuclear Exportome

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Introduction and Objectives: The defining eukaryotic organelle is nucleus, which is separated from cytoplasm by the nuclear envelope. Passive diffusion and active nuclear transport determine how proteins partition across nuclear envelope. Despite the importance of this partition in eukaryotic life, it has not been thoroughly characterized due to the challenges to isolate nuclear proteome without cross-contamination. Therefore, we worked with a model system (giant X. laevis oocytes that have high degree of conservation of cellular and molecular mechanisms with humans) that allows manual micro-dissection to isolate intact nucleus with unmatched purity. Further, to complement nucleocytoplasmic partitioning, we employed affinity chromatography to identify exportome of the most promiscuous exportin (CRM1) in yeast cells, X. laevis oocytes and HeLa cells. **Methods:** X. laevis oocytes were manually micro-dissected to obtain cytoplasmic and nuclear proteomes. UPS2 was spiked to estimate absolute protein concentration. CRM1 immobilized beads were incubated with X. laevis, HeLa and yeast extract in the absence or presence of RanQ69L. X. laevis cytoplasmic and nuclear extracts and eluted proteins from CRM1 affinity chromatography were digested with trypsin and analyzed with LC-MS/MS (Orbitrap Q-Exactive and Orbitrap Fusion). Raw MS data were analyzed with MaxQuant. **Results and Discussion:** We quantified the nucleocytoplasmic partition of ~10000 proteins which revealed trimodal localization distribution showing 6 orders of magnitude in protein abundance. These numbers had interesting implications e.g. for RNA metabolism, protein degradation, and organization of cytoskeleton. Further, hundreds of novel CRM1 export cargoes were identified in each three model organisms.The evolutionary conserved CRM1 exportome include nearly all translation factors, certain cytoskeletal proteins and vesicle coat proteins. **Conclusion:** We present a valuable resource for the nucleocytoplasmic partition of a vertebrate proteome, and provide insights into eukaryotic compartmentation which has direct implications in human. Further, majority of the evolutionary conserved CRM1 exportome relate to cytoplasmic activities that should be (at least temporarily) suppressed inside nuclei.

Keywords: Xenopus laevis, CRM1 exportome, Nucleocytoplasmic partitioning

P22.02 Potential of Exosomes in Relation to Diagnostics in Lung-Related Diseases and Cancers

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Introduction and Objectives: Lung diseases are some of the most common medical conditions in the world. Smoking, infections, and genetics are responsible for most of these. When patients with various lung-diseases come into the clinic, the symptoms they are displaying are very similar and a massive elucidation process to diagnose correctly starts. Hence, there is a great need to optimize the diagnostic tools available and hereby ensure earlier diagnosis and better prognosis. Extracellular vesicles (EVs) are readily available in the blood stream and hereby constitute potential biomarkers of human diseases. Studies have identified plasma EVs as useful markers in several diseases including lung cancer (Jakobsen et al., 2015, JEV). **Methods:** Plasma samples from cohorts of healthy individuals, patients with Chronic Obstructive Pulmonary Disease (COPD), pneumonia, Small Cell Lung Cancer (SCLC), and Non-small Cell Lung Cancer (NSCLC) were analyzed for their content of EVs. The EVs were extensively phenotyped with the use of the EV Array technology (Joergensen et al., 2013, JEV). **Results and Discussion:** Analyzing for more than 30 different EV markers in the plasma samples it was possible to generate specific EV profile signatures for the various lung diseases. Using multivariate data analysis it was possible to distinguish several of the cohorts with sensitivities and specificities above 0.8, and accuracies above 0.75. **Conclusion:** Using the EV Array technology, we demonstrate how it serves as a potential diagnostic tool capable to point out lung-diseased individuals from healthy persons with high accuracy.

Keywords: Extracellular vesicles, Lung disease, diagnostics, lung cancer

P22.03 Reinforcement-Polarity Dependently Regulated Synaptic Proteins in a Learning Paradigm in Mice

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Introduction and Objectives: The identification of molecules and pathways that control synaptic plasticity and memory is a major challenge in neuroscience. A growing number of molecules are already identified to be pivotal in these processes. Surprisingly, only sparse knowledge exists how the polarity of reinforcement (reward or punishment) applied in the different learning paradigms affects the molecular reorganization of the synapse. We have designed an auditory detection paradigm in mice which allows the application of both, appetitive and aversive motivation, in a comparable manner in order to perform a molecular characterization by a proteomic approach. **Methods:** Electrical brain stimulation reward through electrodes implanted in the medial forebrain bundle was delivered as appetitive reinforcement when mice correctly responded to a CS+ tone by crossing the hurdle in a shuttle box. Mild footshock punishment was delivered as aversive reinforcement upon misses and false alarms. Separate groups of mice were trained by appetitive or aversive motivation, respectively and sacrificed for enrichment of postsynaptic densities from four different brain areas. Synaptic protein expression patterns obtained from trained mice, their corresponding controls and from naive mice were compared by a quantitative proteomic approach. **Results and Discussion:** The proteomic screen revealed 102 proteins and 345 phosphopeptides significantly changing their relative synaptic abundance after learning compared to naive controls. Moreover, the relative abundance of 32 proteins and 137 phosphopeptides is differentially regulated between aversive and appetitive reinforcement. Beside others, the SRC kinase signaling inhibitor 1 was found as being significantly regulated between appetitive and aversive reinforcements. Note that this molecule is localized in dendritic spines and is identified as a key element involved in learning and synaptic plasticity. **Conclusion:** The current findings indicate the suitability of the established learning paradigm and the used proteomic approach to render promising

candidates for extended studies of molecular differences in reinforcement polarity-dependent reorganization of synaptic connections.

Keywords: learning and memory, chemical synapse, quantitative proteomics, auditory learning

P22.04 Towards the Elucidation of the Complete Mitochondrial Protein-Protein Interaction Network

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Introduction and Objectives: Mitochondrial dysfunction is linked to a variety of human diseases, in particular neurodegenerative disorders and cancers. Recent exploration of the mitochondria as a viable target for medical intervention has revealed entichment of drug targets for the estimated 1500 mitochondrially-localized genes. However, protein interaction data within the mitochondria remains sparse. Our objective is to characterize the full mitochondrial proteome in S. cerevisiae, and predict human protein complexes for evolutionarily- conserved proteins. **Methods:** Using the GFP-tagged strain collection of the model organism S. cerevisiae, we aim to systematically screen all ~1200 yeast mitochondrial proteins for interacting proteins using an AP-MS strategy. Mitochondria are isolated prior to disruption to avoid non-mitochondrial protein contamination. Improved mitochondrial yield can be achieved using non-fermentative YPEG (ethanol-glycerol) media, and protein recovery is achieved using chemical cross-linking and magnetic anti-GFP beads. Binding proteins are identified using an Orbitrap Elite mass spectrometer. The protein-protein interaction network is then used to identify protein complexes based on clusters of highly-interacting subunits, whuch are then mapped to human homologs to identify probable human mitochondrial complexes. **Results and Discussion:** Currently over 800 proteins have been purified and interacting partners have been identified. Novel members of previously-characterized mitochondrial complexes, such as the MITOS complex, have been selected for further study. The full network of interacting proteins is expected to reach completion in June 2015. **Conclusion:** not applicable

Keywords: Mitochondria, proteomics, Protein-protein interaction, Systems biology

P22.05 The Potential of Exosomes as Diagnostic Markers for Non-Small Cell Lung Cancer

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Introduction and Objectives: Lung cancer is among the leading causes of cancer-related deaths, mainly owing to the fact that more than half of the patients have reached the metastatic state at the time of diagnosis. Non-small cell lung cancer (NSCLC), which accounts for the majority of lung cancer cases, currently has a 5-year survival rate of 11-18%. Hence, there is a great need to optimize the diagnostic tools available and hereby ensure earlier diagnosis and better prognosis. **Methods:** Plasma from 109 NSCLC patients, 110 lung-diseased controls (patients initially suspected of having lung cancer but afterwards diagnosed to be cancer-free) and 161 healthy controls were analyzed using a protein microarray platform optimized for phenotyping extracellular vesicles with exosomal characteristics. **Results and Discussion:** Using the Extracellular Vesicle Array (EV Array) it was possible to determine the presence of 37 exosomal- and cancer-related biomarkers simultaneously using only 10 μL of sample. Allowing biomarker proportions to be included in a multivariate data analysis it was possible to establish a 20-marker model able to distinguish the cancer patients from the healthy controls with a sensitivity of 0.82, a specificity of 0.8 and accuracy of 80.7%. However, distinguishing between the cancer patients and the lung-diseased controls is most accurate using a 30-marker model, though with a slightly poorer outcome (sensitivity 0.75, specificity 0.76 and accuracy 75.3%). **Conclusion:** Using the EV Array technology we demonstrated how it serves as a highly potential diagnostic tool capable to point out NSCLC patients from healthy as well as lung-diseased individuals with high accuracy.

Keywords: diagnostics, Extracellular vesicles, EV Array, lung cancer

P22.06 Multiplexed Quantitative Profiling of Mitochondria from the USP30 Knockout Mouse

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Introduction and Objectives: Impaired mitochondrial degradation is thought to explain the wide array of mitochondria defects seen in Parkinson's Disease (PD) and may be causally linked to neurodegeneration. Recent literature suggests Parkin and PINK1, mitochondria-associated proteins whose genes are known to be mutated in PD, function to clear damaged mitochondria in cells. Data from our group indicates USP30 is a deubiquitinating enzyme (DUB) that antagonizes the Parkin-PINK1 mitochondrial degradation pathway. USP30 overexpression blocks parkin-mediated mitochondria clearance, while knockdown of USP30 rescues mitochondria degradation defects associated with pathogenic Parkin mutations. In an effort to study mitochondrial protein alterations in the USP30 knockout mouse in an unbiased manner, TMT multiplexed mass spectrometry was applied. **Methods:** Crude mitochondria were purified from the dissected brain cortexes of three WT and three USP30 knockout mice and subjected to our standard TMT multiplexed global protein profiling workflow. Briefly, samples were digested in solution with trypsin, labeled with TMT-6-plex, and fractioned using a high pH reverse phase HPLC method. LC-MS analysis was performed either on an Orbitrap Elite mass spectrometer using MS3 scans for TMT quantitation or on an Orbitrap Fusion using Synchronous Precursor Selection mode. Spectra were searched using Mascot and MS3 reporters quantified using the Mojave algorithm. **Results and Discussion:** We were able to successfully identify and quantify 671 mitochondrial proteins from wild type and USP30 knockout mouse brains by applying a TMT reporter ion based quantitative proteomics workflow. We found that the majority of quantified proteins in the brain of the USP30 knockout mouse do not show significant differences in comparison to the wild type. Follow up experiments are currently underway to investigate mitochondrial proteins that were found to be differentially regulated. **Conclusion:** Multiplexed quantitative proteomic workflow allows for profiling of mitochondrial proteins in mouse brains from genetically engineered mouse models and provided meaningful insights into the complex regulation of mitochondrial homeostatis.

Keywords: TMT, Mitochondria

P22.07 Subcellular Proteomic Study of Skeletal Muscle by Evaluated Fatty Acid Level

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Introduction and Objectives: Obesity has a tight association with type 2 diabetes mellitus and elevated plasma free fatty acid level induced insulin desensitization is believed as the link between obesity and T2DM. However, the detailed mechanism of the elevation of plasma free fatty acid level result in insulin desensitization remains to be elucidated. Previous studies demonstrated that, associated with insulin desensitization, we hypothesize that there are some changes inside nuclei which consequently affect the genes expression and DNA methylation which are related to insulin desensitization. **Methods:** A murine skeletal muscle cell line, C2C12 myotubes were established and exposed to first, palmitic acid in order to induce insulin desensitization; and followed by treatment with oleic acid to act as control. To focus on the changes of nuclear proteome in comparing with that of the cytosolic proteomic status, nuclear fractions were enriched by centrifugation for two-dimensional gel electrophoresis (2-DE) based proteomic study. **Results and Discussion:** Five differentially expressed proteins were found after palmitic acid treatment. Among these five proteins, nuclear factor NF-kappa-B (NF-κB) p65 subunit and 60S acidic ribosomal protein P0 were upregulated, after exposed to plamitic acid; while peroxisome proliferator-activated receptor gamma coactivator 1- alpha (PPARGC-1α), cleavage and polyadenylation specificity factor subunit 5 (CFIm25) and prohibitin were downregulated. The 2-DE result was confirmed by western blotting analysis. Inhibiting NF-κB activation could rescue C2C12 myotubes from palmitic acid induced insulin desensitization. Inhibiting NF-κB activation by parthenolide reversed the deleterious effects of palmitic acid on Akt activation and insulin stimulated glucose uptake These results indicated that NF-κB was involved in palmitic acid induced insulin desensitization. **Conclusion:** The nuclear proteomic study indicated that, associated with this desensitization, five nuclear proteins showed changes in expression; Furthermore, palmitic acid induced insulin desensitization was accommodated with NF-κB p65 subunit nuclear translocation; Inhibiting NF-κB p65 subunit nuclear translocation could protect C2C12

Keyword: Nuclear proteomic C2C12 Fatty-Acids

P23: POSTER SESSION - PERSONALIZED MEDICINE

P23.01 Phosphopeptide LCMS Immunoassays to Assess Active Tumor Signaling and Application to Core Biopsies

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Introduction and Objectives: We have clinical need to identify active signaling pathways in patients' tumors from very small biopsy samples to inform treatment decisions. Mass spectrometry-based biomarker assays are well-suited to this task due to their exquisite specificity and ability to be multiplexed. Here we demonstrate a highly sensitive and specific method for capturing phosphopeptides with commercially-available antibodies and quantifying those peptides in multiplexed Selected Reaction Monitoring (SRM) and Single Ion Monitoring (SIM) LCMS assays. **Methods:** All assays were performed using a Q Exactive mass spectrometer with nanoflow chromatography. Native and isotope-labeled versions of each assayed peptide were synthesized and quantified. Peptide immunoenrichment was performed in multiplexed fashion, with dozens of phosphorylation site-specific and peptide-specific antibodies mixed to enable a single immunoprecipitation for and subsequent quantification of multiple target peptides and phosphopeptides. **Results and Discussion:** SRM scans showed linear response over four orders of magnitude in a complex matrix while linear response was observed over five orders of magnitude using the SIM method in all matrices tested, with low amol sensitivity consistently achieved. Instrument settings including gradient length, maximum ion time, and resolution were optimized for sensitivity and specificity. Sensitivity of the immunoenrichment-LCMS assays was determined using the reversed curve strategy in a background of human lung tissue peptides. Multiplexed assays targeting 20-50 phosphopeptides were used to quantify peptides immunoenriched from cell lines and xenograft tumors, demonstrating linear response and reproducible quantitation of phosphopeptides from 5 ug total input protein. This method was also applied to core needle biopsies from xenograft tumors, resulting in successful phosphopeptide quantification from single core biopsies. **Conclusion:** Phosphopeptide quantification through multiplexed LCMS immunoassays allows activity of targeted cell-signaling nodes to be measured from core needle biopsies. This technology utilizes commercially-available reagents, would be complimentary to genetic analysis of tumors for informing treatment decisions, and could determine pharmacodynamic drug responses in clinical trials.

Keywords: Multiplexed assay, Immunoassay, phosphoproteomics, Translational proteomics

P23.02 Development of Companion Diagnostics for RCC (Renal Cell Carcinoma) Treatment with mTOR Inhibitor

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Introduction and Objectives: Temsirolimus is a specific inhibitor of mTOR and an anticancer drug for RCC patients. Although temsirolimus serves as first-line option in RCC treatment, the use of temsirolimus differs in setting and patient population characteristics. Even today, there is no promising biomarker to predict clinical outcome of mTOR inhibitors for patients with RCC. Companion diagnostics provides information about effectiveness and safety of the drugs clinically used, and personalized medicine using such companion diagnostics is desired to improve clinical outcome in RCC treatment. **Methods:** We performed total and phospho proteomic analysis of RCC cell lines that have different temsirolimus sensitivity to find a factor that determines the temsirolimus sensitivity, which could be a biomarker candidate in RCC treatment with temsirolimus. **Results and Discussion:** Expression or phosphorylation of 22 proteins was correlated with the temsirolimus sensitivity and a couple of proteins changed the temsirolimus sensitivity in RCC cell lines, but not other mTOR inhibitors (Torin1, PP242 or KU-0063794) sensitivity. We are currently validating our data in in vivo using mouse xenograft model and investigating the molecular mechanisms of how the temsirolimus sensitivity is regulated by the biomarker candidates. In addition, we are going to see whether the biomarker candidates are correlated with drug efficacy in RCC patient and could be a target of companion diagnostics. **Conclusion:** These findings indicated that proteomic approach identified several biomarker candidates that determine the temsirolimus sensitivity. Further extensive validation is required to see whether the biomarker candidates would be clinically usable for the first time in mTOR inhibitor therapy and could be a target in the development of companion diagnostics.

Keywords: RCC (renal cell carcinoma), personalized medicine, mTOR, temsirolimus

P23.03 Discovery of Biomarker Candidates for NSCLC Treatment Using CTOS (Cancer Tissue-Originated Spheroid)

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Introduction and Objectives: Erlotinib is EGFR-targeted anticancer drug and widely used for patients with NSCLC (non-small cell lung cancer) who have EGFR gene alternation, such as exon19 deletion and exon 21 (L858R) mutation, which has been used clinically as a validated predictive marker. However, the resistance to erlotinib is common feature in NSCLC treatment, and drug efficacy and time to progression differ from person to person. In order for the personalized medicine to improve clinical outcome in NSCLC treatment with erlotinib, we immediately need a biomarker to predict better clinical benefit for patients with NSCLC. **Methods:** We used CTOS (cancer tissue-originated spheroid) method, a primary culture of cancer cells from NSCLC patients, to identify biomarker candidates in NSCLC treatment with erlotinib. We performed proteomic analysis of CTOSs from lung cancer patient samples that have different erlotinib sensitivity, and selected 40 proteins whose expression is correlated with the erlotinib sensitivity as a biomarker candidate. **Results and Discussion:** We found that 9 out of the 40 proteins changed the erlotinib sensitivity in NSCLC cell lines and 7 proteins specifically changed the erlotinib sensitivity, but not the lapatinib sensitivity, another EGFR inhibitor. We are currently validating our data in in vivo using mouse xenograft model and investigating the molecular mechanisms of how the erlotinib sensitivity is regulated by the biomarker candidates. In addition, we are going to evaluate whether the potential biomarker is correlated with drug efficacy or time to progression in NSCLC patient and could be a target of companion diagnostics. **Conclusion:** These findings indicated that CTOS could be an invaluable tool for the discovery of biomarker candidates in clinical samples from cancer patients. Although drug resistance in cancer patients is a huge problem, we believe that CTOS method will help us understand the molecular mechanisms of drug resistance and develop novel treatment to overcome drug resistance.

Keywords: NSCLC, CTOS (cancer tissue-originated spheroid), erlotinib, companion diagnostics

P23.04 Array-Based Signaling Pathway Profiling for Cancer Therapy Personalization

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Introduction and Objectives: Sorafenib is a multi-kinase inhibitor that has been proved to be effective for the treatment of advanced hepatocellular carcinoma (HCC). However, only a small proportion of patients receiving sorafenib obtain the anticipated therapeutic benefits. It would therefore be desirable to clarify the precise molecular mechanisms that confer resistance to sorafenib and identify a predictive biomarker for unresponsiveness to sorafenib. **Methods:** We developed a high-density reverse-phase protein array (RPPA) (1) onto which 3,072 lysate of 95 cancer cell lines derived from 8 different types of cancer, including 23 HCCs, were plotted. We examined the expression of 180 phosphorylated proteins serving as nodes in 120 signaling pathways registered in the NCI-Nature curated database. **Results and Discussion:** We found that the relative level of phosphorylation of ribosomal protein S6 (pS6RP) at the Ser235/236 residues showed the highest correlation with sensitivity to sorafenib among the 180 signaling nodes (Spearman correlation analysis). Immunoblot analysis confirmed that the sorafenib-resistant cell lines had the highest level of S6RP phosphorylation (2). The high expression of p-RPS6 S235/236 was confirmed in biopsy samples obtained from HCC patients who responded poorly to sorafenib. **Conclusion:** The efficacy of molecular therapeutics often varies among individuals, and precise mapping of active signaling molecules in individual patients is now considered essential for therapy optimization. RPPA requires only a small amount of protein and is ideal for application to clinical settings (3). References: 1. Matsubara et al., Mol Cell Proteomics. 2011 2010 9(4):695-704. 2. Masuda et al., Mol Cell Proteomics. 2014 13(6):1429-38. 3. Masuda and Yamada, Biochim Biophys Acta. 2015 1854(6):651-657.

Keywords: RPPA, Sorafenib, Hepatocellular carcinoma, mTOR signaling

P23.05 Targeting Modified Single Amino Acid Variants in Cancer Cell Lines by Parallel Reaction Monitoring

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Introduction and Objectives: Single nucleotide variants (SNVs) are the simplest and most commonly occurring variants in the human genome. Many of the SNVs are non-synonymous (nsSNVs), resulting in altered protein sequences, such as amino acid variants (SAVs) or protein truncations. We have recently showed that many SAVs affect modifiable amino acid residues on key signaling proteins and therefore have potential to rewire signal transduction networks. However, due to low frequency of variant peptides, the extent of modified SAVs in individual proteome requires application of advanced, high resolution targeted proteomic strategies. **Methods:** We devised an MS approach to stratify and target SNV-containing peptides in a complex biological sample using parallel reaction monitoring (PRM) on the Q Exactive HF mass spectrometer. For precise measurements, two acquisition strategies were applied to a set of selected SAV-containing peptides and standards: (I) retention time scheduled data acquisition controlled by retention time calibration peptide mixture and (II) internal standard triggered PRM. **Results and Discussion:** Out of 54,097 variants in a cervical cancer cell line, 9,917 were non-synonymous SNVs, of which 4,042 affected either Ser (39%), Thr (35%), Tyr (9%) or Lysine (17%) amino acid residues. About 20% of the detected nsSNVs fell into defined kinase target motifs and many affected key signaling proteins. We used these facts to prioritize targeted analysis of potentially modified variant peptides. Initial PRM measurements have confirmed presence of several modified variant peptides, some of which were already detected (but not reported) in previous large-scale studies. We are currently optimizing PRM acquisition parameters and performing targeted analyses on additional variant peptides. **Conclusion:** Targeted PRM analysis has a potential to detect and quantify individual SAV-containing peptides in a complex background and therefore has an important future application in personalized proteomics.

Keywords: variant, phosphorylation, PRM, Orbitrap

P23.06 Molecular You Corporation (MYCO): Comprehensive Omic Analysis for Personalized Preventive Medicine

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Introduction and Objectives: Early detection of disease allowing early intervention could dramatically improve health and decrease health costs. The advent of low-cost genomic, proteomic, metabolomic and microbiomic (Omic) analyses has the potential to provide reliable individualized evidence of early stage disease necessary for such intervention. Towards introduction of personalized medicine in Canada, the Molecular You Corporation (MYCO) and the UBC Personalized Medicine Initative will conduct longitudinal studies in large populations that will characterize participants at the molecular level. The current "10 Pathfinders" pilot study is a feasibility study aimed at testing the protocols for collection, storage and analysis of the data from 10 healthy volunteers. **Methods:** Blood, urine and stool will be collected for downstream omics analyses in addition to biophysical, sleep, diet, activity and environmental patterns. Participants' genomes will be sequenced to identify genetic risk factors for disease. Levels of a comprehensive panel of plasma proteins, metabolites and gut microbial species will be analyzed and correlated with the participant's state of health. All data generated will be analyzed using integrated computational biology approaches to characterize health status and to correlate observed transitions from health to disease (or visa versa) with various 'omic' metrics. **Results and Discussion:** Participant data will be evaluated using the latest information available concerning biomarkers of disease as determined in validated clinical studies and the broader scientific literature. Participants will receive regular consultation with a healthcare professional to interpret data and discuss personalized options to safely achieve a healthy state. Examples of preliminary findings are discussed. **Conclusion:** The large database created from this study will be a valuable resource for future development of actionable biomarkers of health and early-stage disease. Such knowledge will provide the basis of new intellectual property, commercialization opportunities and technologies for prevention and treatment of diseases before they become dangerous, chronic and costly.

Keywords: personalized medicine, Integrated omics, molecular you, database

P24: POSTER SESSION - PROTEOGENOMICS

P24.01 LncRNA Proteomics: A Novel Mass Spectrometry Data Identification Database of Long-Noncoding RNA

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Introduction and Objectives: Mass spectrometry based proteomics is one of the most popular life science technologies in the identification, quantification and characterization of the proteins. A general limitation of current mass spectrometry based proteomics methods is their dependence on coding-RNA sequence databases for identifying proteins [1]. Mining more information stored in mass spectrometry data is an important problem in proteomics. Furthermore, huge amounts of long-noncoding RNA (lncRNA) have been found to play an important role in the gene expression regulatory network at all stages, including DNA, transcription, RNA, translation and protein. These discoveries made lncRNA become a hot research topic in the last few years. **Methods:** We have built a novel mass spectrometry identification database on lncRNA, called LCP-MS, which would help connect lncRNA with proteomics research. With 111,685 human annotated lncRNAs downloaded from lncipedia database, LCP-MS consists of 58,886 potential proteins sequences translated by present algorithms with filtration. Not only it was developed as a database to search mass spectrometry data to sequences, but also it annotated the peptides or proteins involve lncRNA information subtracted from other database. **Results and Discussion:** This project concentrates on searching results of mass spectrometry data from different tissues in human against LCP-MS database. In LCP-MS project, a series of data analysis methods have been applied such as lncRNA and coding RNA co-translate analysis, helping us obtain a full understanding of how lncRNA works in human system at proteomics level. **Conclusion:** Future directions for lncRNA proteomics within more bioinformatics methods would also be discussed further and their applications for connecting lncRNA research with proteomics also show a promising outlook for us to explore.

Keywords: long non-coding RNA, search database, Mass spectrometry

P24.02 Profiling Molecular Changes during Malignant Transformation and Response to Different Oxygen Levels

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Introduction and Objectives: Transformation of normal cells to cancer cells that can form tumors and even metastasize is a multi step process where molecular changes of high complexity are acquired. By using a four-stage human cell model that mimics the route to malignancy we demonstrate how a transcriptomics approach with subsequent protein analysis can be used to define the molecular changes that accompany mechanisms related to immortalization, transformation and invasion/metastasis separately. We are currently investigating the impacts of hypoxia by studying differential protein expression and spatial distributions of proteins under different oxygen levels. **Methods:** This project is performed in close collaboration with the Human Protein Atlas (HPA) project and with the use of RNA-seq in combination with the HPA collection of antibodies covering the whole human proteome and high-resolution microscopy, protein expression is studied on a single cell level. **Results and Discussion:** We have previously shown that about 6000 genes are differentially expressed across the cell model with a majority of genes being downregulated, supporting the principle of dedifferentiation en route to malignancy. At the moment we are studying differential gene expression between two oxygen conditions, atmospheric oxygen concentration and 3% oxygen. **Conclusion:** not applicable

Keywords: RNA-seq, Hypoxia, cancer, Cell line model

P24.03 PGA: An R Package for Identification of Novel Peptides by Customized Database Derived from RNA-Seq

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Introduction and Objectives: Peptide identification in mass spectrometry (MS)-based proteomics is generally achieved by comparison of the experimental mass spectra with the theoretical mass spectra that are derived from a reference protein database, however, this strategy could not identify peptide sequences that are absent from a reference database. The customized protein database on the basis of RNA-Seq data was proposed to assist and improve identification of such novel peptides. To make database construction and the relevant peptide identification generally accessible to the broad proteomics community, we have developed an R package, named PGA, which can be used for identification of novel peptides by customized database derived from RNA-Seq. **Methods:** PGA is implemented as a Bioconductor package in R. The workflow of PGA is generally divided to four steps, (1) construction of customized database based on the RNA-Seq data, (2) MS/MS data searching against the customized database by using X!Tandem, or MASCOT, (3) post-processing and (4) generation of the HTML-based report. **Results and Discussion:** The PGA functions were evaluated through a published data set, which contained a set of RNA-seq and proteomic data collected from Jurcat cell line. The FDR threshold for identification of the canonical and novel peptides was set as 1%. A total 632 novel peptides were identified by PGA, including 508 SAP peptides, 2 indel peptides, 53 splice junction peptides and 71 novel transcripts-derived peptides. In addition, the peptide identification based on searching the customized database was compared with that based on searching the reference database. It clearly indicated that more peptides were identified based on the customized database (73459) as compared with that derived from searching the reference database (72956). **Conclusion:** PGA is platform-independent and easy-to-use and was well proven in identification of novel peptides through searching the customized protein database derived from RNA-Seq data.

Keywords: Bioinformatics, Proteogenomics, RNA-seq, protein identification

P24.04 A Proteogenomic Study on p53 Mutant Peptide Detection Reveals Biological and Technical Limitations

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Introduction and Objectives: Many variant peptides corresponding to single nucleotide variations, insertions/deletions, fusions or splice isoforms are associated with specific diseases or outcomes. Identification of novel such alterations at the proteome level may lead to diagnostic or prognostic biomarker development. For this purpose, the emerging field of proteogenomics uses well-established shotgun proteomic workflows in combination with customized genomic-derived databases. Subsequently, variant peptides identified are validated with targeted approaches. Here we have chosen mutated p53 protein as a model to explore the effectiveness of proteogenomic workflows towards the identification and validation of variant peptides in clinical specimens. **Methods:** In this study, 17 variant peptides corresponding to an equal number of TP53 mutations were tracked in a set of 19 cytoplasmic extracts derived from breast tumors. All tissues had previously been sequenced for TP53 gene and one mutation was detected in each sample. Shotgun proteomic data generated from a fractionated pool of 6 extracts was searched against a reference human database with the respective variant peptide entries appended to the database. In addition, 18 individual extracts were assayed by selected reaction monitoring for the respective variant peptides. **Results and Discussion:** None of the 6 mutant peptides was identified with the shotgun approach, whereas targeted SRM detected one of the 6 variant peptides (p.R273P mutation) that were missed. SRM detected another variant peptide (p.R273C mutation) in the remaining 13 cytoplasmic extracts. Data-dependent acquisition is a limiting factor for identification of variant peptides present in a sample. Furthermore, low abundance and possible poor electrospray ionization efficiency of variant peptides hamper both their shotgun identification and targeted validation. **Conclusion:** Current mass spectrometry-based technologies can detect variant peptides successfully. To minimize false positive and false negative proteogenomic identifications, sample-specific customized databases, targeted validation of peptide identifications and the deepest proteome coverage possible should be considered.

Keywords: Proteogenomics, p53, variant peptides, Mass spectrometry

P25: POSTER SESSION - METABOLOMICS AND METABOLIC DISEASES

P25.01 Characterization and Collision Cross Section Determination of Lipids from Obese Mouse Models

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Introduction and Objectives: Obesity is a risk-factors associated with metabolic syndrome, causing excess body fat to be accumulated to the extent that it adversely affects health and life expectancy. This work provides additional characterization of the associated lipids using ion-mobility with collision cross section (CCS) databases. **Methods:** Lipid were extracted from liver tissue of obese and inhibitor treated mice. Extracts were separated over a 20 min reversed-phase LC gradient and data acquired using a data independent acquisition approach utilizing ion mobility. Data were processed and searched using Progenesis QI and dedicated lipid compound databases, providing normalized label-free quantitation results with additional specificity of CCS measurement. **Results and Discussion:** Interrogation of the LC-IM-DIA-MS data revealed over 5000 potential features for further investigation as a result of positive and negative ion acquisitions combined. Data were further interrogated using multvariate statistical analyses, showing clear distinction between control and glucosylceramide inhibitor treated groups. OPLS discriminant analysis revealed 795 potential features that were of significant correlation and covariance. Database searching resulted in 163 candidates. Identifications were scored according to mass accuracy, isotoptic fit, CCS and MS/MS fragmentation. Additional filtering to curate the data was based on mass errors less than 2 ppm, fold change >2, 5% CCS tolerance and ANOVA p-value <6E-06. This resulted in 15 significant identifications including phosphatidylcholines, sphingomyelins, triglycerides and lysophosphatidylcholines. **Conclusion:** Ion mobility-derived CCS measurements allowed for improved specificity with the inclusion of drift time, providing additional confidence in the identifications returned. Pathway analysis

revealed lipid metabolism as a significantly perturbed pathway with mapping highlighting diabetes and inflammatory responses.

P25.02 Analysis of Low-MW Aldoses, Ketoses, and Carboxylates by Single Chemical Derivatization/LC-MS/MS

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Introduction and Objectives: Low-molecular weight (LMW) sugars and carboxylates are the metabolic substrates or intermediates of central carbon metabolism and require sensitive and reliable measurements in biological samples. This is usually done by GC-MS with chemical derivatization. Here, we describe a single chemical derivatization - LC-MS/MS method for simultaneous analysis of these metabolites. **Methods:** Fifteen LMW aldoses and ketoses (sucrose, lactose, maltose, cellobiose, glucose, galactose, fructose, mannose, fucose, rhamnose, ribose, xylose, arabinose, xylulose and ribulose) were reacted with 3-nitrophenylhydrazine as the derivatizing reagent in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide and pyridine. Reaction medium, temperature, and duration were optimized for completeness of derivatization. Quantitation was performed by UPLC/multiple-reaction monitoring (MRM)-MS on a triple-quadrupole instrument. Analytical sensitivity, specificity, precision and accuracy were validated. Isotope-resolved mass analysis of ^{13}C-labeled wild-type versus knockout mouse hearts was conducted by UPLC/high-resolution MS on an LTQ-Orbitrap. **Results and Discussion:** The derivatization was found to be optimal in 60-80% methanol and at 55 °C for one hour. Sucrose cannot be derivatized because it lacks a reacting carbonyl group. With water:methanol:0.1% formic acid as the mobile phase, good separations of sugar structural isomers were achieved on a pentafluorophenyl-bonded LC column. Using ^{13}C-labeled internal standards, the quantifiable sugars showed good linearity within a 512 to 2056-fold concentration range, with low-picomole analytical sensitivities, good precision (CVs ≤11.5%), and good accuracy (88.7% to 113.5%) for quantitation in wine. Assay of sugars using this new protocol and of 11 fermentation-related carboxylates using a previously described protocol with the same derivatization method showed significant compositional differences among wines manufactured in the Okanagan Valley region of British Columbia, Canada. Isotopomer analysis of both the LMW sugars and the tri-carboxylic cycle carboxylates for metabolic flux analysis in the ^{13}C-labeled mouse hearts was successfully achieved by UPLC/high-resolution MS. **Conclusion:** A single LC-MS/MS method was successfully developed for simultaneous analysis of 14 aldoses/ketoses and 11 metabolic carboxylates.

Keywords: chemical derivatization, 3-nitrophenylhydrazine, sugar, isotopomer analysis

P25.03 Creation of Metabolite Libraries for Metabolomics Screening of Yeast Strains Used in Beer Production

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Introduction and Objectives: Beer flavor comprises a combination of cereal, hop-related substances, as well as secreted proteins and metabolites from the yeast strain that create a specific combination of odor and taste impressions. In this study, we tried to decipher the molecular profile of two different yeast strains: WLP001 and WLP300, across the whole fermentation process, to determine the differential metabolites between both strains across 7 time points. Furthermore, we performed metabolite identification using a compound spectral library that contains a repository of accurate masses, retention times and MS2 spectra information, which are acceptance criteria that can improve the confidence in metabolite identification **Methods:** Single cell colony fermentations were carried out in triplicates of each strain in 5% malt extract growing at 25C over 48 hours. 7 different time points were collected. Cells were removed and supernatants were submitted for metabolomic analysis. Metabolites were analyzed using a 15 min LC gradient on a Thermo Scientific Q Exactive mass spectrometer coupled to an Ultimate 3000 UHPLC system. All data were processed using the Thermo Scientific TraceFinder 3.2 software. Statistical analysis was further performed using Perseus. **Results and Discussion:** In the last decades, beer industry has gone through a major transformation trying to provide customers with novel beers that carry unique sensorial characteristics. Principal component analysis (PCA) of data from the LC–MS measurements allows discriminating between both strains. Multivariate statistically analysis uncovered the specific metabolites responsible for the odor and taste of each strain. Over 20 flavanoids responsible for taste and the color were also monitored. **Conclusion:** Overall, this study provides (a) a fast and confident metabolite screening using a metabolomics approach and commercial software, and (b) the means to show the taste differences comparison between different beer strains that contribute to their specific organoleptic characteristics.

Keywords: Metabolomics, Orbitrap, Beer, LCMS

P25.04 Adding Information to Metabolic Pathway Understanding of Rat Liver Tissue Changes on High Fat Diets

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Introduction and Objectives: Pathway meta analyses of liver samples are described in this study using sample-matched proteomic data and a software tool for integrating data from metabolomics & proteomics sources using the Thermo Fisher Cloud platform. High fat intake is known to increase triglycerides (TGs) in liver tissue and may lead to detrimental health events such as diabetes, liver disease and stroke. This study aims to extend a metabolic pathway understanding of liver samples following high and low fat diets. **Methods:** Liver data samples are derived from twenty male Wistar rats fed HF/no carbohydrate, HF/high carbohydrate, low fat or standard chow for 4 weeks (n= 4 per diet group). Bottom-up proteomic data were collected with a LTQ-Velos instrument (Thermo Fisher Scientific) and processed with the Proteome Discoverer 2.0 software (Thermo Fisher Scientific). Normalized and filtered data were imported into the Thermo Fisher Cloud Protein Analysis application where set-enrichment, and pathway mapping were performed. **Results and Discussion:** High-fat liver samples show significant enrichment of the fatty acid degradation pathway (Kegg:rno00071; raw p-value 3.0E-17; BH Adjusted p-value:1.4E-15), the fatty acid metabolism pathway (Kegg:rno01212; raw p-value: 5.1E-12; BH Adjusted p-value:1.6E-10), and the PPAR signaling pathway (Kegg:rno03320; raw p-value: 4.3E-11; BH Adjusted 9.9E-10). Eight of 81 peroxisome pathway proteins are observed in high-fat samples corresponding to an enrichment p-value of 3.7E-06(BH Adjusted 2.6E-05) for pathway rno04146. This corroborates metabolomics observation of elevated glutathione levels; peroxisomal activity and elevation of reactive oxygen species which lead endogenous oxidative stress. The liver isoform of Carnitine O-palmitoyltransferase

is among 11 identified proteins from the fatty acid metabolic pathway (of 45 proteins). This agrees with previous reports showing significant alteration of carnitines and related metabolite levels in high-fat samples. **Conclusion:** This analysis performed with the Thermo Fisher Cloud Protein Analysis application provides corroborating evidence of specific pathways in rat liver that respond to controlled high-fat diets.

Keyword: tissue pathway enrichment integration

P25.05 MetTailor: Post-Alignment Preprocessing Package for Mass Spectrometry Data in Metabolomics

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Introduction and Objectives: Mass spectrometry (MS) coupled with chromatographic separation enables high-throughput analysis of proteins and small molecules. Analysis of MS data in metabolomics is more dependent on accurate cross-sample peak alignment and reliable normalization due to lack of MS/MS-based compound identification. Shortcomings in these processing steps can therefore introduce false positive findings due to misalignments and erroneous normalization adjustments in datasets with a large sample size. **Methods:** We developed a software package MetTailor containing two novel post-alignment data preprocessing steps to remedy potential drawbacks. First, we propose a novel dynamic block summarization (DBS) method for correcting misalignments. In addition, we also provide a tool to extract isotopic intensity patterns and charge states from the raw data, which enables comparison of the isotopic ratio profiles of the re-aligned peaks. Second, we propose a flexible data normalization procedure that adjusts the data against temporal variation along the chromatography retention time (RT). **Results and Discussion:** We demonstrate the DBS method using a publicly available dataset with the gold standard alignment. Comparing with the gold standard, 108/117(92.3%) recovered peaks were mapped to gold standard. We also demonstrated the proposed method using a published dataset from patient sera with primary Dengue infection, including 115 MS runs. Overall, the DBS method corrected ~7,000 misalignments. Comparing the peaks with clear charge states and isotopic patterns, 2107/2380(88.5%) of these re-alignments were of credible quality. In addition, the RT-based normalization algorithm efficiently removed temporal yet systematic variations along the chromatography time, without sacrificing the sensitivity of detecting differentially expressed metabolites. **Conclusion:** Overall, in this work, we provide an open source software package to supplement the popular data extraction tools in the context of large sample experiments. We also provide an alignment quality evaluation protocol to enable cross-sample evaluation of peak alignment quality through charge states and isotopic patterns. All our implementations are freely available at http://mettailor.sourceforge.net/.

Keywords: Metabolomics, Bioinformatics, peak alignment, normalization

P25.06 Application of Quantitative Metabolomics in Maternal, Prenatal and Neonatal Disease Studies

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Introduction and Objectives: Metabolomics has shown significant promise for the discovery of new biomarkers for the detection of a number of complex disorders. The use of metabolomics in obstetrics is however relatively new. The Metabolomics Innovation Centre specializes in quantitative metabolomics. Recently, TMIC has participated in several maternal, prenatal and neonatal disease biomarker studies, including prediction of early and late-onset pre-eclampsia (PE), Trisomy 18, Trisomy 21, and Hypoxic Ischaemic Encephalopathy (HIE). These studies highlight the potential for metabolomic based diagnostic tests, and the potential for contributing to the understanding of maternal and neonatal health. **Methods:** First trimester prediction of early- and late-onset PE, Trisomy 18, and Trisomy 21: NMR based metabolomic analysis was performed on first trimester maternal serum between 11-13 weeks of gestation in a case-control study. There were 30 cases each of early and late onset PE, Trisomy 18, and Trisomy 21, and 60 unaffected controls. The concentrations of 40 metabolites were compared between the two groups. Hypoxic Ischaemic Encephalopathy (HIE): The study population was divided into those with confirmed HIE (n=31), asphyxiated infants without encephalopathy (n=40) and matched controls (n=71). A combined DI and LC-MS/MS assay (AbsolutIDQ p180 kit, Biocrates Life Sciences AG, Innsbruck, Austria) was used for the metabolomic analyses. **Results and Discussion:** Statistically significant differences between cases and controls were observed for 20 metabolites (early PE) and 17 metabolites (late PE); 11 metabolites in trisomy 21; 2 metabolites in trisomy 18; and 5 metabolites in HIE. The markers demonstrated high sensitivity and high specificity. A logistic regression model using 5 metabolites clearly delineated the severity of asphyxia and classifies HIE infants with AUC = 0.92. **Conclusion:** Here we have demonstrated the utility of metabolomics technology for studying prenatal, neonatal and maternal conditions. Metabolomic biomarkers for early and late onset pre-clampsia, trisomy 18, trisomy 21 and HIE have been identified in these studies.

Keywords: Trisomies 18 and 21, pre-eclampsia, Hypoxic Ischaemic Encephalopathy, Metabolomics

P25.07 Global Activity Survey of Transcription Machinery in Circadian Rhythm of Mammals

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Introduction and Objectives: An intrinsic clock enables organism to anticipate environmental changes through aligning the physiological behavior and biochemical processes with the day/night cycle. Transcription factor (TF) and coregulator (CoR) families, whose expressions and transcriptional activities are driven by the core interlocking loops of mammalian circadian clock, maintain the global rhythms of gene expression. However, the comprehensive and dynamic regulations of TF-CoR transcriptional machinery in a day/night circadian clock remained ill-known. **Methods:** By using catTFRE pull-down and combined with a label-free MS-based quantification approach. **Results and Discussion:** We found more than 400 transcription factors (TFs) and the comparable number of coregulators in mouse liver in a light/dark

cycle. Most of them had higher DNA binding activity at the time point of dark to light, including some general transcription related complex that show circadian osilations. In addition, the binding activities of Pol II and histone components bursted at Zeitgeber time (ZT) 12, while mediator complex exhibited a single transient pulse at ZT 0. Our results have demonstrated that DNA binding activities of TFs and CoRs featured obvious circadian rhythmicity. **Conclusion:** Based on the study, we introduced a possible transcriptional working model: general transcription machinery initializes transcription at the time point of light to dark switch; then core clock genes were suppressed and transcription paused; until mediator complex recruits TFs to drive the productive elongation at the time point of dark to light switch; and core clock genes were activated during the day of elongation. We believe that this work can sever as data resource in understanding transcriptional regulation in circadian clock.

Keywords: Circadian clock, General transcriptional machinery, Mediator complex, Core clock genes

P25.08 AltitudeOmics: Red Blood Cell Metabolic Adaptation to High Altitude Hypoxia

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Introduction and Objectives: Adjustment to hypoxia is a key challenge for millions of people either living at or traveling to high altitudes. Exposure to acute hypoxia for unadjusted individuals results in a marked drop in exercise capacity, impaired cognitive function, and possible loss of consciousness, all of which can be reversed upon acclimatization. The AltitudeOmics research program was designed to provide insights into acclimatization and retention of these adaptations upon reascent to high altitudes. Here, we assessed the metabolic response of red blood cells (RBC), key mediators of tissue oxygenation, to high altitude hypoxia. **Methods:** RBCs were collected from 21 healthy volunteers at sea level, after exposure to high altitude (5260m) for 1, 7 and 16 days, and following reascent after 7 days at 1525 m. UHPLC-MS was used for relative quantitation of hydrophilic metabolites across the time points and results were correlated with physiological and athletic performance parameters. **Results and Discussion:** Immediate metabolic adaptations were noted as early as a few hours after ascending to >5000 m and were maintained for 16 days at high altitude. Consistent with the mechanisms elucidated in vitro, hypoxia promoted glycolysis and deregulated the pentose phosphate pathway, as well purine catabolism, glutathione homeostasis, arginine/nitric oxide and sulphur/H2S metabolism. Metabolic adaptations were preserved one week after descent to lower altitude and were consistent with improved physical performances in comparison to the first ascent, thus suggesting a mechanism of metabolic memory. **Conclusion:** Correlations in the levels of purines and triose phosphates suggest strong metabolic linkages between these pathways, indicating possible avenues for therapeutic intervention to promote adaptation to hypoxia. Ultimately insights gained from understanding RBC response to hypoxia not only provide a mechanistic understanding of RBC biology, but can also be applied to practices of transfusion medicine and treatment of trauma/hemorrhagic shock-induced hypoxemia.

Keywords: Metabolomics, Erythrocyte, Hypoxia

P25.09 Qualitative and Quantitative Profiling of Naphthenic Acids in Oil Sands Process Water

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Introduction and Objectives: Classical naphthenic acids (cNAs; CnH2n+zO2) are aliphatic and polycyclic organic acids which occur naturally in crude oils and bitumen. cNAs leaching from bitumen into the oil sands process water (OSPW) have acute or chronic toxicities of cNAs to aquatic organisms. In this study, we demonstrate a polar reversed-phase ultrahigh performance/ ultrahigh-resolution (UPLC-UHR) MS approach for more comprehensive profiling of OSPW cNAs. **Methods:** Two commercial NA standard substances and two representative OSPW samples collected from northern Alberta, Canada, were used. cNAs were extracted by either solid-phase extraction (SPE), liquid-liquid extraction (LLE) or acetonitrile precipitation. Separations of various cNAs were compared on cyano (CN), C4, C8 and C18 UPLC columns. An LTQ-Orbitrap Fusion mass spectrometer was used for compound detection in the Fourier transform (FT) mode. Concentrations of cNAs were determined and the accuracy was measured as the recovery of a spiked-in standard. **Results and Discussion:** The polar CN UPLC column generated the best peak shapes of cNAs and this polar reversed-phase UPLC/FTMS method enabled successful assignments of 448 molecular formulae representing 630 different cNA compounds detected in the OSPW from steam-assisted gravity drainage oil sands extraction process. Reliable identification of cNAs was based on accurate mass measurements; correlation ($R^2 \geq 0.995$) of the LC retention times with the molecular compositions of homologous cNAs; and structural confirmation of the COOH groups in the putative cNAs by chemical derivatization. Our results indicated a more complicated cNA profile of OSPW than that of the commercially available standard mixtures, and two different OSPW samples showed significantly different cNA abundance distributions from the standard substances and from each other. Absolute quantitation with external standard calibration showed the quantitation accuracy in the range of 65% to 80%. **Conclusion:** Comprehensive characterization of classical naphthenic acids (cNAs) in oil sands process water was achieved by polar reversed-phase UPLC-FTMS.

Keywords: Naphthenic acid, UPLC-FTMS

P25.10 SIRT5 Desuccinylates Mitochondrial Isocitrate Dehydrogenase 2 and Regulates Tumorgenesis

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Introduction and Objectives: 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. **Methods:** not applicable

Results and Discussion: 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. 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. **Conclusion:** Therefore, our findings indicate possible important link between SIRT5 and tumorgenesis.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.

Keywords: SIRT5, IDH2, Liver cancer, Succinylation

P26: POSTER SESSION - CHEMICAL PROTEOMICS AND DRUG DISCOVERY

P2: P6.01 Assessing Biotherapeutics Stability Using Raman Spectroscopy

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Introduction and Objectives: Pharmaceutical industries are drifting towards the development of biotherapeutics as they offer better drug efficacy with minimal side-effects. Responding to the need for self-administered drugs, protein-based therapeutics have to be developed at high concentrations. However, the tendency for proteins to aggregate in solution is increased at these high levels (>100 mg/mL). As a result, the formation of aggregates can alter protein structure and influence the bioavailability and the effectiveness of the drug. **Methods:** To this end, our group has investigated the potential of Raman spectroscopy as a non-invasive and label-free tool to assess protein formulations stability. **Results and Discussion:** Results from this study identified specific Raman signature bands that can be used to highlight individual amino acid residues that are responsible for structural changes in proteins. **Conclusion:** not applicable

Keywords: aggregation, proteins, drugs, label-free

P26.02 Cell-Based, High-Throughput Screen to Overcome Drug Resistance by CIN (Chromosomal Instability)

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Introduction and Objectives: Drug resistance is a major problem in anticancer drug therapy and is caused by CIN (chromosomal instability), a hallmark of cancer. CIN is a high rate of gain or loss of whole or parts of chromosomes, which leads to heterogeneity of cancer cells. **Methods:** In order to overcome drug resistance caused by CIN, we performed small molecule library (about 90,000 compounds) screening to identify compounds that kill CRC (colorectal cancer) cell lines that are often used to study CIN. **Results and Discussion:** With HTS, we selected two compounds that kill 14 CRC cell lines exhibited CIN as well as other 26 cancer cell lines. We showed that the compounds induced apoptotic cell death, which was independent of autophagy. In addition, there was more than 20 hold difference of IC50 between CRC and normal cells. IP (immunoprecipitation) -MS and phosphoproteomic analysis revealed that the compounds activated mitotic checkpoint protein, such as NBN, BRCA1, CHK1 and CHK2, but did not activate ATM. Total proteomic analysis showed that the compounds decreased the abundance of mitochondrial protein. The compounds inhibited mTOR signaling leading to the induction of HIF1 protein expression, p27 protein induction and p62 degradation. We are currently focusing on the molecular mechanisms of mTOR pathway inhibition by mitotic checkpoint proteins, the importance of the decreased mitochondrial protein, and the identification of the molecular target of the compounds. **Conclusion:** Our data indicates that chemical proteomic approach leads to the understandings of the mode of action of small molecule even when the target of small molecule is unknown. From these findings, we will try to understand how to overcome drug resistance by CIN, which is often correlated with poor patient outcome in anti-cancer drug therapy.

Keywords: CRC (colorectal cancer), chromosomal instability, drug resistance, High throughput screening

P26.03 Evaluation and Optimization of Immunodepletion of Plasma from Common Preclinical Animal Models

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Introduction and Objectives: Pharmacokinetic measurements of non antibody-based biotherapeutics during early stage drug discovery can be hampered by the complexity of the plasma proteome within animal models; particularly for targets which have no complement antibody for enrichment. In an attempt to simplify PK measurements via LC-MS, we have demonstrated the applicability of immunodepletion technologies, originally designed for use with human plasma, against cynomolgus monkey and beagle, two commonly used animal models. **Methods:** An LC-based immunodepletion column, containing an IgY antibody population specific to the most abundant proteins within human plasma, was tested for efficiency in its use with both cynomolgus monkey and beagle plasma. As a baseline control, column performance using pooled plasma for each species was compared using an equivalent load of human pooled plasma. Quantitative performance of the column was assessed globally using standardized colorimetric and spectrometric assays. Performance towards individual analytes was determined using label-free quantification via LC-MS. Optimizations of resin performance were then undertaken utilizing varying levels of input amounts, as well as looking at alternate depletion strategies and formats. **Results and Discussion:** The depletion column was shown to be effective at removing >90% of protein mass for monkey and beagle plasma, when loaded at approximately 80% and 40% of theoretical column capacity, respectively, as compared to human samples. Remarkably, under these conditions, the most abundant protein within plasma, albumin, was shown to be depleted at efficiencies of >99%. **Conclusion:** The results show that there is significant cross-reactivity of IgY antibodies produced for human targets with mammalian non-human antigens. This finding demonstrates the applicability of immune-based sample fractionation technologies across multiple mammalian species and a potential universal approach for simplification of the analysis of

biotherapeutic samples for LC-MS.

Keywords: biotherapeutic, Depletion, plasma, pharmacokinetic

P27: POSTER SESSION - PROTEOMICS AND CELL IMMUNITY

P27.01 Unbiased & System-Wide Characterization of MHC-Bound Peptides & Epitopes

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Introduction and Objectives: Vaccines are an effective preventative medicine, yet their development remains slow. The challenge is identifying what the host presents on the highly polymorphic major histocompatibility complexes (MHCs). Immunopeptidomics holds promise but conventional data processing schemes yield high error rates which this project aimed to circumvent. **Methods:** MHC-bound peptides from B-lymphocytes of two parents and their five children were eluted from the cell surface using an acidic solution and analyzed on a QExactive mass spectrometer. Peptides that formed nested sets were organized into epitopes with an associated Ei value based on normalized intensities. Epitopes were clustered by Ei and the sequences in each node were aligned then analyzed using Weblogo, revealing MHC binding consensus sequences that either matched the donors' MHC haplotypes, or previously unreported ones. In a cell homozygous for HLA-A2*02, this gene was knocked down to validate its binders and their consensus sequence. **Results and Discussion:** In total, 38814 peptides (1.8% FDR) were identified. In MHCI consensus sequences, binding anchors were at positions 2 and 9 of the core region, and roughly at positions 1, 4, 6, and 9 for MHCII. Peptide intensities could predict the core binding region of each epitope. Lost epitopes in the HLA-A2*02 knockdown matched the HLA-A2*02 consensus sequence. Immunopeptidomic analysis of acid-eluted peptides from cells, followed by data tabulation and unbiased filtering, can identify epitopes bound by specific MHCs and predict their core binding regions. Consanguinity of the cell lines made it possible to deduce the consensus binding sequence for many of their HLA haplotypes. **Conclusion:** This approach identifies MHC-bound peptides without inherent biases associated with MHC immunopurification or validation based on prior consensus motifs. Its ability to analyse many cell lines using low sample amounts in a species-independent manner to reveal immunodominant epitopes is a major breakthrough that paves the way for rational vaccine design.

Keywords: immunopeptidomics, mhc, immunodominance, non-tryptic peptides

P27.02 Profiling of Alum Adjuvants-Induced Host Cell Immune Response, by a Systems Vaccinology Approach

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Introduction and Objectives: Alum adjuvants are used since the 1920's and are the most widely used vaccine adjuvant worldwide. Although Alum is a critical component in many vaccines used today, a comprehensive understanding of the working mechanism of alum adjuvants is still incomplete. Here, we use complementary techniques to unravel the mechanism of action of Alum adjuvant. **Methods:** Monocytes were isolated from human blood donors and cultured with AlOH3 stimulation or in culture medium for 24 and 48 hours. From all cell batches, cell samples were taken for evaluation of selected differentiation markers using FACS analysis, whereas supernatants were used to detect specific excreted immunological markers using Luminex technology. The remaining portions of the cell batches were used for qPCR on 84 genes from the innate and adaptive immune system and for comprehensive proteome analysis using TMT(6) isobaric labeling. ProteinCenter was used to process the data. **Results and Discussion:** Alum stimulation of monocytes initiates the differentiation of monocytes into dendritic cells, even in the absence of antigens. Quantitative profiling using qPCR and mass spectrometry revealed significant regulation of several immunological relevant cellular pathways upon Alum stimulation, including the activation of the inflammasome as revealed by the up-regulation of NOD-like receptors, which regulate the inflammatory response of e.g. dendritic cells. Interestingly, MHC Class-I and Class-II antigen presenting pathways show a combined increased expression profile, without antigens present. Discussion: Our data revealed that upon stimulation with alum monocytes prepare themselves for generating an inflammatory as well as an adaptive immune response, even in absence of antigen. The up-regulation of both MHC I and MHC II presentation pathways indicated that alum is capable of initiating various immunogenic pathways. **Conclusion:** Utilizing complementary techniques (Luminex, FACS, qPCR and mass spectrometry-based proteomics) allowed for the comprehensive molecular profiling of the effect of Alum upon stimulation of human monocytes.

Keywords: alum adjuvant, systems vaccinology, MHC response to alum adjuvants, immune skewing by alum adjuvant, proteomics approach for adjuvant analysis

P27.03 The Human T Cell Secretome. Physiological Responses to ex vivo Transfer, and to in vitro Activation

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Introduction and Objectives: Naive human T-lymphocytes from two adult male donors were harvested from buffy coats, sorted by FACS, and put in primary culture. The changes in protein composition of the cell culture medium was studied over time during T-cell activation by antiCD3 and antiCD28. **Methods:** At 3 time points after activation (6, 24 and 48h), conditioned media were analyzed by conventional (bottom-up) proteomics. After centrifugation of the cell culture medium to remove cell debris, proteins were separated by C5 RP-HPLC. The resulting 13 fractions were individually reduced, alkylated, and digested by trypsin. Tryptic peptides were analyzed by C18 RP nano-LC MS/MS on a linear ion trap-orbitrap hybrid instrument (LTQ Orbitrap Velos, ThermoFisher, Bremen, Germany). Data were searched by 2 different methods, including Mascot against UniProt using spectral counting for protein quantification, as well as Sequest employing 'Quanty', a protein quantitation algorithm developed at Karolinska. **Results and Discussion:** From over 512 hours of LC MS/MS analyses, yielding >300 Gb data, more than 90% of the data matched with bovine proteins, evidently originating in the culture medium additive FCS. Yet the <5% human peptides identified clearly reveal some of the cell biological (re)actions of the in vitro cell to its transfer ex vivo. In addition, clear activation signals are detected,

including various interleukins (IL-2, IL-9, IL-17A, IL17F, IL-37...), GDF9, TNFalpha, IFNG, CCL4, granulins, and others. For several of these secretory proteins their differential presence in the activated versus non-activated cell media was orthogonally validated by ELISA. We want to underline that our primary detection of these activation 'signals' was entirely unbiased, using orbitrap based MS as sole detection device and without employing any antibody. **Conclusion:** We observe that upon rapid adaptation by the ex vivo T-cell to an artificial medium, an optimized secretomics approach allows one to subsequently measure many typical extracellular activation proteins, described earlier as T-cell communication signals.

Keywords: human T-cell activation, secretome, in vitro culture, intercellular communication

P27.04 MS-Based Quantitative Proteomics for Serum Biomarker Discovery in Systemic Lupus Erythematosus

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Introduction and Objectives: Systemic Lupus Erythematosus (SLE) is a multisystem autoimmune disorder where interplay of environmental and genetic risk factors leads to progressive loss of tolerance to self antigens culminating in clinical disease. SLE is largely an unmet medical need with only one new approved drug in the past 50 years. Given the highly heterogeneous nature of this disease, a personalized approach with novel predictive markers is important for the development of new medicines for SLE. Our study was undertaken to develop mass spectrometry-based methodology for serum biomarker discovery in SLE. **Methods:** We coupled serum depletion methods with mass spectrometry based quantitative proteomics. The high abundant proteins were first removed from 10 SLE and 10 healthy control sera via serum depletion columns. Depleted sera were next subjected to digestion and tandem mass tag labeling prior to 2D fractionation and MS/MS run in a Q-Exactive. Mascot/Proteome discoverer was used for peptide/protein identification and quantification. **Results and Discussion:** A total of 1,349 proteins from SLE and healthy control serum samples were identified and quantified. Gene ontology analysis showed that one third of the quantified proteins are intracellular proteins which may be released due to the high apoptosis rates in tissues of SLE patients. Multiple proteins showed higher (n=78) or lower (n=5) levels in SLE than healthy controls ($p<0.05$). Ingenuity pathway analysis (including differentially expressed proteins with p-value<0.05) showed that cell movement of neutrophils, inflammatory and immune cell response, phagocyte and antigen presenting cell activation were over-represented in SLE sera. Furthermore, leukocyte extravasation signalling and interleukin pathways were found to be upregulated in SLE patients. **Conclusion:** In this study, we have developed a successful serum depletion coupled MS based proteomics technique which can be used to discover novel biomarkers from an easily accessible body fluid and this information can be used to enhance our understanding of disease pathophysiology.

Keywords: Systemic Lupus Erythematosus, TMT proteomics, serum depletion, serum biomarker discovery

P27.05 Proteomic Analysis of Serum Antibody Repertoires

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Introduction and Objectives: An individual's antibody repertoire encompasses a large diversity of non-germline encoded immunoglobulin proteins. These antibodies are the primary effectors of the B cell adaptive immune response. As such, direct characterization of the antibody repertoire is paramount for understanding the complex dynamics of the response, for example, to vaccination or infection. Challenges specific to antibody repertoire proteomics preclude the use of standard analysis methods and motivate our development of novel tools and approaches for interpretation of human polyclonal antibody repertoires. In particular the processes of antibody sequence generation and diversification, through which conserved gene framework regions are interspersed with variable CDR regions and mutated, lead to an enormous expansion of highly similar but distinct antibody proteins. The very similar nature of the resultant peptides, where thousands may share the majority of their sequence, brings unique difficulties for proteomic interpretation and FDR estimation. **Methods:** We have developed a combined next-generation sequencing and proteomics workflow for profiling the human serum antibody repertoire. We construct an antibody sequence database through NGS of peripheral B cells and specialized processing of the antibody variable region sequences. In parallel, we isolate serum antibodies from the same donor, affinity purify against the target antigen(s), and observe using tandem mass spectrometry techniques. Spectra are matched to the database, and we apply computational tools and stringent filters developed in response to the challenges mentioned above. **Results and Discussion:** In recent human vaccine studies, we have shown how our methods are able to resolve the dynamics of an immune response from the broad temporal patterns down to the relative abundances of monoclonal antibodies. **Conclusion:** These capabilities provide the means to study directly the functional nature of the adaptive immune system. Applied to medicine, the methods may be used for disease diagnostics, vaccine efficacy and development studies, and therapeutic antibody discovery.

P27.06 Neonatal Screening for Immuno-Deficiencies by Profiling of Dried Blood Spots on Antibody Bead Arrays

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Introduction and Objectives: Defects in the immune system at birth can lead to severe disorders and recognition of such treatable deficiencies at early stage is of critical importance. Nation-wide neonatal screenings are programs that aim at diagnosis of treatable diseases without evident symptoms at birth, which can otherwise lead to irreversible impairment of vital organs or even death. The number of disorders included in such programs is rising thanks to increased availability of appropriate technologies, therefore expanding the subsequent benefits. Identification of immune-deficiencies at birth is particularly valuable and fulfills the criteria to be included in neonatal screening programs. However, many of such disorders have not yet been implemented in any programs worldwide. This is mainly due to the lack of appropriate assays with reliable analysis that can be feasibly applied on large cohorts. **Methods:** Here, we have assessed the antibody bead array systems, to be applied for multiplexed analysis of immune factors in blood. Proteins are eluted from dried blood spot (DBS) samples, capillary whole blood drops dried on filter paper, as the common sample source in neonatal screening programs. **Results and Discussion:** As a proof of concept, screening was performed

on DBS samples from primary immuno-deficient individuals that lack certain protein targets and normal cord blood was used as controls. A panel of 20 immune factors (C1Q, C2, C3, C4, C5, C6, C7, C8, C9, CFH, CFP, CFB, CFI, CFD, CYBA, CYBB, NCF1, NCF4, HAX1 and ELANE) is examined and the results confirm the lack of signal in respective deficient samples. **Conclusion:** This study suggests the reliability of applying antibody bead array systems in identification of immuno-deficiencies at early stage. Considering the fundamental aspects of such technologies including high-throughput systems and possibilities for automation, the presented assay is readily expandable to large-scale profiling for neonatal screening of immuno-deficiencies.

Keywords: Neonatal screening, Primary Immuno-deficiency, antibody bead array, large scale protein profiling

P28: POSTER SESSION - INTEGRATED OMICS

P28.01 Multi-Omics Analysis of Estradiol Treatment Effects in MCF7 Cells as Part of Human Toxome Project

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Introduction and Objectives: MCF7 cells are widely used in vitro system to study estrogenic effects. Using integrated biology approach combining transcriptomics, genomics, proteomics and metabolomics datasets we are developing the framework for mechanistic assessment of Pathways of Toxicity, a key goal of the Human Toxome Project. Here we report one of the first specific findings addressing why an integrated biology approach analyzing results from multiple technologies simultaneously can significantly improve interpretation and guide follow up experiments more quickly. **Methods:** MCF7 cells treated with control, estradiol or propyl pyrazole triol. Transcriptome profiling experiment was performed on Agilent human gene expression microarrays and qPCR. Microarray and RNA-seq gene expression profiling datasets deposited in GEO were downloaded and analyzed as per standard methods. Shotgun proteomics was performed on UHPLC/Q-TOF mass spectrometer using 100min gradient and data was analyzed using SpectrumMill. MRM experiments were performed on LC-QQQ for metabolomics analysis. Data integration was done on GeneSpring Multi-Omic-Analysis software. **Results and Discussion:** Single omics differential pathway analysis of transcriptomics or proteomics data independently shows number of possible targets to validate. Multi-omics-analysis using integrated pathway analysis of transcriptomics with proteomics enables focus on smaller number of nodes for further analysis and validation. Growing the nodes using network analysis can be used for novel interaction analysis of dataset using hypothesis based model. Using this approach we overlaid genes and proteins that are differentially expressed with ER agonist treatment. Gene regulatory network analysis of transcriptomics datasets using differential expression analysis leading to enrichment scores for Transcription Factors found overlapping sets of regulatory pathways in the two technologies, further evidence Multi-Omic-Analysis enabled elucidation of known biology driving confidence toward further analysis for as of yet uncovered relationships and likely pathways of toxicity. **Conclusion:** Using MOA approach we identified histones and Carnitine o-palmytol transferase b which were differentially regulated by ER induction in both genomic and proteomic datasets

Keywords: Jet Stream Proteomics, Multi-Omics, Toxome, Cancer cell

P28.02 Integrated Molecular Phenotyping in Chondrocytes Identifies Pathways Disrupted in Osteoarthritis

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Introduction and Objectives: Osteoarthritis (OA) affects over 40% of individuals over the age of 70, and is a leading cause of pain and loss of physical function. The causal genes underlying the majority of OA susceptibility have not been identified yet. To increase our understanding of the biological aetiology of OA we have generated DNA methylation, RNA sequencing and quantitative proteomics data from OA joint tissue samples in order to obtain a comprehensive molecular portrait of diseased and healthy chondrocytes. **Methods:** We used isobaric labeling proteomics to quantify the relative abundance of over 6000 proteins. For the generation of RNAseq data we sequenced total RNA using the Illumina HiSeq 2000. For genome-wide methylation profiling we used the Illumina 450k methylation array to assay ~480k CpG sites across the genome. **Results and Discussion:** We performed a gene set enrichment analysis and find that several common gene sets are highlighted from the different functional genomics experiments. We identified 19 enriched pathways from KEGG & Reactome at a combined 5% FDR, and 30 GO annotations. We identify several pathways, including "Extra-cellular matrix organisation" and "Collagen formation", that are affected by genes identified in all 3 experiments. This suggests that the 3 assays converge on shared mechanisms. A common theme to the highlighted pathways is cartilage matrix regulation and degeneration. The GO analysis also identified enrichment in genes annotated as involved in the regulation of angiogenesis. Indeed within the samples investigated histological examination displayed greater blood vessel ingrowth in high grade v/s low grade tissues. **Conclusion:** Collectively these results provide the first integrated analysis of the molecular changes involved in OA chondrocytes from 3 important molecular levels. Using this multi-level approach we have provided for the first time a detailed and integrated characterisation of the genomic consequences of this debilitating disease.

Keyword: Osteoarthritis, Proteomics, RNAseq, Genome-wide methylation

P28.03 Mitochondria-Driven Cancer Pathways in Triple Negative Breast Cancer

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Introduction and Objectives: Driver pathways of triple negative breast cancer (TN-BCa) are still poorly understood. Thus, it is important to identify the underlying mechanisms of TN-BCa progression. Mitochondria-nuclear crosstalk is known to affect tumor properties and metastasis. Here we have generated transmitochondrial-cybrid models to understand mitochondria-regulated cancer pathways and performed different experiments to compare the tumor properties of the cybrids. All OMICs approach was used to study mitochondrial regulation of tumor pathways in transmitochondrial-cybrid models. **Methods:** Transmitochondrial-cybrids were generated under common nuclear backgrounds with mitochondria from benign breast and metastatic TN-BCa cells. Colony formation assay, wound healing assay and in vivo tumor mammary gland transplantation were used to understand tumor properties of cybrids. Mitochondrial electron-transport chain activity, mtDNA genomic variations, reactive oxygen species levels, respiration and mitochondrial ATP levels were analyzed. Gene expression profile was performed by established protocols. Shotgun Jetstream proteomics was performed on UHPLC/AJS-iFunnel Q-TOF. MRM experiments were performed on QQQ LC-MS for metabolomics analysis. **Results and Discussion:** In vivo and in vitro analyses confirmed that mitochondria-nuclear crosstalk regulates tumor property of cancer cell. Microarray data suggested that several cancer-related genes are up and down-regulated in cybrids with cancer and benign mitochondria. Pathway analysis suggested that Src oncogenic pathway is one of the critical pathways involved. We performed pathway-based approach to understand mitochondria-mediated regulation of Src in TN-BCa and we observed autophosphorylation at Y419, which is significantly increased in cancer cells and their cybrids. Further mitochondria respiratory complexes inhibitors suggested that mitochondrial electron-transport chain is playing critical role in the regulation of Src autophosphorylation. Shotgun Jetstream proteomics data suggested that several proteins related to oxidoreductase activity, mitochondrion and lipid metabolic process have altered in cybrids and parental cells. **Conclusion:** Combination of cell, molecular biology, biochemical, in vivo, and OMICs approaches allowed us to discover a novel mitochondria-driven pathway that impacts on TN-BCa oncogenesis and metastatic progression

Keywords: Cybrids, Jet Stream Proteomics, Multi-Omics, Phospho

P28.04 Using Integrative Multi-Omics to Detemine Biomolecular Changes in Weight Gain and Insulin Resistance

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Introduction and Objectives: Three million deaths worldwide are attributed to obesity. However, the biomolecular mechanisms that describe the link between adiposity and subsequent disease states are poorly understood. Insulin resistance characterizes approximately half of obese individuals and is a major cause of obesity-mediated diseases such as Type II diabetes, hypertension and other cardiovascular diseases. This study makes use of longitudinal quantitative and high-throughput multi-omics (genomics, epigenomics, transcriptomics, glycoproteomics etc.) methodologies on adipose tissue and blood samples to develop multigenic and multi-analyte signatures associated with weight gain and insulin resistance. **Methods:** Participants of this study underwent a 30-day period of weight gain via excessive caloric intake followed by a 60-day period of restricted dieting and return to baseline weight. We took blood samples and adipose tissue biopsies at three different time points per patient: baseline, peak-weight and post weight loss. We also categorized our patients as either insulin resistant (IR) or insulin sensitive (IS) and then processed their samples using longitudinal multi-omic technologies. **Results and Discussion:** This comparative study revealed a wealth of biomolecular changes associated with weight gain. Pathways of interest included those involved in lipid remodeling, acute inflammatory response and glucose metabolism. Some of these biomolecules returned to baseline levels as the patient returned to normal weight whilst some remained elevated. IR patients exhibited key differences in inflammatory response regulation in comparison to IS patients at all time points. These signatures suggest differential fat metabolism pathways between IR and IS patients. **Conclusion:** We identified biomolecular differences associated with weight gain and insulin resistance on various levels: in gene expression, epigenetic change, transcriptional regulation and glycosylation. However, in this study, we were not only able to contribute to new biology that could be of use in preventing or predicting obesity-mediated diseases, but we also matured novel technologies to produce and process data on so many omics levels.

Keywords: insulin resistance, integrative personalized omics, Obesity

P28.05 Integrating Genomics and Proteomics to Reveal Loci Underpinning Social Immunity in Honey Bees

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Introduction and Objectives: Honey bees are arguably the world's most important pollinator species. Maintaining the health of these species is critical for the beekeeping industry and for the production of food that depends on honey bee pollination. The use of isolated breeding programs to select for health-associated traits has been one successful strategy for mitigating the impacts of disease on honey bee health. One of the most successful disease resistance traits that bee breeders have bred for has been hygienic behavior. This behavior involves nurse bees This behavior involves nurse bees detecting and removing dead or infected larvae from the colony. **Methods:** We integrated the power of both Proteomic and Genomic data sets in order to understand the loci underpinning this trait. Using a shot gun proteomics approach we identified proteins correlated with hygienic behavior (Guarna et al, 2015, BMC Genomics 16:63) and then developed MRM assays for these proteins. The MRM assays were used in parallel with field assays in a selective breeding program for hygienic behavior. After three generations, we were able to increase the average hygiene in the selected population 30% over unselected population's average. Using Illumina HiSeq 2500 Rapid with 150bp paired-ended reads we fully sequenced a total of 126 haploid drones from each population (27 selected colonies and 11 unselected colonies) to a mean depth of 33.07 reads.

Results and Discussion: Using genome-wide scans for positive selection, we identified putative causal loci across the genome and narrowed these regions by associating hygienic behavior and protein expression within these selected regions. **Conclusion:** These associated regions can be used for targeted-marker-assisted breeding programs of honey bee populations and to better understand the evolution of social immunity.

Keyword: honey bees, agriculture, -omics,

P28.06 SIMPLEX - A Multimolecular Omics Approach for Systems Biology

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Introduction and Objectives: Cross-talk between lipids and proteins occurs on multiple layers, however is far from well- understood. We present SIMPLEX (SImultaneous Metabolite, Proteins Lipid EXtraction) – a straightforward protocol enabling quantitative metabolomics, proteomics (including PTMs) and lipidomics from a single sample in one workflow. Performance, sensitivity, and reproducibility of SIMPLEX are comparable to well-established protocols that are limited to individual species. Thus, even for limited sample amounts complex biochemical processes can be studied on multi-molecular levels as demonstrated for PPARG signaling in a model system for adipogenesis. **Methods:** Metabolites, proteins and lipids were simultaneously extracted from OP9 cells using SIMPLEX. Samples were analyzed by high resolution LC-MS, direct infusion and SRM. After demonstrating the high technical reproducibility of SIMPLEX, we studied PPARG signaling in OP9 cells using multi-molecular label free quantitation. **Results and Discussion:** We could quantify 360 lipids, 75 metabolites and 3,327 proteins (2 h single shot, >2 unique peptides quantified) by label free quantitation, using only 1,000,000 cells for all three replicates. We achieved excellent reproducibility, obtaining R^2 values >0.97 on all three omics levels. Relative standard deviations were 5% for lipids, 15% for metabolites, and 18% for proteins – over 3-4 orders of magnitude. Moreover, after SIMPLEX we identified 2,018 high-confidence phosphorylation sites from only 60 μg of protein (2 h analysis). Finally, we used SIMPLEX to study PPARG signaling in OP9 cells, identifying 869 significantly regulated species: 101 lipids, 48 metabolites, and 720 proteins. Our data indicate exciting novel interactions within and between all three classes that would have been concealed from classical workflows. **Conclusion:** SIMPLEX is a reproducible and powerful method for quantitative multi-omics, allowing for the first time to simultaneously study lipids, metabolites and proteins, a clear advantage over classical unimolecular workflows.Thus, SIMPLEX provides completely new insights into the systems biology of health and disease.

Keywords: proteome, lipidome, metabolome, Multi-Omics

P28.07 An Integrated Omics Approach Reveals Synaptic Plasticity Changes in an Alzheimer´S Mouse Model

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Introduction and Objectives: Alzheimer´s disease (AD) is the leading form of dementia within the elderly population and ranks on the fourth death-causing place. As the global prevalence of AD is predicted to increase dramatically to up to 80 million patients by 2040, it is crucial to elucidate the disorder´s molecular mechanism on the level of signalling pathway alterations and their role in AD aetiology. **Methods:** We have studied the neurological-focused miRNAome, synaptic plasticity-targeted transcriptome and proteome with its global phosphorylation and glycosylation pattern in the hippocampus, cortex, olfactory bulbus and brainstem of 12 months old APP/PSEN1 male mice. Signalling pathway analysis to elucidate affected molecular networks and upstream transcription factors was performed using different bioinformatic tools. **Results and Discussion:** Several miRNAs were found to be deregulated associated to neuroinflammation, neuroprotection and synaptic plasticity. Particularly, miR-146 levels were elevated in the hippocampus and cortex acting as a pivotal regulator of the pro-inflammatory NFκB pathway. By transcriptomic, we detected a change in mRNA transcripts levels of the NFκB pathway and neurothrophic molecules, such as an increase in Igf1, only in these two brain regions. Several subunits of neuronal receptors for glutamate were elevated in all the brain regions suggesting defects in neurotransmission on synapses. Investigations on the proteomic and post-translational modification level are currently performed to get more insight into this pathway dysregulation. **Conclusion:** The molecular analysis demonstrated that signalling pathways related to neuroinflammatory and synaptic plasticity dynamics are altered in a symptomatic AD mouse model. The molecular data may link to learning and memory disturbances which are a hallmark in AD patients.

Keywords: NFκB, miRNA, Alzheimer´s, signalling pathway analysis

P28.08 Sportomics: Building a New Concept in Metabolic Studies and Exercise Science

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Introduction and Objectives: Advances in biochemical and biophysical methods have enabled us to better understand cells and organisms. For more than a decade, we have used alternative approaches to understand metabolic responses to physical stress. In addition to classic laboratory studies (cell and animal models), we have used elite athletes and sports to examine metabolic stress. In 2011, in an analogy to other "-omics" sciences, we proposed the concept of Sportomics to mimic the real challenges and conditions that are faced during sports training and competition. **Methods:** Sportomics is non-hypothesis-driven research on an individual's metabolite changes during sports and exercise. Focusing mostly in proteomics and metabolomics (also other "-omics" approaches) Sportomics centers on sports as a metabolic challenge. Our study is holistic and top-down; we treat the data systematically and have generated a large computer-searchable database. We also propose that in-field metabolic analyses are important for understanding, supporting and training elite athletes. **Results and Discussion:** Sportomics is a useful tool for managing athlete training and performance. We showed that collecting and analyzing physiological data during training could provide important information on an athlete's clinical condition and performance. Here we are going to show how Sportomics is helping in preparing world-class athletes for the forthcoming Games. Thus, using Sportomics protocols, we can better understand the metabolic changes that are induced by exercise and sports. **Conclusion:** We believe that this approach can fill a methodological gap between systems biology and translational medicine similar as a bench to the field approach.

Keywords: Sportomics, Metabolomics, proteomics, Sports Science

P28.09 Combined Targeted Analysis of Proteins and Metabolites in Tear Fluid

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Introduction and Objectives: Tear fluid fulfills multiple tasks with regard to the eye's visual functionality. It protects the epithelial cells against dehydration, prevents microbial invasion and carries nutrients. Therefore, its composition is reflective of ocular health. Moreover, given the possibility to measure the glucose concentration in tear fluid as a surrogate for the blood glucose level, tear fluid has the potential to become a matrix for diagnostic analyses of systemic disorders. Since the sampling of tear fluid seems rather variable we sought to establish a standardized protocol for combined proteomics and metabolomics. **Methods:** Tear fluid was taken from human volunteers by special Schirmer strips. For further processing 4 mm punches of the strips were used. A metabolite fraction was generated by liquid extraction either with or without derivatization leaving the precipitated proteins behind. The remaining material was processed for bottom-up proteomics. Both methods were carried out in a targeted fashion using isotope-labelled standards in order to quantify analytes of interest. **Results and Discussion:** Targeted metabolite analysis could be performed for more than 100 analytes, e.g. amino acids, acylcarnitines, glycerophospholipids, etc. Most of the major metabolites exhibited good intra-individual reproducibility (CV < 20 %, e.g. amino acids: 17 of 21). With respect to targeted proteomics an MRM-based assay could be established for ten abundant tear proteins like lactotransferrin, lipocalin-1, hemopexin etc. The proteins were represented by 14 pre-selected proteotypic peptides, for all of which the reproducibility was high (CV < 15 %). Since normalization of tear fluid data is a major issue we have evaluated strategies to use the two different data sets for cross-normalization. **Conclusion:** Despite the minute sample amounts and despite variances in tear secretion rates it has been successfully shown that targeted metabolomics and proteomics of tear fluid can be standardized. This gives hope for future diagnostic applications using tear fluid, which is rather easily accessible.

Keywords: targeted metabolomics, Targeted proteomics, diagnostics, body fluid

P28.10 Visualizing Proteomics Data in Genomic Context Using the UCSC Genome Browser

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Introduction and Objectives: As the comprehensiveness and quality of mass spectrometry peptide identifications improves, researchers outside the proteomics community are increasingly interested in visualizing and analyzing proteomic datasets in genomic context with gene sets, epigenetic profiles, genetic variation, and clinical data anchored to the genome. Those seeking to integrate their proteomics with other 'omics datasets can benefit from existing tools and databases of well-curated genome annotations. The UCSC Genome Browser provides a comprehensive resource of genomes and annotations together with visualization and analysis tools in continuous use by scientists and students worldwide for 15 years. Here we describe recent developments in the browser relevant to the proteomics community, including the incorporation of the PeptideAtlas 2014 human build and the CPTAC cancer proteomics data hub. **Methods:** The UCSC Genome Browser group develops and maintains the database, website, and tools at genome.ucsc.edu and genome-euro.ucsc.edu. Genomes and annotations from NCBI Genbank, Refseq, dbSNP, and ClinVar are hosted alongside gene sets from Ensembl/GENCODE, transcription and regulatory datasets from ENCODE, genetic variation from 1000Genomes, biomedical annotations from literature and curated sources, and comparative genomics from sequence alignments of over 100 species. **Results and Discussion:** Proteomics datasets and displays are new to the browser in 2014. The PeptideAtlas 2014 human build was incorporated into the browser database. CPTAC cancer proteomics public data hub is now available to browser users. Also new this year. the 'Genome Browser In a Box' is a virtual machine image of the UCSC browser that runs on most personal computers (Windows, Macintosh, or Linux). This easily-installed subset of a mirror site is useful to researchers with protected data. For shared data, the 'data hub' feature supports high-performance public access to locally stored user data. **Conclusion:** Integrating proteomics data into the UCSC Genome Browser expands the data utility to users beyond the proteomics community and provides an integration platform for researchers.

Keyword: proteogenomics genomics visualization

P28.11 Integrated Proteomics for Identification of the Specific Network in Cancer Stem Cells

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Introduction and Objectives: Comprehensive analyses for studying cancer related cellular signals to be clinically targeted had been difficult due to limitations in analytical software, biological database and validation standard. We have developed an integrated analysis system using semi-quantitative proteomics, such as LC-shotgun: iTRAQ/SYLAC/IDA etc. and gel-based electrophoresis: 2D-DIGE/1D-PAGE as well as transcriptomics, such as DNA array/qPCR, and following a unique sequential strategy that includes data mining tools called MANGO[1)] and iPEACH[2,3)]. These tools provide the voluminous comprehensive data including protein/gene expressions, post-translational modifications, and several functional information from several types of analyses into a useful data file. In this study, we constructed an iPEACH database for glioma stem cells (GSCs), and used GO and knowledge-based network analyses to extract novel candidate signal networks regulated during their malignant processes. **Methods:** To identify the molecular targets related to the maintenance/differentiation of GSC, we established 9 clones from patient's gliomas having the potential to differentiate into glioblastomas, and subjected to iTRAQ, 2D-DIGE and DNA array based integrated proteomics. **Results and Discussion:** iPEACH analysis of 8,471 proteins and 21,857 mRNAs identified and integrated revealed that ECMs-integrins-RAS-MAPK/PI3K signalings were significantly upregulated, and specific proteoglycans(pGAGs) as well as stem cell markers were obviously downregulated during GSC differentiation. The differentiation was dramatically accelerated by these ECMs, and suppressed by integrin inhibitors/GAGs that raised GSC chemosensitivity. Combination of TMZ and their inhibitors suppressed glioma progression and led the longer survival of mouse xenograft models[3)]. These results demonstrate that GSCs induce/secrete ECMs and their receptors to regulate their stemness and differentiation process

via developping specific "niches" that could be clinically targeted. **Conclusion:** This is the first integrated proteomics providing new therapeutic ideas against the cancer stem cells-associated malignant tumors. Kobayashi D et al. Mol Cell Proteomics 2009, 8(10):2350, Hirayama M. Kobayashi D. et al. Mol Cell Proteomics 2013 12(5):1377, Niibori-Nambu, A. et al. PLOS ONE 2013 8(5):e59558

Keywords: glioma, integrated proteomics, cancer stem cells, network analysis

P28.12 The Principles of the Relationship between Gene Intrinsic Properties and Phenotypic Features

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Introduction and Objectives: How does the information stored in DNA determine the eventual complex phenotypic features is one the fundamental questions in molecular biology. To answer this question, it is necessary to classify the protein-coding genes by their intrinsic properties, such as evolutionary origin time, evolution rate, structural and functional features, etc, and to explore the relationship between these properties and molecular phenotypic features, such as gene expression width, expression level, and so on. Following our previous studies (MBE 2012), here, we report the new results from the analyses based on a series of genomic, transcriptomic, and proteomic data. **Methods:** Over/under representation analysis is based on hypergeometric distribution model and the P values were corrected using Benjamini-Hochberg method. RankSum Test are used to compare two datasets. **Results and Discussion:** Firstly, in the qualitative view of expression, we found that complex genes, such as long genes, genes with multiple cis-regulatory modules, genes encoding long proteins or multi-domain proteins, tend to be utilized preferentially at each developmental stage and each adult OTCs (organ, tissue or cell types). On the other hand, genes with novel properties tend to be expressed at specific developmental stages. These main results are robust across protostomia and deuterostomia regardless of different technologies used to produce the data. These results indicate genes/products' complexity contribute to the complexity of individual proteome at certain states, whereas novelty of genes/products contribute to the diversity of proteomes at different spatial-temporal states. Secondly, In in the quantitative view of expression, we found that bio-complexity-related proteins, including paralogs, multi-domain proteins, proteins containing younger domains, and the sharing domains, all tend to be with lower abundance, and vice versa. **Conclusion:** Our work provides new insights into the general principles for the utilization of genomic complexity factors at molecular phenotypic level both in the qualitative and quantitative views.

Keywords: Protein domain, Expression level, Expression width, intrinsic property

P28.13 Multi-Omic Blood Biomarker Signatures of the Late Phase Asthmatic Response

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Introduction and Objectives: Individuals with allergic asthma respond differently, but reproducibly, to allergen inhalation challenge. Some individuals develop an isolated early response (early responders, ERs) while others also go on to develop a late response (dual responders, DRs). It is not understood why late responses do not develop in all sensitized individuals. **Methods:** 32 individuals participated in the allergen inhalation challenge as part of the AllerGen Clinical Investigator Collaborative. 15 (17) participants were classified as ERs (DRs), respectively. Blood samples were collected prior to and 2 hours after allergen challenge. Measurements of cell counts were obtained using a hematolyzer; gene transcript relative levels using RNA sequencing; metabolite levels using tandem mass spectrometry. Sparse generalized canonical correlation discriminant analysis was used to classify ERs and DRs by integrating all three datasets adjusting for age and sex. Gene Set enrichment analysis was performed using Enrichr. **Results and Discussion:** The pre-challenge multi-signature classifier (error = 30%) out-performed the post multi-signature classifier (error = 50%) in separating ERs from DRs. The cells selected in the pre-challenge multi-signature panel included eosinophils, lymphocytes and neutrophils. The selected metabolites were enriched for glycerophospholipids. The subset of gene transcripts in the multi-signature panel was enriched for the T-cell receptor and co-stimulatory signaling pathway ($p=3.4x10^{-6}$, Wikipathways) and positive regulation of antigen receptor-mediated signaling pathway ($p=5.7x10^{-4}$, GO Ontology). **Conclusion:** This study provides a systems perspective on the deregulated molecular processes between early and dual responses in whole blood.

P28.14 Multi-Omics Analysis of Primary Cytotrophoblasts from Second Trimester and Term Placentas

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Introduction and Objectives: During human pregnancy, a subset of placental cytotrophoblasts (CTBs) differentiates into cells that aggressively invade the uterus and its vasculature, anchoring the progeny and rerouting maternal blood to the placenta. Defects in this process are the hallmark of the pregnancy complication preeclampsia. While disease-associated genes or transcripts may serve as useful biomarkers, they are not necessarily predictive of disease mechanisms. Thus, we performed global proteomic and transcriptional profiling to measure expression patterns of CTBs from second trimester and term normal placentas to gain further understanding of CTB differentiation in healthy pregnancy. **Methods:** Primary CTBs were isolated using collagenase and trypsin digestion and Percoll gradient centrifugation. For proteomics analysis, cells were lysed and digested with trypsin. Variable window SWATH MS data were acquired over a 180 min. gradient using a nanoLC 425 cHiPLC-TripleTOF 6600 System interfaced (Sciex). SWATH data were processed using OneOmics applications in BaseSpace (Illumina). For transcriptomics, a second set of CTBs were analyzed using RNAseq (Kundaje, Nature 2015). iPathwayGuide (Advaita) was used to compare protein and RNA levels for pathway and gene ontology analyses. **Results and Discussion:** Approximately 3000 proteins were quantified and ~400 showed differential expression in second trimester CTBs vs. term. Proteins known to function in CTB differentiation processes, e.g. angiogenesis and hypoxia response, as well as previously uncharacterized processes, e.g., NF-kappaB signaling, metal ion transport and muscle contraction, were altered. Integration with RNAseq data showed variations at the molecular level but concordance among pathways and processes. **Conclusion:** Multi-omics data sets comprised of SWATH MS protein quantification and RNAseq expression results showed gestation age differences in healthy CTB populations corresponding to known and novel processes. Using these methods to study CTBs from patients with preeclampsia is likely to identify aberrations that could contribute to disease

and/or serve as diagnostic markers.

Keywords: SWATH, DIA, Multi-Omics

P28.15 A Component-Based Classifier Can Diagnose AECOPD by Integrating across Omics Data Types

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Introduction and Objectives: Patients with chronic obstructive pulmonary disease (COPD) experience a number of symptoms as a result of fixed airflow obstruction. Acute exacerbations (AECOPD), during which these symptoms worsen significantly, are associated with increased hospitalization, morbidity, and mortality. Yet a ccurate diagnosis of AECOPD remains challenging. The objective of our work was to identify blood-based multi-omics biomarker panels that could be used in the clinical setting to diagnose AECOPD. **Methods:** DNA methylation (Illumina HumanMethylation450 BeadChip), gene expression (Affymetrix Gene 1.1 ST) and protein expression (MRM proteomics) were assayed from blood fractions of 60 COPD patients, part of the Rapid Transition Program cohort, both during an AECOPD and after resolution (convalescent). Biomarker discovery used sparse generalized canonical correlation discriminant analysis (SGCCDA) to identify correlated groups of discriminative features across data types. Out-of-sample performance was estimated using 10 x 5-fold cross-validation. **Results and Discussion:** After pre-processing and normalization of each dataset, the most variable features were submitted to SGCCDA; 18,500 CpG sites, 8,325 probe-sets and 59 peptides, respectively. Ten features were retained from each data type. A 10 x 5-fold cross-validation resulted in an overall estimated out-of-sample error of 14.1±1.9% (20.6±3.7% AECOPD; 9.1±3.2% convalescent), comparable to that obtained using state-of-the-art single omics approaches. **Conclusion:** A panel of 30 features correlated across 3 omics data types can distinguish convalescent from AECOPD, suggesting it may be possible to create a clinically applicable blood test to diagnose AECOPD, and to further elucidate integrated molecular pathways and networks that might be better targets for intervention.

Keywords: proteomics, biomarker, DNA methylation, gene expression

P28.16 Proteomic Identification of Natural Substrates for Matriptase-2

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Introduction and Objectives: The micronutrient iron is essential for all living organisms. It is necessary for blood oxygen transport using the carrier protein hemoglobin and is involved in other metabolic reactions. Iron deficiency can lead to several severe diseases like anemia, while in excess it is toxic. Globally, anemia affects 1.62 billion people worldwide and half of which are preschool-age children. Matriptase-2 (TMPRSS6) is a recently identified member of the type II transmembrane serine protease (TTSP) family but its patho-physiological functions remain unknown. The enzyme is predominantly located at the surface of liver cells but higher mRNA amount have also been found in several cancerous tumors indicating that this enzyme, similar to other TTSPs, will likely have important cell surface associated roles in normal and disease states. CLIP-CHIP microarray analysis of three primary breast cancer tumor cell lines (4T1, 66cl4 and 67NR) also showed up-regulation of the matriptase-2 gene. Recently it has been suggested that protein fragments, generated by cell surface matriptase-2 are able to modulate the iron balance and in "über"-active state can lead to anemia, making matriptase-2 an essential regulator of iron homeostasis through its generation of novel signalling peptides. **Methods:** To identify new natural substrates we isolated proteomes from liver tissues from Matriptase-2-deficient and wild-type mice and compared by using shot-gun proteomics and our in-house developed proteomic method TAILS (Terminal Amine Isotopic Labling of Substrate). Matriptase-2 is a serine protease therefore we analysed the 2 different liver transcriptomes with our in-house developed CLIP-CHIP microarray. **Results and Discussion:** We will present first preliminary results of transcriptomic and proteomic analysis of Matriptase2 deficient mice livers in comparison to its healthy counterpart. **Conclusion:** The proteomic and transcriptomic approaches will hopefully identify the mechanisms by which matriptase-2 influences and regulates iron homeostasis and might lead into the development of new drugs for iron deficiency and toxicity treatment.

Keyword: TAILS degradome Protease CLIP-CHIP-Microarray

P28.17 An Integrated NMR-MS Metabonomic Approach for Classification of Pleural Effusions: New Criteria

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Introduction and Objectives: The first step for evaluating pleural effusions (PE) would rely on Light's criteria for the classification of exudates from transudates. However, this method can potentially misclassify transudates into exudates. Moreover, there is no simple biochemical test that provides a diagnosis for malignant PE (MPE) from tuberculous PE (TPE), the two common causes of exudates. Here, we will describe a novel NMR-MS-based metabonomic approach for the classification of exudates, transudates, MPE and TPE. **Methods:** PE samples were recruited from 67 patients. The diagnosis was based on clinical, histological and radiological findings. NMR experiments were performed using Bruker Avance 600 MHz NMR spectrometer. LC-MS/MS experiments were performed using Agilent 1260 LC and AB SCIEX TripleTOF 5600 system. Data were analyzed using multivariate analysis (SIMCA-P13). **Results and Discussion:** There were 50 exudates (32 MPE and 18 TPE) and 17 transudates. Using NMR spectroscopy, PE lipoprotein was determined to be the best biomarker to differentiate exudates from transudates with an area-under-ROC curve (AUC) of 0.96 (95% confidence interval (CI): 0.89–0.99), sensitivity of 98% and specificity of 88%. The diagnostic performance was superior to Light's criteria which showed a specificity of 65% at the same sensitivity of 98%. Using LC-MS/MS, we determined free fatty acid (FFA) 18:1 to be the best biomarker for MPE with AUC of 0.96 (95% CI: 0.87–1.00), sensitivity of 84% and specificity of 100% and ceramide (d18:1/16:0) to be the best biomarker for TPE with an AUC of 0.85 (95% CI: 0.73–0.94), sensitivity of 91% and specificity of 72%. Using the ratio of FFA 18:1-to-ceramide (d18:1/16:0), the AUC increased to 0.99 (95% CI: 0.91–1.00) with sensitivity of 94% and specificity of 100%. **Conclusion:** We recommend this integrated NMR-MS method and new criteria for clinical classification of PE.

Keywords: Mass spectrometry (MS), Pleural effusions, New criteria, Nuclear magnetic resonance (NMR) spectroscopy

P29: POSTER SESSION - STANDARDIZATION IN PROTEOMICS

P29.01 Study on Gradient Selection for Peptide Separate in RPLC

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Introduction and Objectives: More attentions were paid to RT (retention time) information in label-free LC-MS/MS quantification. Real RT can be obtained in an enough elution time, which offers exchange equilibrium in column, while there were few study focused on it. ACN concentration of peptide RT was figured out in different elution time in this study, and an appropriate elution method for peptide separate was displayed. **Methods:** Dead time of column (Thermo 75 μm × 50 cm, 2 μm, 100 A, C18 packing material) was measured with urasil using 80% ACN and 20% water solvent. Gradient delay time of liquid chromatography (Eksigent 425 system) was measured with a TUV detector (Waters) at 265 nm, using a linear gradient from 100% solvent A (100% water) and 0% solvent B (10% acetone, 90% water) to 25% solvent B over 30, 60 and 90 min. RTs of 12 peptides from Pierce Retention Time Calibration Mixture (Thermo) were collected from 95% solvent A (100% water, 0.1% formic acid) and 5% solvent B (100% ACN, 0.1% formic acid) to 35% solvent B over different times. **Results and Discussion:** Research PeakView (AB SCIEX) was applied to peptide RT detection, and with the data of column dead time and gradient delay time, ACN concentration of peptide RT can be calculated accurately. A figure was plotted, which showed ACN concentration of peptides to elution time. And the best linear gradient of 0.25% ACN/min was found, which showed stable ACN concentration. The gradient of 0.167% ACN/min could also offer a stable ACN concentration of RT, while it lead to a wider peak width. **Conclusion:** Since the 0.25% ACN/min linear gradient method predicted retention time in a coefficient of 0.9844 with the Sequence Specific Retention Calculator, our study showed an appropriate elution gradient for peptide separate in RPLC.

Keywords: Gradient Selection, Gradient Delay Time, Peptide Separate, RPLC

P29.02 The Power of Predicted Spectral Libraries to Unlock Data Independent Acquisition

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Introduction and Objectives: A major obstacle to extracting peptide quantitation information from a data independent acquisition (DIA) experiment is the need to obtain a spectral library containing a peptide's fragments and their intensities. While DIA data can be analyzed without a spectral library, comparison with a known fragmentation pattern provides substantial additional validation. Instead of committing additional time and sample to building a sufficient library or using public libraries with data collected from potentially very different samples under disparate conditions, accurately predicting a peptide's spectrum for any fragmentation or instrument, enables the extraction and quantitation of potentially any and every peptide from a given database. **Methods:** Several discovery experiments were conducted on two non-small cell lung cancer lines, one showing marked resistance to the tyrosine kinase inhibitor Erlotinib on a Thermo Fisher™ Q Exactive™ HF MS. A database containing several hundred proteins of interest was digested in silico and the fragmentation pattern of the several thousand resulting tryptic peptides was predicted. A multiplexed DIA method was run in triplicate. **Results and Discussion:** Preliminary results indicate that the DIA data showed similar correlations (dot products) with predicted spectral library as with the experimental spectral library enabling use of the predicted library as a substitute for the experimentally derived one or until an experimentally derived is available. **Conclusion:** An in silico predicted library enabled the quantitation of many peptides not detected from the search results of several DDA experiments. Quantitation results obtained in this study agreed with previously obtained results but enabled much more comprehensive pathway profiling.

Keywords: predlicted library, DIA, spectral

P29.03 Proteomics Data Analysis Workflow for Accumulated LCMS Files in Public Database

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Introduction and Objectives: Rapid progress has been made in mass spectrometry-based proteomics, and huge numbers of raw LCMS files have been accumulated in public data repository such as Proteome Xchange Consortium. The world-wide efforts to establish proteome maps of various organisms are continuously made and several databases are currently available. However, it is not easy to accumulate the proteome data from different labs with different MS instruments under the different measuring conditions. Here, we established a standard workflow for proteome data analysis of accumulated LCMS files stored in public database. **Methods:** LCMS raw files were obtained from proto-type version of jPOST (Japan ProteOme Standard repository and database), and processed using different peak picking algorisms, followed by the database search using different search engines against different protein databases to identify peptides and proteins. The redundancy of the merged results were removed and the quantitation processes based on MS signals as well as emPAI were conducted. **Results and Discussion:** Phosphoproteomics data from one single human cancer cell line analyzed by Thermo Q-Exactive was used to compare with two different peak-picking algorithms such as ProteoWizard and MaxQuant. By searching with Mascot, 3,620 non-redundant phosphosites were identified by ProteoWizard-Mascot approach, whereas additional 848 phosphosites (27% increase) were identified by MaxQuant-Mascot approach. The same tendency was also observed for global proteome data of HeLa lysate by ABSciex TripleTOF 5600plus, i. e., ProteoWizard generated 5,841 peptides whereas MS converter/ProteinPilot generated additional 1,571 peptides, identified by Mascot. Based on these findings, we optimized a complementary and unbiased data analysis workflow in combination with MSSS (mass spectrum sequential subtraction) approach to minimize the processing time (Genes Cells, 2012, 17, 633-44). **Conclusion:** We successfully established a workflow to accumulate results from different peak-picking algorithms and different search engines to reduce the biases from each approach for proteomics data analysis.

Keywords: standard data analysis, peak picking, search engine, data repository

P29.04 Modified Coomassie Staining Supports Different Imaging Formats for Quantitative Top-Down Proteomics

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Introduction and Objectives: 2DE offers the highest proteome resolution and information content in a single assay, enabling quantitative, top-down discovery proteomics. Continuous improvements to in-gel protein detection yield progressively higher detection sensitivity (DS), improving analysis of resolved proteomes. SDS-PAGE (1DE) of protein standards is often used to determine DS. However, as 2DE 'spots' are more concentrated than 1DE 'bands', DS may be greater for 2DE. Inter-lab variables further confound 1DE assessments, and protein standard purity (and general failure to independently assess this) also likely affect 1DE DS estimates. A modified colloidal Coomassie Brilliant Blue (cCBB) staining protocol, coupled with near-infrared fluorescence detection (IRFD), appears to be a new gold-standard (Gauci et al. 2013), offering cost-effective, high DS. Here we assess the DS of this protocol using 1DE models more closely resembling 2DE, and compare high-resolution densitometry with IRFD. The low purity of commercial protein standards and the importance of standardisation in establishing realistic measures of DS are also noted. **Methods:** Concentration and purity of protein standards were assessed using Beer-Lambert Law and 1DE. To determine DS, narrow loading wells for 1DE were used to yield narrow 'bands' (i.e. 2 mm 'spots'). cCBB-stained gels were imaged via IRFD (FLA-9000, 10-100 μm pixels) and densitometry (LAS-4000, 11 μm pixels). 2DE analysis of native proteomes was carried out to validate improvements to DS. **Results and Discussion:** Concentrating protein 'bands' to emulate 'spots' significantly improved DS of cCBB staining, detecting sub-nanogram protein amounts. High-resolution densitometry offers a lower-cost, high-sensitivity detection alternative to standard IRFD (i.e. 100 μm pixels). Increasing IRFD image resolution further increases DS for 2DE, though noise increases with the currently available instrumentation. **Conclusion:** cCBB staining offers higher DS than previously reported, and can be used with a variety of imaging modalities. We highlight the need to standardise instrument and experimental parameters to further enhance DS.

Keywords: Top-down proteomics, Coomassie, Detection sensitivity, Fluorescence/Densitometry

P29.05 A Universal Method for Peptide Identification

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Introduction and Objectives: 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 and Orbitrap Fusion Lumos 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 and Discussion:** Maximum identifications are obtained by reaching a balance between scan rate and quality of spectra. With 1 microgram HeLa digest, Orbitrap Fusion MS 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. **Conclusion:** 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.

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