CS01.03: Differential Structural Proteome And Interactome Analysis Reveal The Molecular Basis Of Phenotypes Mediated by the Histidine Methyltransferase Hpm1p

Bartolec T[1], Hamey J[1], Keller A[2], Chavez J[2], Bruce J[2], Wilkins M[1]
[1]Systems Biology Initiative, School of Biotechnology and Biomolecular Sciences, The University of New South Wales, [2]Department of Genome Sciences, University of Washington

INTRODUCTION

Hpm1p mono-methylates H243 in the ribosomal protein Rpl3p and represents the only known histidine methyltransferase in Saccharomyces cerevisiae. Systematic screens have identified non-ribosomal phenotypes for the hpm1 knockout strain, most of which remain unexplored at the molecular level. The emerging approach of large scale quantitative cross-linking mass spectrometry (qXL-MS) enables the untargeted comparison of protein structural conformations and protein-protein interactions (PPIs) between complex samples. Here, we explored the orthogonality and utility of using qXL-MS as a tool for the discovery of a protein's molecular or cellular function/s.

METHODS

To investigate how the loss of the hpm1 gene resulted in diverse phenotypes, we employed several experimental approaches including targeted mass spectrometry, growth assays, quantitative differential proteomics and biochemical validation studies. Notably, we successfully adapted large scale qXL-MS to enable comparison of protein structures and PPIs from intact budding yeast by combining 2nSILAC labelling, enzymatic digestion of the cell wall, and BDP-NHP cross-linking.

RESULTS

We confirmed the localisation and stoichiometry of the H243 methylation site, found unreported sensitivities of Δhpm1 yeast to additional non-ribosomal stressors, and identified differentially abundant proteins upon hpm1 knockout with clear links to the coordination of sugar metabolism. We identified 1,267 unique lysine-lysine cross-links within in vivo proteins, and by reproducibly monitoring over 350 of these in wild-type and Δhpm1, we detected changes to protein structures or PPIs in the ribosome, membrane proteins, chromatin and mitochondria. Importantly, these occurred independently to changes in protein abundance and could explain a number of mutant phenotypes. Further to this, several phenotypes which were predicted solely from changes in protein structure or interactions could be experimentally validated using orthogonal techniques.

CONCLUSIONS

Taken together, these studies reveal a broad role for Hpm1p in yeast and illustrate how qXL-MS will be an essential tool for untangling the molecular basis of complex phenotypes.

CS01.04: FLiP-MS: A novel systems biology approach to monitor changes in protein-protein interactions applied to HU-induced DNA-replication stress

Doerig C[1], Marulli C[1], Peskett T[1], Volkmar N[1], Pantolini L[2], Barral Y[1], Picotti P[1]
[1]ETHZ, [2]University of Basel

Introduction

Protein complexes are crucial to the functioning of biological systems. While advances in the analysis of protein complex composition have been made, methods monitoring the assembly and disassembly of protein complexes in response to perturbations suffer from low throughput. Here we introduce FLiP-MS, a structural proteomics workflow that enables probing the occupancy of protein binding interfaces globally and directly from cell lysates.

Methods

First, we generate an atlas of peptides at protein-binding interfaces whose intensity reports on the assembly state of complexes. This is achieved by the serial filtration of a cell lysate followed by limited proteolysis and mass spectrometry (LiP-MS) of the fractions. This detects interfaces based on their differences in protease accessibility between the complex-bound and monomeric forms of proteins separated by size.
Next, lysates from differently perturbed samples are subjected to LiP-MS. Changes in the previously identified interface markers and network analysis allow pinpointing alterations in the assembly states of protein complexes upon perturbation.

Results

Applied to S. cerevisiae, the approach yielded 4693 candidate interface markers from 1086 proteins at 5% FDR. Notably, interface markers are significantly enriched in protein-binding domains.
We used these interface markers to probe interaction changes in yeast grown under DNA-replication stress. We identified changes in protein complexes related to p-body formation and in the SAGA complex. Several of the changes in p-body-related complexes depend on SAGA's acetyltransferase activity. Microscopy analyses confirmed that the dynamics of p-body formation differed between a SAGA acetyltransferase mutant and the wild-type strain, indicating that SAGA's acetyltransferase directly or indirectly regulates p-body formation.

Conclusions

We anticipate that FLiP-MS could be the starting point for monitoring the dynamics of protein complexes upon environmental or genetic perturbations on a proteome-wide scale. Further, our library of complex binding interfaces could be applied to docking analyses and studying disease-associated mutations' effects.

CS01.05: Global in situ analysis of osmolyte mechanisms in regulating protein thermal stability

Pepelnjak M[1], Velten B[2,3], Näpflin N[1], von Rosen T[4], Capasso Palmiero U[5], Arosio P[5], Weber-Ban E[4], de Souza N[1], Huber W[6], Picotti P[1]
[1]Institute of Molecular Systems Biology, Department of Biology, ETH Zurich, [2]Division of Computational Genomics and Systems Genetics, German Cancer Research Center (DKFZ), [3]Cellular Genetics Programme, Wellcome Sanger Institute, [4]Institute of Molecular biology & Biophysics, Department of Biology, ETH Zurich, [5]Department of Chemistry and Applied Biosciences, ETH Zurich, [6]Genome Biology Unit, European Molecular Biological Laboratory

Intro: Osmolytes are small organic molecules used by organisms across all kingdoms of life to stabilise proteins and to adapt to diverse environmental conditions. The capability of osmolytes to stabilise proteins has been largely deduced from in vitro studies on specific purified proteins. However, their general mechanisms of action remain poorly understood and systematic global studies of osmolyte action within the cellular milieu are lacking.

Methods: We performed in situ proteome-wide analyses, using E. coli as model organism, to study protein thermal stabilisation by six osmolytes covering all major classes of these molecules: trimethylamine N-oxide (TMAO), betaine, glycerol, proline, trehalose and glucose. Using limited proteolysis-coupled mass spectrometry (LiP-MS), we systematically probed the effects of osmolytes on protein native structures, protein thermal stability and protein aggregation.

Results: We show that the tested osmolytes stabilise most proteins in the proteome and that the osmolyte TMAO shows the strongest stabilisation effect. Our data indicate that most, but not all, proteins are stabilised by the preferential exclusion of osmolytes from the protein surface. For some proteins, direct binding of osmolytes causes even stronger protein stabilisation; however, direct binding or a change in protein native structure are not necessary for protein stabilisation. Finally, we show that most proteins aggregate at higher temperatures in the presence of osmolytes than in their absence, although TMAO promotes aggregation of some disordered proteins that would not precipitate in its absence.

Conclusions: Our global analysis of osmolyte effects on proteins within the complex cellular matrix provides mechanistic insight into how osmolytes perform their adaptive function and could have important implications for the design of formulations of protein-based drugs. Our findings could also be used to identify the most efficient stabilisers for biotechnologically relevant proteins.

CS02.03: Proteomic basis for understanding the molecular mode of action of clinical ATR kinase inhibitors in pancreatic cancer cells

Hoefer S[1], Frasch L[1], Rogler T[1], Putzker K[2], Lewis J[2], Kuster B[1,3]
[1]Chair of Proteomics and Bioanalytics, Technical University of Munich, [2]Chemical Biology Core Facility, EMBL, [3]Bavarian Biomolecular Mass Spectrometry Center (BayBioMS), Technical University of Munich

Introduction: In pancreatic cancer, the DNA-damaging agent gemcitabine is used as first-line chemotherapy but chemo-resistance is frequently observed. One potential way to overcome gemcitabine resistance is the combination with molecularly targeted agents such as kinase inhibitors. The protein kinase ATR is a key regulator of cellular replication stress response and DNA repair, and the combination of ATR inhibitors (ATRi) with chemodrugs is currently being clinically investigated. Here we present a novel phosphoproteomic approach called decryptM that characterizes clinical ATRi in PDAC cells on a molecular level.
Methods: For target profiling of ATRi using Kinobeads, drugs were allowed to bind kinases from AsPC-1 cell lysate in a dose-dependent fashion, followed by kinase-enrichment and nano-LC-MS/MS. For decryptM experiments, AsPC1 cells were treated with increasing concentrations of ATRi. Samples were processed using SP3 magnetic beads, and each drug dose was labeled using TMT11plex. The pooled sample was deep fractionated using basic-reversed phase chromatography and pooled to 12 fractions, followed by IMAC enrichment and nano-LC-MS/MS.
Results: Proteomic target profiling revealed high selectivity of ATRi elimusertib with a 100-fold higher binding affinity compared to other kinases. DecryptM experiments resulted in the quantification of >15,000 phosphorylation site, of which >200 sites were regulated in a dose-dependent fashion upon ATR inhibition. Potential ATR substrates containing the SQ/TQ motif were found to be inhibited, indicating target engagement and downregulation of replication stress response. Interestingly, downregulation of phosphorylation sites occurred at lower doses of ATRi compared to most upregulation events (mean EC50 <500nM compared to >1uM). This discrepancy in potency may help to distinguish direct from indirect signalling by ATR, and how ATRi can counteract DNA damage response induced by gemcitabine in combination treatments.
Conclusions: Using a novel phosphoproteomic approach called decryptM, this project elucidates the molecular mode of action of ATR kinase inhibitors.

CS02.02: Extracellular PTM Crosstalk in cancer: Proteolysis & Glycosylation

Madzharova E[1], Sabino F, Keller U
[1]Technical University Of Denamark

Introduction: Proteolysis critically regulates important cell and tissue responses and is often disturbed in disease. Crosstalk between proteolytic processing and other major post-translational modifications (PTM) is emerging as an important regulatory mechanism to modulate protease activity and maintain tissue homeostasis.

Methods: With a focus on matrix metalloproteinase (MMP)-mediated cleavages and mucin-type O-glycosylation, two major PTMs of proteins in the extracellular space, we investigated the influence of truncated O-glycan trees resulting in Tn antigen expression, as often found in aggressive tumors in general, and MMP9-specific proteolytic processing by combining advanced cellular engineering and mass spectrometry-based quantitative analysis of the proteome and N-terminome using terminal amine isotopic labeling of substrates (TAILS) technology.

Results: Our analysis revealed significant changes to the proteome in MDA-MB-231 breast cancer cells upon inactivation of the C1GALT1 specific chaperone 1 (COSMC). We identified novel ectodomain shedding events modulated by O-glycan truncation and revealed enhanced proteolysis by MMP9 in Tn antigen expressing extracellular proteomes.

Conclusion: Together, we demonstrate the importance of mature O-glycosylation for proteome homeostasis and fine tuning of proteolytic processing, indicating a critical influence on cancer disease phenotypes.

CS02.04: Modulation of reversible cysteine-modifications upon brief T cell activation

Mandal K[1], Jensen P[1], Larsen M[1]
[1]Syddansk Universitet / University Of Southern Denmark

Background: T cell signaling in known to be fast and abundant. The mechanism involves complex interplays of feedback and feedforward loops, parallel pathways and PTM crosstalk. Proteins orchestrating these events must be fast and reversible, function which could be attributed to its dynamic post translational modifications (PTM's). Protein phosphorylation events are well established to drive the T-cell signaling, however, in this study, we have uncovered an additional layer of T-cell signaling through reversible cysteine (Cys) modifications integrated with protein phosphorylation.

Methods: Jurkat cells were stimulated for 0S, 30S, 60S and 300S using 1:1 mixture of pre-crosslinked anti-CD3 and anti-CD28. This was done with and without TPEN, an intracellular membrane-permeable ion chelator. The free cysteines were blocked with CysPAT and the proteins were digested with trypsin after precipitation. Tryptic peptides were labeled using TMT16plex. The peptides containing reversible Cys modifications were isolated using thiopropyl sepharose beads (SH-beads, 3S). Finally, the CysPAT labeled cysteines and phosphopeptides were isolated with TiO2 beads. All enriched fractions were subjected to high pH followed by LC-MS/MS analysis and bioinformatic analysis.

Results: A large coverage of the phosphosites in the T-cell signaling pathways was obtained. Interestingly, activation of T cells resulted in a significant modulation of specific cysteines in proteins known to be involved in the T cell signaling pathways. Especially we found Cys changes in proteins know to be regulated by forming conjugations with Zinc. After treatment with TPEN, which chelate the internal Zinc pool we observed a change in the T cell activation, supporting the modulation of Zinc mediated activation of proteins in the T cell activation pathways.

Conclusion: We have identified numerous phosphosites and reversible cysteine modification sites to be important for T cells signaling pathways. The results show that the crosstalk between the PTMs is crucial for the mechanism of T-cell activation.

CS02.05: Debugging the bacterial phosphoproteome

Potel C[1], Le Sueur C[1], Burtscher M[1], Becher I[1], Beltrao P[2], Typas A[1], Savitski M[1]
[1]EMBL, [2]ETH

Introduction

Reversible protein phosphorylation is involved in the regulation of virtually every cellular process. It is now possible to routinely identify tens of thousands of phosphorylation events in eukaryotic cells by mass spectrometry-based phosphoproteomics. However, the same is not true for prokaryotes owing to technical challenges which severely limit the numbers of identified phosphorylation sites. Here, we present a universal high-throughput phosphoproteomics workflow leading to a 10-fold increase in bacterial phosphoproteomes coverage.

Methods

We have previously shown that contaminants, in particular, nucleic acids prevent in depth identification of phosphopeptides in bacterial samples [1]. Here, we show that selective capture of nucleic acids prior to protein precipitation solves this problem. We designed the sample preparation as well as the phosphopeptides enrichment to be fast, cost-effective and compatible with a 96-well plate format, making it possible to process hundreds of samples in parallel.

Results

We identified more than 100,000 unique phosphopeptides in over 50 organisms, confirming that the extent of Ser/Thr/Tyr phosphorylations in prokaryotes has largely been underestimated. We were able to gain insights in the evolution of phospho-mediated signaling by (i) analyzing the phosphorylated Ser/Thr/Tyr distribution in different phyla as well as (ii) the conservation of phosphorylation sites through evolution, (iii) identifying both eukaryotic-like and new prokaryotic phosphorylation motifs and (iv) studying the structural context of the identified phosphorylations using Alphafold.

Conclusions

The developed method paves the way for high-throughput identification and quantification of the dynamics of the long neglected phosphorylation events in prokaryotes. In addition, the data generated will be a valuable resource for researchers interested in cell signaling and its evolution.

References

[1] Potel, C.M. et al 2018 Nature Methods 15, 187–190

CS02.06: Hybrid-DIA Combines Targeted and Discovery Phoshoproteomics to dissect Cancer Cell Signaling Networks

Martinez-Val A[1], Fort K[2], Franciosa G[1], Koenig C[1], Moehring T[2], Ishihama Y[3], Chen Y[4], Xuan Y[2], Makarov A[2], Olsen J[1]
[1]Novo Nordisk Foundation Center for Protein Research, University of Copenhagen, [2]Thermo Fisher Scientific (Bremen) GmbH, [3]Graduate School of Pharmaceutical Sciences, Kyoto University, [4]Institute of Chemistry, Academia Sinica

Introduction: Achieving sufficient coverage by MS to cover all regulatory phosphorylation sites of interest is challenging due to their low stoichiometry and the high complexity of the phosphoproteome. Here, we present a Hybrid-DIA method to extract precise quantitative information of >100 selected phosphopeptide targets in parallel with profiling the global phosphoproteome in just one MS run per sample.
Methods: The hybrid-DIA strategy combines the best of targeted and discovery proteomics through the utilization of the Application Programming Interface (API) to dynamically intercalate data-independent acquisition (DIA) scans with accurate triggering of predefined (phospho)peptide targets by analyzing MS1 scans for heavy-labeled (phospho)peptides, which then trigger multiplexed (MSX) targeted quantitation scans of heavy-labeled and endogenous peptide pairs. Different perturbed cancer cells were harvested in boiling SDS. Protein aggregates were captured on magnetic microparticles and on-bead LysC/Trypsin digested overnight. 0.05 pmol of 130 Phosphopeptide Standards was spiked before phosphopeptide-enrichment using Ti-IMAC-HP beads. Samples were analyzed using hybrid-DIA and SureQuant on an ExplorisTM 480 coupled to Evosep One with high sensitivity Whisper LC methods. Raw files were processed using python/R, Spectronaut and Skyline.
Results: We analyzed perturbed cancer cell phosphoproteomes using hybrid-DIA analysis and developed a computational workflow for processing, quantifying and visualizing the multiplexed targeted data. We demonstrate that Hybrid-DIA is comparable to SureQuant in sensitivity and quantitative accuracy for targeted phosphopeptides derived from low microgram of cancer cell lysates while simultaneously identifying global phosphoproteomes of 5000+ phosphorylation sites from ‘background’ DIA data. 100+ phosphorylation sites covering seven major signaling pathways in cancer cells (EGFR, RAS-MAPK, PI3K-AKT-mTOR, AMPK, apoptosis and stress response) were accurately quantified across cancer cells treated with different kinase inhibitors recapitulating known downstream kinase dynamics and cell states.
Conclusions: Novel hybrid-DIA combines targeted and discovery proteomics workflow for biomarker discovery and sensitive cancer cell signaling analysis.

CS03.03: Multi-omics Analysis for the Differential Diagnosis of Inflammatory Arthritis and the Advance in Precision Medicine Strategies

Ruiz-romero C[1,5], Rocha B[1], Calamia V[1], Fernández-Puente P[4], Cillero-Pastor B[2], Illiano A[3], Lourido L[1], Paz-González R[1], Quaranta P[1], Celis R[6], Pinto G[3], Amoresano A[3], Cañete J[6], Heeren R[2], Blanco F[1,4]
[1]Unidad de Proteómica - Grupo de Investigación de Reumatología (GIR), Instituto de Investigación Biomédica de A Coruña (INIBIC), Complexo Hospitalario Universitario de A Coruña (CHUAC), Sergas , [2]The Maastricht Multimodal Molecular Imaging Institute (M4I), Division of Imaging Mass Spectrometry, Maastricht University, [3]Department of Chemical Sciences, University of Naples "Federico II", [4]Universidad de A Coruña, [5]CIBER-BBN, [6]Arthritis Unit, Rheumatology Department, Hospital Clinic and IDIBAPS

Introduction: Differential diagnosis of Psoriatic arthritis (PsA) and Rheumatoid Arthritis (RA) is often difficult, especially in seronegative patients, due to the similarity of symptoms and the unavailability of reliable clinical biomarkers. Molecular alterations contribute to pathophysiological processes in the joint, and it is known that chronic inflammation induces significant changes in the synovial tissue (ST) and synovial fluid (SF) lipidome and proteome. We evaluated whether differences in the molecular profiles from ST and SF could support the differential diagnosis of these diseases.
Methods: ST frozen samples (n=28) of patients affected by RA, PsA and control donors were compared using MALDI-MSI for spatially resolved lipid analysis. A targeted approach based on MRM-MS was then performed to validate the alteration of 84 phospholipid species in SF (n=52). Finally, the quantitative proteomic analysis was carried out by nLC-MS/MS on FFPE ST (n=34).
Results: Lipidomic profiles in ST from PsA and RA were unequivocally distinguished by MALDI-MSI followed by PCA-DA. Sphingomyelins, phosphatidylcholines and phosphatidylethanolamines (PE) presented the greatest separation power to classify RA and PsA tissue samples, being most of them significantly increased in PsA compared to RA and controls. The distribution of the PE species was associated with ST areas with increased vascularity and inflammatory cell infiltrates. RA and PsA patients were also classified based on the SF levels of all quantified lipid species. Finally, the proteomic analysis quantified around 2,500 distinct proteins in the ST, including several related with lipid metabolism. Thirty-six of them discriminated RA and PsA patients with high statistical significance.
Conclusion: Our study shows distinct molecular profiles between RA and PsA synovial tissue and synovial fluid. It reports potential useful lipid and protein markers for the differential diagnosis of these diseases, which will facilitate the development of precision medicine strategies aiming to improve their clinical management.

CS03.04: Targeted proteomics to verify the association between translational control pathways and capivasertib response in cancer cell lines and patient tumours

Sobsey C[1,2], Mitsa G[1,2], Popp R[3], Awan A[4], Aguilar-Mahecha A[5], Basik M[6], Koromilas A[4,7], Borchers C[2,3,4], Batist G[5,8]

[1]Division of Experimental Medicine, McGill University, [2]Segal Cancer Proteomics Centre, Lady Davis Institute, Jewish General Hospital, McGill University, [3]MRM Proteomics Inc, [4]Gerald Bronfman Department of Oncology, Jewish General Hospital, McGill University, [5]Segal Cancer Centre, Lady Davis Institute, Jewish General Hospital, [6]Department of Surgery, Jewish General Hospital, McGill University, [7]Lady Davis Institute for Medical Research-McGill University, Sir Mortimer B. Davis-Jewish General Hospital, [8] Department of Oncology, Faculty of Medicine, McGill University, [9]Exactis Innovation

Introduction: We previously analyzed formalin-fixed paraffin-embedded (FFPE) PIK3CA-mutant breast and gynecological tumours from patients enrolled in a clinical trial of the AKT inhibitor, capivasertib. Our data showed differential proteomic profiles between those patients who demonstrated a clinical benefit in response to capivasertib treatment as compared to those who did not. The proteins of interest mapped to pathways involved in cancer-associated translational control including EIF2 signalling and EIF4 activation. We therefore developed targeted assays that can be used to validate the association between the proteins of interest and capivasertib sensitivity in other sample sets and models.

Methods: Targeted MRM- and PRM-MS assays were developed using 98 synthetic proteotypic peptides and corresponding stable-isotope labelled standard peptides for 75 proteins of interest. Synthetic peptides were optimized on two different systems: (1) a UPLC + Agilent 6495B-QQQ-MS and (2) an EvopSep One + Q-Exactive Orbitrap-MS to maximize sensitivity for clinical samples. Following peptide optimization, assay performance was characterized according to CPTAC guidelines.

Results: A set of 3 PIK3CA-mutant and 3 PIK3CA-WT breast cancer cell lines were tested for sensitivity to capivasertib using a cytotoxicity assay. Samples were collected at baseline and at pre-specified timepoints after capivasertib administration. Protein expression in sensitive vs. resistant cell lines will be compared to confirm the relationship between the markers of interest and capivasertib sensitivity. Since most of the tumour samples available for cancer research are stored as FFPE, we are also applying the assays to tumour blocks (n=33) obtained with patient consent under Research Ethics Board approval at the Jewish General Hospital. Conclusions: Together, these analyses will permit us to assess the markers' relationship to capivasertib response and tumour genetics in new models, characterize intra- and inter-tumour variability, evaluate different sampling approaches, and thereby provide key data to assess feasibility for use in clinical tumour samples.

CS03.05: Proteomic Changes Associated with Racial Background and Survival Outcomes of Sepsis Patients with Primary Urinary Tract Infection

Kapp K[1,2], Choi M[1], Arul A[1], Zhang K[3], Du L[3,4], Yende S[5,6,7], Kellum J[6], Angus D[5,6,7], Peck-Palmer O[5,6,7,8], Robinson R[1,2]
[1]Department of Chemistry, Vanderbilt University, [2]The Vanderbilt Institute of Chemical Biology, Vanderbilt University, [3]Department of Biostatistics, Vanderbilt University Medical Center, [4]Vanderbilt Center for Quantitative Sciences, Vanderbilt University Medical Center, [5]The Clinical Research, Investigation, and Systems Modeling of Acute Illnesses (CRISMA) Center, University of Pittsburgh, [6]Department of Critical Care Medicine, University of Pittsburgh School of Medicine, [7]Department of Clinical and Translational Science, University of Pittsburgh, [8]Department of Pathology, University of Pittsburgh

Introduction: Socioeconomic factors do not fully explain why African American/Black (AA/B) patients have higher mortality rates from sepsis than Non-Hispanic Whites (NHW), creating the need to characterize the effect of race on sepsis survival on a molecular level. We have previously identified inflammatory proteins whose levels are dependent on both race and survival outcome in patients with intra-abdominal sepsis, but infection source can influence patient response. We hypothesize that proteins impacting survival outcomes have different expression levels between NHW and AA/B patients with urinary tract infections (UTIs), another leading cause of sepsis. Therefore, we subjected plasma samples from a racially diverse cohort of sepsis patients with UTI to bottom-up proteomics analysis.

Methods: Upon hospital admission, plasma samples (N = 166) from NHW and AA/B sepsis patients with primary UTI were obtained from the Protocolized Care for Early Septic Shock cohort. Survival outcomes were assessed 90 days post-admission. Plasma samples were stratified into four groups based on patients' race and survival outcome and subjected to immunodepletion, tryptic digestion, and tandem mass tag (TMTpro) labeling. Each TMTpro batch contained 15 samples and a pooled quality control sample. The 12 batches were fractionated and analyzed with high-resolution liquid chromatography-tandem mass spectrometry (LC-MS/MS). Linear regression models assessed survival's effects on protein levels with and without stratification by patients' self-identified race.

Results: LC-MS/MS analysis identified ~2,000 proteins per batch and overall, ~3,500 unique proteins. These proteins are primarily involved in liver X receptor/retinoid X receptor activation, clathrin-mediated endocytosis, acute phase response, and actin cytoskeleton signaling, all related to functions implicated in previous studies. Results of the linear regression models will be discussed, emphasizing proteins whose levels are dependent on both patients' race and survival.

Conclusions: Plasma proteomics of a racially diverse cohort furthers the understanding of disparities related to sepsis survival on a molecular level.

CS03.06: Meta-analysis of 9 clinical trials reveals challenges and opportunities of using deep plasma proteomics to enhance clinical trials outcomes

Feng C[1], Wang Y[1], Nanasi T[1], Lohr S[1], Szoke B[1], Minguet C[2], Gonzalo R[2], Ortiz A[2], Costa M[2], Cerezo C[1], Braithwaite S[1], Lehallier B[1]
[1]Alkahest, [2]Grifols

Introduction: Clinical proteomics offer a unique opportunity to study effects of treatments directly in patients, help uncover mechanisms of action and identify new biomarkers. However, getting access to tissues to do proteomics profiling is challenging due to invasiveness. Recent methodological developments allow now measurements of several thousands of proteins in the blood with very high sensitivity and specificity. Still, many confounders can affect the quality of plasma proteomics signals. In this study, we analyzed data from 9 phase 1/2/3 clinical trials and identified main challenges and opportunities in using deep plasma proteomics in clinical trials.
Methods: We examined effects of biological and technical factors in 11 plasma proteomics datasets from 9 clinical trials performed by Alkahest and Grifols, covering 5 different disease areas using two high throughput proteomics platforms (SomaLogic and Olink) with dominance analysis.
Results: Our integrated analysis of multiple clinical proteomics datasets demonstrate the versatility of deep plasma proteomics to identify biological signals and new biomarkers in clinical trials. On the other hand, we identified the most important contributors to the variance of the plasma proteomics signal such as inconsistencies in sample collection, storage conditions and high inter-subjects' variance. We identified several confounders consistent across studies but also confounders that are specific to unique diseases. In addition, our analyses demonstrate that the choice of the analytical methods has to be driven by population structure, sample size and missingness but also reveal the complementarity of different proteomics platforms to unveil biological signals.
Conclusions: Our study demonstrates the value of deep plasma proteomics to enhance the understanding and values of clinical trials but also emphasize the importance of the standardization of plasma sample collection, processing and storage. We also provide recommendations on confounders to be included in clinical proteomics analysis to reduce variance and bias, and to increase statistical power.

CS03.07: Exploring the autoantibody repertoire in autoimmune inflammatory conditions

Pin E[1], Bayati S[1], Nilsson P[1]
[1]Department of Protein Science, SciLifeLab, KTH Royal Institute of Technology

Introduction: Autoantibodies are a hallmark of autoimmune diseases and play an important role as biomarkers. However, the currently available autoantibody biomarkers are in many cases not disease specific, and can often be identified also in healthy individuals. High throughput multiplex technologies such as protein arrays allow to profile a large number of autoantibodies, with the potential to identify new specific and selective candidate biomarkers. We are aiming to identify new candidate biomarkers to improve the management of autoimmune inflammatory conditions, with a special focus on early diagnosis, patients´ subclassification, definition of prognosis, and overtime monitoring of disease activity.

Methods: In-house developed planar and bead-based antigen arrays were generated using protein fragments representing 18.000 unique human proteins, as well as 2000 full-length versions of secreted proteins from the Human Protein Atlas collection (www.proteinatlas.org). Through an optimised workflow and data analysis pipeline, we performed a proteome-wide screening and verification of autoantibodies in plasma and serum samples from patients with vasculitis and systemic sclerosis. Deeply characterised sample cohorts were provided through well established international collaborations with expert clinicians.

Results: A screening of serum samples from systemic sclerosis patients allowed to identify novel fibrosis-associated autoantibodies. Among these, we identified potential biomarkers to improve diagnosis and subclassification of patients with skin and lung fibrosis. On the other hand, our studies on ANCA-associated vasculitis identified previously unreported autoantibodies that could represent new candidate biomarkers for diagnosis and prediction of risk of relapse.

Conclusions: The combination of clinical expertise and high throughput cutting-edge technology for autoantibody profiling allowed us to identify novel autoantibodies in the context of autoimmune inflammatory conditions. These data are being verified on independently collected sample cohorts. Moreover, a newly developed protocol for antibody enrichment will allow us to test the role played by molecular mimicry in the generation of these autoantibodies.

CS03.08: Clinical cancer research on metastases from refractory colorectal cancer using a multi-omics approach to improve current precision oncology treatment.

Mitsa G[1,2], Chaplygina D[3], Qasrawi D[1], Merza R[4,5], Nagaria T[4], Smolar Bocher J[5], Papadakis A[4,5], Tourcotte S[6,7], Spatz A[4,5], Petrotchenko E[1], Batist G[5,8,9], Zahedi R[1,10,11], Borchers C[1,9]
[1]Segal Cancer Proteomics Centre, Lady Davis Institute, Jewish General Hospital, McGill University, [2]Division of Experimental Medicine, McGill University, [3]Center for Computational and Data-Intensive Science and Engineering, Skolkovo Institute of Science and Technology, [4]Department of Pathology, Jewish General Hospital, McGill University, [5]Segal Cancer Centre, Lady Davis Institute, Jewish General Hospital, McGill University, [6]Centre de Recherche du Centre hospitalier de l'Université de Montréal (CRCHUM), [7]Institut du cancer de Montréal, [8]Exactis Innovation, [9]Gerald Bronfman Department of Oncology, Jewish General Hospital, McGill University, [10]Department of Internal Medicine, University of Manitoba, [11]Manitoba Centre for Proteomics and Systems Biology

Introduction
Colorectal cancer (CRC) is probably the best described cancer on a genomic level. However, medical treatment has not changed for the last 20 years, and there is a lack of translation of genomic-centered research into the clinic. CRC remains within the top-3 cancers worldwide, with incidences in younger population (<50 years) increasing.
Clinical research mainly focuses on primary disease, but ~75% of CRC patients ultimately develop metastases (mCRC). The 5-year survival then drops to only 12%.
Treatment response to current precision oncology is ~20% with progression-free-survival between 5 months to 2 years. To date there is no clear definition of (targeted) therapy for metastatic disease.

Methods
Ninety-seven liver mCRC tumors (45 KRAS-wildtype, 46 KRAS-mutated, 6 unknown KRAS status) were assessed for tumor cellularity and viability. Whole exome sequencing data was used to identify tumor-specific somatic mutations and to guide TMT-based (phospho)proteomic database searches. The multi-omics data was analyzed using machine-learning approaches to identify correlations between multi-omics signatures and treatment response for better stratification of tumors.

Results
In depth multi-omics covering 7 orders-of-magnitude of the mCRC liver proteome quantified ~9,000 proteins with at least 1 protein unique peptide and 1% FDR on the protein level. Differential analysis identified significantly enriched new therapeutic drug candidates (>2-fold change, student t-test $p<0.01$), and functional enrichment analysis discovered significantly enriched (FDR 1%) pathways that have not yet been experimentally described in human subjects of mCRC.
Clinical data is currently used for prioritization and evaluation of identified drug target candidates. Metabolomics, targeted proteomics and functional genomics will be used for validation of identified drug candidates in a larger validation group.
Conclusion
Clinical proteomics is here used for the first time on liver metastases from refractory mCRC, identified new drug candidates and suggests off-label use of known drugs for advanced CRC independent of KRAS mutation status.

CS04.02: Mutated proteome analysis of circulating extracellular vesicles enabled sensitive and effective liquid biopsy for renal cell cancer

Hakozaki Y[1], Yamada Y[2], Kume H[2], Ueda K[1]
[1]Cancer Proteomics Group, Cancer Precision Medicine Center, Japanese Foundation For Cancer Research, [2]Department of Urology, Graduate School of Medicine, The University of Tokyo

Introduction

Exosomes are nano-scaled extracellular vesicles secreted from any types of cells into various body fluids. Especially, cancer cell-derived exosomes are considered to have a great potential as biomarker carriers. Since no effective biomarker is available for diagnosis of clear cell renal cell carcinoma (ccRCC) in clinical use, we aimed to develop a novel liquid biopsy method for ccRCC by targeting mutated proteins encapsulated in circulating exosomes.

Methods

The ccRCC and matched normal tissues were collected from 11 patients who received partial or radical nephrectomy at the University of Tokyo Hospital. Whole exome sequence analysis was performed using tissue samples to construct personalized amino acid sequence databases containing somatic mutations (neo-sequences) by the R package, Neoantimon. The tissues were then analyzed by Orbitrap Fusion Lumos-FAIMS Pro LC/MS system to identify mutated proteins. Plasma and urine samples were collected from each patient before and 2-4 months after surgery. We constructed the multiple reaction monitoring (MRM)-based absolute quantification method for detection of the ccRCC mutated protein panel and analyzed exosomes isolated from plasma or urine samples.

Results

Whole exome sequence analysis of cancer tissues and matched normal samples identified 63.5 nonsynonymous and 16.0 frameshift mutations per sample on average. Of these mutations, 7.5 or 5.0 mutated proteins were identified per sample from plasma or urine exosomes, respectively. Furthermore, absolute quantification measurement of the mutated protein panel for pre/post operative samples showed that drastic reduction or complete disappearance of EV mutated proteins were observed in all post-operative cases. Interestingly, the level of exosomal mutated proteins was reascended in a case diagnosed with recurrent cancer within lung 8 months after surgery.

Conclusions

Cancer-specific mutated proteins were detectable in plasma or urine exosomes, suggesting that our circulating mutated protein-based liquid biopsy could serve as an effective tool for diagnosis of kidney cancer detection or monitoring.

CS04.03: Proteogenomic Landscape of East Asian Breast Cancer Highlights Distinct Subtypes to Guide Prognostic and Therapeutic opportunities

Chang Y[1], Chen Y[1], Han C[2], Lin K[1], Lu Y[3], Su[4], Cehn H[4], Yu J[5], Yu S[4], Chen Y[1], Chen H[1]
[1]Academia Sinica, [2]Taipei Medical University, [3]National Taiwan University Hospital, [4]National Taiwan University, [5]Tri-Service General Hospital

Breast cancer (BC) is a heterogeneous disease reflecting on various clinical and pathological factors. BC in East Asia is characterized by remarkably higher and increasing incidence of younger generations, high prevalence in luminal type compared to Western woman. However, the etiology and molecular mechanisms underlying the disease remain poorly understood. We presented a deep proteogenomics landscape to reveal age-related pathogenesis and nominate biomarkers for risk assessment and treatment innovations.

Proteogenomics profiling, including WES, RNA-seq, proteomics and phosphoproteomics analysis, were obtained for 138 prospectively collected paired tumor and adjacent normal tissues. The integrated multi-omics dataset and clinical feature were used to derive carcinogen imprint and endogenous mutation signature, identify subtypes and their molecular characteristics, and druggable pathways.

Deep genomic analysis revealed endogenous and environmental carcinogen-associated mutagenesis and linked APOBEC and DBAC signatures to significantly elevated hormone biosynthesis and chemical carcinogenesis in younger patients. A novel subset was also identified with high mutation burden and immune escape potentially beneficial from combined epigenetic therapy and immunotherapy. A proteomics-informed classification distinguished the distinct clinical characteristics of BC patients with luminal subtype and young females. The multi-omic analysis delineated the association of exogenous factors among a unique group of younger female patients, and corresponding molecular mechanisms for the accompanying high-grade risk, tumor invasion and lower CDK expression. Integrated protein network provides new strategies for patient stratification and their actionable pathways beyond the conventional IHC staging.

This study reveals the distinct genetic profile of the Asian cohort, highlights key signatures associated with young females, and provides a transformative view on the potential treatment strategy of early stage BC patients.

CS05.02: Combining MSFragger Glyco Search with O-Pair Localization of O-Glycopeptides in FragPipe

Polasky D[1], Lu L[2], Yu F[1], Teo G[1], Shortreed M[3], Nesvizhskii A[1]
[1]University Of Michigan, [2]University of California, San Francisco, [3]University of Wisconsin, Madison

Introduction

MSFragger glyco search provides fast and sensitive analysis of N- and O-linked glycopeptides. However, confident localization of O-glycosites typically requires a separate electron-based activation scan due to the propensity of O-glycans to dissociate completely from the peptide during collisional activation. The O-Pair method provides an elegant solution to this problem, using fast collisional activation scans to identify peptide backbones and a follow-up electron-activation scan to localize the glycosite(s) using a graph-based algorithm. Here, we incorporate O-Pair into FragPipe and adapt it to work with MSFragger glyco search results.

Methods

A standalone C# executable version of the O-Pair algorithm was developed and adapted to access the data formats used by MSFragger and FragPipe. MSFragger was used to search collisional activation scans to identify the peptide sequence and total glycan mass, using the previously described MSFragger glyco search. O-Pair was then used to localize the O-glycan(s) using the electron-based activation scan paired with each collisional scan.

Results

The resulting MSFragger-OPair workflow in FragPipe runs all steps of O-glycoproteomics analysis, including searching collisional and electron-based activation scans, PSM validation and FDR control, O-glycan localization, and quantification (if desired), with a single click. We validated that the O-Pair executable provides identical results to the original O-Pair package in MetaMorpheus, then expanded to compare against other software tools for O-glycoproteomics analysis. We analyzed previously annotated O-glycopeptide data to evaluate the sensitivity, speed, and localization accuracy of our MSFragger-OPair method to that of other software tools. Finally, we assess the utility of adding a composition-level glycan assignment method developed for N-glycopeptides to validate O-glycan assignments using glycan fragment ions from the collisional activation scan.

Conclusions

We have incorporated the O-Pair algorithm for localizing multiple O-glycans into FragPipe, allowing for sensitive and fast O-glycopeptide searches with MSFragger to include complete O-glycosite localization.

CS05.03: Applying transcriptomics to study glycosylation at the cell type level

Dworkin L[1], Clausen H[1], Joshi H[1]
[1]Copenhagen Center for Glycomics, Department of Cellular and Molecular Medicine, Faculty of Health Sciences, University Of Copenhagen

Introduction

The complex multi-step process of glycosylation occurs in a single cell, yet current analytics generally cannot measure the output (the glycome) of a single cell. The glycome is not directly translatable from the genome as it is synthesised through the activity of a metabolic network of enzymes. Previously, we assembled information on the contributions of glycosylation and modification enzymes to the glycome into an atlas of cellular glycosylation pathways[1–2], which we can use to infer glycosylation capacity from the repertoire of expressed enzymes. Here, we tested usage of single cell transcriptomics to break the single cell barrier for glycomics by estimating glycosylation capacities in individual cell types[3].

Methods

Leveraging single cell RNA-seq atlases, we investigated how this data can be used to characterise the state of the glycosylation machinery and metabolic network in single cells. Addressing the challenge that single cell transcriptomics data presents for quantification of the expression level of metabolic network members, we developed methods to reliably extract this information. Using our atlas that comprises 214 glycosylation and modification enzymes, we predicted cellular glycosylation capacities.

Results

We studied differential mRNA regulation of enzymes at the organ and single cell level, finding that most of the general protein and lipid oligosaccharide scaffolds are produced by enzymes exhibiting limited transcriptional regulation among cells. We predict key enzymes within different glycosylation pathways act as regulatable hotspots of the cellular glycome, with distinct expression patterns across over 200 cell types.

Conclusions

Available as Glycopacity (https://glyco.me), investigators can extract and interpret glycosylation information from their data. Cell-type deconvolution of glycosylation reveals that the apparent complexity can be managed, and together with further single cell atlases or proteomics, this method sets the foundation for a new field of in-silico glycomics to help uncover cell-specific functions of glycans.

References

1—https://doi.org/10.1038/s41580-020-00294-x
2—https://doi.org/10.1093/glycob/cwy015
3—https://doi.org/10.1016/j.isci.2022.104419

CS05.04: Differential glycosylation of the spike protein from the Gamma variant of SARS-CoV-2

Pegg C[1], Modhiran N[1,2], Parry R[1], Schulz B[1], Watterson D[1,2]
[1]School of Chemistry and Molecular Biosciences, University of Queensland, [2]Australian Institute for Bioengineering and Nanotechnology, University of Queensland

Introduction: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) - the cause of the ongoing COVID-19 pandemic - has the capacity to generate variants with major antigenic changes in the surface exposed spike glycoprotein. The emergence of SARS-CoV-2 variants threatens current anti-viral and preventative strategies, including monoclonal antibodies and vaccines. The trimeric spike protein of SARS-CoV-2 mediates binding to and fusion with host cells and is covered with a "glycan shield" of ~66 N-glycosylation sites per trimer. Glycosylation is required for efficient folding and maturation of spike, and glycan sites and structures can alter protein conformation and protein-protein interactions, thereby guiding viral infectivity and immune evasion. To gain a better understanding of the role glycosylation plays in SARS-CoV-2 variants, we examined the antibody and glycosylation profiles of Gamma spike which has mutations that introduce two new N-glycosylation sequons (NxT/S; x≠Pro) in the N-terminal domain of the protein.
Methods: We expressed and purified pre-fusion trimeric spike from the ancestral strain (Wu-1), Gamma variant and Gamma with single, double or triple mutations at positions L18F, T20N or R190S in CHO cells. We analysed mAb binding profiles and performed glycoproteomic analysis to elucidate changes in site-specific N-glycosylation occupancy and heterogeneity.
Results: The introduction of a N-linked sequon through T20N resulted in full occupancy at N20 in preference of the native site N17. This site-switching altered antibody binding profiles in the N-terminal domain. We also observed a dramatic change in glycosylation across the protein with decreased sialylation compared to Wu-1. We confirmed changes in sialic acid was not driving changes in mAb binding profiles.
Conclusions: This work highlights biochemical validation is required to confirm site-occupancy in variants with changes in N-linked sequons. N-linked site-switching can alter antibody binding and sequence changes in proteins can dramatically alter the glycosylation profile.

CS07.02: Investigation of the Effect of Staurosporine Induced Kinase Inhibition on HeLa using Top-down Proteome Profiling and Thermal Proteome Profiling

Cupp-sutton K[1], Chowdhury T[1], Guo Y[1], Wu S[1]
[1]University Of Oklahoma

Introduction

Enzymatic phosphorylation/dephosphorylation of proteins is involved in nearly every cellular process; abnormal phosphorylation is implicated in human disease such as cancer. Many diseases have also been linked to mutations in protein kinases that catalyze protein phosphorylation. Staurosporine (STP) is a broad-spectrum kinase inhibitor that induces cellular apoptosis using a pathway that is only partially understood. We have applied quantitative top-down proteomics and thermal proteome profiling (TD-TPP) techniques to investigate the effect of STP treatment on proteoform expression and thermal stability.

Methods

HeLa cells were cultured in 0.5 uM STP or DMSO vehicle for 6 hours followed by lysis and extraction of soluble proteoforms. 1D RPLC-MS analysis was used for label-free quantitation of intact proteoforms. 2D high-pH/low-pH RPLC-MS (TMT) was used to investigate proteoform thermal stability with TD-TPP.

Results

We detected and quantified 1,847 proteoforms using 1D RPLC-MS with label-free quantitation and 3,847 proteoforms using a 2D TMT-based TD-TPP approach to evaluate the proteoform thermal stability. One protein group found to have differential expression was the Jupiter microtubule associated homolog 1 (JUPI1). The JUPI1 protein is known to be involved in the GSK3β pathway and its presence facilitates degradation of β-catenin, which is required for proliferation of cancer cells; however, the effect of differential phosphorylation on intact JUPI1 has not been studied. We observed 3 proteoforms of JUPI1: intact, truncation after residue 46, and intact with 1 phosphorylation. The relative ratio of unphosphorylated proteoforms were higher in the STP treated lysate compared with the phosphorylated proteoform. Furthermore, we found that the intact proteoforms, phosphorylated or unphosphorylated, were more stable than the truncated, unphosphorylated proteoform.

Conclusions

We applied top-down proteomics methods to study the effect of kinase inhibition on phosphorylation and proteoform thermal stability. To our knowledge, the first high-throughput top-down proteomics examination of kinase inhibition using the intact human proteome.

CS07.03: Top-Down Mass Spectrometry Enables Comprehensive O-Glycoform Structural Elucidation of SARS-CoV-2 Spike RBD Variants

Roberts D[1], Mann M[2], Brasier A[2,3], Jin S[1], Ge Y[1,4,5]
[1]Department of Chemistry, University of Wisconsin-Madison, [2]Department of Medicine, School of Medicine and Public Health, University of Wisconsin-Madison, [3]Institute for Clinical and Translational Research, University of Wisconsin-Madison, [4]Human Proteomics Program, School of Medicine and Public Health, University of Wisconsin-Madison, [5]Department of Cell and Regenerative Biology, University of Wisconsin-Madison

Introduction:
The rapid emergence of new SARS-CoV-2 variants of concern (VOC) have destabilized global efforts to combat COVID-19. Recent VOCs, such as Omicron, possess multiple spike receptor-binding domain (S-RBD) mutations and show enhanced transmissibility and evasion of neutralizing antibodies. But exactly how protein-level changes in the new COVID variants enhance viral escape from immunological protection remains to be understood. Here we use top-down mass spectrometry (MS) to provide a comprehensive analysis of the molecular variations and post-translational glycosylation of S-RBD VOCs.

Methods:
S-RBD variants wild-type (WA1/2020), Delta (B.1.617.2), and Omicron (B.1.1.529) were analyzed using a TriVersa Nanomate system coupled to a solariX XR 12-Tesla Fourier Transform Ion Cyclotron Resonance mass spectrometer (FTICR-MS). Trapped ion mobility spectrometry (TIMS)-MS analysis was performed using a timsTOF Pro. MASH Explorer was used for MS/MS analysis.

Results:
We developed a hybrid top-down MS approach by combining ultrahigh-resolution FTICR-MS, to elucidate the sites and structures of the O-glycoforms, and native TIMS-MS, using a Bruker timsTOF Pro, to reveal and characterize O-glycoform structural heterogeneity. Top-down MS/MS analysis revealed a novel O-glycosite for the Omicron variant and new changes to the molecular stoichiometry of eight O-glycoforms compared to WT or Delta. The molecular abundance of Core 2 O-glycan structures was enhanced in Omicron and featured a 70:30 molecular ratio in Core 1 to Core 2 O-glycan structures as compared to WT (82:18) or Delta (87:13). The Core 1 glycan structure, GalNAcGal(NeuAc)2, was found to be the most abundant O-glycoform across all variants (> 60%). Furthermore, GalNAc(GalNeuAc)(GlcNAcGalNeuAc) was found to be the most abundant Core 2 O-glycan structure (>10% of total O-glycoform abundance for Omicron).

Conclusions:
This hybrid top-down MS approach enables accurate determination of the structures and proteoforms of the complex glycoproteins, and provides a necessary molecular foundation to accurately distinguish molecular changes in emerging S variants.

CS08.02: Induction of senescence alters the immunopeptidome and renders cancer cells highly immunogenic for effective immunotherapy

Caron E[1], Marín I, Boix O, García A, Sirois I, Caballe A, Zarzuela E, Ruano I, Stephan-Otto Attolini C, Prats N, López Domínguez J, Kovatcheva M, Garralda E, Muñoz J, Abad M, Gros A, Pietrocola F, Serrano M

[1]CHU Sainte-Justine Research Center, University Of Montreal

Introduction: Cellular senescence is a stress response that activates innate immunity. However, the interplay between senescent cells and the adaptive immune system remains largely unexplored.

Methods and Results: Here, we show that senescent cells display enhanced MHC class I (MHC-I) antigen processing and presentation, and a unique immunopeptidome. Immunization of mice with senescent syngeneic fibroblasts generates CD8 T cells reactive against both normal and senescent fibroblasts, some of them targeting senescence-associated MHC-I-peptides. In the context of cancer, we demonstrate that senescent cancer cells trigger strong anti-tumor protection mediated by antigen-presenting cells and CD8 T cells. This response is superior to the protection elicited by cells undergoing immunogenic cell death. Finally, induction of senescence in patient-derived cancer cells exacerbates the activation of autologous tumor-reactive CD8 tumor-infiltrating lymphocytes (TILs) with no effect on non-reactive TILs.

Conclusion: Our study indicates that immunization with senescent cancer cells strongly activates anti-tumor immunity, and this can be exploited for effective immunotherapeutic interventions against cancer.

CS08.03: Metabolic oxidative stress triggers qualitative and quantitative changes in the mouse DC proteome and immunopeptidome displayed by I-Ab MHC II

Clement C[1], Nanaware P[2], Stern L[2], Soni R[3], Santambrogio L[1]
[1]Weill Cornell Medicine, [2]University of Massachusetts Chan Medical School, [3]Columbia University Irving Medical Center

Introduction
Environment-driven lipotoxicity and glucotoxicity induces liver damage that promotes dendritic cell adjuvanticity and generates MHC-II immunopeptidomes enriched with peptides derived from proteins involved in cellular metabolism, oxidative phosphorylation, and stress responses. We employed complementary proteomics and immunopeptidome analyses aimed to reveal the impact of oxidative stress on the antigen processing machinery and epitopes presentation by I-Ab MHC-II of dendritic cells (DCs) from B6 mice on standard (Ctr) and high fructose/high fat (HF) diet.
Methods
DCs harvested from mice on HF vs Ctr diet (n=6 biological replicates) were subjected to processing for proteomic analysis using an optimized FASP assay. HCD MS/MS sequencing on a Q Exactive HF quadrupole orbitrap and (DDA) mode generated the spectral libraries. One-shot DIA data acquisition for each biological replicate was performed using the Orbitrap Fusion Tribrid mass spectrometer. DIA-MS samples were analyzed in Scaffold DIA, and PEAKS X-Pro. The immunopeptidomes were obtained using immunoaffinity-purified MHC-II proteins from the DCs of Ob/Ob mice, as well as B6 mice on Ctr or HF diets followed by peptide elution and analysis using LFQ DDA and DIA complementary approaches.
Results
Label-free DIA quantitative analysis retrieved 744 proteins exhibiting statistically significant quantitative changes in the proteomes of DCs harvested from mice on HF vs Ctr diet (ANOVA, $p<0.05$ and attained FDR<1%). Bioinformatics mapped quantitative changes in energy and stress-related proteins associated with several metabolic pathways including glycolysis, lipolysis, and mitochondrial oxidative phosphorylation. Parallel reaction monitoring (PRM) with stable-isotope-labeled peptides standards, followed by analysis in Skyline enabled the comparative quantification of I-Ab-eluted self-epitopes originating from metabolic enzymes such as (malate dehydrogenase) MDH, aldolase (ALDOA), pyruvate kinase (PKM) and protein disulfide-isomerase 3 (PDIA3).
Conclusions
The qualitative and quantitative changes in the DC proteomes elicited by metabolic insults were mirrored in the landscape of immunopeptidomes eluted from I-Ab MHC-II molecules on DCs.

CS08.04: Thunder Isolation DDA PASEF enables high coverage immunopeptidomics and identifies naturally presented MHC class I ligands from SarsCov2 spike protein

Gomez-Zepeda D[1,2], Arnold-Schild D[2], Kumm E[2], Schild H[1,2], Tenzer S[1,2]
[1]Helmholtz Institute For Translational Oncology (hi-tron), [2]Institute for Immunology, University Medical Center of the Johannes Gutenberg University Mainz

Introduction:
Major histocompatibility complex class I (MHCI) peptide ligands (MHCIp) can be exploited for the development of vaccines and immunotherapies against infectious pathogens or cancer. Nevertheless, their characterization is challenging due to their high diversity and low abundance. Here, we developed a highly sensitive and reproducible method for the identification of HLAIps using liquid chromatography-ion mobility-tandem mass spectrometry (LC-IMS-MS/MS).
Methods:
Cell pellets of JY and Raji human cell lines, wild-type or expressing the SARS-Cov-2 spike protein, were lysed and MHCI-peptide complexes were immunoaffinity-enriched using W6/32 anti-human-MHC-A/B/C antibody. Then, peptides were eluted (0.2% TFA), ultrafiltrated (10 kDa cutoff) and desalted. NanoLC-MS analysis was performed in DDA-PASEF using a nanoElute coupled to timsTOF-Pro-2 with a 2-hours RP separation. Peptide identification was performed in PEAKS XPro using unspecific cleavage, rescored with MS2Rescore and filtered to 1% FDR. MHC-binding affinity was predicted using NetMHCpan 4.1.
Results:
The optimized method, named Thunder DDA-PASEF, uses high resolution IMS separation and an adapted isolation polygon to selectively fragment peptides of the expected size for MHCIps. This effectively doubled the number of peptides identified, including singly-charged ions comprising more than 30% of the identifications. Additionally, MS2Rescore further boosted the confident identifications (1% FDR) by 20%. Analysis of the equivalent to 15 million cells resulted in >13,000 peptides per run and >20.000 peptides mapping to > 4500 proteins per cell line, including 85% predicted to bind human MHCI complexes. Thunder DDA-PASEF allowed an unprecedented coverage of predicted MHCIps including possible antigens from the SARS-Cov-2 Spike protein, counting ten previously reported as immunogenic.
Conclusions: We developed a highly sensitive and reproducible DDA-PASEF immunopeptidomics method for the identification of MHC class I ligands which will enable the study of neoantigens of cancer-related mutations from scarce-samples such as tumors and cancer biopsies, constituting a step towards the development of personalized immunotherapies.

CS08.05: Discovery and Validation of HTLV-1 specific HLA-I associated peptides from HTLV infected cell lines and ATLL patient samples

Srivastava M[1], Van Meter M[1], Kuhnert F[1], Copin R[1], Olsen O[1], Olson W[1], Salzler R[1]
[1]Regeneron Pharmaceuticals

Introduction:
Human T-lymphotropic virus (HTLV-1) is an oncogenic retrovirus that is associated with the development of Adult T-cell Leukemia/ Lymphoma (ATLL) and HTLV-1 associated myelopathy (HAM). Disease progression primarily depends on the proviral load and the efficiency of the CD8+ cytotoxic T lymphocyte responses to HTLV-1 infection. Here we characterize the immunopeptidome of HTLV-1 infected cell lines and ATLL primary samples with the goal to identify HTLV-1 unique HLA-associated peptides that can be utilized as targets for immunotherapy.

Methods:
HTLV-1 (+) T cell lymphoblastic cell lines C8166, MT-2, MT-4, C5/MJ, and primary ATLL samples were subjected to immunopeptidomics workflow. Briefly, HLA class I/peptide complexes from cell lysates were enriched using Pan HLA class I (W6/32) affinity purification, followed by C18 reverse phase-based separation of peptides and LC-MS analysis.

Results:
We detected more than 13000 HLA-associated peptides from MT-4 cell line and 9000 – 10000 peptides from the additional three HTLV-1-transformed cell lines. This includes about 10 – 30 unique HTLV-1 HLA-associated peptides from each cell line. Specifically, we detected multiple peptides from Pol, Env and the tax, the protein implicated in oncogenesis, from the four cell lines. Next, we investigated the HLA-I presented HTLV-1 peptides from ATLL primary cells: ATL43T+, ATL55T+, ATL43Tb-, ED40515+, ED40515- and ED41214+. We detected HLA-associated peptides from Gag-Pro-Pol polyprotein and tax from ED41214- cells. Current efforts are towards discovering HLA-I-associated HTLV-1 unique peptides from the ATLL patient samples.

Conclusions:
Our discovery of HTLV-1 peptides will assist in the development of bispecific antibody, CAR-T or vaccine based immunotherapeutics against HTLV-1 infections.

CS09.03: Protein signatures across neurodegenerative diseases

Bergström S[1], Mravinacová S[1], Olofsson J[1], Remnestål J[1], Anggraeni Andersson M[1], Månberg A[1]
[1]KTH - Royal Institute of Technology, SciLifeLab

Background

The identification of disease-associated protein signatures could contribute to an increased understanding of neurodegenerative disorders. Some key features have been identified, but much remains unknown about the disease pathogenesis. We hypothesize that there are complex patterns and associations to be discovered, specific to and similar between different diseases. To reveal such patterns, it is crucial to investigate many proteins in several independent cohorts.

Methodology

Our in-house developed affinity proteomics method allows for measurement of hundreds of proteins in hundreds of samples in the same assay. This is enabled by biotinylation of samples and a single binder setup with antibodies immobilized onto magnetic color-coded beads. We primarily use antibodies produced within the Human Protein Atlas project (proteinatlas.org) and the selection of proteins to be analyzed is tailored for each project, allowing for a high flexibility and adaptability.

Results

Over the years, we have analyzed more than 3000 CSF samples and almost 5000 plasma samples from cohorts including patients with Alzheimer's disease, Parkinson's disease, frontotemporal dementia, and amyotrophic lateral sclerosis. Additionally, we have analyzed samples from neurologically healthy and cognitively impaired controls. In total, we have utilized over 1500 antibodies to profile almost 1000 proteins. The majority of the proteins are brain-enriched according to tissue transcriptomics and we have assessed their association to diagnosis, disease progression and other clinically relevant parameters.

Conclusion

To identify disease-relevant protein signatures, it is crucial to analyze large and independent cohorts including different neurodegenerative diseases. These signatures could potentially aid in finding multifactorial differences and similarities between diseases and help understanding the underlying mechanisms. We also explore the data with the aim to find novel subgroups of patients with relevance for clinical outcome and care.

CS09.04: AI-based Proteomic Prediction of Cancer Type from 1,289 Human Tissue Samples and 949 Cancer Cell Lines

Noor Z[1], Cai Z[1], L Boys E[1], Xavier D[1], Lucas N[1], Williams S[1], M S Koh J[1], C Poulos R[1], G Hains P[1], Aref A[1], J Robinson P[1], Reddel R[1], Balleine R[2], Zhong Q[1]
[1]ProCan®, Children's Medical Research Institute, Faculty of Medicine and Health, The University of Sydney, Westmead, [2]Westmead Institute for Medical Research, Faculty of Medicine and Health, The University of Sydney, Westmead

Introduction: Cancer type is determined by expert pathologists using tumour morphology which may be aided by immunohistochemical staining. Development of deep learning (DL) models using histology slides has powered the image-based prediction of site of origin in cancer of unknown primary (CUP). Here, we present a DL-based method to predict cancer type from a pan-cancer cohort consisting of 1,289 human tissue samples spanning 44 cancer types and 26 different tissues based on proteomic data.

Methods: We quantified 9,051 proteotypic proteins from 795 tumour and 494 tumour-adjacent "normal" samples, using data-independent acquisition (DIA) mass spectrometry. We processed the proteomic data of 3,864 runs with DIA-NN and used DL to train and test the models. We used top-k accuracy as the evaluation metric, computing how often the correct cancer type class (defined by histopathologic evaluation) is among the top k classes predicted.

Results: We trained DL models on 80% of the tumour samples and tested the model on the remaining 20%, achieving a top-3 accuracy of 98.6% for cancer type prediction. We also trained models on a pan-cancer proteomic dataset (ProCan-DepMapSanger) of 949 human cancer cell lines1 and tested the final models on the tumour samples, yielding an average top-3 accuracy of 98.9%. This indicates that DL models trained on highly enriched cancer cell populations, can predict cancer types in complex tumour tissue samples. Differential expression analysis of both tumour and "normal" samples revealed significant proteins and biological pathways expressed in each cancer type and tissue type.

Conclusion: Our proteomic-based multinomial DL models can predict cancer type in concordance with existing histopathological classification. It can also assign multiple probabilities to sites of tumour origin, potentially enabling classification of CUP in future work.

1 Emanuel Gonçalves*, Rebecca C. Poulos*, Zhaoxiang Cai* et al., 2022 Cancer Cell, doi:10.1016/j.ccell.2022.06.010

CS09.05: Proteomic Characterization of High-Grade Endometrioid Endometrial and Uterine Serous Cancers

Bateman N[1,2], Makohon-Moore S[1,2], Taqee S[1,2], Oliver J[1,2], Conrads K[1,2], Hood B[1,2], Casablanca Y[1,3], Phippen N[1,3], Tarney C[1,3], Darcy K[1,2], Elshaikh M[4], Ali-Fehmi R[4], Cote M[4], Maxwell G[1,5], Conrads T[1,5]
[1]Gynecologic Cancer Center of Excellence, [2]The Henry M. Jackson Foundation for the Advancement of Military Medicine Inc., [3]Murtha Cancer Center Research Program, [4]Wayne State University, [5]Women's Health Integrated Research Center

Introduction: Recent efforts focusing on proteomic characterization of endometrial cancers (EC) have largely characterized low-grade EC tumors and analysis of high-grade EC tumors is needed as high-grade disease portends worse disease outcome. To address this, we describe a deep proteomic characterization of >300 high-grade endometrial endometrioid carcinoma (EEC) and uterine serous carcinoma (USC) tumors.

Methods: Formalin-fixed, paraffin-embedded tumors from patients with high-grade (G3) EEC (n=145) or USC (n=165) were assembled at Wayne State University. Tumor cells were collected using laser microdissection on an LMD7 microscope (Leica Microsystems) and processed by barocycler-assisted digestion (2320EXT, Pressure BioSciences). Samples were labelled with tandem mass tags (TMTpro) and analyzed by nanoflow liquid chromatography (EASY-nLC 1200) mass spectrometry (Q-Exactive HF-X, Thermo Fisher Scientific). Data was searched using Proteome Discoverer (Thermo Fisher Scientific) and Mascot (Matrix Sciences).

Preliminary Data: Global proteomic analysis quantified 5,219 proteins across >300 tumor tissues collected from endometrial cancer patients. Comparison of putative biomarkers for USC and high-grade EEC (PMID: 30550483) showed protein levels of beta-catenin as significantly elevated (LIMMA adjusted $p < 0.05$) in EEC tumors and multiple proteins including tumor protein P53 as significantly elevated in USC tumors, consistent with previous observations. Differential analysis identified 107 protein alterations between USC and EEC tumors correlating with enrichment of pathways regulating anchoring fibril formation in USC tumors and membrane trafficking in EEC tumors. Protein alterations between USC and high-grade EEC tumors with public proteome data for a cohort of USC (n=14) and low-grade EEC tumors, i.e. n=37 G1, n=40 G2, and n=8 G3 (PMID: 32059776) identified 110 proteins significantly co-altered that exhibited high quantitative correlation (Spearman Rho=0.864, P<0.0001).

Conclusions: Our efforts describe deep, proteomic characterization of high-grade EEC and USC tumor tissues and highlights include identification of protein alterations between EEC and USC tumors that are highly conserved, irrespective of disease grade.

CS09.06: A clinically relevant rapid sample preparation method and prognostic signature identification for HPV-Related Oropharyngeal Squamous Cell Carcinoma

Williams S[1], Jackson C[2], Liu J[1], Anees A[1], Noor Z[1], Hains P[1], Porceddu S[2], Liu H[2], Ladwa R[3], Whitefield J[4], Simpson F[4], Reddel R[1], Zhong Q[1], Panizza B[5], Robinson P[1]
[1]Children's Medical Research Institute, [2]Princess Alexandra Hospital, [3]University of Queensland, [4]The University of Queensland Diamantina Institute, [5]The Kinghorn Cancer Centre
Introduction: The last several decades have seen an epidemic-like increase in HPV-associated oropharyngeal squamous cell carcinoma (OPSCC). Outcome prediction is crude and treatment protocols are generic. We developed a new high-throughput proteomics workflow, to identify a proteomic signature for HPV-positive OPSCC recurrence risk using the smallest possible formalin-fixed paraffin embedded (FFPE) samples.
Method: Our new workflow was first developed to utilise the smallest viable FFPE samples from tissue microarrays (TMAs) or cores sections cut from blocks. It incorporated Pressure Cycling Technology to provide low microgram peptide yields from 10 µm thick sections which were cut from 1 or 2 mm diameter TMAs for single MS runs. However, the smallest sample size for sufficient peptide yield to support replicate runs on by microflow data-independent acquisition mass spectrometry (DIA-MS) was larger - 2x2 mm sections.
We analysed 139 FFPE specimens from 124 patients with locally advanced HPV-positive OPSCC, treated with chemoradiotherapy at the Princess Alexandra Hospital (Brisbane, Australia) 2007-2019, including 50 non-responders (recurrence <5-years from diagnosis) and 74 matched responders. Data was searched using DIA-NN and data filtering was developed to identify differentially expressed biomarker candidates.
Results: We quantified 5,903 proteins and 38,709 peptides. Cox regression models and machine learning were used to generate a 16-protein signature and a 21-peptide signature associated with 5-year recurrence-free survival. The signatures will be tested against a future validation cohort.
Conclusions: Our rapid and robust sample preparation method is suitable for high-throughput MS-based proteomics from TMA sections or small tumour cores, and offers a new path for proteome analysis of small samples when there is a limited tumour sample source. Using core biopsies the approach was successfully used to risk stratify patients and identify those at high risk of recurrence. This may help inform future clinical trials to better tailor upfront therapy.

CS09.07: Peptide based proteomics screen to study mutated phosphorylation sites within intrinsically disordered regions

Rrustemi T[1], Meyer K[1,2], Uyar B[1], Imami K[3], Ishihama Y[3], Selbach M[1]
[1]Max Delbrück Center For Molecular Medicine, [2]University of Zürich, Molecular Life Sciences, [3]Kyoto University, School of Pharmaceutical Sciences

Introduction: With the recent advances in sequencing technologies, the number of identified pathogenic mutations has increased drastically and surpassed our ability to functionally characterize them. Many disease causing missense mutations affect intrinsically disordered regions (IDRs). IDRs often contain short linear motifs (SLiMs) that mediate interactions with other proteins and/or are substrates of posttranslational modifications such as phosphorylation. As SLiMs are crucial for protein-protein interactions (PPIs), studying how mutations in IDRs alter PPIs can provide information on disease mechanisms.

Methods: In this project, we employed a peptide-based screen to assess how pathogenic mutations of known phosphorylation sites affect PPIs. To this end, we selected 38 disease-causing mutations, germline and somatic, from the PTMVar database of PhosphositePlus. Peptides corresponding to the mutated regions were synthesized on cellulose membranes in the wild-type, mutated, and phosphorylated form and used to pull-down interacting proteins from cellular extracts.

Results: We find that the interactome of the phosphorylated and mutated peptides differed markedly, with most of the interactions being lost upon mutation, in several cases due to the destruction of a phosphorylated SLiM. We have used proximity labeling-mass spectrometry, BioID, to validate some screen results. Both the screen and BioID results imply a role of SYNRG in clathrin-mediated endocytosis, in concordance with the literature. Moreover, we observed that a phosphorylated peptide derived from the zinc-finger protein GATAD1 interacts specifically with 14-3-3 proteins while the corresponding mutated peptide does not. The mutation of this phosphorylation site causes dilated cardiomyopathy. We are investigating if a potential disruption of GATAD1/14-3-3 interaction could lead to GATAD1 mislocalization.

Conclusion: This proteomics screen is an efficient method that allows the study of hundreds to thousands of point mutations simultaneously by investigating their impact on PPIs. This information could lead to new insights into disease development mechanisms.

CS10.03: Mapping the diversity in spatiotemporal regulation of G protein-coupled receptors

Li Q[1,2,3], Polacco B[1,2,3], Marley A[3,4], Patil T[1,2,3], Li J[3,4], Blythe E[3,4], Xu J[1,2,3], Zhong X[1,2,3], Krogan N[1,2,3], Von Zastrow M[3,4], Huttenhain R[1,2,3]
[1]Department of Cellular and Molecular Pharmacology, UCSF, [2]J. David Gladstone Institutes, [3]Quantitative Biosciences Institute (QBI), UCSF, [4]Department of Psychiatry, UCSF

G protein-coupled receptors (GPCRs) regulate diverse physiological processes in response to hormones, neurotransmitters, odorants, and light, making these receptors drug targets for many diseases. Despite their relevance, our understanding of their signaling is mostly limited to known signal transducers, such as heterotrimeric G proteins and Arrestins, and second messengers. These are however insufficient to explain the diverse physiological effects mediated by GPCRs, suggesting additional specialization on the cellular level. Here, we leverage a quantitative proteomics strategy for a diverse set of clinically relevant GPCRs to generate the first systematic, time-resolved map of their cellular response to activation in living cells.

Specifically, we used our recently developed proximity biotin labeling method based on the engineered peroxidase APEX combined with quantitative proteomics (GPCR-APEX-MS) for a panel of eleven GPCRs expressed along the pain pathways. Taking snapshots of their proximal proteome over a time course of activation, we demonstrated that GPCR-APEX-MS has the capacity to simultaneously capture interaction networks and cellular location of the activated receptors with high temporal resolution.

Our spatiotemporal GPCR map revealed (1) a striking diversity in receptor localization and trafficking of the pain-related GPCRs and (2) a combined interaction network containing shared and receptor-specific interactors. For example, we discovered a novel interaction specific for the RF-amide receptor NPFFR1 with the CUL1-FBXW11 E3 ligase complex. We found that this interaction depends on a phosphodegron in the C-terminal tail of NPFFR1 which becomes phosphorylated by G protein-coupled receptor kinases (GRKs) upon activation. Both mutation of the phosphodegron and inhibition of GRKs stabilized the receptor, indicating that CUL1-FBXW11 may be involved in degradation of the activated NPFFR1.

These results (1) exemplify the power of quantitative proteomics to illuminate GPCR biology and (2) suggest that cellular compartmentalization of signaling and receptor specific interactions contribute to the diversity of GPCR cellular responses.

CS10.04: Robust and streamlined Deep Visual Proteomics enables characterization and stratification of colorectal adenomas

Kabatnik S[1], Drici L[1], Strauss M[1], Snejbjerg Bartels A[2], Mund A[1], Moreira J[2], Mann M[3]
[1]NNF Center for Protein Research, Faculty of Health Science, University of Copenhagen, [2]Section of Molecular Disease Biology, Department of Drug Design and Pharmacology, Faculty of Health and Medical Sciences, [3]Department of Proteomics and Signal Transduction, Max Planck Institute of Biochemistry

Introduction: Colorectal adenomas (CRA) are benign tumors that may progress to adenocarcinomas. As a result of national screening programmes, colorectal cancer incidence has declined in connection with increases in adenoma detection rates. Being able to predict disease recurrence or progression from adenoma stratification would benefit patients and the healthcare system. To meet this clinical need, we applied Deep Visual Proteomics (DVP), a method which combines machine learning-based image analysis, automated single cell laser microdissection and ultra-high sensitivity mass spectrometry on formalin-fixed, paraffin-embedded (FFPE) CRA tissues. We wished to develop a robust and streamlined workflow that could be broadly applied on clinically established staining for molecular characterisation.

Methods: We analyzed 9 CRA FFPE tissues by DVP, immunohistologically stained for CDX2. Nuclei-based segmentation and classification resulted in four relevant classes which were automatically excised at single cell level with a fixed offset. 1,000 contours of each biologically fractionated class, equivalent to 120-200 cells, were pooled and then analyzed by the Evosep One system at 30 samples per day in combination with diaPASEF on the Bruker single cell timsTOF. Data analysis was performed with DIA-NN against our deep library.

Results: We created a deep CRA library with a depth of 12,300 unique protein groups, using extensive fractionation and the Evosep 30 samples per day method, which allowed fast analysis and boosted identification of low-input DVP samples. While bulk tissue proteomics provided limited biological insights about our patient's disease recurrence, DVP, successfully identified region and cell class-specific pathobiological proteome changes within one and across all 9 patient tissues.

Conclusion: We developed a streamlined and robust DVP workflow for low-input samples that requires no change to current pathology practice and only readily available commercial equipment. It provided biological insights into spatially and morphologically different tissue areas across CRA patients with known cancer recurrence history.

CS10.05: High-resolution complexome of mitochondria reveals quality control pathways of protein import

Schulte U[1], den Brave F[2], Haupt A[1], Gupta A[2], Song J[2], Müller C[1], Engelke J[2], Mishra S[2], Mårtensson C[3], Ellenrieder L[3], Priesnitz C[3], Straub S[3], Doan K[3], Kulawiak B[3], Bildl W[1], Rampelt H[3], Wiedemann N[3], Pfanner N[3], Fakler B[1], Becker T[2]
[1]Institute Of Physiology, University Of Freiburg, [2]Institute of Biochemistry and Molecular Biology, University of Bonn, [3]Institute of Biochemistry and Molecular Biology, ZBMZ, University of Freiburg
Mitochondria contain ~1,000 different proteins that often assemble into complexes and supercomplexes such as respiratory complexes and preprotein translocases. However, the complexome organization of major parts of the mitochondrial proteome is poorly understood. We report a quantitative mapping of mitochondrial protein assemblies by high-resolution complexome-profiling of >90% of the yeast mitochondrial proteome, termed MitCOM. Analysis of MitCOM resolves >5,200 protein peaks and demonstrates an unexpected complexity of mitochondrial protein assemblies with distinct appearance of respiration, metabolism, biogenesis, dynamics, regulation and redox processes. The identification of quality control factors operating at the mitochondrial protein entry gate reveals pathways for preprotein ubiquitylation, deubiquitylation and degradation. MitCOM, accessible through an interactive profile viewer, serves as comprehensive resource for analyzing the functional organization and interaction of mitochondrial machineries and pathways.

CS11.03: Region-resolved Proteomic Profiling of a Postmortem Human Brain

Tueshaus J[1], Delbrige C[2], Schlegel J[2], Kuster B[1]
[1]Technical University Munich, Chair of Proteomics and Bioanalytics, [2]Technical University of Munich, Institute of Pathology

The brain is the most complex organ of the human body. Over the last decade, great effort has been expended to unravel the architecture of the brain using imaging and omic approaches. However, little is understood about regional differences especially on protein level. Therefore, we optimized a micro-flow timsTOF-HT setup in order to generate deep proteome profiles of thirteen brain regions.

Aiming for deep proteome coverage, we developed a workflow for formalin fixed brain tissue including lipid depletion. High efficient protein extraction and de-crosslinking was achieved by boiling the tissue in 4% SDS, Tris buffer. Proteins were digested using the SP3 approach on automated liquid handling platforms ensuring high reproducibility. Desalted peptides were fractionated using basic reversed phase liquid chromatography. Samples were analyzed on a micro-flow LC coupled to a timsTOF-HT mass spectrometer. Settings of the VIP-HESI source and the timsTOF-HT were optimized in order to cope with the challenges of micro LC flowrates (50 µl/min).

We optimized a micro-flow LC timsTOF-HT setup including ESI, ion mobility and fragmentation parameters leading to rapid, robust and sensitive proteome analysis. For example, we identify >5,000 protein groups supported by > 25,000 peptides in single-shot HeLa runs using a 30 min gradient. With this setup at hand, we analyzed 48 fractions of thirteen postmortem human brain regions. Bioinformatic analysis uncovered profiles distinguishing regions by proteome composition and highlighted candidates of regional driver proteins. While a substantial core proteome was shared between all brain regions, we also identified unique fingerprints pointing towards specific functions and cellular composition of particular regions.

Within the scope of this project, we, for the first time, established the power of combining micro-flow LC with a timsTOF-HT which turned out to be a fast, robust and sensitive LC-MS setup. This enabled the region-resoled elucidation of the human brain proteome.

CS11.04: Mapping the 3D Proteome using Surface Accessibility in Animal Models of Disease

Yates J[1], Son A, Bamberger C, Pankow S
[1]The Scripps Research Institute

Methods to determine the surface accessibility of amino acids on proteins can provide information about conformational changes to proteins because of gene mutations, drug treatment, or other environmental perturbations. Methods such as FPOP, SPROX or LiP are collectively described as protein footprinting methods. Mostly these methods are applied in vitro although FPOP is being developed for in vivo applications in C. elegans. We have been developing a strategy, Covalent Protein Painting (CPP), with in vivo application in animal models of diseases.

Methods
CPP uses formaldehyde to label accessible lysine residues in non-denatured proteins which are then converted to dimethyl tags using cyanoborohydride. As has been used in quantitative proteomics, different stable isotope elements can be used in formaldehyde and cyanoborohydride to create different weight tags and thus a secondary labeling after proteolytic digestion of proteins using different weight reagents labels lysine residues that were not labeled in the first labeling step. When performed in vivo this method can provide a measure of how surface accessibility changes in proteins based on a perturbation. A method to perfuse reagents into mice has been developed.

Results
After the reactions are complete all of the major organs (8 in total) are removed, homogenized, lysed and prepared for digestion. A chymotrypsin digestion is then performed. A thorough and comprehensive analysis of peptides from each organ is performed with the intent to measure dimethyl tagged peptides. An initial comparison of a 3-month-old mouse to a 9-month-old is performed. The hypothesis is older animals should show more perturbations to conformations of proteins as proteostasis systems start to fail with age. An Alzheimer's disease mouse model (APPNL-F/NL-F) is then analyzed and compared to an age and sex matched control.

Conclusion
A new method to study the 3D proteome in animal models of diseases is presented.

CS11.05: Optimizing Acoustic Ejection Mass Spectrometry (AEMS) for Ultra-high Throughput Peptide Quantification Studies

Hunter C[1], Wang O, Hoedt E, Fu Q, Van Eyk J
[1]SCIEX, [2]Cedars-Sinai Medical Center

Introduction

Acoustic droplet ejection (ADE) platforms have been widely adopted in high-throughput screening labs to rapidly, accurately and precisely transfer nanoliter volumes of liquids between plates. More recently, this dispensing technique has been coupled with Open port interface (OPI) in order to transfer the samples to a mass spectrometer enabling rates of sample analysis of up to 1 sec/sample. Here, we explore and optimize the acquisition conditions for peptide quantification, assessing on sensitivity and reproducibility, with a focus on the SISCAPA workflow as the upfront automated sample preparation strategy.

Methods

A variety of stable isotope labeled peptides and the equivalent unlabeled peptides were used to test the Echo MS system for peptide quantification. Matrix conditions were varied, various carrier solvents and flow rates were explored, and droplet counts were optimized. Sensitivity, reproducibility of raw peak areas and peptide ratios, and repeatability were then evaluated.

Results

Peptide performance was evaluated across various experimental conditions. Current best acquisition strategy uses a carrier solvent of acetonitrile with medronic acid, samples diluted in water/formic acid with low amount of CHAPS (<0.0003%), flow rates of ~450-500 µL /min and 40 nL ejections. Using these conditions, calibration curves were generated to assess the average lower limits of quantification (typically 1 fmol/µL in plate). A large-scale reproducibility study was performed, two light peptides in 384 well plates, across 27 plates. 10,2600 samples were analyzed in 5 hours of acquisition time, providing intra-plate CVs of <10%. Equivalent LC-MS time for same # of samples would take 45 days (up to 230x faster with AEMS). Currently, best conditions for heavy/light ratio reproducibility are under determination.

Conclusions

Ultra-high throughput peptide quantification using Echo MS system with a turnaround time of ~1 second/sample for biomarker research.

CS11.06: Thinking BIG: Setting up a High-throughput High-volume Proteomics Lab

Viode A[1], Fatou B[1], Smolen K[1], van Zalm P[1], Stevenson D[1], Jha M[1], Ahmed S[1], Steen J[1], Steen H[1]
[1]Boston Children's Hospital and Harvard Medical School

Introduction

Proteomics has the reputation of being a low throughput and expensive technology. When the world was at a grinding halt in the midst of a worldwide pandemic, the Steen Lab was tasked to increase its throughput 10-fold to support the nationwide COVID-19 research. Within 3 months, the Steen lab leveraged commercially available hardware and in-house developed methodology to analyze thousands of samples in a high-throughput fashion.

Methods

Scaling-up the throughput of our pipeline 10-fold required optimization and streamlining of supply chain and sample management, sample processing, sample analysis, data analysis, and robust hardware. All aspects of the pipeline were equally important as any bottleneck would have affected the throughput. Given the special circumstances and the short time frame, the pipeline was built around two mass spectrometers, and commercially available consumables, reagents, and solutions.

Results

Pre-barcoded tubes compatible with multi-titer plates were co-opted from other high throughput omic technologies. However, irrespective of the use of LIMS and advanced barcode readers, sample management remains a time-consuming but crucial undertaking. Successful supply chain management requires careful planning of the consumable and reagent needs. Liquid handling-based sample processing significantly improves the reproducibility of the simple and robust protocols for the processing and depletion of thousands of samples. Robust LC and MS instrumentation is a must to minimize any maintenance-associated downtime and batch effects. Handling and analyzing terabytes of data require high-performance computing as local servers are underpowered.

Conclusions

High-volume high-throughput proteomics on many different body fluids is achievable with commercially available consumables, reagents and solutions, and reasonable hardware requirements. Attention to detail is critical as seemingly small problems can result in unsalvageable problems downstream. Currently, pilot studies with 100 samples are the norm, and genetic scale sample numbers of 10,000 plasma samples/year analyzed on a single mass spectrometer are now feasible.

CS11.07: Maximizing information content in ion mobility-enhanced DIA using overlapping, ion mobility-encoded quadrupole windows

Distler U[1], Łącki M[1], Startek M[1], Teschner D[2], Brehmer S[3], Decker J[3], Raether O[3], Tenzer S[1]
[1]Universitätsmedizin der Johannes-Gutenberg Universität Mainz, [2]Johannes-Gutenberg Universität, [3]Bruker Daltonics GmbH & Co. KG

Introduction

Cycling deterministically through segments of a precursor m/z range, data-independent acquisition approaches provide comprehensive records of all detectable precursor and fragment ions. Standard dia-PASEF methods utilize the correlation of molecular weight and ion mobility in a trapped ion mobility device (timsTOF Pro 2) to extend sensitivity and specificity of DIA by the additional ion mobility dimension. Here, we present a novel DIA scan mode, using mobility-specific micro-encoding of overlapping quadrupole windows to maximize information content in DIA acquisitions, providing parallel improvements in cycle time (-50%) and sensitivity (+150%) compared to dia-PASEF.

Methods

Overlapping ion-mobility-dependent quadrupole windows were defined based on spectral libraries to provide >99% library coverage over the full mass range. Datasets were acquired on a modified timsTOF Pro 2 platform in dia-PASEF and several variations of the novel acquisition scheme.

Results

The novel acquisition mode utilizes ion-mobility-based scanning of quadrupole windows, thereby encoding precursor position in the datasets. Using overlapping quadrupole selection windows between successive individual TIMS-frames provides a 2.5-fold increase in fragment ion sensitivity compared to dia-PASEF reference runs, while covering the entire precursor ion mass range without sacrificing cycle time.

To process the resulting high-complexity datasets, we developed algorithms for multidimensional peak detection. Highly specific pseudo-MSMS spectra were generated based on multidimensional deconvolution (RT, IMS, quad window), matching the intensity distribution of a given fragment to the one of a target precursor. Pseudo-MSMS spectra were converted into mgf format and searched directly with PEAKS and Mascot.

Preliminary data acquired indicate that the novel acquisition mode enables a deconvolution-based refinement of precursor-fragment ion relationships to below 2 Th, providing the basis for DDA-like fragment spectrum quality over the entire mass range (300-2000 m/z).

Conclusions

Novel quadrupole scanmode enables comprehensive, fast and highly sensitive DIA, surpassing classical diaPASEF in terms of sensitivity, speed and selectivity

CS11.08: Proteome wide, real-time spectral library matching to improve sensitivity and efficiency of quantitative proteomics workflows.

Mcgann C[1], Schweppe D[1]
[1]University Of Washington

Introduction. Real-time data acquisition methods increase protein identifications, quantitative accuracy, and instrument efficiency. Previous work has shown proof-of-principle utility of real-time database searching (RTS, Schweppe et al. 2020), now we demonstrate the utility of an integrated real-time library searching method (RTLS) to selectively trigger MS3 scans downstream of high-confidence peptide spectra matching. We tested the potential and benefits of coupling hardware improvements of a new modified Orbitrap instrument with improved real-time informatics (RTLS) for sample multiplexed quantitative proteomics.

Methods. A two proteome HyPro standard (90% human, 10% yeast) was used to assess sensitivity and quantitative accuracy. Unfractionated peptides were injected into a new modified Orbitrap instrument running new instrument control software and data was compared to those from an Orbitrap Eclipse. We employed both empirical spectral libraries (SpectraST; human, yeast) and predicted TMTpro libraries (Gessulat, et al. 2019). Custom library software was written in R.

Results. In proof-of-principle demonstrations, we show that RTLS can efficiently match spectra both from predicted and empirical libraries in real time with instrument acquisition median search times for all methods under 15ms. These speeds enable RTLS to match against libraries ranging from small, targeted libraries with a few hundred library spectra to a proteome wide library consisting of >2 million spectra. To facilitate library building and rapid prototyping, we built a custom library-processing tool to convert common spectral library formats to be compatible with the modified instrument control software. RTLS consistently improved selective triggering of HyPro yeast peptides by at least 35%. We also observed improved instrument efficiency and sensitivity of the modified Orbitrap instrument in terms of quantified peptides per hour.

Conclusion. We establish the utility and extensibility of intelligent data acquisition on a modified Orbitrap instrument to improve sample multiplexed quantitative proteomic sensitivity and accuracy.

CS12.02: Organism-wide secretome mapping of tissue crosstalk in exercise

Riley N[1], Wei W, Lyu X, Shen X, Guo J, Zhao M, Moya-Garzon M, Basu H, Tung A, Li V, Huang W, Svensson K, Snyder M, Bertozzi C, Long J
[1]Stanford University

Introduction

Exercise is a powerful physiologic stimulus that provides benefits to multiple organ systems and confers protection against disease. These effects are in part mediated by blood-borne factors that mediate tissue crosstalk and function as molecular effectors of physical activity. To globally understand how physical activity reshapes cellular secretomes, here we use a proximity biotinylation approach to profile cell type-specific secretomes following treadmill running in mice. This organism-wide, 21-cell type, 10-tissue secretome atlas reveals complex, bidirectional, and cell type-specific regulation of secreted proteins following exercise training.

Methods

An engineered biotinylation enzyme TurboID was delivered into the secretory pathway of cells via adeno-associated virus (AAV) transduction. Cell type-specific labeling is achieved genetically because the expression of the TurboID is restricted to those cells expressing cre recombinase. Biotinylated and secreted plasma proteins can then be purified directly from blood plasma using streptavidin beads and analyzed by LC-MS/MS using data-independent acquisition with DIA-only chromatogram libraries generated through gas-phase fractionation.

Results

In total across all samples (N = 3 mice/condition x 2 conditions x 21 genotypes), we detect 1,272 unique cell type-protein pairs with ≥ 2 peptides detected in all 3 replicates of at least one condition. Exercise significantly altered 256 cell type-protein pairs (20.1% of the entire dataset, adjusted P-value < 0.05). We identify a gradient of secretome responses across cell types, with secretomes from Pdgfra+ being one of the most exercise-responsive in the entire dataset. Peptide-level correlation analysis uncovers exercise regulation of cell type-specific secreted proteoforms. Finally, we show that exercise-inducible, liver-derived CES2 proteins modulate systemic energy metabolism and suppress obesity in high fat diet-fed mouse models.

Conclusions

Together, our studies map exercise-regulated cell types and secreted proteins and illuminate the dynamic remodeling of cell and tissue crosstalk by physical activity.

CS12.03: Identifying plasma predictors of cardiac remodeling after myocardial infarction using proteomics

Chalise U[1,2], Becirovic-Agic M[1,2], Rodriguez-Paar J[1,2], Konfrst S[1,2], de Morais S[1,2], Johnson C[1], Flynn E[3], Hall M[3], Anderson D[1], Cook L[1], DeLeon-Pennell K[4,5], Lindsey M[6,7]
[1]University of Nebraska Medical Center, [2]Nebraska-Western Iowa Health Care System, [3]University of Mississippi Medical Center, [4]Medical University of South Carolina, [5]Ralph H. Johnson Veterans Affairs Medical Center, [6]Meharry Medical College, [7]Nashville VA Medical Center

Introduction: Loss of cardiomyocytes after myocardial infarction (MI) induces cardiac remodeling causing infarct wall thinning and dilation.
Methods: We used three targeted proteomic platforms that combined measured 165 unique proteins to identify plasma proteins that mirror cardiac remodeling across the MI time course. We retrospectively interrogated the plasma proteome of the day (D)0 control (n=16, 8M/8F) and D3 MI (n=15, 4M/11F) from adult C57/BL6 mice. We correlated plasma protein expression with cardiac physiology variables (dimensions and volumes at systole and diastole, infarct wall thickness, and ejection fraction) measured by echocardiography. Prospectively in a validation cohort of mice (n=20, 9M/11F), we tested the hypothesis that the plasma candidates identified in the D3 retrospective cohort mirror cardiac physiology at an extended timepoint of MI (D7). Further, we examined a human plasma glycoprotein array dataset from healthy controls (n=18, 4M/14F) and patients 48h after the presentation for MI (n=41, 23M/18F) to evaluate the enrichment of signaling pathways and transcription factors associated with the identified proteins.
Results: Retrospectively, we identified 5 plasma markers as strong predictors ($r \geq 0.60$ and $p < 0.05$) of adverse cardiac remodeling at MI D3 (Apolipoprotein A1 (ApoA1), Haptoglobin, Immunoglobulin A (IgA), Interleukin (IL)-17E, and Tissue Inhibitor of Metalloproteinase-1 (TIMP-1)). Prospectively, in the validation cohort, ApoA1, IgA, IL-17E, and TIMP-1 mirrored cardiac remodeling at D7, indicating a linear response over MI time. Haptoglobin was a time-specific marker, showing elevated levels in the retrospective D3 dataset and reduced levels in the validation D7 cohort. From the human glycoproteomics protein-protein interaction analysis, ApoA1, IL-17E, Haptoglobin, and TIMP-1 all associated with cytokine-cytokine receptor signaling as the most enriched KEGG pathway.
Conclusion: We identified four plasma proteins (ApoA1, IgA, IL-17E, and TIMP-1) involved in MI wound repair that mirror current and predict future adverse cardiac remodeling.

CS12.04: Precise phenotyping on the dynamic architecture of the circulating proteome

Bendes A[1,2], Dahl L[1,2,3], Dodig-Crnkovic T[1,2], Dale M[1,2], Sahi M[1,2], Bauer S[1,3], Roxhed N[1], Fredolini C[1,2], Schwenk J[1,2]
[1]KTH Royal Institute of Technology, [2]Science for Life Laboratory, [3]Wallenberg AI, Autonomous Systems And Software Program

Recent advances in technologies have enabled us to study the proteins circulating in human blood at a yet unprecedented depth and precision. Together with the availability and growth in complementary data, we are now in an era to further unlock the potential of plasma proteomics and investigate its constituents in detail and at scale.

The presentation will touch upon our insights and lessons learned from large-scaled explorations of circulating proteomes aiming to characterize states of human health and disease. Engaging in a variety of large-scale disease efforts, profiling 100s of proteins circulating in 1000s of samples has become increasingly feasible. This includes studies on regular plasma samples from clinics or biobanks, as well as microsampling or dried blood spots collected in the general population. Over the last decade, we continuously developed validation schemes for affinity proteomics efforts and started to include a variety of data types to create knowledge out of our observations. This includes using genetic data to infer protein binding and regulation.

A remaining challenge is to better account for the variety of pre-analytical, biological, and clinical parameters affecting the dynamic architectures of the circulating proteomes. Besides using proteomics data in larger multi-omics efforts, we will present findings from longitudinal analyses of person-specific proteomes, profiling of self-sampling blood, and the implementation of emerging data-driven analysis strategies. The latter has allowed us to obtain new perspectives useful for patient stratification in the context of health monitoring, disease progression, or treatment response.

CS12.05: Discovery of specific molecular signatures useful for patients stratification in a cohort of osteoarthritis patients with associated comorbidities.

Calamia V[1], Fernandez Puente P[2], Quaranta P[1], Lourido L[1], Paz Gonzalez R[1], Ruiz Romero C[3], Blanco Garcia F[4]
[1]INIBIC - FPNS, [2]CICA - UDC, [3]INIBIC - SERGAS, [4]UDC - SERGAS
Introduction: Comorbidities in osteoarthritis (OA) determine the appearance of distinct clinical phenotypes associated with specific molecular features or OA endotypes. To date, these phenotypes and their constituent molecular endotypes have not yet been clearly characterized. The main objective of the present study is to discover specific molecular signatures useful for patients' stratification in a cohort of OA patients with associated comorbidities.
Methodology: We have analyzed a cohort of 4010 patients composed by 5 sub-cohorts: Osteoarthritis, Dyslipidemia, Diabetes, HIV and Cardiovascular disease cohorts. Finally, 35 OA plasma samples from 7 patients of each cohort were selected. 10uL of each sample were depleted with immunoaffinity spin columns. Ten µg of the depleted samples were digested with trypsin. The digested peptides were desalted and the peptide mixtures (200 ng) were loaded in a nano-flow LC coupled to a high-resolution timsTOF Pro (Bruker). Peptides were analyzed in data-independent mode with the Parallel Accumulation–Serial Fragmentation (DIA-PASEF) enabled. MS raw files were processed with PEAKS Studio 10.6 (Bioinformatics Solutions Inc.) for protein identification and quantification.
Results: The joint bioinformatic analysis of the previously collected clinical and genomic data allowed the selection of a group of patients on whom to perform the proteomic study. The shotgun proteomic analysis resulted in the identification of 802 proteins and 578 protein groups (11,757 peptides) in the OA patient's plasma. Thirty-four proteins were significantly altered (fold change ≥1.5, p ≤0.05) when comparing OA patients from the five sub-cohorts. Most of them were related with complement and coagulation cascades. Other modulated proteins were extracellular matrix structural constituent.
Conclusions: The protein profile heatmap revealed the presence of different endotypes among the OA patients of our cohort, strictly related with their comorbidities. We are now developing a Big Data platform for further prediction of the occurrence of OA phenotypes using machine learning techniques.

CS13.02: Proteomics analysis of the major seed storage proteins across genetically diverse narrow-leafed lupin varieties

Tahmasian A[1,2], Broadbent J[2], Juhász A[1], Nye-Wood M[1], Le T[3], Colgrave M[1,2]
[1]Edith Cowan University, [2]CSIRO Agriculture and Food, [3]Auckland University of Technology

Introduction:
Lupin seeds possess a unique nutritional profile including high protein and fibre content and negligible carbohydrate and fat. Furthermore, several bioactive properties have been ascribed to lupin, including prevention of obesity, hypertension, and type 2 diabetes. Despite its potential as a sustainable source of plant-based protein, lupin seeds are under-utilised as food ingredient and are mainly grown as stockfeed and green manure. One constraint to widespread adoption of lupin in food products is because it is acknowledged as an allergen and is subjected to mandatory labelling on food products in many countries. This highlights the importance of the reliable and robust methods for lupin allergen detection and quantitation, as well as the identification/development of the hypoallergenic lupin varieties.

Methods:
Herein, a combination of discovery and quantitative proteome measurements were employed for evaluation of the four major seed storage protein families (α-, β-, δ-, and γ-conglutins) across a panel of 46 genetically diverse narrow-leafed lupin (NLL) genotypes. The targeted MRM assay developed for this study allowed the differentiation and quantitation of the 16 known conglutin subfamilies.

Results:
This comparative study revealed a larger variability for the β- and δ-conglutin content across the genotypes under study. Notably, the β-conglutin proteins, which are the major allergens from lupin, were substantially reduced within several of the domesticated cultivars, wherein some degree of compensatory elevation of the bioactive γ-conglutin proteins was noted.

Conclusion:
These potential hypoallergenic lupin genotypes are more suited to be cultivated for food purposes or prioritised in breeding programs for development of lupin varieties with optimal nutritional composition. The outcome of this research opens new vistas for further exploitation of the potential of this emerging legume as a complementary plant-based protein source.

CS13.03: Global profiling of dehydration-induced chloroplast dynamics and defense response in chickpea

Chakraborty N[1]
[1]National Institute Of Plant Genome Research

Introduction

Elucidation of molecular basis of dehydration-induced responses aids understanding of crop adaptation and has direct implications towards fortification of sustainable agriculture. Previously, we demonstrated the dehydration-induced alterations in chloroplast proteome and reprogramming of cellular metabolism in developing chickpea. Next, we investigated the dehydration-responsive behaviour of a novel chloroplast protein, CaPDZ1 and interactive partners towards adaptation under water-deficit conditions.

Methods

Three-week-old chickpea seedlings were subjected to progressive dehydration, and integrity and purity of chloroplast fraction was evaluated. Protein identification was carried out at 1% FDR. Gene characterization in the native system was carried out under the control of rd-29 promoter.

Results

The screening of a dehydration-responsive chloroplast proteome of chickpea led us to identify and investigate the functional importance of an uncharacterized protein, designated CaPDZ1. In all, we identified 14 CaPDZs, and phylogenetic analysis revealed that these belong to photosynthetic eukaryotes. The global expression analysis showed that CaPDZs are intimately associated with various abiotic stresses and phytohormone responses. The CaPDZ1-overexpressing chickpea seedlings exhibited distinct phenotypic and molecular responses, particularly increased photosystem efficiency, ETR and qP that validated its participation in PSII complex assembly and/or repair. The investigation of CaPDZ1 interacting proteins through Y2H library screening and co-IP analysis revealed the interacting partners to be PSII associated CP43, CP47, D1, D2 and STN8. These findings supported the earlier hypothesis regarding the role of direct or indirect involvement of PDZ proteins in PS assembly or repair. Moreover, the GUS-promoter analysis demonstrated the preferential expression of CaPDZ1 specifically in photosynthetic tissues. We classified CaPDZ1 as a dehydration-responsive chloroplast intrinsic protein with multi-fold abundance under dehydration stress, which may participate synergistically with other chloroplast proteins in the maintenance of the photosystem.

Conclusions

We identified a dehydration-responsive chloroplastic protein, CaPDZ1 which provides new insights elucidating the stress adaptive role of PDZ domain-containing proteins in plants.

CS13.04: Novel antimicrobial peptide families identified in Capsicum plants via in silico prediction and targeted peptidomics

Culver K[1], Hicks L[1]
[1]University Of North Carolina At Chapel Hill

Introduction: Antimicrobial peptides (AMPs), host defense peptides expressed by all forms of life, have promising antibacterial properties which can potentially help circumvent the rise of antimicrobial resistance through novel therapeutics. We recently discovered two novel AMPs within ghost pepper (Capsicum chinense x frutescens), CC-AMP1 and CC-AMP2, with activity against clinically relevant pathogens. These peptides each contain novel cysteine motifs which preclude them from classification into known plant AMP families. In silico AMP predictions applied to all UniProt Solanaceae proteins indicated likely AMPs with the same cysteine motifs expressed across related species, suggesting that these belong to two previously unidentified AMP families.

Methods: Aleppo pepper plants (Capsicum annuum) and Criolla Sella pepper plants (Capsicum baccatum) were grown and leaf tissue was harvested. Plant material was subjected to an optimized peptidome extraction using 10% acetic acid followed by filtration and cleanup steps. Extracts were fractionated with reversed-phase LC and the fractions were assayed for antibacterial activity against E. coli ATCC 25922.

Results: C. annuum and C. baccatum fractions which displayed antimicrobial activity were subjected to LC-MS/MS analysis and the masses correlating to the observed bioactivity were identified. These peptides were targeted for both top-down and bottom-up peptidomics analyses and their full sequences were determined. One CC-AMP1-like peptide and one CC-AMP2-like peptide was identified in C. annuum, while four CC-AMP1-like peptides and three CC-AMP2-like peptides were identified in C. baccatum.

Conclusions: Five new CC-AMP1-like peptides and four new CC-AMP2-like peptides were identified in Capsicum plants, with their sequences fully characterized via top-down and bottom-up peptidomics. The proliferation of their associated novel cysteine motifs across Solanaceae suggest that these peptides belong to two previously unidentified AMP families. Future work will involve biological characterization in order to assess and compare their therapeutic potential.

CS14.02: An Update on the HPP Pathology Pillar: Transforming Theranostics for the Benefit of Patients

Nice E[2], Roehrl M[1]
[1]Memorial Sloan Kettering Cancer Center, [2]Monash University

Introduction

Current diagnostics in medicine, especially oncology, remains focused on detecting genomic and transcriptomic alterations at the nucleic acid level. Recognizing that virtually all precision medicine drugs today interact directly with proteins in a patient, the HPP Pathology Pillar was created to support the development of a new, proteome-based diagnostic paradigm.

Methods

The HPP Pathology Pillar uses outreach and teaching programs to engage the broader clinical medical community (pathologists, oncologists, surgeons, etc.) and to introduce the concepts, tools, and opportunities for proteomics in clinical medicine. In addition, the HPP Pathology Pillar has created an international venue for identifying and addressing high-impact clinical needs (such as risk stratification of cancers or companion diagnostic development).

Results

Our presentation will update the HUPO/HPP community on progress in outreach (with examples from Australia, the US, and Europe). We will also illustrate specific examples of proteome-based diagnostics from medical centers that serve as prototypes for the impending “Proteome-Based Precision Medicine” (PBPM) revolution.

Conclusions

Establishing proteomics as a novel and powerful tool in clinical medicine is only at the beginning, and the HPP Pathology Pillar has been designed as a conduit to accelerate this development internationally. Pathologists, other physicians, and healthcare providers all need to become familiar with, and trained in, the application of proteome-based diagnostics. We predict that new drug development, clinical trials, and companion diagnostics will be rapidly transformed by the implementation of proteome-based diagnostics and therapy response monitoring.

CS14.03: A global, multi-project initiative to identify and annotate non-canonical human translated sequences.

Mudge J[1], Ruiz-Orera J[2], Prensner J[3], Deutsch E[4], Jungreis I[3], Frankish A[1], Moritz R[4], van Heesch S[5]
[1]EMBL-EBI, [2]Max Delbrück Center for Molecular Medicine in the Helmholtz Association , [3]Broad Institute of MIT and Harvard, [4]Institute for Systems Biology, [5]Princess Máxima Center for Pediatric Oncology

Introduction. Against the backdrop of ongoing Human Proteome Organisation (HUPO) Human Proteome Project (HPP) efforts to identify, validate and classify human protein-coding genes, it has become clear that extensive translation occurs outside of 'canonical' reference protein and gene annotation catalogs. Especially, hundreds of 'non-canonical' translations have been captured by Ribosome Profiling / Ribo-seq, which identifies translated ORFs by sequencing RNA undergoing ribosomal processing. Ribo-seq can help identify missing protein-coding genes, especially those hard to identify in annotation workflows; e.g. microproteins and evolutionarily young proteins. However, as Ribo-seq does not confirm protein existence, research efforts typically integrate mass spectrometry methods, including more recently the interrogation of immunopeptidomics datasets. While such work has the power to transform our understanding of translation - with significant clinical implications - questions on the technical utility and biological interpretation of such data have limited their incorporation into reference annotation projects.
Methods. Our HUPO-HPP endorsed project1 unites 16 laboratories and databases. We are sharing expertise in Ribo-seq, mass spectrometry-based proteomics and immunopeptidomics, molecular evolution, and gene and protein-annotation, in order to drive solutions for the identification, interpretation and standardized annotation of non-canonical translations.
Results. We offer the first publicly available reference annotation catalog of human non-canonical translations, containing 7,264 ORFs identified from published Ribo-seq datasets mapped to Ensembl/GENCODE. To support the deeper functional interpretation of these ORFs, we are developing a stringent analytical framework for proteomics and immunopeptidomics datasets, and in parallel a new evolutionary model for non-canonical ORF evolution.
Conclusions. As our resources are produced within the reference annotation 'ecosystem', this is the point at which non-canonical translation enters the mainstream of genomic and medical science. Nonetheless, these are the early steps of a long-term, multi-faceted project, and we are keen for other groups to join our efforts.

1. Mudge, JM et al, Nat Biotech, 2022

CS15.02: iMetaLab Suite: A one-stop toolset for metaproteomics

Ning Z[1], Li L, Cheng K, Zhang X, Simopoulos C, Figeys D
[1]University Of Ottawa

Introduction

Metaproteomics is a recently thriving technique that studies the collection of proteins in complex microbiomes of the human, animal, plant, and environment. The bioinformatics workflow required for metaproteomics research, from the database search and protein quantification to downstream functional and taxonomic analysis has been challenging and thus limiting the accessibility of metaproteomics to microbiome researchers.

Methods

To overcome these challenges, we have developed a set of tools named iMetaLab Suite. iMetaLab Suite includes the following components: (1) MetaLab Desktop, an automated database search software that facilities proteins identification, quantitation taxon and function analysis from microbiomes; (2) the automated iMetaReport that allows users to quickly access database search results and data set profiles; and (3) an interactive online toolset, iMetaShiny, covering most frequently used functional, taxonomic, and statistical analysis in metaproteomics.

Results

iMetaLab Suite is free, easily accessible, and under actively development, available to assist researchers to explore metaproteomic data.

Conclusions

With iMetaLab Suite, we aim to maximize the accessibility of metaproteomic bioinformatics workflow to scientists with all levels of bioinformatics expertise in the field of microbiome research, as well as those in conventional proteomics/systems biology. We are actively developing novel database search workflows and strategies, as well as more statistical approaches for downstream functional, taxonomic, and ecological analysis of the metaproteomics data. These will be actively updated into the iMetaLab Suite and we welcome feedback and suggestions from users to improve the user experience and performance of the tools.

CS15.03: Mistle: Metaproteomic index and spectral library search engine

Nowatzky Y[1], Benner P[1], Reinert K[2], Muth T[1]
[1]Bundesanstalt für Materialforschung und -prüfung (BAM), [2]Free University of Berlin

Introduction:
With the introduction of accurate deep learning predictors, spectral matching applications might experience a renaissance in tandem mass spectrometry (MS/MS) driven proteomics. Deep learning models, e.g., Prosit, predict complete MS/MS spectra from peptide sequences and give the unprecedented ability to accurately predict mass spectra that may arise from any given proteome. However, the amount of spectral data is enormous when querying large search spaces, e.g., metaproteomes composed of many different species.
Current spectral library search software, such as SpectraST, is not equipped to meet run time and memory constraints imposed by such large MS/MS databases, covering several millions of peptide spectrum predictions.

Methods:
Inspired by the fragment index data structure that had been introduced with MSFragger, we implement an efficient peak matching algorithm for computing spectral similarity between query and library spectra. Mistle (Metaproteomic index and spectral library search engine) uses index partitioning and SIMD (Single instruction, multiple data) intrinsics, which greatly improves speed and memory efficiency for searching large spectral libraries. Mistle is written in C++20 and highly parallelized.

Results:
We demonstrate the efficiency of Mistle on two predicted spectral libraries for the lab-assembled microbial communities 9MM and SIHUMIx. Compared to the spectral library search engine SpectraST, Mistle shows a >10-fold runtime improvement and is also faster than msSLASH, which uses locality-sensitive hashing. Although Mistle is slower than MSFragger, Mistle‘s memory footprint is an order of magnitude smaller. Furthermore, we find evidence that the spectral matching approach to predicted libraries identifies peptides with higher precision. Mistle detects peptides not found by database search via MSFragger and in turn uncovers unnoticed false discoveries among their matches.

Conclusion:
In this study, we show that predicted spectral libraries can enhance peptide identification for metaproteomics. Mistle provides the means to efficiently search large-scale spectral libraries, highlighted for the microbiota 9MM and SIHUMIx.

CS15.04: A Bioinformatic Workflow for Metaproteomic Analysis of Host-Microbe Dynamics in Clinical Samples

Jagtap P[1], Kruk M[1], Bihani S[2], Mehta S[1], Johnson J[1], Wagner R[1], Laguna T[3], Srivastava S[2], Griffin T[1]
[1]University Of Minnesota, [2]Indian Institute of Technology, [3]Ann & Robert H. Lurie Children's Hospital of Chicago

Introduction: Clinical metaproteomics has the potential to offer insights into host-microbiome interactions. However, the field faces challenges in the detection of low-abundance microbial proteins. As a solution, we have developed an integrated workflow coupling deep mass spectrometry-based analysis, with customized bioinformatic processing of microbial proteins. We have utilized this workflow in ongoing projects to identify microbial peptide panels for a) cystic fibrosis (CF) disease progression studies; and b) co-infection status during COVID-19 pandemic waves.

Methods: The bioinformatics workflow uses software tools within the Galaxy platform such as MetaNovo (to generate a reduced protein database), SearchGUI/PeptideShaker, MaxQuant and FragPipe (to generate peptide-spectral matches (PSMs) and quantitation), PepQuery (to verify the quality of PSMs), and Unipept and BLAST-P (for taxonomy and functional annotation).

Results: In the CF study, broncho-alveolar samples from pediatric CF and disease control patients were characterized for their microbial diversity. Rigorous taxonomic, functional and quantitative analysis of verified microbial peptides generated a peptide panel of 87 microbial peptides. This includes 24 taxonomy-specific peptides; 20 peptides from three protein cluster groups, and the rest with ambiguous taxonomy but known functions. This analysis also detected human peptides from CF-enriched proteins. We will analyze this microbial and human peptide panel, using targeted analysis to track host-microbe protein dynamics during CF progression.
In another study, nasopharyngeal swabs from SARS-CoV-2 infected patients from a hospital in India were analyzed to identify microbial peptides corresponding to potential co-infecting microorganisms during the pandemic waves (July 2020 and May 2021). The study detected several peptides belonging to opportunistic bacterial and fungal pathogens. We will present a quantitative analysis of the expression of these microbial proteins and their potential as co-infecting pathogens during the pandemic waves.

Conclusion: We have developed a bioinformatics workflow to generate well-characterized peptide panels for targeted investigation of host-microbe dynamics in disease.

CS16.02: Myddosome dynamics during the innate immune response

Gillen J[1], Bridgwater C[1], Tucholski T[1], Nita-Lazar A[1]
[1]Functional Cellular Networks Section, Laboratory of Immune system Biology, NIAID, NIH

Introduction: Critical for the innate immune response to pathogen-associated molecular patterns (PAMPs), the myddosome is a complex of proteins characterized by the presence of myeloid differentiation factor 88 (Myd88). The myddosome transfers signals from the toll-like receptors (TLRs) to TNF receptor-associated factor 6 (TRAF6). In addition to Myd88, the myddosome contains multiple copies of the interleukin-1 receptor associated kinases (IRAK) 2 and 4 and is theorized to form following stimulation of the TLR proteins.

Methods: We used affinity purification – mass spectrometry analysis (AP-MS) in coordination with stable isotope labeling by amino acids in cell culture (SILAC) to identify Myd88-associated proteins in mouse immortalized bone marrow-derived macrophages during the response to lipopolysaccharide (LPS) treatment. Because of the changes in the Myd88 interactome during the response to LPS, we examined mechanisms regulating the activity of the myddosome. We used proteasome inhibitors to determine proteasome dependence of the regulatory interactions. We evaluated the role of phosphorylation on myddosome activity. We examined the changes in myddosome-associated protein localization during TLR4 stimulation.

Results: Prior to LPS treatment, we found the stable association of Myd88 to several downstream effector proteins, (e. g. IRAK4, TRAF6). Following LPS treatment, Myd88 associated with additional downstream effector proteins, (IRAK1, IRAK2) and inhibitory proteins (IRAK3, Tnip1). In addition, RNA regulatory proteins (Dicer1, Eif2ak2), were found to associate with Myd88 throughout the LPS-response. Some of the associations (e.g. Myd88-IRAK4) were proteasome-dependent. We found that MyD88 and IRAKs changed their cellular localization following TLR4 stimulation.

Conclusions: The pre-formed myddosome exists prior to TLR stimulation. The signaling cascade activation is achieved through the removal of inhibitors and assembly of downstream activators and regulated by proteasome-dependent and -independent mechanisms, including PTM and protein localization changes.

This research was supported by the Intramural Research Program of the NIH, National Institute of Allergy and Infectious Diseases.

CS16.03: Large clostridial toxins reorganize the proteome and phosphoproteome of target cells

Pich A[1], Schweitzer T[1], Stieglitz F[1], Wulf A[1], Gerhard R[1], Genth H[1]
[1]Hannover Medical School

Large clostridial toxins (LCTs) have been identified as primary virulence factors in various animal and human diseases such as C. difficile infection (CDI), wound-associated gas gangrene or toxic shock syndrome. Classification of toxins as LCTs was made basing on a high sequence homology and similar structural set up as well as their ability to induce pronounced changes in cell morphology. The group of LCTs include toxins TcdA and TcdB from Clostridioides difficile, TcsL and TcsH from Paeniclostridium sordellii, TcnA from Clostridium novyi and Clostridium perfringens' TpeL.
Proteome and phosphoproteome responses of C. difficile toxins and C. novyi's TcnA were analyzed in cell culture to elucidate unknown functions of these pathogens and their toxins. A combinational approach of biochemical analysis and different MS techniques were used to investigate effects of TcdA, TcdB, CDT and TcnA on target cells in vitro. Cell-cell junction, cytoskeleton organization and cell death were found to be toxin-responsive. Toxic effects of TcdA could be traced back to its glucosyltransferase activity while in case of TcdB a catalytically inactive variant also altered the proteome of target cells and induced early cell death. By infection of mice with C. difficile spores various biological processes related to immune response were identified as enriched while metabolic processes were found to be down-regulated.
Utilizing various MS techniques it was possible to illuminate so far unknown effects of C. difficile and its toxins as well as C. novyi's TcnA on cellular processes of the host.

CS16.04: Phosphoproteomic Evaluation of Mycobacterial PknG Inhibition during Alveolar-like Macrophage Infection

Baros-Steyl S[1], Ganief T[1,2], Iradukunda J[1], Schell D[1], Soares N[3], Blackburn J[1,2]
[1]University Of Cape Town, [2]Institute of Infectious Disease and Molecular Medicine, [3]University of Sharjah

Introduction:
Pathogenic mycobacteria, such as Mycobacterium tuberculosis, modulate the host immune system to evade clearance and promote long-term persistence, resulting in disease progression or latent infection. The early interactions between M. tuberculosis and the host innate immune system largely determine the establishment of tuberculosis infection and disease development. Protein kinase G (PknG) in pathogenic mycobacteria has been shown to play an important role in avoiding clearance by macrophages by blocking phagosome-lysosome fusion; however, the exact mechanism is not completely understood. Inhibition of PknG results in mycobacterial transfer to lysosomes and cell death. A better understanding of the signalling dynamics within immune cells together with the specific mechanisms that PknG manipulates to contribute to the pathogenesis of tuberculosis may reveal potential host-directed therapeutic targets for promoting clearance of M. tuberculosis and limiting its survival in vivo. However, further development of this new strategy is limited by the lack of detailed knowledge on the mechanisms of action of PknG during infection and the identity of its host targets.

Methods:
Here, to further investigate the role of mycobacterial PknG during early macrophage infection, alveolar-like macrophages were infected with M. bovis BCG followed by treatment with a PknG-specific inhibitor. After proteolysis and digestion, phosphopeptides were enriched using Zr-IMAC HP magnetic microparticles and subjected to LC-MS/MS to identify differentially phosphorylated peptides between the untreated and treated infected macrophages.

Results:
A total of 5766 phosphosites on 2174 unique proteins were identified. Following phosphoproteome normalisation and differential expression analysis, a total of 77 phosphosites were differentially phosphorylated in the treated versus untreated infected macrophages. A subset of 49 phosphosites was differentially up-regulated in the presence of PknG activity.

Conclusion:
Functional analysis of our data revealed that PknG kinase activity reprograms normal macrophage function through interfering with host cytoskeletal organisation, lysosomal cholesterol metabolism, and programmed cell death.

test

Moir J[1]
[1]Ics Events
test

CS17.03: Unravelling tissue-specific protein patterns using machine learning and public data

Claeys T[1], Menu M, Gevaert K[1], Martens L[1]
[1]VIB - UGhent

Introduction

The first step towards thorough knowledge on the proteomic behaviour of a tissue in diseased state, is the understanding of its proteomic composition in healthy circumstances. Possible downstream information such as biomarker and tissue leakage protein identification, and drug targets depend on the knowledge of healthy tissue-specific protein expression patterns. Using a myriad of experiments, from available public data, we trained a machine learning model to unravel these complex protein patterns on both tissue and cell type level.

Methods

A total of 183 PRIDE projects were searched with ionbot and tissue annotation was manually added. The data was annotated on the level of 68 tissues, 99 cell types and disease status. Healthy data were randomly split into 85 and 15 percent for train and test set, respectively which was used to train a RandomForest model on protein abundances to classify samples in tissues and cell types. Subsequently, one-vs-all classification and the feature importances as SHAP- and f-scores are used to analyse the most important protein abundances.

Results

With solely protein abundance, the model was able to predict tissues with 98 percent accuracy and cell types with 97 percent accuracy. We identified approximately 5 000 proteins crucial for classification. The SHAP- and f-scores revealed tissue-specific proteins and relevant biological insights. Application of the model onto diseased and cancerous samples show a drop in accuracy to approximately 67 percent.

Conclusions

Public proteomics data and state-of-the-art machine learning algorithms allowed for highly accurate classification models for tissues and cell types. Furthermore, the models allowed for revealing the protein expression patterns of these classes. Future research will include post-translational modification data thus allowing even higher accuracy and understanding of modification patterns. Moreover, further in depth analysis of the output from this model might lead to new biological insights.

CS17.04: The coding potential of pseudogenes: between relics and new beginnings

Brunet M[1], Lapointe X[1], Leblanc S[1]
[1]University Of Sherbrooke

Introduction: Recent advances have revealed pervasive translation throughout the genome. These novel sequences are found overlapping canonical open reading frame (ORF) or in non-coding regions, such as pseudogenes. Pseudogenes are thought defective copies of protein-coding genes. They are identified as any genomic sequence similar to another but that is defective and often without introns. However, these criteria are ill-advised to predict the functionality of a gene or absence thereof.

Methods: We developed the first proteogenomics resource endorsing a multi-coding annotation of eukaryotic genomes, OpenProt. OpenProt cumulates experimental evidence for all predicted ORF from an exhaustive transcriptome. Using OpenProt, machine-learning and multi-omics, we evaluated the coding nature of human pseudogenes.

Results: Large-scale mining of omics datasets highlighted 4,478 novel ORF in the human genome detected in at least 3 independent Ribo-seq datasets or with at least 2 unique peptides within at least 2 independent proteomics datasets. Within GAPDH affinity purification datasets, we identified unique peptides for 9 proteins from different pseudogenes of the GAPDH family. Spectra were validated using synthetic peptides, and AlphaFold2 simulations further supported the interaction with the parental GAPDH. Out of 15,152 pseudogenes in human, 3,448 lacked any ORF but 1,717 presented robust experimental evidence. We built an ensemble learning algorithm, CoP3E, to predict the coding potential of human pseudogenes. With a 90% accuracy, our model largely outperforms existing ones and identified 228 pseudogenes as coding with a confidence over 80%. From these, 140 were identified with at least 2 unique peptides within functional protein complexes using MS2rescore. Interestingly, 30% of pseudogene-encoded proteins have no significant homology with the parental protein, which supports novel gene birth from pseudogenes.

Conclusions: As pseudogenes are deemed non-functional, they are excluded from protein databases. Our results highlight the overlooked coding nature of pseudogenes, their role in cellular mechanisms and gene evolution.

CS17.05: MSReact: A Novel Software Solution Facilitating the Development of Intelligent Data Acquisition Methods Through Real-Time Control of MS Instruments

Udvardy Z[1], Wilhelm M[2], Burlet-Schiltz O[1], Bouyssié D[1]
[1]Institut de Pharmacologie et de Biologie Structurale, Université de Toulouse, CNRS, [2]TUM School of Life Sciences, Technical University of Munich

Introduction: The analysis of proteomics samples can benefit from using sophisticated, intelligent and customised MS acquisition methods. The ability to control MS instruments in real-time is key in creating such methods [1,2]. Developing software tools directly interfacing with instruments requires a lot of knowledge and effort, but there are no open-source solutions aiding this process. We propose MSReact, an easy to use framework for the creation of intelligent acquisition methods.

Methods: MSReact has two components, a .NET-based server and a Python-based client communicating through a websocket interface. The server is responsible for the direct control of the MS instruments using the Thermo Instrument Application Programming Interface (IAPI). The client provides a framework as a Python package to create novel acquisition methods facilitating the analysis of incoming scans and, in response, triggering custom scans in real-time.

Results: Using the Python language on the client side unlocks the possibility to use third party scientific and data analysis libraries (NumPy, SciPy), as well as the most popular machine learning frameworks (TensorFlow, PyTorch) for real-time data analysis and decision making. Furthermore, it promotes the integration with other Python-based MS analytical tools such as Prosit, pyOpenMS and DeepLC.
The server currently supports the control of two different MS instrument families of Thermo Scientific (Exactive, Tribrid), and also provides a simulation mode, aiding the testing of the newly developed acquisition methods. The software is used to implement an advanced detection algorithm of crosslinked peptides and an intelligent two-stage PRM acquisition method.

Conclusion: MSReact provides an easy way to define and create novel intelligent MS acquisition methods. It constitutes a useful open-source framework for software developers, benefitting from its client-server architecture and built-in simulator.

References:
1. Wichmann, C. et al., MCP, 2019
2. Erickson, B. K. et al., JPR, 2019

CS17.06: Tripartite graph modeling enables comprehensive protein isoform characterization in shotgun proteomics

Dou Y[1], Yi X[1], Olsen L[1], Zhang B[1]
[1]Lester and Sue Smith Breast Center; Department of Molecular and Human Genetics, Baylor College Of Medicine

Introduction: Most peptides detected in mass spectrometry (MS)-based shotgun proteomics map to multiple protein isoforms, which complicates protein inference and quantification. The current best practice is to collapse proteins with the same or subset of supporting peptides into a minimal list of protein groups, and for quantitative rollup, peptides shared by multiple proteins are assigned only to the ones with the most identification evidence. Although practically useful, this parsimonious approach greatly limits the potential for protein isoform characterization. Here, we present a tripartite graph modeling (TGM) approach to address this limitation.

Methods: A tripartite graph was built with three vertices sets representing all peptides identified in a study, proteins the peptides can be mapped to, and host genes of the proteins, respectively. The vertices were connected by edges indicating their mapping relationships. Peptides that are connected to exactly the same set of protein vertices were defined as a structurally equivalent peptide group (SEPG). All identified SEPGs passing SEPG level false discovery rate (FDR) cutoff of 1% were quantified by rolling up the abundance of their associated peptides and then classified into five classes based on their patterns of connections to source proteins and genes in the tripartite graph for reporting.

Results: Applying the TGM method to an iPSC cell line dataset identified more than 100 genes with isoform-level regulation during cardiomyocyte differentiation. Applying the method to two human liver tumor datasets identified hundreds of protein isoform level regulatory events with significant associations to tumor development and prognosis. Most of these findings could not have been obtained using the parsimonious approach.

Conclusions: Using SEPGs instead of parsimonious protein groups as the reporting and quantification units, the TGM method maximizes protein isoform information that can be extracted from shotgun proteomics data to boost discoveries in biological and translational research.

CS17.07: MSBooster: Flexible PSM Rescoring with Deep Learning in FragPipe

Yang K[1], Yu F[2], Teo G[2], Demichev V[3,4,5], Nesvizhskii A[1,2]
[1]University Of Michigan, Department of Computational Medicine and Bioinformatics, [2]University Of Michigan, Department of Pathology, [3]Department of Biochemistry, Charité – Universitätsmedizin Berlin, [4]Molecular Biology of Metabolism Laboratory, The Francis Crick Institute, [5]Department of Biochemistry and Milner Therapeutics Institute, University of Cambridge

Introduction

Accurate peptide-spectrum matches (PSMs) are essential for peptide and protein-level identification and quantification. In order to increase the number of PSMs at a set false discovery rate (FDR), data analysis pipelines have turned to incorporating machine learning-based predictions of MS/MS spectra and retention times to provide additional information on top of the database search score for determining the validity of PSMs. Feature scores can quantify the similarity between the observed and predicted MS2 spectra and retention times (RT). Such PSM rescoring can be adapted to various types of experimental setups.

Methods

MSFragger searches the data. MSBooster extracts only proposed peptides (not all peptides from in-silico proteome digestion) and formats them into an input file for a deep learning model. MSBooster calculates feature scores based on observed-predicted similarity. Percolator rescores PSMs. Philosopher performs protein inference and FDR control.

Results

MSBooster provides significant gains in peptide/protein identifications for HLA/DIA/single cell/TMT data. It supports spectral and RT predictions from various models. PredFull provides further gains in HLA rescoring with non-y/b fragment ions. Retraining DIA-NN to predict TMT spectra and RT improves upon previous versions of FragPipe with PSM rescoring based on models trained on unlabelled peptides. Use of pDeep3 improves phosphopeptide identifications using neutral loss fragments, with DeepLC providing more accurate RTs. We also present the first results of rescoring of ETD data using PredFull predictions.

Conclusions

PSM rescoring with deep-learning libraries boosts identifications across a myriad of experimental workflows. While previous rescoring pipelines only considered y and b ions, we highlight the potential improvements by using other fragment ion types. For next steps, we hope to extend MSBooster to rescoring PSMs from large search spaces, namely metaproteomics and proteogenomics data. MSBooster is conveniently packaged within FragPipe, or as a standalone Java tool with increased functionality.

CS18.03: Development of integrated strategies for streamlined single-cell and nanoscale phosphoproteomics analysis

Gebreyesus S[1], Ali A[1], Muneer G[1], Chen E, Chen C[1], Chen Y[1], Tu H[1]
[1]Institute Of Chemistry, Academia Sinica

Introduction

Proteomics profiling has become a key tool for dissecting cellular and molecular network of biological systems. Analyzing proteome and its functional forms, such as phosphoproteome, from low-input cells down to a single-cell is critical to advance our understanding of cellular heterogeneity especially for mass-limited samples. Yet such molecular profiling requires ultrahigh sensitivity and its development is still limited. Here, we report integrated workflows combining miniaturized devices and data-independent acquisition mass spectrometry (DIA-MS) for single-cell proteomics (SCP) and microscale phosphoproteomics down to 10 cells.

Methods

We developed microfluidic devices coupled with DIA-MS for sensitive SCP and phosphoproteomics from low-input samples. These chips are designed as a one-stop station, offering features including cell capture and lysis, protein digestion and peptide cleanup. This device was implemented for phosphoproteomic processing by incorporating an enrichment module. By constructing sample size-compatible library, DIA-MS is used to offer improved proteomic and phosphopeptide coverage.

Results

Using this workflow, ~1500 and ~450 protein groups were robustly identified from a single lung cancer and leukemia cell, respectively. Furthermore, the results revealed decent proteome coverage, good quantification range and reproducibility with low missing values. Meanwhile, phosphoproteomics analysis showed ~15, 400 and 3500 phosphopeptides could be identified from 10, 100 and 1000 cells with 1% FDR. Importantly, the results demonstrated good mapping coverage in lung cancer and B-cell receptor pathways, and decent linearity on the quantitation of biomarkers at the 1-100 cells level, suggesting potential application of this strategy for basic research and translational applications.

Conclusion

The Chip-DIA based approach is versatile and can be implemented to post-translational modifications or to accommodate additional microfluidics functionality to achieve biochemical analysis under desired context. This strategy is anticipated to be particular useful for mass-limited samples, and shall motivate research effort for developing miniaturized platforms featuring sensitive and functional SCP profiling.

CS18.04: High-throughput platform for automatic single cell proteomics sample preparation

Matsumoto C[1], Bogosavljevic M[1], Gao Y[1]
[1]University Of Illinois At Chicago

Introduction

One of the major challenges in single-cell proteomics is the sample loss during sample preparation and the relative low-throughput of the process. Here we present a novel platform that utilizes a containerless method to automatically process single-cell samples in a high-throughput fashion. In our testing, our prototype system is able to achieve 300 samples per day with improved sensitivity compared to the existing methods.

Methods

Our containerless system eliminates the liquid-solid interface to minimize sample loss due to non-specific binding between the plastic or glass surface and the proteins. The free movement of the cell in the air allows us to manipulate the cell in many different ways, including lysis, reduction, alkylation, digestion, and TMT labeling. When these processes are streamlined, the whole single-cell sample preparation method is fully automated. This highly paralleled method allowed us to process multiple cells at the same time, minimize disturbance to the cells, and therefore improved both sensitivity and throughput of the single-cell sample preparation.

Results

Comparing existing methods such as SCOPE2-MS, we can consistently identify 20-40% more PSMs in our tests. Sample preparation of a single cell can be done fully automatically within 30 minutes. With simple parallelization, we can achieve a throughput of >300 samples per day (less than 5 minutes per sample). For each cell without MBR (match between runs), we are able to consistently identify 500-1000 proteins with TMT labeling and DDA. Our system showed a higher consistency when processing multiple cells (50-100) and larger heterogeneity when processing single cells.

Conclusions

In conclusion, our newly developed system allows us to sample a large number of cells at single-cell resolution with higher efficiency and lower cost.

CS18.05: Hands-free Processing of Single Cells using the CellenONE and the timsTOF SCP in a Label-free Proteomics Approach

Adams C[4], Krisp C[1], Hartlmayr D[2], Seth A[2], Tourniaire G[2], Ledertheil T[1], Greisch J[1], Lubeck M[1], Kruppa G[3]
[1]Bruker Daltonics GmbH & Co. KG, [2]Cellenion, [3]Bruker s.r.o., [4]Bruker Scientific, LLC

Introduction

Single cell proteomics is a rapidly developing field with the potential to make important contributions to the understanding of cellular heterogeneity. Single cell protein extraction, minimal exposure of samples to surfaces and optimal storage and transfer conditions are crucial for loss-less single cell proteome analyses. Recent enhancements in trapped ion mobility spectrometry (TIMS) coupled to fast and sensitive mass spectrometry established in the timsTOF SCP paired with automated single cell sorting and sample preparation realized with the cellenONE® platform allows for sensitive proteome analyses at the single cell level.

Methods

Single HeLa cells were sorted into the label-free proteoCHIP®, directly lysed, and proteins digested at 50°C with high humidity on deck using the cellenONE platform. The label-free proteoCHIP with tryptic peptides was placed into the nanoElute series autosampler, peptides injected onto a 25 cm x 75 µm Aurora C18 column (IonOpticks) and eluted into a timsTOF SCP. dia-PASEF® mode was used and analyzed with TIMS-DIA-NN on PaSER® using a library generated from a deeply fractionated human cell line.

Results

Sample pick-up directly from the label-free proteoCHIP was assessed with HeLa lysate digests (Pierce) showing excellent reproducibility at various concentrations. Injections of 1 ng of HeLa peptides on column (1 µL in well) resulted in 23000 peptides from 3600 proteins which was matched by 1 ng HeLa peptides injected from a vial (1 ng/µL).
We then analyzed single HeLa cells which were directly sorted and prepared in the label-free proteoCHIP and identified in average more than 2000 proteins per single cell. Quantitative comparison of the single cells demonstrated good reproducibility with some variations depended on cell size, elongation, and cell cycle state.

Conclusion

A fully hands-free and label-free analysis workflow reproducibly identifys >2000 proteins from single HeLa cells, using the CellenONE platform with the lable-free proteoCHIP and the timsTOF SCP.

CS18.06: Proteome asymmetry in mouse and human embryos before fate specification

Petelski A[1], Iwamoto-Stohl L[3], Meglicki M[3], Zernicka M[3,4], Slavov N[1,2]
[1]Northeastern University, [2]Barnett Institute, [3]University of Cambridge, [4]California Institute of Technology

Introduction: While asymmetry in early embryonic development has been extensively characterized in non-mammalian species such as the well-known Bicoid gradient in Drosophila, such pre-patterning was thought not to exist in very early mammalian embryos. However, the potential for early functional specialization in mouse and human embryos has been suggested through reports of distinct developmental fates starting at the 2-cell stage and heterogeneous abundance of certain mRNAs. Nevertheless, the extent of the earliest intra-embryo differences remains unclear and controversial.
Methods: By utilizing multiplexed (SCoPE2) and label-free single-cell proteomics by mass-spectrometry, we were able to investigate the potential of sister cell asymmetry on a global proteome scale. The developmental stages studied in mouse were early 2-cell, late 2-cell, and 4-cell stages, for a total of 57 embryos. We additionally studied 13 human 2-cell stage embryos.
Results: We show that sister cells as early as the 2-cell stage of murine development cluster into 2 groups. From these two groups, we determined that hundreds of proteins are consistently differential between sisters, with enrichment for protein transport and protein degradation. Because of these striking differences, we termed the 2 groups as alpha and beta cells. This asymmetric proteome signature extends to the 4-cell mouse embryos. When we additionally processed human 2-cell embryos and split mouse zygotes into two pieces, we also found asymmetric protein abundance recapitulating alpha and beta blastomeres.
Conclusions: We have demonstrated evidence for consistent differential proteome at the very first developmental stages in mammalian embryos. The asymmetric protein abundance between alpha and beta blastomeres that is found in early 2-cell, late 2-cell, and 4-cell mouse embryos is recapitulated in the zygote, suggesting that proteome localization is differentia at the zygote stage. Human 2-cell embryos also exhibit biclustering behavior between sisters, sharing proteome concordance with the alpha-beta clusters in mouse.

CS19.03: Chemoproteomic target deconvolution of metallo-enzyme drugs

Lechner S[1], Kuster B[1], Médard G[1]
[1]Technical University Of Munich

Introduction: Metal ions are essential cofactors in enzyme active sites. To inhibit such metallo-enzymes, many natural or synthetic molecules exhibit metal-binding warheads such as thiols or hydroxamates. Since metal-warhead interaction is often the main contributor to overall binding affinity, the inhibitors are prone to be unselective. Here, we present the chemoproteomic target identification and affinity measurement of more than 80 metal-binding drugs.

Methods: An affinity-based chemoproteomic competition assay is used, where drugs of interest are set to compete for target binding against novel metalloprotein affinity matrices. In a first step, cell lysates are incubated with increasing doses of a drug to reach binding equilibrium. In a second step, residual unbound proteins are pulled down by the affinity matrix, then identified and quantified by bottom-up proteomics. Dose response curves are derived from the nanoLC-MS/MS data, whose inspection reveal the targets of the drugs together with the affinity of each drug-target pair interaction.

Results: The target landscape of >80 small molecules featuring metal-binding warheads was established, comprising affinity values for more than 1200 drug-target interactions. It includes 9 zinc-dependent HDACs, other metallo-enzymes as well as several off-targets not described as metalloproteins. Our results question claimed target spaces and selectivities of widely used drugs or chemical probes. Strikingly, over 40% of the hydroxamates, including approved drugs, potently bind and inhibit ill-annotated metalloprotein MBLAC2, whose inhibition or knockdown lead to extracellular vesicles accumulation. Moreover, our profiling uncovered the molecular targets of a drug approved for more than 30 years with undefined mechanism of action.

Conclusions: Our chemoproteomics assay can systematically probe thousands of drug-protein interactions for each of dozens of drugs, allowing to discover unsuspected targets of metallo-enzyme drugs and modes of action.

Reference: Lechner S, et al. Nat Chem Biol. (2022) doi: 10.1038/s41589-022-01015-5

CS19.04: Proteomics-based Thermal Shift Assay Could Identify Novel Protein Targets of Metabolic Disrupting Chemicals in Cells

Carrasco del Amor A[1], Munic Kos V[2], Cristobal S[1]
[1]Department of Biomedical and Clinical Sciences, Cell Biology, Medical Faculty, Linköping University, [2]Department of Physiology and Pharmacology, Karolinska Institute

The molecular interaction between chemicals with proteins could alter protein solubility and structural conformation. The consequences of the interaction can result in changes in protein activity, intracellular location or interaction with other macromolecules, and initiates most of the alteration of cellular functions. Therefore, identifying protein targets of chemicals is an initial step to predict any impact of chemical exposure on human health. Our aim is advancing in developing methods for a high-throughput target identification of metabolic disrupting compounds (MDC)s that could be used to map the intertwined mechanisms of action affected by those molecular interactions. We have recently demonstrated the applications of this methodology, proteome integral solubility alteration, to identify the protein targets of different chemicals using a zebrafish embryo model [1]. Here, we show the proteome-wide target identification for 3 metabolic disrupting compounds: bisphenol A (BPA), tributyltin chloride (TBT) and perfluorooctanoic acid ammonium salt (PFOA) in the 3T3-L1 preadipocytes and discuss it in the context of the possible impact on obesity-related pathways. We remark a few of novel targets of BPA, such as SUMO conjugating enzyme Ubc9, which could affect PPARγ activity and adipocyte development or regulate subcellular localization of GLUT4. In the TBT study, heterogeneous nuclear ribonucleoprotein U is one of the target proteins that could modulate human adipocyte differentiation and lipid metabolism via interaction with lincRNAs. For PFOA, the target histone acetyltransferase has a role in the regulation of the development and function of thermogenic adipocytes. The results predict the impact of MDCs on cells. In toxicology, protein target identification is an initial step to elucidate mechanisms of action of chemicals and therefore to predict their possible impact on cellular function.

[1] V. Lizano-Fallas, A.C. et al. Journal of proteomics (2021) 104382.
Funding: European Union’s Horizon 2020, GOLIATH No 825489; IKERBASQUE, Basque Government grant IT-971-16.

CS19.05: One-minute Machine-gun Proteomics Facilitates Biomarker Discovery for Drug Development

Ishihama Y[1,2], Nakai A[1], Tanaka S[1], Kanao E[1,2], Ogata K[1], Xuan Y[3], Olsen J[4], Chen Y[5]
[1]Graduate School of Pharmaceutical Sciences, Kyoto University, [2]National Institutes of Biomedical Innovation, Health and Nutrition, [3]Thermo Fisher Scientific (Bremen) GmbH, [4]Novo Nordisk Foundation Center for Protein Research, University of Copenhagen, [5]Institute of Chemistry, Academia Sinica

Introduction: High-throughput analysis of clinical samples such as tissues and body fluids is essential for biomarker discovery and their validation, but the high speed of LC/MS/MS leads to decreased sensitivity and identification efficiency. Here we show how a capillary LC/MS/MS system using sub-minute gradient elution can achieve a throughput of 1000 samples per day without sensitivity loss. We will also present a method for targeted quantification of membrane proteomes by Cys chemical conversion and its application to an extremely hydrophobic peptide containing EGFR-T790M, which has never been reported before.
Methods: Proteins were reduced with dithiothreitol, alkylated with 2-bromoethylamine. For capillary LC/MS/MS, a tapered C18-modified monolithic silica capillary (100 µm diameter) was used with sub-minute gradient, and PRM or DIA mode was employed.
Results: Careful optimization of flow rate, gradient time, and MSMS acquisition parameters resulted in the development of a capillary LC/MS/MS system without sacrificing high speed and sensitivity. Compared to nanoflow conditions, the ESI sensitivity at around 10 µL/min was reduced by a factor of 3 to 5. However, by making the gradient steeper, the peak was sharpened to achieve high-speed separation without loss of sensitivity. In addition, the use of the capillary monolithic column allowed the flow rate to be increased up to 10 µL/min at less than 200 bar at 25 ˚C. We applied this system to monitoring the EGFR-T790M mutation, the target of a new generation of resistant EGFR therapies in NSCLC. The extremely hydrophobic mutant peptide with 27 residues was shortened to 16 residues. By adding isotope-labeled internal standards, the absolute amounts of various EGFR mutant peptides were quantified, including the T790M-peptide, which have not been reported previously.
Conclusions: Novel capillary-LC/MS/MS technology achieving both high-speed and high-sensitivity facilitates the biomarker discovery through high-throughput analysis of 1000 samples per day.

CS20.02: The post-COVID associated autoantibody repertoire

Jernbom Falk A[1], Skoglund L[1], Andersson M[1], Olofsson J[1], Hellström C[1], Sjöberg R[1], Tegel H[2], Hober S[2], Havervall S[3], Thålin C[3], Pin E[1], Månberg A[1], Nilsson P[1]
[1]Department of Protein Science, SciLifeLab, KTH Royal Institute of Technology, [2]Department of Protein Science, AlbaNova, KTH Royal Institute of Technology, [3]Department of Clinical Sciences, Karolinska Institutet, Danderyd Hospital

Introduction

After the acute phase of COVID-19 infection, many individuals experience persistent symptoms known as post-acute sequelae of COVID-19 (PASC). Long-term symptoms vary considerably, ranging from enduring anosmia to neurocognitive deficits, and may cause considerable disability and suffering. Although the underlying and likely diverse causes of PASC remain unknown, pre-existing or new-onset autoantibodies are major hypotheses. Therefore, we aim to analyze the autoantibody repertoire in 525 extensively clinically characterized healthcare workers (HCW) and inpatients followed over 3-5 visits, spanning 8-16 months of the pandemic.

Methods

In the initial phase of the study, we analyzed 32 HCW with long-term symptoms on two sets of in-house developed planar protein arrays. The untargeted array contains 42000 protein fragments representing approximately 18000 human proteins, while the targeted array contains 1500 full-length secreted or extracellular proteins representing the human secretome. Antigens corresponding to detected autoantibodies were coupled to 363 color-coded magnetic microspheres which were used to characterize the longitudinal autoantibody profiles of the entire cohort.

Results

Early results reveal diverse autoantibody dynamics during COVID-19 infection. Patterns of autoantibody dynamics include repertoire-wide increase of autoantibodies, as well as increase of single or multiple specific autoantibodies, or a combination. Autoantibody profiles are often individual, but shared new-onset autoantibodies are also occurring. Clustering analyses to reveal subgroups of autoantibody repertoires are ongoing.

Conclusions

We have analyzed the autoantibody dynamics before, during, and after COVID-19 in a large and extensively clinically characterized cohort using both wide and focused protein arrays. Early results show diverse and shared new-onset autoantibodies in SARS-CoV-2 infection. These data will be thoroughly analyzed and be combined with post-COVID symptom data to begin to uncover the role of new-onset autoantibodies in the development of post-COVID syndrome.

CS20.03: High-throughput large-scale plasma proteomics on 1000+ COVID-19 patients allows for prognostication of immune response and outcome

Viode A[1,2], Smolen K[2,3,4], van Zalm P[1,2], Fatou B[1,2,3], Stevenson D[1,2], Jha M[1,2], Steen H[1,2,3], on behalf of the IMPACC Network
[1]Department of Pathology, Boston Children's Hospital, [2]Harvard Medical School, [3]Precision Vaccines Program, Boston Children's Hospital, [4]Division of Infectious Diseases, Boston Children's Hospital
Introduction: Our efforts are part of the IMmunoPhenotyping Assessment in a COVID-19 Cohort (IMPACC) study. IMPACC enrolled >1300 hospitalized COVID-19 patients at 15 different medical centers across the USA. One of the hallmarks and challenges of COVID-19 is its highly heterogenous manifestation affecting many organ systems with outcomes ranging from asymptomatic cases to hospitalization and death. However, the mechanisms associated with disease manifestation lack temporal understanding.

Methods: In this study, we analyzed 2985 longitudinally collected plasma samples from 1059 patients. Up to 6 plasma samples/patient were collected during hospitalization. Patients were classified in 5 trajectory groups based on their disease severity and outcome. To increase analytical depth, plasma samples were depleted using our perchloric acid-based precipitation. This low cost, very robust method has significantly advanced the field of large scale, high-throughput LC/MS based proteomics.

Results: We quantified 2900 proteins including the SARS-CoV-2 Nucleoprotein (NP), as it was shown to be an early marker of disease severity. We performed a daily temporal analysis and observed a dysregulation of the host immune response in severe COVID-19. Our findings showed that the fatal outcome (<28 days) is characterized by persistent up-regulation of aspects of the immune system and accumulation of organ damage markers. Some of these markers allow for stratification of patients with similar severe disease trajectories but different ultimate outcome, i.e., death vs. survival. In milder COVID-19, serving as controls, these pathways are following opposite trends. From this analysis we derived prognostic biomarkers of outcome at hospital admission.

Conclusions: High throughput large-scale plasma proteomics study on almost 3000 samples provided information about changes to the plasma proteome of >1000 hospitalized COVID-19 patients with daily resolution. We achieved in-depth longitudinal characterization of pathways linked to disease severity, identifying prognostic biomarkers of disease outcome.

CS20.04: Pulmonary and Renal Long COVID at Two-year Revisit

Wang J[1], Liang X[2], Zheng Y[1], Zhu Y[2], Zhou K[1], Wu X[1], Sun R[2], Hu Y[3], Zhu X[1], Chi H[1], Chen S[3], Lyu M[2], Xie Y[2], Yi X[3], Liu W[3], Cai X[2], Li S[2], Zhang Q[3], Wu C[3], Shi Y[2], Wang D[1], Peng M[1], Zhang Y[1], Liu H[4], Zhang C[4], Quan S[4], Kong Z[4], Kang Z[4], Zhu G[1], Zhu H[1], Chen S[1], Liang J[1], Yang H[1], pang J[1], Fang Y[1], Chen H[1], Li J[1], Xu J[1], Shen B[1], Guo T[2]

[1]Taizhou Hospital of Zhejiang Province affiliated to Wenzhou Medical University, [2]Westlake University, [3]Westlake Omics Biotechnology Co., Ltd., [4]Key Laboratory of Digital Clinical Diagnostics Technology of Zhejiang Province, Calibra Lab, DIAN Diagnostics

Introduction

More than 500 million individuals have recovered from COVID-19, but little is known about the host responses to long COVID.

Methods

We performed proteomic and metabolomic analyses of 991 blood and urine specimens from 144 COVID-19 patients with comprehensive clinical data and up to 763 days of follow up, from the disease onset, recovery and discharge, one-year revisit, to two-year revisit.

Results

Abnormal clinical indices were detected in over 30% of the patients, involving lung, kidney, and liver. Pulmonary and renal long COVID of one-year revisit can be predicted by a machine learning model based on clinical and multi-omics data collected during the first month from the disease onset with an ACC of 87.5%. Proteomics revealed that lung fibrous stripes was related to continuous down-regulation of SFTPB in the sera, which might be a potential therapeutic target for pulmonary long COVID. Notably, our data show that all the patients with persistent pulmonary ground glass opacity or patchy opacity lesions developed into pulmonary fibrous stripes at two-year revisit.

Conclusions

This study depicts the longitudinal clinical and molecular landscape of COVID-19 with up to two-year follow-up and presents a method to predict pulmonary and renal long COVID.

CS20.05: Novel insights in SARS-CoV-2 infection by characterization of humoral immune response dynamics

Fuentes M[1]
[1]Centro De Investigación Del Cáncer. University of Salamanca-CSIC. IBSAL
Background: the humoral immune response remains a key element in the diagnosis and follow-up of patients infected with SARS-CoV-2. Specifically, the autoimmune response, the immununoglobulin (Ig) neutralizing profile against the virus and other soluble factors such as acute phase reactants (APR) can serve as a basis for determining a dynamic in patients with SARS-CoV-2 infection.
Methods: In-depth characterization of humoral response of more than 4000 COVID-19 patients with different symptoms and severity was performed. Multiplex high throughput analysis has led to more than 620000 immunoassays that have been used to characterize the humoral dynamics of patients.
Results: The Ig production against SARS-CoV-2 proteins drew distinct profiles at different severity levels of COVID-19 infection as well as APR and autoantibodies (AAB) profiling reveals key players of dynamic humoral response at different COVID-19 disease stages. Monitoring of humoral response against SARS-CoV-2 infection convalescence reveals potential long-COVID-19 biomarkers and widespread changes in the functional coordination of these factors in COVID-19 patients.
Conclusions: Several humoral response proteins have been found to be significant and allow differentiation of the severity and evolution of infected patients in ICU as well as provide clues about the follow-up of patients in post-acute sequelae of SARS-CoV-2 infection (PACS).

CS21.02: Deep Proteomics Profiling and Multi-omics Data Integration Reveal Sinus Nodal Dysregulation Behind Bradycardia in Heart Failure

Kahnert K[1], Wilson C[2,3], Mills R[1], Hansen F[1], Maurya S[1], Sorrentino A[1], Hu W[4], Zi M[2], Olesen M[1], Zhang H[4], Jensen T[5], Boyett M[1,2], Lundby A[1]

[1]Department of Biomedical Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, [2]Division of Cardiovascular Sciences, University of Manchester, [3]Institute of Systems, Molecular & Integrative Biology, University of Liverpool, [4]Biological Physics Group, Department of Physics & Astronomy, University of Manchester, [5]The Heart Center, Rigshospitalet

Introduction

Heart failure (HF) is a major health problem affecting ~64 million people worldwide. In 6% of the patients HF is accompanied by bradycardia, and this is an important predictor of mortality. At present, the molecular underpinnings of bradycardia in HF are little understood. To identify the molecular dysregulation behind the bradycardic phenotype in HF we set out to investigate changes in the sinus node, which dictates the intrinsic heart rate, in an animal model.

Methods

We applied isobaric labelling and state-of-the-art LC-MS/MS to acquire deep, quantitative sinus node proteomes and phosphoproteomes from a mouse model of HF with bradycardia. These data were used for biophysically detailed mathematical modelling of the mouse sinus node action potential. To prioritize among all regulated proteins those of importance for human pathophysiology, we performed multi-omics data integration across orthogonal datasets. We intersected our acquired data with human genomics data on heart rate, single-cell RNA sequencing data of failing human hearts as well as pharmacovigilance data on bradycardia.

Results

Deep proteome and phosphoproteome measurements identified 6849 proteins and 6286 phosphorylation sites, respectively, with an overlap of >99% across all samples. Our data revealed dysregulation of 573 proteins and 586 phosphorylation sites. Mathematical modeling based on measured protein abundance changes recapitulated a reduced heart rate as observed clinically. Our multi-omics data integration strategy shortlisted key proteins with genomics and pharmacological evidence of importance for the heart rate in humans. Ultimately, we highlight downregulation of six proteins in cardiomyocytes and upregulation of one protein in fibroblasts, as key contributors to the bradycardic phenotype in HF.

Conclusions

By combining a global proteomics analysis of sinus nodal remodeling in bradycardic HF in a mouse model with a multi-omics data integration approach, we pinpoint seven specific proteins that are critical for the bradycardic phenotype in humans.

CS21.03: Protein expression landscape of the human brain based on transcriptomics analysis of 200 micro-dissected region

Mulder J[1], Mitsios N[1], Zhong W[2], Oksvold P[2], Lindskog C[4], Renner E[3], von Feilitzen K[2], Fagerberg L[2], Hökfelt T[1], Palkovits M[3], Uhlen M[2]
[1]Department of Neuroscience, Karolinska Institute, [2]Science for Life Laboratory, Royal institute of technology, [3]Human brain tissue bank, Semmelweis university, [4]Rudbeck laboratory, University of Uppsala

Introduction: The Human Protein Atlas (HPA; www.proteinatlas.org) is a public online database that provides an integrated overview of protein expression and distribution in all major human tissue types, including brain. As part of that, a comprehensive overview of gene and protein expression in the main anatomical structures of the mouse, pig and human brain is provided, by combining in-house generated and publicly available transcriptomic data.
Methods:In the current version (HPA21.1), released in May 2022, the brain section of the portal includes additional in-house generated RNA sequencing data, based on regional and subregional expression of protein-coding genes in 967 samples from 202 human brain regions, microdissected in the Human Brain Tissue Bank, Budapest.
Results: The current collection of samples presented in HPA21 include 10 samples from different basal ganglia areas, 16 thalamic and 9 hypothalamic nuclei, 9 samples from the hippocampal complex, 5 from the amygdala, over 70 from brainstem (midbrain, pons, medulla) and 5 cerebellar cortical and nuclear samples. In addition, from the cerebral cortex, more than 70 areas, gyri and subregions have also been characterized.
Conclusion: Altogether, the HPA brain section provides gene-centric pages that give an extensive and integrated overview on transcript expression across regions and subregions of the brain and allow comparison between species. Furthermore, we classify all protein coding genes based on regional distribution and co-expression, thus providing lists of genes associated to brain regions and functions. As an example, the piriform cortex appears to have a unique transcriptomic signature, quite different from the rest of the cerebral cortex while the claustrum, although it anatomically belongs to the cortical subplate, its transcriptional profile shares more common characteristics with the subregions of the cortical plate. All data presented are freely accessible based on the FAIR Data Principles.

CS21.04: Spectrum is All You Need: Direct Disease Classification from Raw Mass-Spectrometry data using Self-Supervised Deep Learning

Kim E[1], Park J[2], Lee M[3], Eum J[3], Jung G[3], Kang U[3], Kim S[1]
[1]Bertis Bioscience Inc., [2]Bertis Inc., [3]Bertis R&D Division

Introduction

Deep learning has made great strides in many areas, but in proteomics, the adaptation has been limited to a small number of applications, such as prediction of chromatographic retention time and product ion intensities. We gauge an untapped potential of deep learning by building a model that classifies cancer patients from normal subjects directly from data-independent acquisition (DIA) data, without resorting to proteomics knowledge.

Methods

Transformer encoders were used for encoding DIA data. To process large data, the encoders were laid out in a hierarchy. The level-1 transformer encodes one MS/MS spectrum, and the level-2 transformer encodes a sequence of level-1 outputs. Both encoders are trained in a self-supervised fashion – learning the distribution itself without labels. In the self-supervised training, novel optimization objectives were added on top of the typical objective of predicting hidden input. After training each level in sequence, the top-level classifier is fine-tuned along with the level-2 transformer. The labels used in the final fine-tune step are the only external information injected to the model.

Results

We tested our method on two DIA datasets – ovarian cancer with 157 samples and pancreatic cancer with 118 samples, each of which contains ~50% of healthy samples as control. The datasets were split into 80% training, 10% evaluation and 10% test sets. For the split, both models achieved an area under curve of 1.0 in classifying the cancer and normal samples.

Conclusions

The results hint that deep learning architectures and training regimes that are successful in other domains are applicable to mass spectrometry-based proteomics. Minimal domain knowledge was used, suggesting that the method can be extended to other mass spectrometry-based omics technologies (e.g., metabolomics and lipidomics) and their integrations. This work paves a way toward making sense of underutilized information, such as post-translational modifications.

CS21.05: Spatial exploration of ovarian proteome throughout the lifespan of women

Méar L[1], Sutantiwanichkul T[1], Östman J[1], Lindskog C[1]
[1]Uppsala University, Dept. of Immunology, Genetics and Pathology

Introduction

One of the most dynamic organs in the human body, the ovary, plays a crucial role in the production of both hormones and gametes. From puberty to menopause, it undergoes major structural changes, monthly, to release the oocyte. Moreover, ovarian aging correlates with a decrease of fertility. Due to the heterogeneous distribution of follicles and their limited number, ovarian tissue remains highly challenging to study. As part of the Human Protein Atlas (HPA), mRNA and protein expression profiles are compiled in several tissues. For the ovary, however, there is no complementary information regarding age-related expression. Our aim is to identify and localize proteins potentially associated with the main changes in ovary function across women's lifespan such as the menopause.

Methods

To identify genes differentially expressed by age group, RNA sequencing data from GTEx available on HPA was re-analysed. Thus, nTPM values were quality controlled and feature selected (Hartigan's dip test of unimodality). Then, multiple analysis of variance (MANOVA) was used to identify potential age markers. The results were validated with immunohistochemistry (IHC) on a unique ovarian tissue microarray covering all the age groups. Finally, multiplexed immunofluorescence imaging was used to map the details of ovarian structure and the resulting images were automatically analyzed.

Results

MANOVA resulted in potential 111 genes linked to changes in age groups. Among them, 69 had available antibodies as part of the HPA project, including well-known markers as well as proteins with unknown function in the ovary.

Conclusions

This study presents a data-driven approach combining machine learning on transcriptomics data and resourceful image analysis for multiplexed spatial proteomics. By using the HPA data, we will gain a unique insight into the spatial proteome of ovary from the span of reproductive to post-menopausal ages.

PP01.001: Deep Proteome Profiling of Formalin-fixed Paraffin-Embedded Heart Tissue

Achter J[1], Bilir S[1], Pisano P[2], Jensen T[3], Wierer M[2], Lundby A[1]
[1]Department of Biomedical Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, [2]Proteomics Research Infrastructure Facility, Faculty of Health and Medical Sciences, University of Copenhagen, [3]Department of Pathology, Rigshospitalet, Copenhagen University Hospital
Introduction: Formalin fixation and paraffin-embedding (FFPE) serves as a gold standard for tissue preservation and clinical diagnosis of biopsy specimens. It is estimated that over a billion FFPE tissue samples are currently stored in biobanks and hospital archives, providing an invaluable resource for clinical proteomics. To this end, we investigated the performance of two popular MS workflows (isobaric labeling quantification (ILQ) and data-independent acquisition (dia-PASEF)) for proteome profiling of human FFPE heart tissue.
Methods: Proteomic measurements were performed on matched flash frozen tissue and FFPE tissue collected from the left ventricle of five patients. Samples were solubilized in high concentrations of SDS and processed with S-Trap technology. DDA raw data from the ILQ workflow were analyzed with FragPipe, while diaPASEF data were processed in DIA-NN using FragPipe-generated spectral libraries.
Results: Our results provide collective evidence for >6600 proteins quantified from cardiac FFPE tissue specimens. We observed excellent correlation between FFPE and matched flash frozen tissue and high reproducibility of our proteomic workflows. In combination with in-depth project-specific spectral libraries, dia-PASEF measurements showed substantial overlap and strong correlation with the TMT dataset. Both methods were able to capture characteristic proteomic signatures.
Conclusions: FFPE tissue represents a valuable alternative to flash-frozen tissue and holds great potential for cardiovascular research. Neither formalin fixation nor the choice of MS acquisition impede proteomic analysis and enable comprehensive patient phenotyping in challenging samples. While the ILQ workflow produced higher proteome coverage and fewer missing values, the dia-PASEF approach is particularly suited for analyzing large cohorts of clinical FFPE tissue.

PP01.002: Parkin Activator, PR-364 Protects Cardiomyocytes Post-Heart Attack: Increased Mitochondrial Function and Translational Reprogramming

Ai L[1], Stotland A[1], Kreimer S[1], Ayres M[1], Butt T[2], Kumar S[2], Gottlieb R[1], Van Eyk J[1]
[1]Cedars-Sinai Medical Center, [2]Progenra Inc.

Introduction: ~30% of survivors following myocardial infarction (MI) develop heart failure, due in part to dysfunctional mitochondria accumulation, which can exacerbate myocardial damage. Our group has shown a single dose (2hrs post-MI) of a selective Parkin activator, PR-364 preserved cardiac ejection fraction and halted HF progression by Day28 in mice (n=20-30/group). Between Day3-7 post-MI, 50% of vehicle-treated mice died while 100% of PR-364 mice survived (n=5-8/group). To elucidate the initial protective mechanism of PR-364, we deployed a multi-omic strategy in myocardial ventricular AC16-cells and mice models.

Methods: Adult mice underwent sham or permanent coronary artery ligation (PCAL, an MI model), followed by single injection of 50nMol PR-364 or vehicle/DMSO at 2hrs. Non-ischemic heart tissues were collected on Day3 and Day7 (n=5-8/group) and analyzed using targeted metabolomics (iMRM, 6490 MS, Agilent), mitochondrial function (Seahorse) and proteomics (DIA-MS, Lumos Fusion MS, Thermo) with data analysis by DIA-NN. AC16s were treated vehicle/DMSO or 3µM PR-364 for 6hrs (n=6/group) and analyzed by proteomics.

Results: At Day3 post-surgery, although 18% proteome were significantly altered by PCAL, PR-364 treatment maintained similar “normal” expressions of the 70 differentially expressed proteins (DEPs) in the post-PCAL compared to sham/vehicle mice; by day7 with HF, the DEPs increased to 286, including those involved in nuclear and mitochondrial translation (5+7), TCA (5), ETC (7). In AC16-cells, PR-364 also altered nuclear and mitochondrial translational pathways (6+6) and mitochondrial Ca2+ transport (5). Based on flow cytometry and western blots, PR-364 affected AC16s by downregulating MICU1, the gatekeeper for mitochondrial Ca2+ uptake, which elevated mitochondrial [Ca2+]m and correlated to an increased ATP production and mitochondrial function.

Conclusion: Our study indicates PR-364 may have a two-pronged effect triggering concurrently i) translational reprograming of the proteome and ii) modulation of mitochondria-Ca2+ preserving mitochondrial function and resetting the injured myocardium toward a “healthy protected state”.

PP01.003: Proteomic and Phosphoproteomic Profiling of Head and Neck Cancer Patient-Derived Xenografts Highlights CDK6 as a Potential Drug Target

Alp M[1,2,6], Klinghammer K[3], Beier C[1,2], Tinhofer I[4], Keilholz U[5], Mertins P[1,2]
[1]Max Delbrück Center for Molecular Medicine, [2]Berlin Institute of Health, Charité - Universitätsmedizin Berlin, [3]Charité - Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, Department of Hematology, Oncology and Cancer Immunology, [4]Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, Department of Radiooncology and Radiotherapy, [5]Charité Comprehensive Cancer Center, Charité - Universitätsmedizin Berlin, [6]Humboldt-Universität zu Berlin, Faculty of Biology

Introduction: There is an unmet need for improved treatment stratification and predictive biomarkers for head and neck squamous cell carcinoma (HNSCC) patients. Previously, Klinghammer et al. showed that the basal subtype defined by transcriptomics is predictive of response to EGFR blockade by cetuximab treatment in a set of 28 patient-derived xenografts (PDX). To improve tumor subtyping, and define potential drug targets and predictive biomarkers, we performed proteomic and phosphoproteomic profiling of an extended set of HNSCC PDX tumors that have been characterized for their response to multiple drug compounds including cetuximab.
Methods: 64 HNSCC PDX tumors were analyzed by established Tandem Mass Tag (TMT)-based mass spectrometry workflow. Bioinformatic analysis was performed by integrating proteomics and phosphoproteomics with clinical annotation, drug response data, gene expression, and mutation profiles of these tumors.
Results: More than 8,500 human proteins and 15,000 phosphosites were quantified across the 64 PDXs. NMF clustering on proteome and phosphoproteome levels resulted in three distinct clusters overlapping partly with RNA-based classification termed classical, mesenchymal, and basal subtypes. Differential expression analysis validated clinical biomarkers for human papillomavirus (HPV) status and cetuximab response and highlighted additional proteins so far not connected to HNSCC. Interestingly, CDK4 expression was associated with HPVpos tumors, whereas the homolog kinase CDK6 was found to be upregulated in HPVneg tumors belonging to the basal-like proteomic subtype. Dependency Map (DEPMAP) analysis validated that CDK6 has strong codependency with EGFR in cell lines from the upper aerodigestive tract including HNSCC.
Conclusions: Integrative proteogenomic tumor analysis is a powerful tool for cancer classification, may predict the efficacy of targeted therapies more accurately, and more importantly, displays new subtype-specific druggable protein targets. Our proteomics investigation of well-defined HNSCC PDX models yields a better understanding of the co-dependency of different signaling pathways and helps to select new candidates for synergistic treatment strategies.

PP01.004: Leveraging Proteomic Profiles Identifies Dupilumab Treatment-Associated Biomarkers for Atopic Dermatitis

An L[1], Hulse K[2], Mack M[2], Weidinger S[3], Bryce P[2], Zhang B[1]
[1]Precision Medicine and Computational Biology, Sanofi, [2]Immunology and Inflammation, Sanofi, [3]University Hospital Schleswig-Holstein

Atopic dermatitis (AD) is a highly heterogenous, chronic, relapsing inflammatory skin disease that affects children and adults. The causes of AD are multifactorial, including both genetic and environmental factors. While new treatment options have recently shown success in treating many patients with AD, it has become clear that there are different endotypes of AD, but the molecular mechanisms remain unclear. Many studies have used transcriptomics analysis of blood or skin biopsies from patients with AD, but these studies have practical limitations, as obtaining skin biopsies is not feasible for many patients, and gene expression levels do not always correlate with protein expression. To date, no deep proteomic profiling studies have been performed to assess treatment response and define endotypes in AD patients. We sought to identify protein biomarkers of disease severity and treatment response using deep proteomic profiling of AD patient serum samples.
Matched serum samples were collected at baseline (pre-treatment) and 3 months post treatment (Dupixent (n=61) or Ciclosporin (n=14)) from patients participating in the TREATGermany cohort. Biognosys HRM LC MS/MS protein profiling was used to process the samples, and on average, >1,200 proteins were quantified per sample.
19 proteins were upregulated (FDR < 0.05, Log2FC > 0.5) in AD patients with severe disease pre-treatment (EASI >24.1; n=17), compared with moderate disease (EASI <24.1; n=57). Two of the 19 proteins, LCP1 (L-plastin) and TNC (Tenascin), were also positively associated with treatment response to Dupilumab (both AUC > 0.76). Moreover, LCP1 was a biomarker predicting Dupilumab treatment response (AUC = 0.7) but not cyclosporin response (AUC = 0.59), while TNC was a treatment response biomarker in both groups.
Overall, this study identified serum biomarkers that can be easily used to identify AD patients with severe disease, as well as those who are likely to respond to specific therapies.

PP01.005: Autoantibody profiling Uilizing Secretome Arrays

Anggraeni Andersson M[1], Olofsson J, Hellström C, Skoglund L, Jernbom Falk A, Månberg A, Pin E, Sjöberg R, Nilsson P
[1]SciLifeLab & KTH Royal Institute Of Technology

Background and Aims

There is a great need for access to diverse and highly comprehensive representations of large number of human proteins in order to explore the autoantibody repertoires in health and disease. Aiming to complement the proteome-wide coverage of E. coli expressed protein fragments, a complete set of full-length human secretome1 proteins have now been expressed in mammalian cells within the Human Protein Atlas (HPA) (www.proteinatlas.org). We here describe the development of secretome arrays enabling autoantibody screening, as well as antibody validation, that meets the contemporary demands in both throughput and multiplexing.

Methods

Suspension bead arrays and planar arrays are generated using the secretome proteins produced within the HPA project. Based on our previously optimized technology for protein fragments (median length of 80 aa), the full length proteins were immobilized on color coded magnetic beads (suspension bead array) or on epoxysilane coated glass slides with a non-contact printing technique (planar array). These arrays have been tested and validated using protein specific antibodies and human plasma.

Results

Highly multiplexed arrays with up to 1500 unique full length secretome proteins are being developed and utilized for the profiling of autoantibody repertoires in plasma, serum and CSF samples as well as extensive analysis of selectivity and specificity of monoclonal and polyclonal antibodies.

Conclusions

The new full length protein arrays, as a complement to our protein fragment arrays, can be used for antibody validation and autoantibody screening with a high multiplex capacity and high sample throughput.

References

1 Uhlén et al, The human secretome (2019), Science Signaling

PP01.006: Integrative Proteomics and Pharmacological Analysis of Colon Cancer Reveals the Classical Lipogenic Pathway with Prognostic and Therapeutic Opportunities

Barpanda A[1], Srivastava S[1]
[1]Indian Institute Of Technology Bombay

Introduction: Despite recent advancements in colon cancer (CAC) research, the high mortality rate remains a significant concern. Identifying protein markers could be a great asset in disease management. Numerous studies have reported increased de novo lipogenesis (DNL) activity in CAC progression, but only a few have comprehensively investigated its role in disease pathobiology. Hence, this study aims to establish the prognostic as well as the therapeutic potential of DNL and associated signaling cascades in CAC.
Methods: Two key lipogenic enzymes, FASN and ACLY, were quantified in tumors and matched controls using Parallel Reaction Monitoring (PRM). Peptide intensities of both the proteins were used for class prediction analysis. Furthermore, DNL was inhibited in the HCT 116 cell line using cerulenin, and in silico molecular docking-based drug repurposing was performed to find potential drug candidates.
Results: PRM analysis of FASN and ACLY revealed significant dysregulation of fatty acid production in CAC. A panel containing 12 unique peptides was identified with the ability to differentiate between CAC and peritumoral tissues with an accuracy of ~0.8. Cerulenin-mediated suppression of the fatty acid synthesis pathway in HCT 116 cells revealed the multifunctional significance of de novo lipogenesis in cancer cell proliferation and metastasis. DNL inhibition affected various cellular signaling pathways, including cancer metabolic processes. PRM analysis also revealed upregulation of 26s proteasome machinery. In silico molecular docking of FASN identified five drugs, i.e., Entrectinib, Imatinib, Risperidone, Axitinib, and Cabozantinib, with potential roles in CAC therapeutics.
Conclusions: The study identified and validated a peptides panel linked to DNL having the potential to differentiate the CACs from controls. We have also shown that inhibition of DNL resulted in proteasomal activation followed by apoptosis induction in CAC cells. The therapeutic potential of DNL and the mechanistic insights of the pathway in cancer development and metastasis.

PP01.007: Profiling the Autoantibody Repertoires in ANCA-associated Vasculitis with Multiplex Antigen Arrays

Bayati S[1], Scott J[2], Little M[3], Nilsson P[1], Pin E[1]
[1]Dep. Protein Science, Scilifelab, Kth Royal Institute Of Technology, [2]Trinity Health Kidney Centre, TCD, Dublin, Ireland, [3]Trinity Translational Medicine Institute, TCD

Introduction: Anti-Neutrophil Cytoplasmic Antibody (ANCA) Associated Vasculitis (AAV) is a rare autoimmune disorder causing severe inflammation of blood vessels. AAV is characterized by two major autoantibodies targeting anti-proteinase 3 (anti-PR3) and anti-myeloperoxidase (anti-MPO). The difficulty in predicting relapse, and selection of patients in whom immunosuppression may be stopped early, is an important unmet need in the management of patients with AAV. We aimed to identify autoantibody repertoires associated with long-term-remission-off-therapy (LTROT) patients and patients experiencing flares.

Methods: Pooled samples provided by the Irish Rare Kidney Disease (RKD) biobank, were screened on planar protein fragment arrays representing 18000 unique human proteins by the Human Protein Atlas (www.proteinatlas.org). Based on the results of this untargeted screening combined with a literature study, 346 protein fragments were selected for targeted analysis. The bead array was generated and 21 plasma samples (13 LTROT and 8 relapse) obtained during periods of stable remission were tested to find novel autoantibodies capable of distinguishing those who remained in remission from those who suffered a relapsing course.

Results: We found IgG reactivity towards 192 out of 346 protein fragments of which the highest and lowest reactivity numbers belonged to relapse and LTROT samples respectively. Patients in the relapse group had a greater prevalence of reactivity to four protein fragments. The combination of the targets into a panel showed that most relapsing patients have more than one of the four autoantibodies. The analysis also showed that there was no sex-related difference in the number of reactive antigens.

Conclusions: We have identified several putative candidate autoantibodies that classify those patients with vasculitis at high risk of long-term relapse. The results are currently under further verification on a new sample collection including 102 plasma samples from 26 LTROT and 76 relapse AAV patients.

PP01.008: A highly Predictive Autoantibody-Based Biomarker Panel for Prognosis in Early-Stage NSCLC with Potential Therapeutic Implications

Patel A, Middleton G, Blackburn J
[1]University of Cape Town

Introduction: Lung cancer is the leading cause of cancer-related death worldwide. Surgical resection remains the definitive curative treatment for early-stage disease, offering an overall 5-year survival rate of 62%. Despite careful case selection, a significant proportion of early-stage cancers relapse aggressively within the first year post-operatively. Identification of these patients is key to accurate prognostication and understanding the biology that drives early relapse might open up potential novel adjuvant therapies. Here, we carried out MS-free quantitative autoantibody profiling to identify and validate pre-operative biomarkers of 5 year survival following surgery.

Methods: Baseline serum samples from 157 study participants (NSCLC stage I–IIIa; 92 survivors; 65 non-survivors) were utilised for proteomic analysis. Antigen-specific IgG titres were determined for >1600 serum-based autoantibody biomarkers using Sengenics iOme arrays. Unsupervised interrogation of the data was performed using an iterative machine-learning algorithm. Further interrogation of this signature was carried out by generating a continuous risk score for every individual on the basis of model coefficients. Using these individual risk scores, survival analyses and multivariate Cox proportional hazards modelling was performed. A custom protein array comprising the top 60 autoantigen markers was fabricated and used for validation in an independent cohort.

Results: We identified a 13-biomarker signature that is highly predictive for survivorship in post-operative early-stage lung cancer; this outperforms currently used autoantibody biomarkers in solid cancers. Our results demonstrate significantly poor survivorship in high expressers of this biomarker signature, with an overall 5-year survival rate of 7.6% and a Hazard ratio of 19.6 (sensitivity 84.2%, specificity 74.1%). The identity of the individual biomarkers provides mechanistic insight.

Conclusions: We anticipate that the data will lead to the development of an off-the-shelf prognostic panel and further that the oncogenic relevance of the proteins recognised in the panel may be a starting point for a new adjuvant therapy.

PP01.009: Next Generation Blood Proteome Profiling for Pan-Cancer Precision Medicine

Bueno Alvez M[1], Edfors F, Ponten F[2], Fagerberg L[1], Uhlen M[1,3]
[1]Science for Life Laboratory, Department of Protein Science, KTH-Royal Institute of Technology, [2]Department of Immunology, Genetics and Pathology, Uppsala University, [3]Department of Neuroscience, Karolinska Institutet

Introduction

Cancer is a highly heterogeneous disease in need of accurate and non-invasive diagnostic tools. A deep characterization of the blood proteome profiles in cancer patients can contribute to a better understanding of the disease, resulting in earlier diagnosis, risk stratification and better monitoring of the different cancer subtypes. Here, we have characterized the plasma profiles of 1,463 proteins from 1,500 cancer patients to explore the proteomic signatures from altogether 12 different cancer types.

Methods

The levels of 1,463 proteins have been measured in less than 10 microliters of blood plasma using the Proximity Extension Assay (PEA) technology. Applying disease prediction models based on all measured proteins allowed us to identify a set of proteins associated with each of the analyzed cancers. Additionally, differential expression analysis was used to identify whether the protein candidates were up- or down-regulated in each of the cancer types. Finally, a new prediction model based on a restricted set of upregulated proteins was built to evaluate the accuracy of the classification of pan-cancer samples.

Results

The pan-cancer analysis showed that each cancer has a distinct plasma proteome profile, characterized by up- and down-regulated of sets of proteins. Moreover, AI-based prediction models and differential expression revealed common protein signals for some of the cancers, such as colorectal and lung cancer. By combining the results from all cancer types, we identified a panel of proteins which resulted in a promising classification of all cancer samples in the different cancer types.

Conclusions

Next-generation plasma proteome profiling has the potential to identify proteins with distinct profiles in a pan-cancer cohort based on minute amounts of blood samples, guiding the AI-based selection of a protein panel to allow precision medicine diagnosis of patients with different cancer origin.

PP01.010: Machine-learning Guided Proteomic Prediction of Diverse Incident Diseases

Carrasco Zanini Sanchez J[1], Pietzner M, Wheeler E, Kerrison N, Koprulu M, Wareham N, Langenberg C
[1]University Of Cambridge

Proof-of-concept studies of broad-capture, high-throughput proteomic technologies have shown the promise of the plasma proteome for disease prediction from a single blood test but have been limited to selected diseases. Here, we derived sparse predictive protein models for the onset of 24 diseases based on 2,925 plasma proteins in a case-cohort design with >17,778 person years of follow-up (case numbers 21-250, cohort controls 289-758). Adding as few as five proteins to health-derived information models including age, sex, body mass index and smoking significantly improved predictive performance for 18 out of 24 diseases including venous thrombosis, breast cancer and prostate cancer. Changes in concordance index (C-index) ranged from 0.05 – 0.27 and net reclassification improvement (NRI) from 0.024 – 0.40. Increased performance was achieved by correct reclassification of cases rather than controls, which demonstrates the potential clinical utility of plasma proteomics for better identification of individuals at highest risk through increased sensitivity. By comparison, polygenic risk scores performed poorly in general and significantly improved basic models for only 3 of the 24 diseases. Our results illustrate the potential of single time-point plasma proteomics to improve prediction of diverse diseases with varying frequency and multiple aetiologies.

PP01.011: High Sensitivity Immunopeptidomics on the timsTOF SCP Mass Spectrometer

Adams C[1], Marx K[2], Tailor A[3], Ternette N[3], Parker R[3]
[1]Bruker Daltonics GmbH & Co. KG, [2]Bruker Daltonics GmbH & Co.KG, [3]The Jenner Institute, University of Oxford

Introduction

Mass spectrometric characterization of immunopeptides is essential to understanding infection, cancer and autoimmunity. Due to their low abundance, non-tryptic nature and high-complexity, comprehensive LC-MS analysis remains challenging. Many peptides ionize as singly charged species hampering their identification. Here, we use the timsTOF SCP system as a highly sensitive instrument with trapped ion mobility separation for in depth analysis and show unprecedented coverage on low sample input of immunopeptide-like standard samples and real immunopeptidome.

Methods

40ng HeLa protein elastase digest was used to optimize the instrument settings in terms of precursor selection, MS/MS spectra quality and collision energies. The optimized parameters were applied to a dilution series of 40ng - 20pg to check the sensitivity. The results were further validated with a synthetic peptide library consisting of 2000 synthetic peptides and a dilution series of 9.36pg – 46.8ng Jurkat immunopeptidome. Chromatographic separation was performed using a nanoElute with gradient times of 20 min, 36 min and 66 min coupled to a captive spray ionisation source. Raw data were processed in PEAKS Xplus.

Results

Inclusion of singly charged peptides in precursor selection improved the number of identified unique sequences by 20-30%. In addition, overall CE optimization resulted in a modest 8% improvement over default strategy developed for proteomics. Validation and assessment of sequencing bias in synthetic HLA peptide standard indicated that this method was more effective in identifying hydrophobic sequences that bind A02:01 HLA compared to other methods. Furthermore, HLA-peptides could be detected with a peptide load of 0.00936ng Jurkat immunopeptidome sample (20,000 cells), with an exponential increase that appeared to reach saturation at 9.36ng (20,000,000 cells).

Conclusions

High sensitivity detection of immunopeptides could be reached out by using a high sensitivity mass spectrometer with ion mobility separation. Optimization of precursors selection and collision energies significantly improved immunopeptide identification.

PP01.012: Functional Diagnostics for Congenital Disorders of Glycosylation from Plasma Glycopeptide PASEF-DDA Data

Wessels H[1], Kulkarni P[1], Zijlstra F[1], Post M[1,2], Bärenfänger M[2,3], Kruppa G[4], Marx K[5], van Gool A[1], Lefeber D[1,2]
[1]Translational Metabolic Laboratory, Department of Laboratory Medicine, Radboud Institute for Molecular Life Sciences, Radboud university medical center, [2]Department of Neurology, Donders Institute for Brain, Cognition, and Behavior, Radboud university medical center, [3]Division of BioAnalytical Chemistry, AIMMS Amsterdam Institute of Molecular and Life Sciences, Vrije Universiteit Amsterdam, [4]Bruker Daltonics GmbH & Co. KG, [5]Bruker Daltonics GmbH & Co. KG

INTRODUCTION

Molecular diagnostics is on the verge of implementing high-throughput functional Omics data in routine clinical practice for high-precision personalized healthcare. Glycoproteomics in blood plasma offers unique possibilities for clinical diagnostics. Both biomarker discovery and diagnostics can be performed using the same PASEF-DDA data. Here, we will share results for diagnosis of Congenital Disorders of Glycosylation (CDG) obtained via optimized PASEF-DDA methods for untargeted biomarker discovery and target biomarker measurement.

METHODS

Patient and control plasma samples were digested (trypsin) and enriched for glycopeptides (Sepharose). Glycopeptides were separated with gradient times of 15min. Eluting peptides were analyzed by DDA-PASEF on a timsTOF Pro 2 with tuning optimized for glycopeptide analysis. Raw data were processed with MS Fragger and glycopeptides of TRFE, IGHG1 and IGHG2 were selected for subsequent targeted data extraction using Skyline based on signal intensities and subsequently processed into glycoform and glycan trait fractions (%) for downstream analyses.

RESULTS

100 healthy individuals and 150 CDG patient samples with primary defects in the N-glycosylation pathway were analyzed. A target list of 217 TRFE, IGHG1 and IGHG2 glycopeptide precursors which include the vast majority of glycoforms required for CDG diagnosis was generated to extract signal intensities and subsequent glycoform and glycan trait fractions. Shifts in glycoform distributions could be explained by the genetic defect underlying glycobiology changes: e.g. loss of fucosylated glycans (SLC35C1 GDP-fucose transporter deficiency), increase in galactose lacking glycans (B4GALT1 galactosyl transferase deficiency), or increase in hybrid and high-mannose glycans (MAN1B1 mannosidase deficiency). Furthermore, the diagnostic performance of this method is compared to the current CDG diagnostics standard that is based on immunocaptured transferrin glycoform profiling by intact protein mass spectrometry.

CONCLUSION

Based on obtained results, this glycopeptide technology will be implemented in routine patientcare for CDG patients at the Radboud university medical center.

PP01.013: Identification of T Cell Epitopes for Cancer Immunotherapy Approaches using Data-Independent Acquisition Immunopeptidomics

Becker J[1,2], Förster J[1,2,4], Foehr S[1,2], Helm D[3], Riemer A[1,2]
[1]Division of Immunotherapy & Immunoprevention, German Cancer Research Center (DKFZ), [2]Molecular Vaccine Design, German Center for Infection Research (DZIF), [3]Proteomics Core Facility, German Cancer Research Center (DKFZ), [4]Faculty of Biosciences, Heidelberg University

Introduction

Presentation of tumor-specific peptides by HLA class I molecules to CD8+ T cells is the foundation of epitope-centric cancer immunotherapies such as therapeutic vaccines or TCR-transgenic T cells. Currently, the only available technique to provide a direct proof of actual peptide presentation at the cell surface and thus of actionable targets is mass spectrometry-based immunopeptidomics.

Methods

For the establishment of a DIA methodology, we investigated the HPV16+ cervix carcinoma cell line CaSki. HLA class I-presented peptides were purified by high-throughput immunoprecipitation optimized for the recovery of Cysteine-containing peptides, which are often neglected in immunopeptidomics. Next generation sequencing data was used to enable the identification of mutation-derived neoepitopes. MS raw data was acquired on an Orbitrap Exploris 480 connected to an Ultimate3000 RSLCnano system equipped with a FAIMS module and using an optimized DIA scheme. The recorded data was analyzed using the Spectronaut software combining library generation directly from DIA data, in silico prediction of fragment spectra using PROSIT and library generation from synthetic peptide data.

Results

Using the established workflow, we reliably identify more than 9000 unique HLA class I-presented peptides from a single injection corresponding to as little as 25 million cells. The use of our reduction and alkylation protocol led to a more than 10-fold increase in identifications of Cysteine-containing peptides. Additionally, combining library generation from DIA data for wildtype peptides with in silico predicted and/or synthetic peptide libraries boosts the number of identified tumor-specific mutation- and virus-derived epitopes when compared to DDA analysis of the same sample.

Conclusions

We established a high-throughput immunopeptidomics workflow utilizing DIA mass spectrometry for the identification of mutation- and virus-derived epitopes. In the future, the described workflow will be applied to the analysis of clinical samples to identify actionable targets for the development of epitope-centric cancer immunotherapies.

PP01.014: A Classification Model for Peptides with the Potential to Trigger Coeliac Disease

Escobar-Correas S[1,2], Gardner B[1], Broadbent J[1], Juhasz A[2], Colgrave M[1,2]
[1]CSIRO, [2]Edith Cowan University

Coeliac disease (CD) is a condition in which an autoimmune and inflammatory reaction arises from the ingestion of gluten proteins from cereal grains. It is critical to provide food safety for these people through the detection of gluten in established foods as well as determination of candidate immune-reactive peptides that could cause illness in emerging cereal grain and crop weeds. The identification of potentially immunogenic peptides requires a series of time-consuming steps. Accordingly, we recognized the opportunity to develop an accurate classification tool that can identify candidate CD peptides.

Herein, a peptide database (positive training set) was constructed of CD immune reactive epitopes using IEDB (Immune Epitope Database). A negative training set was built with non-reactive peptides. The optimal peptide length was defined as 9-33 amino acids, where the core was 9 amino acids. A four-step processing method was performed on the sets: 1-Deamidation: for each epitope we created a subset of peptides with the deamidated versions; 2- Search for the core region: for each epitope, a sliding window of 9 amino acids was used to iterate the whole peptide length with each iteration scored according to CD epitopes characteristic from the literature to select the best core; 3- Calculate physicochemical properties using Biopython for each of the core generated in step 2; 4- A classification model was created using XG boost-gradient booster machine in Python.

The model was tested using ryegrass — a recently discovered grass as a source of gluten-like proteins. Discovery proteomics revealed 17 gluten-like proteins. Using the classification model, a list of possible reactive peptides was created. The ten best candidate peptides (score >90%) were selected for the next stage: peptide synthesis and immune assay. The classification model reduced the time and cost of analysis in searching for new epitopes.

PP01.015: Identification and Validation the of HLA-I Proteasomal Spliced Epitopes by Immunopeptidomics

Faridi P[1]
[1]Monash University

Antigen recognition by CD8+ T cells is primarily governed by the pool of peptide antigens presented on the cell surface in the context of Human Leukocyte Antigens class I (HLA-I) complexes. Recent studies have shown that a fraction of all presented peptides are generated through proteasome-mediated splicing of non-contiguous regions of proteins to form novel peptide antigens. Even though there are many debates on the proportion of spliced peptides in HLA-I immunopeptidomics, accumulating data show their presence and contribution to immune recognition of tumours, viral infection and self-recognition in auto-immune diseases.

I will highlight recent advances in identifying spliced by immunopeptidomics and bioinformatics workflows. I will discuss different methods for validating the authenticity of identified sequence and source antigen(s) for spliced peptides and evaluation of the immunogenicity of identified spliced peptides. I will also introduce novel spliced peptides derived from tumour-associated antigens that are shared across different types of tumours. We identified these spliced peptides by immunopeptidomics analysis of nine different HLA-A*02:01patient derived cell lines with different kinds of cancer such as melanoma, glioblastoma, triple-negative breast cancer and colorectal cancer. We validated the presence of this set of spliced peptides on patients' tumour biopsies and confirmed their immunogenicity by using peripheral blood mononuclear cells from patients and healthy donors. Of note, a set of these spliced peptides are currently in Phase-I clinical trial for melanoma vaccination.

PP01.016: Targeted Detection of Ultra-low Abundance Human Papillomavirus Epitopes for Therapeutic Vaccine Development by Parallel Reaction Monitoring

Förster J[1,2,3], Salek M[1,2], Vuckovic N[1,2,3], Föhr S[1,2], Bonsack M[1,2], Riemer A[1,2]
[1]Division of Immunotherapy & Immunoprevention, German Cancer Research Center (DKFZ), [2]Molecular Vaccine Design, German Center for Infection Research (DZIF), [3]Faculty of Biosciences, Heidelberg University

Introduction

High-risk types of human papillomavirus (HPV), including HPV16 and HPV18, cause more than 600,000 anogenital and oropharyngeal cancer cases worldwide per year. While prognosis for HPV-driven cancers in the early stages is favorable, late stages are often resistant to standard therapy. For the development of a therapeutic vaccine against HPV16, we aim to identify viral epitopes of the oncoproteins E6 and E7 that are bona fide presented on the surface of HPV16-transformed cells using a highly sensitive targeted immunopeptidomics assay.

Methods

Synthetic peptides were used to acquire peptide LC-MS characteristics including fragmentation response to a range of collision energies, permissive FAIMS CV, LC retention time and dominant precursor charge state. This enabled fine-tuning of PRM acquisition on the Orbitrap Exploris 480 mass spectrometer to each precursor, allowing us to far exceed the sensitivity of untargeted approaches. Peptide alkylation and oxidation during the assay was implemented to ensure sensitive detection of all peptides containing cysteine and methionine, respectively. A high quality spectral library and stringent detection criteria are then used to validate epitope detection.

Results

In preparation of the assay, we characterized an extensive set of close to 250 possible HPV16 epitopes. The peptides were targeted on various HPV16-transformed cell lines of different HLA backgrounds and malignancies, leading to the validation of a range of new epitopes covering a set of crucial HLA types.

Conclusion

The extensive characterization of all targets revealed the need for tuning of collision energies per precursor beyond basic normalization. The targeted approach allowed for detection of several peptides based on trace-level signal that is unattainable for untargeted methods. The definition of validated HPV16 E6- and E7-derived epitopes establishes a HPV16 epitope repertoire and will contribute to the development of a therapeutic HPV16 vaccine as a treatment option for persistent HPV16 infections and HPV16-driven malignancies.

PP01.017: Peaks Online DeepNovo Peptidome: A Comprehensive Workflow for Immunopeptidomic Analysis

Hoffman K[1]
[1]Bioinformatics Solutions Inc

Introduction

Immunopeptidomics is the study of the repertoire of peptides displayed by human leukocyte antigen (HLA) proteins as part of the adaptive immune response and is promising for cancer vaccine development. This involves multiple software tools for database searching, sorting/filtering peptides, and predicting HLA binding affinity and immunogenicity, which is time consuming and computationally intensive. Here, we developed a tool for streamlined immunopeptidomic analysis that uses deep learning technology to improve the accuracy and sensitivity for HLA peptide identification. The platform is implemented with high-performance computing technology, allowing high-throughput analysis for clinical applications.

Methods

Samples were collected from Tongji Medical College of Huazhong University of Scoience and Technology. Peptides were isolated following methods described in Pandey et al. (2021) and analyzed on a ThermoFisher Orbitrap Eclipse instrument. Data analysis was done using Peaks Online DeepNovo Peptidome workflow. Precursor and fragment ion mass error tolerances were 10 PPM and 0.02 Da, respectively. Oxidation of methionine and N-terminal acetylation were set as variable modifications. Unique peptides 8-12 amino acids in length and passing a peptide FDR of 1% were considered.

Results

Over 3000 peptides per sample were identified in only 20% of the time taken for MS experiments, removing computational bottlenecks. We improved the accuracy of HLA peptide predictions directly from de novo sequencing results by up to 10% when implementing an FDR estimation. A homology-based search in DeepNovo Peptidome workflow revealed mutated peptides representing potential neoantigens. A peptide list categorized by identification type and ranked by HLA binding affinity is easily exported for downstream immunogenicity experiments.

Conclusion

Taken together, DeepNovo Peptidome workflow is an efficient and comprehensive solution for HLA peptide identification from LC-MS/MS data, providing a tool to help in the development of peptide-based cancer vaccines.

PP01.018: Class II Presentation at the Mucosal Surface of the Gut

Holiar V[1], Novogroder N[1], Gradchenko P[1], Levin Y[2], Samuels Y[3], Biton M[1]
[1]Department of Immunology and Regenerative Biology, The Weizmann Institute Of Science, [2]The de Botton Institute for Protein Profiling, The Nancy and Stephen Grand Israel National Center for Personalized Medicine, Weizmann Institute of Science, [3]Department of Molecular Cell Biology, Weizmann Institute of Science

Introduction

The gut is one of the largest interfaces of our body with the outside world. As such, intestinal epithelial cells (IECs) and immune cells are needed to maintain a delicate balance between immune activation and tolerance to various foreign substances. The major histocompatibility complex (MHC) presentation pathway is an integral part of this process that recognizes pathogens and establishes tolerance to harmless agents. However, disruption of the MHC II presentation pathway may lead to different intestinal pathologies, including autoimmunity or cancer. IECs and immune cells express MHC II and interact with CD4+ T cells in an antigen-specific manner. Nevertheless, gut MHC II-associated antigen identity and diversity were not characterized to date. Therefore, we aimed to explore the epithelial and immune MHC II-associated antigen repertoire of the intestine under homeostasis and inflammation.

Methods

Using liquid chromatography coupled to high-resolution mass spectrometry (LC-MS/MS), we analyzed the MHC II-bound peptides presented by intestinal IECs and lamina propria (LP) professional antigen-presenting cells (APCs).

Results

We discovered a broad range of self-antigens originating from proteins from both extra- and intracellular compartments. The immunopeptide repertoire of both IECs and LP immune cells reflected their anatomical location as well as the molecular characteristics of these cells. LP immune cells were confirmed to present antigens of epithelial origin which may play a role in establishing peripheral tolerance. Additionally, we identified IEC- and LP immune cell-specific peptides of commensal bacteria with immune-modulating potential.

Conclusions

Characterization of the gut MHC II-associated peptidome can shed light on the regulation of immune activation versus tolerance in the intestine. Understanding the antigen-presentation capabilities of the intestine may lead to the development of novel therapeutic strategies aimed to treat gut-related pathologies.

PP01.019: FAIMS Improves Identification of MHC-II Tumor Neoantigens and Enables Physiological-Level Detection

Meng W[1], Schreiber R[1,2], Lichti C[1,2]
[1]Department of Pathology and Immunology, [2]Bursky Center for Human Immunology and Immunotherapy Programs

Introduction

Recently, FAIMS was shown to improve the detection of MHC-I tumor neoantigens, but a similar in-depth profiling of the MHC-II immunopeptidome has not been reported to date. Herein, using a T3 mouse sarcoma cell line carrying the well-defined MHC-II neoantigen mutant integrin beta-1 (mItgb1), we report our detailed MHC-II FAIMS optimization and demonstrate that FAIMS improves MHC-II neoantigen identification at physiologically relevant levels.

Methods

I-Ab peptides from T3-CIITA cells were analyzed by nLC-MS/MS on an Orbitrap Fusion Lumos equipped with a FAIMS-Pro interface (Thermo Fisher Scientific). Samples were analyzed without FAIMS and at compensation voltages CVs ranging from -40V to -85V. Data files were searched against a Uniprot/SwissProt mouse database appended with predicted T3 class II neoantigen sequences, using PEAKS Studio Xpro (BSI). Deamidation (NQ) and oxidation M were set as variable modifications, with tolerances of 10 ppm and 0.02 Da at the parent and fragment ion levels, respectively. FDR calculation was enabled, and peptides were filtered at a -10lgP score of 15.

Results

Application of FAIMS CVs from -40V to -85V revealed that mItgb1 was detected as doubly charged 14mer, 16mer and 17mer at -40V, while triply charged 17mer and 18mer were detected at -65V. Without FAIMS, all were detected as doubly charged peptides. For all, an approximate twofold improvement in signal was seen with FAIMS. Using the optimal CVs determined for our system -40V, -65V, a dilution series was performed to elucidate the lower limit of detection. By DDA, mItgb1 could be reliably identified in peptides eluted from 45 million cells. By PRM, the lower limit of detection was further improved tenfold.

Conclusions

We demonstrated that optimal FAIMS CVs improve detection of tumor-specific MHC-II neoantigens at physiologically relevant levels. This could be particularly meaningful when working with scarce clinical samples.

(References optional)

PP01.020: Analysis of HLA-DR Peptide Repertoires Unveils Novel T-cell Epitopes in Rheumatoid Arthritis

Maggi J[1,2], Carrascal M[1], Soto L[2,3], Neira O[4], Cuéllar M[4], Aravena O[2], Abian J[1], Jaraquemada D[5], Catalán D[2], Aguillón J[2]

[1]Biological and Environmental Proteomics Group, Institute of Biomedical Research of Barcelona (IIBB) - CSIC, [2]Immune Regulation and Tolerance Research Group, Universidad de Chile, [3]Hospital Clínico de la Universidad de Chile, [4]Hospital del Salvador, [5]Immunology Unit, Universidad Autónoma de Barcelona

Introduction: Rheumatoid arthritis (RA) is a chronic autoimmune disease, triggered and sustained by autoreactive CD4+ T cells recognizing self-peptides (epitopes) presented by human leukocyte antigen (HLA)-class II molecules disposed on the surface of antigen-presenting cells (APCs). In this work we aimed to identify HLA-DR-naturally presented self-epitopes recognized by CD4+ T cells from RA patients. Methods: HLA-DR/peptide complexes were isolated from RA synovial tissue (ST; n=3), as well as from monocyte-derived dendritic cells carrying RA-susceptible HLA-DR molecules, pulsed with synovial fluid (n=7) or ST (n=2). Peptide sequencing was performed by mass spectrometry, and the immunostimulatory capacity of selected peptides was evaluated on peripheral blood mononuclear cells from RA patients (n=29) and healthy controls (HC; n=12) by flow cytometry. Results: Between 103 and 888 peptides were identified per sample. We selected 37 native and six citrullinated (cit)-peptides for stimulation assays. Six of these peptides increased the expression of CD40L on RA CD4+ T cells, and specifically triggered IFN-γ production on RA CD4+ T cells compared to HC. Finally, both the frequency of IFN-γ-producing CD4+ T cells specific for a myeloperoxidase-derived peptide and TNF-α-producing CD4+ T cells specific for a histone H4-derived peptide, were correlated with disease activity. Conclusions: We expanded the immunopeptidome presented by HLA-DR molecules in a physiologically relevant context, identifying six new epitopes recognized by RA CD4+ T cells. This information is essential for a better understanding of the disease immunopathology, as well as for designing tolerizing antigen-specific immunotherapies. Funding: Fondecyt 1181853, Redes 180028, ANID-PFCHA/DoctoradoNacional/2018-21181538.

PP01.021: An Effort to Catalog the Cancer Driver Mutation-carrying Neoantigens for the Frontier of Immunotherapy

Minegishi Y[1], Kiyotani K[1], Nagayama S[1,2], Ueda K[1]
[1]Japanese Foundation For Cancer Research, [2]Uji-Tokusyukai Medical Center

Introduction: With the rapid progress in cancer immunotherapy such as the new generation of CART-Ts and the bispecific antibodies, the information about optimal antigens to benefit by these therapies is required never before. Identifying the presented antigens by mass spectrometry (MS), called immunopeptidomics, is currently the sole approach in gaining that knowledge. While, it has been a longtime challenge to improve the identification efficiency of neoantigens especially from limited amount of samples, such as endoscopic biopsy from patients. We have introduced differential ion mobility (DIM)-MS in immunopeptidomics analyses to overcome that task. Here, we show our latest results to catalog the shared neoantigens that carry major cancer driver mutations for upcoming cancer immunotherapy by exploring the immunopeptidomes from various samples including clinical tissues.

Methods: Immunopeptide samples were prepared by immunopurification by W6/32 antibody. For cell line samples, we used 1e8 cells per analysis. For tissue samples, we used around 40 mg of tissue per analysis. DIM-based immunopeptidomics was conducted by Orbitrap Fusion Lumos Tribrid Mass Spectrometer with FAIMS-Pro interface. By this DIM-based immunopeptidomics, we screened actually-presented neoantigens that carry cancer driver mutations.

Results: Among immunopeptidome of total 44,815 unique sequences from tumor tissues of colorectal cancer (n = 17), private neoantigens including oncogenic KRAS (G12V)-carrying neoantigen were identified. This implies many of neoantigens with oncogenic mutations have yet to been identified. We also analyzed the cancer cell lines that have known to carry cancer driver mutations and found that the different sequences of oncogenic KRAS-carrying neoantigens were presented by those cell lines. These neoantigens were further confirmed as true identification by targeted-MS approach with synthetic peptides.

Conclusions: These results show that immunopeptidomics by DIM-MS is of use to screen the shared neoantigens carrying oncogenic mutations from various materials to catalog the actually-presentable neoantigens for emerging cancer immunotherapies.

PP01.022: DIA Analysis with Variable Isolation Windows for Multiple Drug Targets Detection in Lung Cancer

Chiu H[1,2], Chan H[1,2], Chen L[1,2], Hsu H[1], Chen Y[1,2]
[1]Institute of Chemistry, Academia Sinica, [2]Department of Chemistry, National Taiwan University

Introduction: Currently, molecular genotyping and immunohistochemistry are served as gold standard tools to determine treatment decisions for cancer patients. However, most targeted therapy is designed based on the overexpression or mutation of protein. Due to the low correlation between genomic and proteomic expression profiles, the DNA genotyping results can't truly predict the actual cancer phenotype. And the need for various antibodies in immunoassay restricts the throughput of multi-target analysis. Though mass spectrometry-based proteomics has demonstrated large-scale identification, protein-based detection remains to be further developed, we developed a high-sensitivity targeting DIA assay to multiplexed detection and quantification of the FDA-approved drug targets in lung cancer.

Methods: The workflow include membrane protein extraction and multi-protease digestion on lung cancer cell lines. To enhance the drug target detection, an isotope-based DIA assay was designed with variable window width to analyze FDA-approved lung cancer drug targets. Library-based DIA approach was used for protein identification. Isotopic peptide standards were used for protein quantification.

Result: Using PC9 lung cancer cells as a model, the preliminary results show the identification of 3525 proteins, including 14 (42%) FDA-approved druggable targets for lung cancer. Compared to conventional DIA, our approach significantly improved an average of 1.9-fold signal-to-noise ratio of the targeted peptides. Those targets are associated with abnormal cell proliferation such as PI3K-PKB/Akt and MAPK Pathways (e.g. EGFR, KRAS, and BRAF). Further, an average of 10 unique peptides per protein target was detected, which can be used for acquiring reliable quantitative information on the drug-targeted proteins. The quantitation experiment for different cell lines is ongoing.

Conclusion: These results demonstrated the potential to develop a sensitivity DIA protein assay to measure the drug target protein abundance. Further application on tumor tissues may provide precise information to correlate with drug response that will complement conventional gene testing and immunochemical assay.

PP01.023: Recombinant Expression, Modeling, and Epitope Prediction of two Allergens from Carya Lllinoinensis Pollen

Morales Amparano M[1], Pastor Palacios G[2], Cárdenas Conejo Y[3], Llamas Gárcia M[4], Lara González S[4], Ramos-Clamont Montfort G[1], Vázquez Moreno L[1], Vizuet de Rueda J[5], Terán Juárez L[5], Huerta Ocampo J[6]

[1]Centro De Investigación En Alimentación Y Desarrollo, A.c., [2]Instituto Politécnico Nacional, [3]Universidad de Colima, [4]Instituto Potosino de Investigación Científica y Tecnológica, [5]Instituto Nacional de Enfermedades Respiratorias, [6]CONACYT-CIAD A. C

Introduction: Tree pollen is the main source of aeroallergens; however, many have not been well characterized and others remain unexplored. Until recently, this was the case of Carya illinoinensis pollen, a species widely distributed in North America for its fruit, the pecan nut. Previously, our group reported the first identification of C. illinoinensis pollen allergens by immunoproteomics approach. In this work we selected two pecan tree pollen allergens and expressed them by recombinant means, their allergenicity was confirmed, and epitope prediction was performed by computational tools.

Methods: After synthesizing the nucleotide sequences of the two selected allergens, enolase and profilin, they were used to transform BL21(DE3)pLysS cells to overexpress them. After purification, their identity and allergenicity were confirmed by mass spectrometry and IgE Western blot, respectively. Allergen epitope prediction was performed by combining several bioinformatic tools for modeling, and ABCpred, BepiPred, Immunomedicine, and ElliPro to predict linear epitopes.

Results: Identity of both recombinant allergens was confirmed by LC-MSMS and bioinformatic analysis, whereas Western blot corroborated that the IgE of sensitive patients recognizes them. Linear epitopes composed of 5 to 25 sequential amino acid residues (4 for profilin and 14 for enolase) were predicted by a consensus of at least three of the four tools used.

Conclusions: We overexpressed the first two recombinant allergens of Carya illinoinensis pollen and obtained the prediction of 4 and 14 linear epitopes for profilin and enolase, respectively. These data are of relevance for areas of diagnosis and treatment of respiratory allergy. Revealing of epitopes in clinical significance, and previously unexplored allergenic sources opens the doors to the implementation of novel immunotherapeutic strategies.

PP01.024: First-in-class Inhibitors of ERAP1 have the Potential to be a Transformative Immunotherapy in Oncology

Patel M[1], Leishman A, Paes W, Reelfs O, Anderton K, Pinggera M, Sette J, Ternette N, Morgan J, Shiers J, Quibell M, Joyce P
[1]Grey Wolf Therapeutics Limited

Clinical data demonstrates neoantigen-based therapies have the potential to turn “cold” tumors into “hot” ones and their association with immune checkpoint inhibitors’ therapeutic responses in cancer patients. In addition, increased HLA heterozygosity and HLA evolutionary diversity are non-overlapping factors which further diversify the immunopeptidome and improve clinical response to checkpoint therapies. Endoplasmic reticulum aminopeptidase 1 (ERAP1) is an enzyme that trims peptides loaded into classical and nonclassical MHC Class I molecules. Grey Wolf Therapeutics have developed highly potent and selective ERAP1 inhibitors to directly alter tumour cells, generating entirely novel neoantigens to illuminate tumours and drive durable responses in cancer patients. These inhibitors demonstrate significant modulation of the cancer-related antigen repertoire across diverse ERAP1 and HLA genotypes and cancer-type backgrounds, both in vitro and in vivo. These changes in the antigen repertoire drive changes in T cell activation and response, leading to increased T cell infiltration into CT26 syngeneic tumors and T cell receptor (TCR) diversification when combined with anti-PD-1. Consistent peptide length changes in the immunopeptidome, caused by ERAP1 inhibition, is a proof of mechanism biomarker, whilst tumour immunohistochemisty, TCR repertoire analysis and RNA sequencing are potential proof of principle biomarkers that can all be translated into the clinic. These data provide the foundation from which we will be exploring the potential of our first-in-class ERAP1 inhibitor development candidate in the clinic in Q1 2023. In parallel, extensive assessment of a modular and extendable Bioinformatics framework for analyzing and integrating immunopeptidomes with different omics layers is ongoing.

PP01.025: Complementarity of Class I and II Neoantigen Mapping in MSI-high Colorectal Cancer in Needle Biopsy Size Tissue Samples

Shapiro I[1], Räss L[1], Tognetti M[1], Temu T[1], Bernhardt O[1], Bruderer R[1], Woodsmith J[2], Reiter L[1]
[1]Biognosys AG, [2]Indivumed GmbH

Introduction
Immunopeptides play an essential role in adaptive immunity by activating and ensuring T-cell specificity. Mass spectrometry is currently the only technology that can reliably measure and identify the immunopeptide profiles of biological samples at a large scale. However, studies are frequently limited by sample input and poor scalability. Here, we introduce a semi-automated workflow requiring low sample input to robustly identify immunopeptides from cultured cells and tissue samples and apply it to a cohort of colorectal cancer samples for immunopeptide profiling and neoantigen identification.
Methods
Native lysis and the immunoprecipitation workflow were optimized while ensuring scalability and reproducibility. 15 mg of fresh frozen tissue was processed in duplicates for sequential class-I/class-II immunopeptide enrichment. FAIMS Data-Independent-Acquisition was performed and supported by a high-pH-reversed-phase FAIMS Data-Dependent-Acquisition library. Data analysis was performed with SpectroMine and Spectronaut (Biognosys) with 1% FDR at the PSM, peptide and protein group level. Whole genome sequencing on both tumor and associated normal tissue was used for high-confidence somatic variation calling (Indivumed) and neoantigen definition.
Results
On average, we identified 9,767 class-I and 16,445 class-II immunopeptides. Class-II identification numbers were more variable than class-I, likely linked to immune infiltration levels. In total, we characterized 131,578 unique immunopeptides that map to 12,488 genes. We identified 16 class-I and 29 class-II neoantigens, covering 87% of the microsatellite instability-high (MSI-H) samples in the cohort. Coverage of both classes was essential to increase neoantigen discovery as 53% MSI-H samples had neoantigens mapping exclusively to either class-I/-II.
Conclusions
Overall, we have established a scalable, efficient pipeline for cell line and tissue immunopeptidomics for both class-I and II immunopeptides. Our pipeline generates high-quality identifications from minimal starting material and can be deployed to help shed light on immunopeptidomics heterogeneity through large-scale profiling of patients as exemplified in the case of MSI-H colorectal cancer.

PP01.026: Immunoproteomics Approach to Discover Immunogenic Infectious Virus Antigens for Vaccine Development and Diagnosis Platform

Yu J[1], Ahn H[2], Jeong yeon H[3], Oh Y[3], Kim K[1,2,3]
[1]Asan Institute for Life Sciences, Asan Medical Center, [2]Convergence Medicine Research Center, Asan Medical Center, [3]Department of Biomedical Sciences, University of Ulsan College of Medicine
Immunoproteomics with high resolution LC-MS platform have been one of effecvtive way to discover immunopeptide features from various viral infectious disease such as Severe fever with thrombocytopenia syndrome (SFTS) and COVID-19. Among many viral infectious diseas, SFTS is an emerging infectious disease in Asia area from 2013. The major clinical symptoms of SFTS are fever, vomiting, diarrhea, multiple organ failure, thrombocytopenia, leucopenia and elevated liver enzyme levels, showing its fatality rates ranging from 12% to as high as 30%. SFTS virus is a phlebovirus in the family of Bunyaviridae, and consist of 3 gene segments, large (L), medium (M) and small (S). And 6 proteins have been identified—an RNA dependent RNA polymerase (RdRp), a glycoprotein precursor (M), a glycoprotein N (Gn), a glycoprotein C (Gc), a nuclear protein (NP) and a non-structural protein (NSP). In this study, using immunoproteomics approach coupled with high resolution LC-MS platform, several highly immunogenic SFTSv antigens were discovered.
Those antigens are expected to be applied in vaccine development and rapid detection kit development. In this study, two kinds of immunoproteomics approaches were applied on serum and PBMC to discover SFTS virus antigens, one is humoral immunity-based one and the other is antigen presenting cell immunity-based way. The MHC bound SFTS viral antigen peptides were identified using high resolution LC-MS system using immunoprecipitation of MHC-peptide complex. The range of peptide length was from 12 to 14 amino acids.
We hope this immunoproteomic approach shows its possibility as a useful tool for antigen discovery for other infectious disease such as COVID-19.

PP01.027: A Bioinformatics Approach to Search for Microbial Proteins in Human Samples: Covid-19 as an Example.

Alnakli A[1], Heng B[1], Ranganathan S[3], Ahn C[1], Guillemin G[1], Mohamedali A[2]
[1]Macquarie Medical School, Macquarie University, [2] School of Natural Sciences, Macquarie University, [3]Applied Biosciences

Introduction: Mass spectrometry (MS) is one of the key technologies used in proteomics. The majority of studies carried out using proteomics have focused on identifying proteins in biological samples such as human plasma to pin down prognostic or diagnostic biomarkers associated with particular conditions or diseases. The aim of this study was to analyze microbial (viral and bacterial) proteins in healthy human plasma and in diseased samples.

Methods: MS data of healthy human plasma were searched against the complete proteomes of all available viruses and bacteria. With this baseline established, the same strategy was applied to characterize the protein profile of different SARS-CoV-2 disease stages in the plasma of patients. Two SARS-CoV-2 proteins were detected with high confidence and could serve as the early markers of SARS-CoV-2 infection. The complete bacterial and viral protein content in SARS-CoV-2 samples was compared for the different disease stages. The strategy used bioinformatics tools to facilitate the analysis of the large-size microbial databases so that the search can be conducted using affordable computational machines. Also, the automation of the data filtering and cleaning processes was also recently achieved to increase pipeline efficiency.

Results: the study identified 18 bacterial and 11 viral proteins aligning with the stringent guidelines laid out by the human proteome project (HPP) in healthy human plasma. On the other hand, the number of viral proteins was found to increase significantly with the progression of SARS-CoV-2 infection, at the expense of bacterial proteins. The strategy is now being applied to characterize the microbial proteome of other disorders.

Conclusions: This strategy has been implemented to aid in the development of early diagnostic and prognostic tests for infectious diseases based on microbial biomarkers in human tissue and plasma samples.

PP01.028: MetaLab-MAG: a Metaproteomic Data Analysis Platform for Species-Level Characterization of Microbiomes from the Metagenome-Assembled Genomes Database

Cheng K[1], Ning Z[1], Li L[1], Zhang X[1], Serrana J[1], Mayne J[1], Figeys D[1]
[1]University Of Ottawa

Introduction

The studies of microbial communities have drawn increased attention in various research fields such as agriculture, environment, and human health. Recently metaproteomics has become a powerful tool to interpret the roles of the community members by investigating the expressed proteins of the microbes. However, analyzing the metaproteomic datasets is still challenging because of the lack of proper bioinformatics tools. Here we develop MetaLab-MAG, a specially designed tool for the characterization of microbiomes from metagenome-assembled genomes databases.

Methods

We developed a complete metaproteomics data processing platform, termed MetaLab-MAG (metagenome-assembled genomes), which used the public available MAGs as the resource for peptide/protein identification and taxonomic/functional annotation. Currently four MAGs databases in MGnify including Cow Rumen v1.0, Unified Human Gastrointestinal Genome (UHGG) v2.0, Human Oral v1.0, and Marine v1.0 were supported. To accelerate and improve the data processing, a high-abundant protein (HAP) database searching strategy was utilized to generate the sample-specific database. MetaLab-MAG was not only used for qualitative analysis, both label-free and isobaric labeling-based quantification strategies were supported to provide the researchers with accurate quantitative information.

Results

We evaluate MetaLab-MAG by analyzing various human gut microbiota datasets and find that the performance is comparable to or better than searching the sequencing read-based protein database directly. The reliability of the results had been confirmed by comparing the data from searching the reference or multi-omics database. The identified peptides and proteins could be attributed to corresponding genomes directly, which made the taxonomic assignment more precise. The exported data tables of peptides, proteins, genomes, taxa, and functions with quantitative information were ready for various kinds of post-analysis.

Conclusions

MetaLab-MAG removes the obstacles of metaproteomic data analysis and provides the users with in-depth and comprehensive information from the microbiomes. We believed that MetaLab-MAG could help researchers from various fields using metaproteomic to investigate microbiomes.

PP01.029: Applying 16s Sequencing, in-Silico Spectral Library Generation and SWATH for Deep Clinical Metaproteomic Analysis

Ganief T[1], Radzey N[1], Abrahams A[1], Edupuganti L[3], Buck G[3], Edwards D[3], Smit J[4], Strauss J[3], Ahmed K[5,6], Beksinska M[4], Serrano M[3], Bailey V[5], Morrison C[2], Deese J[7], Masson L[1,8], Blackburn J[1]
[1]Institute Of Infectious Disease And Molecular Medicine, [2]FHI 360, [3]Virginia Commonwealth University, [4]MatCH Research Unit (MRU), Department of Obstetrics and Gynaecolgy, University of the Witwatersrand, [5]Setshaba Research Centre, [6]Department of Medical Microbiology, University of Pretoria, [7]RTI International, Research Triangle Park, [8]Life Sciences Discipline, Burnet Institute

Introduction

As the role of microbiomes in human health and disease has been highlighted in recent years, interest in their analysis has significantly increased. While DNA sequencing approaches remain the gold standard for microbiome taxonomic identification, metaproteomics is a powerful tool for understanding microbiome functionality. However, unlike typical proteomics workflows, the taxonomic composition of microbiome samples are often unknown and, therefore, determining the database composition for spectral matching is challenging. Many popular metaproteomic database search methods have been introduced in recent years, many of which rely on two or more successive, dependent searches starting with a large database. Here, we present a 16s curated, machine learning database construction and short SWATH analysis for deeper metaproteome coverage.

Methods

Digested host and microbial proteins recovered from vaginal swabs were separated using the pre-programmed 40SPD EvoSep one method and analyzed on a SCIEX 6600 triple-TOF mass spectrometer in SWATH mode and a Q-exactive mass spectrometer using typical DDA methodology. DDA data were searched with MaxQuant using a custom MetaNovo database or 16s curated databases. The SWATH data was searched against a 16s curated, machine-learning generated spectral library in DIA-NN.

Results

We compared three database search approaches using data generated on two distinct mass spectrometry platforms. SWATH allowed >5X deeper peptide identification per sample compared to Q-exactive DDA data in the same analysis time and increased data completeness significantly. SWATH analysis using a 16s curated database further allowed for more accurate taxonomic abundance estimation.

Conclusions

A 16s curated machine learning generated spectral library allows for deep and rapid metaproteomic analysis using SWATH. The analysis depth further facilitates more accurate taxonomic composition and abundance determination. Most importantly, SWATH facilitates more detailed functional metaproteomic analyses enabled by the deeper, more complete curated dataset in less than 30 minutes of analysis time per sample.

PP01.030: The Correlation Between Human Gut Microbiome, BMI, and Cardiovascular Disease. A Mass Spectrometry-based Proteomics Study.

Hakimi A[1], Schauer K[1], Renuse S[1], Brown K[2], Seitz V[3], Broeckling C[2], Prenni J[2], Lopez-Ferrer D[1]
[1]Thermo Fisher Scientific, [2]Colorado State University, [3]Department of Horticulture and Landscape Architecture, Colorado State University

Introduction

There is a growing appreciation for the role of microbes and lifestyle in controlling the manifestation of different diseases including atherosclerotic cardiovascular disease (ACVD) which is the leading cause of death globally with a disproportionate effect on women, a historically understudied population in medicine. Here we investigate the correlation between the gut microbiome and the BMI of 23 female patients using an end-to-end proteomics workflow including the AccelerOme™ system, a newly developed nLC column with extensive peak capacity on a Vanquish Neo UHPLC system hyphenated to a High-Field Asymmetric Waveform Ion Mobility Spectrometry (FAIMSTM) on a Thermo ScientificTM Orbitrap EclipseTM TribridTM mass spectrometer to increase the reproducibility and maximizes the proteome coverage of microbiome samples with high protein diversity.

Methods

Proteins were extracted from 23 human fecal samples and prepared for mass spectrometry analysis using the Accelerome platform. 1µg of peptides were separated using an Easy-Spray™ PepMap™ Neo column on a Vanquish Neo UHPLC system coupled to an Orbitrap Eclipse Tribrid MS with FAIMS. The final FAIMS MS method was set to switch between different CVs with a top-speed method in a 3-second total cycle time over a 136 minutes gradient. The raw files were processed using Proteome Discoverer 3.0 utilizing, Sequest HT, and INFERYS re-scoring algorithm.

Results

Using a complete end-to-end workflow enables researchers to focus more time on the research and less on instrument optimization. Using the above workflow we identified up to 10000 proteins and more than 33000 peptides from different patients. This data represents a preliminary analysis of the protein level variation resulting from varying gut microbial communities of women with different BMI statuses. Future work will link this proteomics data to the previously generated metagenomic, metabolite, and 16S data using the same samples to better resolve microbial contributions to cardiovascular disease in women.

PP01.031: Data-independent Acquisition Mass Spectrometry for Metaproteomics of the Human Intestinal Microbiota

Henry C[1], Berland M[2], Vitali G[2], Plaza Oñate F[2], Langella O[3], Millan-Oropeza A[1], Sokol H[4,5,6], Juste C[6]
[1]Université Paris-Saclay, INRAE, AgroParisTech, PAPPSO, [2] Université Paris-Saclay, INRAE, MGP, [3]Université Paris-Saclay, INRAE, CNRS, AgroParisTech, GQE - Le Moulon, PAPPSO, [4]Sorbonne Université, INSERM, Centre de Recherche Saint-Antoine, CRSA, AP-HP, Saint Antoine Hospital, Gastroenterology department, [5]Paris Center for Microbiome Medicine (PaCeMM) FHU, [6] Université Paris-Saclay, INRAE, AgroParisTech, Micalis Institute

Introduction : Metaproteomics of intestinal microbial communities is the necessary complement of metagenomics to reveal the functions that are really expressed in health and disease, discover predictive/prognostic markers, and move towards modulations of the microbiota as curative or preventive measures. To date, the preferred approach in a handful of labs over the world, has been data-dependent mass spectrometry (DDA-MS), which however cannot capture the tremendous complexity of the microbiome in a single run. Here we present outcome results focused on data-independent acquisition mass spectrometry (DIA-MS), for a more in-depth analysis of human gut metaproteomes, exemplified by the MICI-Pep cohort (20 individual envelope-enriched metaproteomes from well-characterized intestinal bowel inflammation phenotypes).
Methods : The direct DIA (without creating a spectral library acquired in DDA, which is time consuming and limited to peptides identified by DDA) was assessed using the timsTOF Pro mass spectrometer (Bruker). Data were analysed in a library-free approach and with MetaprotR. For comparison, DDA-MS identifications were achieved by iterative interrogation of the gut metagenomic database IGC2 (10.4 millions of genes annotated using X!Tandem, proteins were grouped with I2MassChroQ (Langella et al. 2017), XIC were quantified with MassChroQ (Valot et al. 2011) and data were analysed with MetaprotR (http://pappso.inrae.fr/).
Results : In comparison with classical DDA, DIA allowed a deeper quantitative insight into the taxonomy and functional features of the microbiome with a high reproducibility, and allowed us to extract new peptide and protein signatures of disease states.
Conclusion : In addition to bringing unprecedented insights into the functions truly expressed by the intestinal microbiome, this approach makes it possible to discover variables or cluster of variables that efficiently classify patients into clinical groups. If validated for their predictive value in at risk patients through targeted proteomics, they could be used as new biomarkers, targets or therapeutic molecules.

PP01.032: Histone PTM Profiling Of Human Heart Failure Biopsies Reveals H3K27ac-mediated Metabolic Dysfunction

Yoo E[1,2], Guo A[2], Kim J[1], Hahn V[2], Graff S[1], Aguilan J[1], Cutler R[1], Jani V[2], Demianova Z[3], Sharma K[2], Kass D[2], Sidoli S[1]
[1]Albert Einstein College of Medicine, [2]Johns Hopkins Hospital, [3]Preomics

Introduction

The Heart Failure with Preserved Ejection Fraction (HFpEF) syndrome is a disease that represents the greatest unmet therapeutic need in cardiology given its intimate relationship to obesity and metabolic syndrome. Using a multi-proteomics approach, we investigate the hypothesis that obesity alters histone PTMs such as H3K27ac, driving aberrant gene expression programs that regulate myocardial metabolism and worsen HFpEF.

Methods

Endomyocardial biopsies were prospectively obtained from human HFpEF patients (n=32) and donor controls (n=12). We performed metabolomics, proteomics, and histone PTM analysis. ChIP-seq analysis was performed to determine gene promoters targets of H3K27ac. Small molecule histone PTM modulators were screened in vitro using phosphoproteomics to identify therapeutic targets in cardiomyocytes. iST-NHS kits and TiO2 phosphoenrichment strategies were used for all sample preps.

Results

5,000 proteins were identified from each 300 ug biopsy. Pathway analysis revealed suppression of the TCA cycle consistent with our metabolomics data. We observed downregulation of oxidative phosphorylation and the electron transport chain. Histone PTM profiling revealed a 16-fold upregulation of H3K27ac ($p<0.001$) and significant upregulation of other active transcriptional markers like H3K36me3 and H3S28ph. H3K27ac ChIP-seq promoter gene ontology analysis revealed enrichment of RNA processing categories and genes related to fatty acid uptake, as well as major cardiac transcription factors (CD36, GATA4). Analysis of HFpEF-obesity animal models revealed that the L-NAME/HFD mouse had striking histone PTM similarity to human HFpEF (-77 integrated distance units, $p < 0.005$). Small-molecule inhibitor phosphoproteomics screens identified histone modulators that could potentially be therapeutic. Taken together, we have elucidated novel mechanisms by which metabolites modulate histone PTMs and gene expression in HFpEF and identified relevant animal models and small molecules for future studies.

Conclusions

A multi-omics approach reveals that H3K27ac is deposited as consequence of metabolic changes and regulates protein expression in human HFpEF.

PP01.033: Probability Based Taxonomic Profiling of Microbiome Samples using PepGM and Unipept

Holstein T[1,3,4], Verschaffelt P[2,3], Martens L[3,4], Muth T[1]
[1]Bundesanstalt für Materialforschung und - prüfung, [2]Ghent University, [3]VIB - UGent Center for Medical Biotechnology, VIB, [4]Department of Biomolecular Medicine, Ghent University

Introduction: Taxonomic inference in mass spectrometry-based metaproteomics is complex. The exact composition of metaproteomic samples is usually unknown, requiring large reference databases for peptide identification. The presence of proteins and corresponding taxa is then inferred from the identified peptides, which is complicated by protein homology: many proteins do not only share peptides within a single taxon but also between different taxa. Current taxonomic profiling approaches rely on strategies such as peptide-spectrum-match counting or the use of unique peptides . The metaproteomic analysis tool Unipept relies on a precomputed database of tryptic peptides and performs taxonomic identifications based on a threshold of peptide-taxon matches. While widely used, it does not provide confidence estimates for its taxonomic assignments.

Methods: We present PepGM, a graphical model that uses belief propagation to compute the marginal distributions of peptides and taxa. Developed for the probability-based taxonomic identification of viral samples against a large reference database background, we additionally extended it to the analysis of metaproteomic samples. Taxonomic information for peptides identified is now retrieved using the database provided by Unipept. As a multitude of taxa may be present, this streamlines the process of obtaining the peptide-taxon relationships required by PepGM. To address the complexity of the taxonomic composition of metaproteomes, results can be aggregated at different taxonomic levels.

Results: PepGM successfully classifies viral samples searched against unconstrained viral database references with strain-level resolution providing meaningful confidence estimates. Combined with Unipept, PepGM is additionally evaluated using a metaproteomic dataset, resulting in taxonomic profiles for metaproteomes with statistically computed confidence values.

Conclusion: PepGM enables a robust taxonomic analysis of metaproteomic samples supporting statistically sound inference of taxa in mass spectrometric datasets. This eliminates the need for error-prone heuristics, providing the added benefit of confidence estimates for taxonomic assignments.

PP01.034: MetaproDec: A New Algorithm to Appraise the Quantitative Composition of a Microbiome based upon Metaproteomes

Mu J[1], Xu H[1], Zhang Y[2], Deng Y[2], Yan K[2], Zhao Y[2], Liu S[2], Zhu G[2], Qi D[2]
[1]College of Life Sciences, University of Chinese Academy of Sciences, [2]Department of Proteomics, Beijing Genomics Instituion (BGI)-Shenzhen

Introduction: The traditional method of metaproteome for assessing the species population relies on the abundance addition of the corresponding annotated proteins derived from individual species, which likely results in a quantitative bias.
Methods: A new algorithm was proposed to address such problem. A test database was first generated that contained proteomes generated from the isolated bacterium or mixed bacteria, then a deconvolution model was developed to treat the metaproteome matrix. A total of 31 bacteria were obtained from cell culture. The samples of the sole bacterium or mixed bacteria according to certain proportions were treated by protein extraction and the protein were delivered to LC-MS/MS to identify the proteins.
Results: In total of 4,6147 proteins were identified and quantified in these bacteria. Based on the list of total unique proteins, maximum 50 ones per bacterium were selected as the bacterial features according to the abundance ranking in the certain bacterium. A matrix with bacteria and the corresponding protein features (31x50) was generated. And the protein features in an individual sample were picked up and regressed against the matrix of bacterium features. SVR as a core algorithm was employed with multiple optimizations of parameters to solve the matrices and to find appropriate regression coefficients. The pipeline consisting of matrix generation of bacteria features and SVR optimization thus is termed MetaproDec.
Conclusions: Comparison of the bacteria composition estimated by the traditional method and MetaproDec for the test database, the estimation errors were being increased responding to the diluted extent of a bacterium, the more dilutions, and the larger errors, regardless of which estimation method was taken. However, the error extent made by MetaproDec was significantly smaller than that derived from traditional way in most bacteria and varied dilutions. Therefore, MetaproDec was primarily proven acceptable in estimation of bacteria population in a microbiome.

PP01.035: Multi-omics Signature of Autism Spectral Disorder Reveals Novel Microbial Macromolecules

Osama A[1], Mostafa Anwar A[1], Ezzeldin S[1], Ali Ahmed E[1], Mahgoub S[1], Magdeldin S[1]
[1]Children Cancer Hospital In Egypt (CCHE-57357)

Introduction: Autism Spectrum Disorder (ASD) is a class of neurodevelopmental disorders that affects communication and behavior. Changes in gut microbiota have been implicated in the pathophysiology and cognitive capabilities of patients who have ASD. Yet, factors that mediate this interaction remain not fully understood.
Materials and methods: we analyzed 60 stool samples from severe autistic pediatric patients and healthy individuals. We used multi-omics platforms to characterize stool microbiota diversities, bacterial proteins (metaproteome), and possible altered metabolic pathways. At the metagenomic level, we demonstrated the microbiota's significant inequality between species abundances among autistic and control groups.
Results: At the meta-proteomic level, bacterial meta-proteins are associated with aberrant bidirectional host-microbiota crosstalk; especially the lpp protein showed to be highly abundant in severe autistic patients. Furthermore, we revealed some metabolic pathways that have a role in the three main pathways signaling pathway, lipid metabolism, and transporters.
Conclusion: Our findings support that profiling gut microbes has potential and promises to merit further study to develop tools to assess the possible role of the Microbiota-Gut-Brain-Axis in Autism Spectrum Disorder.

PP01.036: MS2Rescore: using Predicted Fragment Ion Intensities and Retention Times to Increase Identification Rates and Taxonomic Confidence in Metaproteomics

Van Den Bossche T[1,2], Gabriels R[1,2], Mesuere B[1,2], Muth T[3], Martens L[1,2]
[1]Ghent University, [2]VIB - UGent Center for Medical Biotechnology, [3]Section eScience (S.3), Federal Institute for Materials Research and Testing

Introduction

The field of metaproteomics, the study of the collective proteome of whole (microbial) ecosystems, has seen substantial growth over the past few years. Despite its high relevance, the field still suffers from low identification rates in comparison to single-species proteomics. The underlying challenge here is a lack of sequence resolution and statistical validation in the current identification algorithms, which are typically designed for single-species proteomics [1,2].

Methods

To solve this issue, we applied the machine learning-based MS²Rescore algorithm on several multi-species, metaproteomics datasets. In this (updated) version of MS²Rescore [3], the search engine-dependent features of Percolator [4] were replaced with MS2 peak intensity features by comparing the PSM with the corresponding MS²PIP-predicted spectrum [5]. In addition, features from DeepLC [6], a novel deep learning retention time predictor were added.

Results and Conclusion

When the updated MS²Rescore algorithm (with all features combined) is applied on metaproteomics datasets, our results show that MS²Rescore leads to an increased identification rate, while at the same time the false discovery rate (FDR) threshold can be lowered to 0.1% for large datasets. As a consequence, we eliminate most false positive taxonomic annotations, leading to a higher taxonomic confidence. The FDR at PSM level remains under control as validated in an entrapment experiment [7].

References

[1] Colaert, N.., et al. 2011. Journal of Proteome Research 10 (12): 5555–61.
[2] Muth, T. et al. 2015. Proteomics 15 (20): 3439–53.
[3] C. Silva, A.S., et al. 2019. Bioinformatics 35 (24): 5243–48.
[4] Käll, L. et al. 2007. Nature Methods 4 (11): 923–25.
[5] Gabriels, R., et al. 2019. Nucleic Acids Research 47 (W1): W295–99.
[6] Bouwmeester, R., et al. 2021. Nature Methods 18: 1363-1369.
[7] Vaudel, M., et al. 2012. Journal of Proteome Research 11 (10): 5065–71.

PP01.037: Next-generation Metaproteomics Data Analysis using Unipept Desktop

Verschaffelt P[1,2]
[1]Ghent University, [2]VIB - UGent Centre for Medical Biotechnology
Introduction: Metaproteomics is increasingly being used by researchers for different kinds of studies. Its possibilities are leveraged for complex environmental and biotechnological studies performed on, for example, ocean water and leaf litter, but also for clinical research concerning the human gut, urine or oral microbiomes. Over the last years, the size of samples that require downstream analysis has increased rapidly, increasing the gap between actual sample size and the size of samples that can easily be analyzed using the currently available software tools. Furthermore, protein reference databases, such as UniProt, are constantly increasing in size, increasing the risk of false-positive matches (and decreasing analysis accuracy).

Methods and Results: Unipept is an ecosystem of tools aimed at the taxonomic and functional analysis of metaproteomics samples. Recently, we developed the new Unipept Desktop application which provides support for the analysis of increasingly larger samples and the analysis of novel proteogenomics samples.

Proteogenomics is a novel research discipline in which a metagenomics experiment is performed prior to a metaproteomics experiment. The information derived from this metagenomics experiment is then leveraged to construct a custom, more-refined, protein reference database. In the next step of the analysis, this custom database will be used in the metaproteomics analysis experiment, which ideally yields better analysis results.

Conclusions: Unipept Desktop provides support for the analysis of larger metaproteomics samples and the construction of custom protein reference databases, making it the ideal candidate for the analysis of next-generation metaproteomics samples and a key tool for the further development of the proteogenomics research field.

PP01.038: Antibodies Good to go - the Aftermath of Adopting Enhanced Antibody Validation Strategies

Al-khalili Szigyarto C[1], Sivertsson Å[2], Von Feilitzen K[2], Uhlen M[2]
[1]KTH - Royal Institute of Technology, [2]Science for Life Laboratory, Division of Systems biology

Introduction

Researchers rely on experimental evidence when choosing antibodies as reagents for proteomics applications. Antibodies have to be thoroughly validated since their performance is application and context dependent. As the number of antibodies continuously increases, sharing experimental results that confirm or disprove their specificity constitutes a necessity in finding the right antibody for the right application. The Antibodypedia database was created as an open source repository of antibodies and a tool that allows scientists to compare and select the most appropriate antibody for a specific application.

Among the different strategies for enhanced validation of antibodies, designed by the International Working Group for Antibody Validation1, orthogonal validation allows confirmation of protein profiles across several samples obtained by antibody-based methods with those generated using a non-antibody based method. Consequently, Antibodypedia has developed tools to highlight and facilitate enhanced validation of antibodies by using existing transcriptomics datasets.

Methods

The Antibodypedia database has been developed to store gene expression datasets and display through the interface gene expression profiles for comparison with antibody validation results.

Results

Antibodypedia, currently comprises 4 567,265 reviewed antibodies and corresponding 2 173,660 validation experiments. Data regarding expression of all human genes, in 55 tissues and 69 cell lines is displayed enabling user-conducted orthogonal validation of antibodies. The ability of the antibodies to detect the intended target in human tissues and cell lines can be assessed by comparison with the gene expression profiles across the same biological samples. In addition the portal also allows filtration of antibodies with enhanced validation and side-by-side comparison of antibodies.

Conclusions

Although the number of antibodies with enhanced validation remains limited the developed tools promote enhanced orthogonal validation of antibodies. Antibodypedia has adopted the enhanced validation strategies to aid user in selection of validated antibodies.

1. Uhlen et al 2016, Nature Methods 13:823–827.

PP01.039: Systematic Validation of GPCR antibodies

Bendes A[1], Kotliar I[2,3], Dahl L[1], Dodig Crnković T[1], Uhlen M[1], Sakmar T[2,4], Schwenk J[1]
[1]Science for Life Laboratory, School of Engineering Sciences in Chemistry, Biotechnology and Health, KTH Royal Institute of Technology, [2]Laboratory of Chemical Biology and Signal Transduction, The Rockefeller University, [3]Tri-Institutional PhD Program in Chemical Biology, [4]Department of Neurobiology, Care Sciences and Society, Section for Neurogeriatrics, Karolinska Institutet

Introduction

Affinity reagents are essential tools for modern biomedicine with a usage spanning from simple assays to complex applications in clinical care. Despite this potential, their analytical performance and target selectivity can vary by application and sample preparation. Hence, there is a need to validate antibodies prior to drawing bioanalytical conclusions or deploying these as therapeutic agents. Proteins of particular interest are the membrane-bound G protein-coupled receptors (GPCRs): GPCRs are therapeutic targets with > 450 FDA-approved drugs acting at 108 unique family members [Hauser et al. (2017) Nat Rev Drug Discov]. However, the GPCR's hydrophobic nature, high sequence homology to closely related members, and multitude of partly unknown ligands make them difficult to study.

Method

To expand the use of antibodies for studying the biology of GPCRs, we scaled and applied our previously established concept [Lorenzen et al (2019) Sci Adv]. Our approach uses 215 GPCRs that are epitope-tagged, ectopically expressed, and detergent-solubilized in combination with 408 polyclonal antibodies [Uhlen et al (2015) Science] in multiplexed protein capture assays. To judge GPCR expression and antibody selectivity, we host the data in an interactive ShinyApp.

Results

After testing each antibody against its intended GPCR and other probable and over-expressed off-target GPCRs, we found 248 antibodies (61% of all) with purely on-target recognition of 154 unique GPCRs (72%). A subset of 13 antibodies co-recognized other homologous GPCRs, while 24 antibodies detected off-target GPCRs due to excessive protein expression. A minor fraction of 31 antibodies (8%) did only detect off-target GPCRs, and 42 GPCRs were not recognized by any of the used antibodies.

Conclusion

Our multiplexed and high-throughput method validated antibodies for the detection of solubilized GPCRs. This opens their utility to studying the biology of GPCRs in greater detail, which is of great interest for drug discovery.

PP01.040: Development and Characterization of Multi-Affinity Probes for Protein Identification by Short-epitope Mapping

Wilcox S[1], Anderson E[1], Bartnicki F[1], Boyce H[1], Burke K[1], Cunningham T[1], Devine J[1], Docouto K[1], Egertson J[1], Hoehn B[1], Ikwa E[1], Kashkina E[1], Leano J[1], Li C[1], Martinez A[1], McGinnis J[1], Nicastro J[1], Panakkadan J[1], Rabkin B[1], Ravuri S[1], Saini T[1], Sanford A[1], Schwientek P[1], Steiner N[1], Tonapi S[1], Villanueva J[1], Watts S[1], Patel S[1], Mallick P[1]
[1]Nautilus Biotechnology

Introduction: Obtaining biological insight from the majority of the 20,000+ proteins in the human proteome is difficult to achieve with most traditional proteomic analysis techniques. Methods are plagued by either technical challenges driven by the protein diversity in biological samples or the need to develop highly specific affinity reagents to every proteoform of interest. We present our approach to generating non-traditional multi-affinity binding reagents which enable decoding the human proteome without these limitations.

Methods: We have developed a diverse library of affinity binding reagents with high affinity and intentionally low specificity to bind short epitopes present across multiple proteins in a given sample. The platform used to detect the presence of these short epitope targets is agnostic to the affinity reagent scaffold, such that we can employ aptamers, antibodies, and less traditional affinity reagents. Selection targets are most commonly tripeptides, and we screen for candidates that ideally recognize between 5-50 biosimilars to the specified target. Once we have identified reagents with the appropriate specificity, we incorporate them into nanoparticles designed for detection on our platform and confirm performance using Protein Identification by Short-epitope Mapping (PrISM).

Results: We have developed nucleic acid and protein-based affinity reagents that bind short epitope targets with picomolar to nanomolar affinities. We have determined epitope profiles for these reagents in order to identify and quantify proteins they interact with, and have demonstrated the performance of these reagents using the PrISM approach. The ability of these reagents to recognize short epitopes across many proteins in a sample enables detection of >95% of the human proteome with as few as 300 reagents.

Conclusions: Diverse affinity reagents can be developed to bind short peptide epitopes. Characterization of the binding profiles and performance of these reagents for PrISM enables identification and quantification of far more human proteins than previously possible.

PP01.041: The Molecular Contribution of Doublecortin like Kinase 1 (DCLK1) to Gastric Cancer Progression.

Carli A[1,2], Afshar-Sterle S[1,2], Adnan N[1], Patel O[3], Lucet I[3], Ferguson F[4], Gray N[4], Faou P[2], Rajapaksha H[2], Steel J[5], Schittenhelm R[5], Ernst M[1,2], Buchert M[1,2]
[1]Olivia Newton-john Cancer Research Institute, [2]La Trobe University, [3]Walter and Eliza Hall Institute, [4]Dana-Faber Institute, [5]Monash University

Introduction: Gastric cancer (GC) is one of the most aggressive and 3rd in cancer related deaths worldwide. Since the cancer genome atlas molecularly profiled GCs many new promising targets have emerged for Eppstein-Barr virus positive, microsatellite-, and chromosomal instable GCs. Unfortunately, no new therapeutic targets have emerged for the genomic stable (GS) subtype, which has the lowest overall survival. However, Ser/Thr-protein kinase Doublecortin like kinase 1 (DCLK1) is significantly upregulated in GS GCs. DCLK1 is a microtubule associated protein family member, hence important for cellular shape, polarity, migration, mitosis, and vesicular transport. DCLK1 has been shown to promote epithelial-to-mesenchymal transition and inducing migration and invasion in many different solid cancers. Therefore, we aimed to investigate whether DCLK1 could be a potential therapeutic target for GS GCs and determine how molecularly DCLK1 contributes to gastric cancer progression and identifying kinase substrates.

Methods/Results: DCLK1 overexpression resulted in increased cellular protrusions and cell migration in vitro, which was reversed upon kinase inhibition with 1 μM small molecule inhibitor DCLK1-IN-1. Gene-ontology overrepresentation analysis of significantly differentially expressed total- and phospho-proteomics based mass spectrometry analysis revealed changes in RNA-processing, cell-cell adhesion, cell cycle processes, cellular matrix organization, chromatin organization, and vesicular transport upon DCLK1 overexpression or inhibition. Next we identified 91 potential interactors of DCLK1 wild-type, kinase dead, and DCLK1-IN-1 inhibitor using probabilistic scoring of affinity purification coupled to mass spectrometry (SAINT). Next we compared the interactome and phospho-proteome data and identified 22 overlapping potential DCLK1 kinase substrates. These 22 proteins are again involved in membrane trafficking, RNA polymerase II transcription, RNA processing, ribosome biogenesis, cytoskeletal-, mitotic spindle-, and chromosome organization.

Conclusion: Our comprehensive proteomics approach revealed novel reversible processes for DCLK1's contribution to cancer progression and identified potential kinase substrates. Together, this study establishes DCLK1 as a promising targetable regulator of GC.

PP01.042: Machine Learning Model to Predict the 3D Architecture of Protein Complexes.

Claussen E[1], Merlos C[1], Drew K[1]
[1]University of Illinois Chicago

INTRODUCTION

Protein complexes are essential to cellular function and when disrupted can cause disease. Knowledge of the 3D-structure of protein complexes illuminates their function and disease etiologies. Unfortunately, many of these 3D-structures are unsolved. Existing high-throughput proteomics methods, such as co-fractionation/mass spectrometry, allow us to determine co-complex interactions; however, it remains difficult to infer direct protein-protein contacts. We propose using machine learning to predict the direct contact networks of protein complexes, by mining high throughput mass spectrometry data. These direct contact networks coupled with AlphaFold2 structure prediction allow for the construction of 3D structural models.

METHODS

In order to implement this machine learning approach, AutoGluon, an automated machine learning tool, was used to generate the learned models. We used existing data from hu.Map 2.0 and Bioplex 3 to generate a feature matrix which informed the model. Our hu.Map 2.0(http://humap2.proteincomplexes.org/) is an existing human protein complex map created by integrating many large scale experiments including affinity purification/mass spectrometry (AP/MS), biochemical fractionation, proximity labeling, and RNA hairpin pulldown. Bioplex 3.0 is an updated AP/MS dataset. We used precision-recall analysis to evaluate model performance, and compare to previous methods for predicting direct contacts.

RESULTS

Compared to our previous methods(1) for predicting direct protein-protein contacts, our machine learning model is more accurate. In the high confidence regions of the precision-recall curve our learned model has a precision twice that of the previous method(approximately 0.8 vs 0.4).

CONCLUSION

High throughput proteomics data sets are critical in predicting the 3D-architecture of protein complexes. Our learned model can make direct contact predictions that will inform protein complex mapping endeavors. These structures will be vital in generating hypotheses related to disease research and drug discovery.

(1) DrewK, MüllerCL, BonneauR, MarcotteEM(2017) Identifying direct contacts between protein complex subunits from their conditional dependence in proteomics datasets. PLOS Computational Biology13(10):e1005625. https://doi.org/10.1371/journal.pcbi.1005625

PP01.043: Circulating Glycopeptide Markers Differentiate between Early- and Late-Stage Epithelial Ovarian Cancer

Dhar C[1], Ramachandran P[1], Zhou B[1], Srinivasan A[1], Xu C[1], Pickering C[1], Čaval T[1], Lindpaintner K[1], Schwarz F[1]
[1]Intervenn Biosciences

Introduction

GLORI is the world's first glycoproteomic laboratory-developed test that utilizes liquid-chromatography/mass spectrometry with artificial intelligence/neural networks to quantify serum glycoproteins at the intact glycopeptide level. Using GLORI one can now differentiate between benign and malignant adnexal masses with a very high level of sensitivity and specificity. Yet, little is known about the biological implications of these glycoproteomic alterations.

Methods

To deconvolute the mechanisms of these alterations we used reverse translational tools on retrospective and prospective GLORI clinical study data. Briefly, glycopeptide fold change plots and machine learning tools were used to analyze these data, and Qiagen's Ingenuity Pathway Analysis (IPA) was used on peptide-level data to identify upstream effectors.

Results

We noticed a distinct fucosylation signature on circulating N-glycoproteins with a higher relative abundance in late-stage (metastatic disease of stage III/IV) epithelial ovarian cancer (EOC). Fucosylation differences were marked when examined in glycopeptides that had tri-/tetra-antennary glycans. A classifier was then built to identify a subset of fucosylated and non-fucosylated tri-/tetra-antennary glycopeptides that best differentiates between early- (stage I/II) and late-stage EOC. IPA predicted that the markers we noticed werere downstream of cytokine signaling, particularly Interleukin-6. Notably, patients with late-stage EOC had higher levels of Interleukin-6 compared to patients with early-stage EOC.

Conclusions

The late-stage EOC classifier - if validated in prospective clinical studies - may help in developing a blood-based test for staging/treatment recommendations and to preempt recurrence and metastatic transformation of EOC. Our markers suggest the presence of the sialyl Lewis X epitope on N-glycans of certain liver-derived circulatory glycoproteins likely downstream of Interleukin-6 signaling particularly in metastatic EOC. Sialyl Lewis X may therefore be an attractive target to prevent and/or to treat metastatic epithelial ovarian cancer, a disease that continues to see dismal survival rates and limited treatment options.

PP01.044: Spatially-Resolved Tissue Proteomics of a Human Brain Tumour

Davis S[1,2], Scott C[3], Oetjen J[4], Charles P[1,5], Kessler B[1,2], Ansorge O[3], Fischer R
[1]Target Discovery Institute, Centre for Medicines Discovery, Nuffield Department of Medicine, University of Oxford, [2]Chinese Academy for Medical Sciences Oxford Institute, Nuffield Department of Medicine, University of Oxford, [3]Academic Unit of Neuropathology, Nuffield Department of Clinical Neurosciences, University of Oxford, [4]Bruker Daltonics GmbH & Co. KG, [5]Big Data Institute, Nuffield Department of Medicine, University of Oxford

Introduction:

Cellular protein expression profiles within tissues are key to understanding disease pathology, and their spatial organisation determines cellular function. To precisely define molecular phenotypes in the spatial context of tissue, there is a need for unbiased, quantitative technology capable of mapping the expression of many hundreds to thousands of proteins within tissue structures. Laser capture microdissection (LCM) in combination with LC-MS/MS-based proteomics is well-placed to meet this need.

Methods:

We used LCM to isolate tissue pixels from 10 µm-thick sections of a human brain tumour in a gridded pattern. Captured tissue was processed using an optimised SP3 protocol prior analysis by LC-MS/MS on an Orbitrap Fusion Lumos using 60-minute gradients or on a timsTOF Pro using 17-minute gradients. Quantified proteins were tested for spatial autocorrelation using a Moran's I test. The spatial expression profiles were used for unbiased spatial clustering by affinity network fusion, ANOVA significance testing and pathway analysis between spatial clusters. We performed validation by immunohistochemistry and lipid MALDI imaging (timsTOF Flex).

Results:

Spatial proteomics was performed at two resolutions: 350 µm and 833 µm from adjacent tissue sections. As the spatial sampling information is retained, quantitative protein measurements can be plotted in their spatial context to generate proteomic tissue maps. 1,375 and 3,212 proteins show significant evidence of spatial autocorrelation, respectively. Protein expression is consistent between both datasets. Immunohistochemistry staining for three highly spatially autocorrelated proteins is consistent with the proteomic datasets. Unbiased spatial clustering generates clusters of samples which co-cluster in space, reflecting the prominent pathology visible in H&E-stained sections along with further nuances. MALDI Imaging of lipid distribution within the tissue reflects the proteomic patterns.

Conclusions:

These and other spatially-resolved proteomics methods which spatially profile thousands of proteins will push the boundaries of understanding tissue biology and pathology at the molecular level.

PP01.045: Differential Fractionation (DIFFRAC): Systematic Characterization of Macromolecular Protein Complexes

Drew K[1]
[1]University Of Illinois At Chicago

Introduction: Macromolecular protein complexes carry out a wide variety of biological functions including essential cellular functions such as replication, transcription, translation, and protein degradation (e.g., MCM-ORC, RNA polymerase, ribosome, proteasome). Importantly, complexes are implicated in most human diseases spanning from hereditary disease to physiological diseases. Characterizing protein complexes in terms of their compositions, stability, and regulation provides valuable insights into their biological function. Unfortunately, we lack high throughput tools to systematically characterize the thousands of protein complexes currently identified.

Methods: To address this gap, we've developed Differential Fractionation (DIFFRAC) which measures the sensitivity of protein complexes in lysate to a given treatment using native size-exclusion chromatography followed by mass spectrometry.

Results: We've first applied this technology to observe complexes sensitive to RNAse treatment (RNA-DIFFRAC). Using a custom computational framework we identified over 1,400 ribonucleoprotein complexes in human and mouse embryonic stem cells [1]. We next applied RNA-DIFFRAC to vertebrate embryonic tissue, and identified novel RNA-associated proteins related to ciliary beating and localizing to liquid-like organelles [2]. We've extended the DIFFRAC framework further to observe complexes sensitive to phosphatase treatment including the FACT complex and the spliceosome (Phospho-DIFFRAC) [3] providing understanding of posttranslational modification's effects on complex assembly. We are now applying the DIFFRAC framework to characterize the sensitivity of protein complexes with respect to known denaturants and chelators gaining insight into the stability landscape of macromolecular assemblies of the cell.

Conclusions: The DIFFRAC framework is allowing the elucidation of general principles of protein self assembly and stability, and is proving a powerful hypothesis generating resource for the broader scientific community.

[1] Mallam et al. Cell Reports 2019
[2] Drew et al. Developmental Biology 2020
[3] Floyd et al. J. Proteome Res. 2021

PP01.046: PIPENN — Protein Interface Prediction from Sequence with an Ensemble of Neural Nets

Feenstra K[1], Stringer B[1], De Ferrante H[1], Abeln S[1], Heringa J[1], Haydarlou R[1]
[1]Vrije Universiteit Amsterdam

Motivation: The interactions between proteins and other molecules are essential to many biological and cellular processes. Experimental identification of interface residues is a time-consuming, costly and challenging task, while protein sequence data are ubiquitous. Consequently, many computational and machine learning approaches have been developed over the years to predict such interface residues from sequence. However, the effectiveness of different Deep Learning (DL) architectures and learning strategies for protein–protein, protein–nucleotide and protein–small molecule interface prediction has not yet been investigated in great detail. Therefore, we here explore the prediction of protein interface residues using six DL architectures and various learning strategies with sequence-derived input features.
Results: We constructed a large dataset dubbed BioDL, comprising protein–protein interactions from the PDB, and DNA/RNA and small molecule interactions from the BioLip database. We also constructed six DL architectures, and evaluated them on the BioDL benchmarks. This shows that no single architecture performs best on all instances. An ensemble architecture, which combines all six architectures, does consistently achieve peak prediction accuracy. We confirmed these results on the published benchmark set by Zhang and Kurgan (ZK448), and on our own existing curated homo- and heteromeric protein interaction dataset. Our PIPENN sequence-based ensemble predictor outperforms current state-of-the-art sequence-based protein interface predictors on ZK448 on all interaction types, achieving an AUC-ROC of 0.718 for protein–protein, 0.823 for protein–nucleotide and 0.842 for protein–small molecule.
Availability and implementation: Source code and datasets are available at https://github.com/ibivu/pipenn/

PP01.047: Systematic Characterization of Protein Complex Stability

Fischer S[1], Mallam A[2], Drew K[1]
[1]University Of Illinois At Chicago, [2]University of Texas at Austin

Introduction: Protein complexes are large molecular assemblies which are responsible for performing most cellular processes. Some key examples include, the proteasome, a complex essential for protein degradation, the MCM complex, an assembly involved in DNA replication, and the RNA exosome, an exoribonuclease complex that engages in RNA processing and degradation. In order to execute their functions, protein complexes need to be properly assembled into stable architectures. However, the structure of many complexes remain unknown, and further those with resolved structures do not have their stability well-characterized. Here, we explore the stability of protein complexes using a high throughput co-fractionation mass spectrometry (CF-MS) framework treated with urea, a known denaturant.

Methods: Through a series of biochemical size-exclusion separation studies, we identify co-eluting proteins under increasing concentrations of urea. In comparing these elution profiles to those of known complexes we are able to identify protein subcomplexes, elucidating complex architecture. Moreover, we have developed the Complex Stability Score which is a composite of subunit pairwise correlations and Shannon entropy to evaluate and categorize individual complex stability.

Results: Currently, we are able to confidently rank the relative stability of nearly 400 assemblies. We also observe an expected negative correlation between complex molecular weight and urea concentration, suggesting the global destabilization of complexes. Within this framework, we are able to identify ~7700 unique proteins and capture the stability of those proteins in over 4500 complexes.

Conclusions: We developed a method in which to rank protein complexes by their stability within the human proteome and submit this as a resource to the community. Assessment of complex stability will be a valuable tool for informing therapeutic design and will contribute to greater understanding of human disease.

PP01.048: Optimized Sample Preparation of Phospho-Enrichable Crosslinkers for Mass Spectrometry

Foster L[1], Flora A[1], Nandhikonda P[1], Etienne C[1], Bomgarden R[1], Viner R[2]
[1]Thermo Fisher Scientific, [2]Thermo Fisher Scientific

Introduction:
Crosslinking mass spectrometry is a powerful method for determining protein-protein interactions but suffers from identification rates of <1% of total peptides in complex samples. We developed a crosslinking workflow using enrichable crosslinkers PhoX (DSPP) and TBDSPP, an Fe-NTA magnetic bead, and optimized cleanup for simple, efficient magnetic phosphoenrichment of crosslinked peptides. TBDSPP is a tert-butyl-protected PhoX that is membrane-permeable for in vivo crosslinking that requires acid cleavage before enrichment. We optimized both protein and peptide-level cleanup to remove unreacted crosslinkers, reduce endogenous phosphopeptide contaminants, and increase crosslinked peptide yields from complex samples.

Methods:
Enrichable crosslinkers PhoX or TBDSPP were used to crosslink proteins, organelles, and cells. Cross-linked samples were phosphatase-treated, cleaned, and digested before peptide-level cleanup, acid-deprotection, and magnetic resin phosphoenrichment. Multiple protein and peptide-level cleanup chemistries were evaluated and modified to improve sample quality and yield. Samples were separated by HPLC using a 50cm analytical column and a 180min gradient, followed by mass spectrometry analysis. Data were analyzed using Proteome Discoverer 2.5 software and XlinkX 2.5.

Results:
Samples crosslinked with PhoX or TBDSPP using optimized cleanup methods and Fe-NTA magnetic enrichment resulted in 4-fold increase crosslinked peptide yield, 5-fold increase in crosslink identifications, and >2-fold decrease in phosphopeptides and contaminants. To increase crosslink peptide yields, we modified traditional peptide clean-up methods to improve the elution of the larger, higher-charged crosslinked peptides. Compared to traditional workflows with non-enrichable crosslinkers, our optimized workflow increased crosslinked peptide identifications by 5 to 10-fold in complex samples.

Conclusions:
Enrichment of crosslinked peptides is necessary for complex samples. Phospho-enrichable crosslinkers have high enrichment specificity, increasing crosslinked peptide identifications. Magnetic resins are ideal for high sample capacity, ease of use, and automation. Our crosslinker workflow was shown to crosslink proteins in vivo, and our optimized cleanup strategies in combination with enrichment improved crosslink identifications.

PP01.049: The Characterization of the Human Fallopian Tube-specific Proteome by Transcriptomics and Affinity-based Proteomics

Hikmet F[1], Digre A[1], Méar L[1], Schutten R[1], Uhlen M[2,3], Lindskog C[1]
[1]Department of Immunology, Genetics and Pathology, [2]Department of Protein Science, Science for Life Laboratory, KTH - Royal Institute of Technology, [3]Department of Neuroscience, Karolinska Institutet

Introduction: The fallopian tube (FT) is essential for successful human reproduction and allows for the transportation of male gametes to the oocyte. Both ciliated and secretory cells play important roles in generating tubal fluid flow by the beating motion of numerous motile cilia on the ciliated cells. Motile cilia are large microtubule-based complexes, and in addition to the FTs, motile cilia are found in other parts of the body where transportation of cells and particles is necessary. This study aimed to characterize the spatial expression of the FT-specific proteome in FT and other tissues with motile ciliated cells.
Methods: Transcriptomic analysis based on Human Protein Atlas identified 315 genes with elevated expression in FT compared with other human tissues. After stringent assessment, 132 FT-elevated proteins were analyzed by immunohistochemistry with highly validated antibodies.
Results: Most FT elevated genes were associated with cilia functionality and related to the different parts of the cilia, e.g. axonemal and dynein complex. As expected, FT elevated genes were often simultaneously elevated in other reproductive tissues, e.g. endometrium, cervix and epididymis, but especially in testis, where the testicular spermatids have a specialized type of cilium (flagellum). Proteomic analysis showed that the proteins were mainly localized to the cilia and cilia roots in FT, but also in other tissues. These findings were validated with available single-cell RNA-seq data, confirming expressions restricted to ciliated cells.
Conclusions: Here, we present a detailed map of the FT-specific proteome with particular emphasis on cilia-associated proteins. Our approach allowed us to identify several poorly-studied cilia-related proteins, and we were able to describe their spatial expression in ciliated epithelia. Understanding the molecular functions and pathways of human cilia proteins is crucial for revealing the mechanistic pathways behind infertility and other cilia-related dysfunctions.

PP01.050: PD-1 Inhibits PIEZO1 on CD8+ T Cells to Impair Antitumor Immunity

Kalocsay M[1,5], Gaudiano E[2,3,4], Clark M[2,3], Eisert R[5], Bradshaw G[5], Montero Lopis P[7], Vikas Anekal P[7], LaFleur M[2,3], Kalyviotis K[8], Markson S[2,3], Buck J[2,4], Sorger P[5], Freeman G[2,3], Pantazis P[8,9], Sharpe A[2,3,4]
[1]Department of Experimental Radiation Oncology, The University of Texas MD Anderson Cancer Center, [2]Department of Immunology, Blavatnik Institute, Harvard Medical School, [3]Evergrande Center for Immunological Diseases, Harvard Medical School and Brigham and Women's Hospital, [4]Department of Chemical Biology, Harvard Graduate School of Arts and Sciences, [5]Laboratory of Systems Pharmacology, Department of Systems Biology, Harvard Medical School, [6]Broad Institute of MIT and Harvard, [7]MicRoN Core, Harvard Medical School, [8]Department of Bioengineering, Imperial College London, [9]Department for Biosystems Science and Engineering (D-BSSE), ETH Zurich

The PD-1 pathway plays a critical role in mediating T cell exhaustion and blockade of this pathway can promote antitumor immunity. While PD-1 inhibitors are revolutionizing cancer therapy, only a subset of patients respond and show durable remission, highlighting the need to better understand the basic mechanisms by which the PD-1 pathway suppresses T cell functions. Here, we quantified PD-1/PD-L1 ligation-induced, dynamic changes of the local proteome proximal to PD-1 by unbiased multiplexed proximity proteomics and identified the mechanosensitive cation channel PIEZO1 as a primary target of PD-1-mediated inhibition. Stimulation of CD8+ T cells through TCR and CD28 engagement triggered the activation of PIEZO1, while simultaneous PD-1 ligation countered this activation. Mice lacking PIEZO1 selectively on CD8+ T cells exhibited increased tumor growth marked by impaired CD8+ T cell function, which could not be rescued by PD-1 blockade. Conversely, CD8+ TILs from mice treated with PIEZO1 agonist showed greater functionality compared to controls. Coadministration of PIEZO1 agonist and anti-PD-1 significantly reduced tumor burden and improved survival in a PD-1 blockade-unresponsive tumor model. These findings identify inhibition of PIEZO1 as an important mechanism by which PD-1 signaling regulates CD8+ T cell functions and suggests that modulating PIEZO1 activity may be a novel approach to augment cancer immunotherapy.

PP01.051: Dissecting Time and Space Dynamics within the EGF Receptor Signalosome

Martin C[1], Knight J[1], Rathod B[1], Larsen B[1], Wong C[1], Gingras A[2]
[1]Mount Sinai Hospital, Lunenfeld-Tanenbaum Research Institute, [2]University of Toronto, Department of Genetics

Introduction: Epidermal growth factor receptor (EGFR) is a key regulator of cell proliferation and cancer development. Despite intense study over the past 60+ years, the mechanisms governing EGFR signaling remain unclear.

Methods: BioID is a proximity-dependent footprinting technique used to identify protein-protein interactions when coupled to LC-MS detection. BioID is particularly useful for characterizations of unstable or insoluble protein complexes, such as those restricted to membranes. However, its utility in the characterization of dynamic signaling events has historically been limited by the relatively slow biotin labelling by the original BirA* enzyme (6-24 hours). The molecularly evolved miniTurbo/TurboID enzymes have recently emerged as promising candidates for faster BioID labeling. Here, we optimize biotin labelling times in HeLa cells stably expressing EGFR-TurboID or EGFR-miniTurbo and, using nanoLC-MS/MS-SWATH acquisition on a Sciex TripleTOF 6600 instrument, analyze the EGFR signalosome.

Results: While TurboID's high avidity for biotin impeded its use in studying dynamics, miniTurbo efficiently detected EGFR proximal interactors within 15 minutes of labelling. Using 15-minute frames in >300 BioID samples, we profiled the temporal nature of EGF-ligand-dependent partner recruitment, which revealed the spatiotemporal dynamics of >200 EGFR neighbours that typify its signaling, endocytosis and trafficking. The spatial organization and interdependence of these relationships was clarified by reciprocal BioID characterizations of EGFR neighbors and by coupling BioID with CRISPR-Cas9 gene editing or bait mutagenesis. These strategies revealed a temporally distinct cluster of ubiquitin modifiers associating with tyrosine-phosphorylated EGFR. Individual disruption of these neighbours, including the novel EGFR interactor and ubiquitin-binding protein SPATA2, profoundly impacted EGFR degradation in cells. SPATA2 amplifications are common across cancer types (Cbioportal), and ongoing efforts aim to determine the mechanism by which this might contribute to disease.

Conclusion: Altogether, these data demonstrate the proficiency of miniTurbo BioID in short timescales and in the investigation protein complex rewiring following cellular stimulations.

PP01.052: Identification of 3’UTR Localization Sequence Motifs using a Protein Co-localization Network

Nadeau R[1,2,3], Lavallée-adam M[1,2,3]
[1]University Of Ottawa, [2]Department of Biochemistry, Microbiology and Immunology, [3]Ottawa Institute of Systems Biology

Introduction: The localization of proteins to their appropriate subcellular compartment is essential to accomplish their function. Protein localization is regulated through various mechanisms. Among these, sequence motifs in mRNA 3’ Untranslated Regions (3’UTRs) are involved in the transport of transcripts to their subcellular compartment where protein translation will then often occur. However, most mRNA localization sequence elements remain uncharacterized. Proximity labelling, such as BioID, coupled with mass spectrometry has generated protein co-localization networks that can help address this need. Indeed, proteins that are densely connected in such networks are likely to be located in similar cell compartments. If such proteins share a common mRNA sequence motif, this motif could be directly or indirectly related to their localization.

Methods: We therefore developed a novel graph theory-based software package to identify 3’UTR sequence motifs for which the associated proteins are locally enriched in a protein co-localization network. Our method uses Monte Carlo sampling to determine if proteins sharing a sequence motif are more densely connected than what is expected by chance in the network. Our computational approach was used to mine the Human Cell Map protein co-localization network from Go et al., 2021.

Results: Our tool identified 31 sequence motifs for which the associated proteins are significantly clustered in the network. These proteins are enriched for Gene Ontology cellular compartment annotations involved in transport, including the endomembrane system and vesicles. Four of these motifs are known RNA-protein and micro-RNA binding sites. All discovered motifs tend to cluster at similar positions within 3’UTR sequences, further hinting at their binding site role. Furthermore, the motifs identified by our method were not detected by a state-of-the-art motif discovery tool, highlighting the novelty of our approach.

Conclusions: Our work demonstrates the ability to detect functional mRNA sequence motifs through the mining of protein co-localization networks.

PP01.053: Time-resolved Interactome Profiling to Deconvolute Protein Quality Control Dynamics

Plate L[1], Wright M, Garcia Lopez V, Timalsina B
[1]Vanderbilt University

Introduction:

The proteostasis network (PN) has become an attractive target for therapeutic intervention to combat protein misfolding disease states. Yet, one of the bottlenecks in the implementation of such strategies is a lack of understanding in the timing and coordination of PN-client protein interactions as there are no methodologies available to identify and quantify transient protein-protein interactions with time-resolution at an organelle-wide scale. To address this challenge, we developed a quantitative mass spectrometry-based method, time-resolved interactome profiling (TRIP), to characterize the temporal interactions between client proteins and the PN to better understand protein quality control processes.

Methods and Results:

We focus on hypothyroidism stemming from mutations in the thyroglobulin (Tg) gene, which encodes a large 330 kDa thyroid-specific, heavily modified secreted prohormone. Patient-derived missense mutations across various domains of Tg lead to a loss of protein secretion, and subsequent hormone production. TRIP has allowed us to identify and deconvolute the altered timing of key interactions across the PN that correlate with hypothyroidism pathophysiology. We identified mutation-specific alterations across pathways such as N-glycosylation, Hsp70/90 assisted folding, disulfide/redox processing, and degradation. We have coupled this methodology with RNAi screening to understand the functional implications of PQC regulators on the folding, processing, and secretion of Tg. This screen has revealed key protein degradation components, including the AAA+ ATPase p97/VCP and ER-phagy receptor TEX264, whose inhibition rescues mutant Tg secretion.

Conclusions:

Our findings highlight the utility of TRIP to not only investigate the implications of many PN components on Tg processing, but also identify interventions able to rescue mutant Tg secretion. Ultimately, our results may reveal protein quality control mechanisms available for therapeutic targeting and provide insight into the coordination of the PN that have broader applicability in other protein misfolding diseases.

PP01.054: Redefining Serological Diagnostics with Immunoaffinity Proteomics

Fu Z[1], Rais Y[1], Dara D[1], Drabovich A[1]
[1]Department of Laboratory Medicine and Pathology, University Of Alberta

Introduction

Serological diagnostics, including identification of specific infectious disease antibodies, is an essential tool in clinical diagnostics. Current serological tests utilize immunoassays and focus on convenient assay development and high throughput measurements. Limitations of such tests include semi-quantitative measurements, lack of standardization, cross-reactivity, and inability to distinguish between the human antibody subclasses. Advances in affinity proteomics and standardization of protocols, including our recent studies [1-3], facilitated development of sensitive and reproducible assays for quantification of low-abundance proteins and circulating antibodies. Here, we suggested that immunoaffinity proteomics would enable rational design and implementation of serological diagnostics of infectious diseases, such as COVID-19.

Methods

Immunoaffinity enrichments of specific antigen-binding antibodies from the patient plasma and saliva were followed by differential quantification of isotypes (IgG/A/M/E/D) and subclasses (IgG1-4, IgA1-2) with targeted proteomic assays. Carefully designed synthetic peptides targeting Constant Heavy chains enabled absolute quantification and standardization. Simple design, fast microflow separations (120 samples/day), and sensitive measurements provided high reproducibility, throughput, and sensitivity. Finally, immunoaffinity-shotgun proteomics identified circulating immunoglobulin-Fc receptor interactomes.

Results

Evaluation of 36 antigen-antibody subclass combinations revealed SARS-CoV-2 receptor-binding domain (RBD)-IgG1 as a combination with the highest diagnostic specificity and sensitivity. Anti-RBD IgG1, IgG3, IgM and IgA1 levels were significantly elevated in COVID-19-convalescent plasma and saliva, while IgG2, IgG4, IgA2 were not informative. Anti-RBD IgG1 (408 ng/mL diagnostic cut-off; N=224 patients) provided 99.3% specificity at 89% sensitivity to detect COVID-19 plasma [4].

Conclusions

Immunoaffinity proteomics as a platform for serological diagnostics will facilitate standardization and improvement of the existing serological tests, enable rational design of novel tests, and offer tools for precision immunology and investigation of antibody subclass cooperation in immunity response.

References

[1] Fu et al. Mol. Cell. Proteomics 2021, 20, 100075
[2] Drabovich et al. Mol.Cell.Proteomics 2019, 18, 1807
[3] Fu et al. medRxiv doi:10.1101/2021.10.25.21265408 and Anal.Chem. 2022, in revision.

PP01.055: Interactomic Analysis of Human LINE-1 Retrotransposons: Understanding the Roles of IGF2 Binding Proteins

Rosas Bringas O[1], Oghbaie M[2], Jiang H[2], LaCava J[1,2]
[1]European Institute for the Biology of Ageing, University Medical Center Groningen, [2]The Rockefeller University

Introduction: Retrotransposons are mobile DNA sequences that proliferate in the host genome via RNA intermediates. The long interspersed element-1 (LINE-1 or L1) is the only autonomous, protein coding family of retrotransposons present in the human genome. Loss of L1 suppression, and the genomic instability it creates via new insertions, is considered a hallmark of cancer and a poor prognostic factor. Although many in the L1 field focus on new insertions as a source of cellular insult, L1's lifecycle presents numerous opportunities to induce stress and toxicity: we hypothesize that the accumulation of L1 ribonucleoprotein (RNP) in cytoplasmic 'granules' creates molecular sponges that sequester host proteins and nucleic acids away from their typical cellular roles. We and others have observed that the proteins IGF2BP1-3 are present in L1 RNPs enriched from model cell lines, and we have seen the same in colorectal cancers (1, 2). IGF2BPs are known components of cytoplasmic RNA granules that interact with RNAs via consensus sequences and/or N6 methyladenosine (m6A) chemical modifications. We are compositionally and functionally dissecting L1 RNPs and granules – including the roles of IGF2BPs in mediating protein-protein and protein-RNA interactions.

Methods: We use IP/MS screening to explore how L1 protein-protein interactions are influenced by different physicochemical environments (3,4). As pathobiological models of L1 expression, we use resected patient colorectal cancer tissues and embryonal carcinoma cell lines (N2102Ep, NTERA1, PA-1). To complement protein interactions studies, we identify RNA components of L1 RNPs by RIP-seq.

(1) PMC5821459
(2) PMC6937734
(3) PMC4449307
(4) PMC7544204

PP01.056: In-depth Characterization of Native Protein Interactomes by Multi-epitope Affinity Purification Mass Spectrometry (meAP-MS)

Schulte U[1], Müller C[1], Jordan J[1], Haupt A[1], Bildl W[1], Schwenk J[1], Fakler B[1]
[1]Institute Of Physiology, University Of Freiburg

Affinity-purification coupled to mass spectrometric analysis (AP-MS) still represents the gold standard for identification of protein-protein interactions in native cells and tissues. Its performance critically depends on antibodies and stringency of the controls used. In reality, the results are impacted by adverse effects that have not yet been quantitatively assessed. Using two comprehensively determined and functionally as well as structurally confirmed interactomes as reference, we demonstrate that individual, even stringently controlled antibodies exhibit significant false-positive and false-negative error rates. We demonstrate that combined evaluation of antibody APs targeting distinct epitopes (meAP-MS) can successfully overcome the shortcomings of individual antibodies. This required introduction of new target and background-normalized specificity measures as well as non-linear visualization and clustering methods, integrated into an interactive software suite termed BELKI. A general workflow combining these elements is provided that allows for confident, straight-forward determination of protein interactomes in various use cases. These comprise analyses of well defined target complexes, multiple assemblies associated with overlapping targets, settings when target knockout controls are not available or the number of suitable antibodies is limited.

PP01.057: Protein Footprinting Reveals the Dynamic Conformational Changes of Proteome in Progressing Alzheimer's Disease

Son A[1], Kim H[1], Diedrich J[1], Bamberger C[1], Yates J[1]
[1]The Scripps Research Institute

Introduction

Alzheimer's disease (AD) is a progressive neurological disorder of which pathological hallmarks are an aggregation of beta-amyloid and tau neurofibrillary tangle. The abnormal conformation of proteins induces neuronal cell death in the later stage of AD. However, the structural changes of proteomes are still unveiled in progressing AD. Here, we present the structural alterations of proteins in multiple organs of mice with progressing AD. In particular, we utilized covalent protein painting (CPP) that is able to quantify and compare the 3D proteome in progressing AD by dimethyl-labeling.

Methods

Seven organs were collected from mice with progressing AD, and age-matched wild-type mice were used as the control. For dimethylation, a solution for 1st dimethyl-labeling (light) was directly injected into the heart of a mouse and passed through a capillary in the tissue using a perfusion-based approach. The passing solution through the capillary labels the accessible lysine sites on the surface of proteins via dimethylation. Following digestion with Chymotrypsin, the non-modified lysine sites were newly exposed and labeled with dimethylation (heavy).

Results

We quantified the dimethyl-labeled peptides and determined the structural change of proteins as processing AD from the multiple organ samples, allowing us to confirm our hypothesis that the dimethyl-labeled proteins exhibit different solvent accessibility with respect to the level of AD. For example, more than 4,500 labeled peptides were quantified from the brain and exhibited a consistent tendency in a decrease in accessibility with aging. The proteins mapped via labeled peptides with this decrease were found to be enriched in neurodegenerative diseases by GO analysis.

Conclusion

Our dimethylation strategy combined with perfusion demonstrated the feasibility of quantifying the conformational changes of the proteome by labeling intact forms of protein. This study highlights the organ-specific conformational changes of proteins with progressing AD as a valuable strategy for understanding protein-protein interactions.

PP01.058: Decoding Functional Surfaceome Residing Protein Interaction Communities using CSI-MS

Steiner S[1,2], Zwicky A[3], Bode J[3], Wollscheid B[1,2]
[1]Institute of Translational Medicine (ITM), Department of Health Sciences and Technology (D-HEST), ETH Zurich, [2]Swiss Institute of Bioinformatics (SIB), [3]Laboratory for Organic Chemistry, Department of Chemistry and Applied Biosciences, ETH Zürich

Introduction: Protein-protein interactions (PPIs) within the cellular surfaceome and with extracellular ligands enable cells to integrate extracellular signaling cues and elicit cellular responses. Functional surfaceome PPIs are orchestrated and organized into dynamic molecular interaction networks on a global, and on nanoscale level. Currently, we have tools in place to analyze the intracellular interactome. However, a more detailed understanding of surfaceome signaling architecture requires further technology development. Here, we developed Cell Surface Interaction MS (CSI-MS), an opto-proteomic surfaceome cross-linking strategy enabling the discovery of surfaceome PPIs and cellular signaling architecture.

Method: CSI-MS combines utilization of light-induced cross-linking sites on cell surface receptors, chemical cross-linking and mass spectrometry. In the CSI-MS strategy, the cellular surface of live cells is coated with Singlet Oxygen Generators (SOGs) that enable the light-controlled photo-oxidation of proximal cell surface proteins. Short illumination of SOGs leads to the oxidation of specific amino acids within cell surface proteins which can then be utilized as “induced cross-linking sites” for selective protein-protein cross-linking of receptor complexes.

Results: First, we assessed the efficiency of our light-mediated cross-linking site induction on B lymphocytes. Using imaging we found a global induction of cross-linking sites on cell surface receptors within 30 seconds of illumination. Upon MS analysis, 205 cell surface proteins were identified bearing light induced cross-linking sites which can be used for subsequent cross-linking. To capture surfaceome PPIs we synthesized a novel tri-functional cross-linker molecule with an affinity handle and two reactive moieties targeting the light introduced sites. Using model peptides we confirmed the reactivity of our cross-linker molecule and established a bioinformatic pipeline for PPI analysis. Pilot experiments using CSI-MS to map surfaceome PPIs on B lymphocytes provide insights into surfaceome signaling architecture.

Conclusion: We have set the foundation for a light-mediated cross-linking technology enabling the systematic mapping of the surfaceome interaction networks.

PP01.059: A Cloud-scalable Software Suite for Large-Scale Proteogenomics Data Analysis and Visualization

Auluck H[1], Donovan M[1], Gajadhar A[1], Berk Y, Lou Y[1], Platt T[1], Batzoglou S[1]
[1]Seer

Introduction

Assessment of the flow of genetic information through multi-omics data integration can reveal the molecular consequences of genetic variation underlying human disease. Integration of proteomics and genomics data requires many tools and complex workflows that can act as a barrier for researchers to adapt new analysis tools. The Proteograph™ Analysis Suite (PAS), a cloud-based, proteomics and proteogenomics data analysis software enables the identification and exploration of peptide variants arising from allelic variation or other user-defined protein sequence altering genetic variants through the integration of Proteograph proteomics data with NGS variant information.

Methods

PAS features integration of stablished proteomics database search algorithms, experiment data management system, analysis protocols, result visualizations, and setup wizards for seamless generation of results. PAS can support both Data Independent Analysis and Data Dependent Analysis workflows and is compatible with variant call format files. Using the Proteogenomics module in PAS, we performed a customized database search to identify variant peptides, including results interpretation using the Variant Peptide Browser and Proteogenomics Data Explorer.

Results

We demonstrate the Proteogenomic features of PAS by analyzing plasma proteome data generated from Proteograph™ Product Suite and corresponding variant data from healthy controls and Alzheimer's Disease (AD) patients. We highlight an interactive Variant Peptide Browser tool for examining peptide variation in the samples. These results include filtering and grouping features to identify qualitative and quantitative trends in single samples or healthy/disease groups. Further, we demonstrate the Proteogenomics Data Explorer tool, which provides a view of the genomic variant coordinates in relationship to the peptide/protein data. Detected variants can be reviewed in the context of gene structure, protein structure, and functional domains so biological insight can easily be gained from the proteogenomic data.

Conclusions

PAS providing an easy-to-use and efficient suite of tools for seamless and fast proteogenomic data analysis.

PP01.060: Deciphering the Ghost Proteome on the Acute Myeloid Leukemia

Cardon T[1], Garcia Del Rio D[1], Ghetti M[2], Simonetti G[2], Roucou X[3], Fournier I[1], Salzet M[1]
[1]Univ. Lille, Inserm, U1192—Proteomique Reponse Inflammatoire Spectrometrie de Masse (PRISM), F-59000 Lille, France., [2]IRCCS Istituto Romagnolo per lo Studio dei Tumori (IRST) "Dino Amadori"-Immuno-Hematology Unit - Biosciences Laboratory, [3]Département de microbiologie, Faculté de Médecine et des Sciences de la Santé, Université de Sherbrooke

Introduction: The ghost proteome, is composed by proteins from regions described as non-coding such as the 3'&5'UTR parts, non-coding RNAs, or even a reading frame shift. The translation capacity of these proteins has been described by RiboSeq analysis, however to identify them in mass spectrometry analyses it is necessary to have databases, which is possible thanks to prediction on database like OpenProt. The consideration of this proteome leads to many conceptual changes, the first being the possibility that an RNA can be translated into several proteins. Similarly, the presence of several ORF can change the nature of certain mutations described as silent because they have no impact on the RefProt.
Method: Proteogenomic study in the context of acute myeloid leukemia (AML), is apply on model cell under hypoxia or normaxia condition and inhibiting PVT1 an lncRNA oncogene. The construction of in-house proteins databases from RNAseq integrate mutation, new isoform and AltProt. These are then applied in large-scale MS proteomics on cells in order to track an extended proteome and mutations.
Result: This study shows us the impact of a hypoxic environment on cultured cells with a first observation of a variation in the mutation rate observed in RefProt but interestingly also in AltProt. Such as AltProt present intercellular functions still misunderstood but impacted by cellular phenomena.
Conclusion: AltProts have already been described as involved in signalling pathways of cell motility or protein translation. Today the identification of AltProt opens the field of possibilities with in particular the demonstration of the presence of post-translational modifications, such as glycosylation. These AltProt are for us the possibility of discovering new biomarkers with the identification of specific mutations linked to cells but also to cell surface specificities. In conclusion, AltProt are a promise of new specific molecules thanks to unconsidered mutations or particular PTMs.

PP01.061: Proteogenomic Analysis Reveals Translation of Noncanonical ORFs from lncRNA in Human Breast Cancer

Cheon S[1,2], Kim S[1,3], Kim H[1,3], Park M[1,3], Lee G[1,3], Jeon E[1,3], Han D[1,3]
[1]Proteomics Core Facility, Biomedical Research Institute, Seoul National University Hospital, [2]School of Biological Sciences and Technology, Chonnam National University, [3]Transdisciplinary Department of Medicine and Advanced Technology, Seoul National University Hospital

Introduction

Long noncoding RNA, a class of RNAs longer than 200 nucleotides which is defined as non-translational transcripts, are known to be involved in various biological funtions on transcriptome levels. But lncRNAs have been tranditionally disregarded to have protein coding potential in current genome annotation procedures which have relied on rules such as the minimum length of translated products. Recent studies of mass spectrometry-based proteomics and deep-sequencing of ribosome profiling have identified unannotated ORFs from non-coding regions across prokaryotes to eykaryotes. However, systematic characterization of functional ORFs from noncoding regions remains still challenging.

Methods

To identify lncRNA-encoded ORFs, we first established a pipeline for customized database from potential translating regions in human lncRNA transcript sequences combined with high-throughput RNA sequencing and deep sequencing of ribosome profiling data to verify expression of unannotated ORFs. For bottom-up proteomics approaches, human breast cancer cell lines were analized by isobaric label and label-free quantification methods to gather in-depth proteome data sets. The mass spectrum data were processed by ProteomeDiscoverer with customized database including Uniprot human proteome.

Results

In this study, we identified 12,386 human proteins from CDS in breast cancer cell line dataset. Several hundread peptide sequences from lncRNA-encoded ORFs were identified using our customized database. Furthermore, fragmented peptides from predicted noncanonical ORFs in lncRNA transcripts were quantified in both bottom-up proteomic approaches. Finally, we identified several peptides derived from lncRNAs that are ubiquitously or subtype-specificially translated ORFs in breast cancer cell lines.

Conclusion

Proteogenomic approaches can discover novel peptides with potentially translational regions in lncRNAs. The robustness of our pipelines identified lncRNA-encoded peptides with in-depth LC-MS/MS proteomics data of human breast cancer cell lines. Further investigation of these identified novel peptides from lncRNAs can pave a way to discover potential dual roles at the RNA and protein levels.

PP01.062: pXg: Comprehensive Identification of Noncanonical Peptides from De Novo Peptide Sequencing Using RNA-Seq Reads

Choi S[1], Paek E[1,2]
[1]Department of Computer Science, Hanyang University, [2]Department of Artificial Intelligence, Hanyang University

Introduction

Major histocompatibility complex I (MHC-I) molecules play a critical part in the recognition of CD8+ T cells to initiate an immune response in cancer immunotherapy. Recent studies have reported that MHC-I associated peptides (MAPs) could originate from both canonical and noncanonical peptides. We describe an innovative software tool, called pXg (proteomics X genomics) that can reliably identify cMAPs (canonical MAPs) and ncMAPs (noncanonical MAPs) from de novo peptide sequencing using RNA-Seq reads.

Methods

pXg directly matches de novo peptides to six-frame translation of reads to generate interpretable peptides at genomic level. As a negative control, the peptides are also matched to the reverse sequences of six-frame translation of reads. Based on the negative control, pXg calculates null distributions of matches between peptide and read to filter out random matches. Then, a target-decoy approach is applied to identify reliable PSMs by using spectra matching the reverse sequences as decoy PSMs. The PSMs that pass all filtering processes are considered reliable and are reported in tab-delimited format.

Results

Using MHC-I immunopeptidome and RNA-Seq data sets, we analyzed four Epstein-Barr virus-transformed B-lymphoblastoid cell lines and could identify comprehensive ncMAPs such as translation from UTRs, noncoding RNAs, frameshift of protein coding genes and even CDR3 region of an antibody. pXg outperformed the previous studies in its number of cMAPs and ncMAPs. In addition, the proportion of MAPs in the identified peptides was significantly higher than what has been previously reported, suggesting that pXg can reliably identify more MAPs.

Conclusions

pXg matches de novo peptides directly to reads, including unmapped reads. This approach permits the discovery of comprehensive ncMAPs beyond the search space defined by the reference genome. In MHC-I immunopeptidomics, pXg can be a useful tool for discovering the repertoire of ncMAPs.

PP01.063: Nitrogen Concentration Modifies Transcriptomic and Proteomic Profiles in the Marine Diatom Chaetoceros Muelleri

De Jesús Campos D[1], Bojórquez-Velázquez E[2], Elizalde Contreras J[2], García Ortega L[3], Huerta Ocampo J[4], Fimbres Olivarría D[1], Hayano Kanashiro Á[1]
[1]Departamento de Investigaciones Científicas y Tecnológicas de la Universidad de Sonora. , [2]Instituto de Ecología, A.C. (INECOL) , [3]Centro de Investigación y Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV), [4]CONACYT-Coordinación de Ciencia de los Alimentos. Centro de Investigación en Alimentación y Desarrollo, A.C (CIAD)

Introduction. Chaetoceros muelleri is characterized by lipids and carbohydrates accumulation under nitrogen stress that compromises the cellular growth of the microalgae. Knowledge of metabolic pathways involved in this accumulation is required for the biotechnological use of microalgae. “Omics” approaches can elucidate the molecular mechanism and pathways involved, so the aim of this project is to study the transcriptomic and proteomic responses of microalgae growing under different nitrogen concentrations.
Methods. C. muelleri was cultured using f media as a control (1.76 mM) and two nitrogen-limiting conditions (0.44 mM and 0.18 mM). RNA was extracted and purified following TRIzol reagent protocol and RNeasy MinElute Cleanup kit (QIAGEN). Transcriptome sequencing was conducted by Illumina Next-Sequencing platform (paired-end, 150 bp read length). Filtered reads (Q>20) were used for de novo transcriptome assembly using Trinity software and functional annotation was carried out using public databases. Proteins (three biological replicates per treatment) were extracted using a phenol-based protocol1 and quantitated by the Bradford method. Protein quality was evaluated through SDS-PAGE. Proteins were digested with MS-grade trypsin, peptides labeled with Amine-reactive TMT 10plex and fractionated by SCX. Protein identification and relative quantitation analysis was performed by synchronous precursor selection (SPS)-MS32 using an Orbitrap and raw-data were processed with Proteome Discoverer 2.1 (Thermo-Fisher) using Sequest-HT and Mascot search engines against an in-house database containing the protein sequences of C. muelleri obtained from transcriptomic analysis.
Results. Limiting conditions activated genes involved in neutral lipid synthesis and on alternative pathways to face nitrogen deficiency. Protein profiles showed significant differences among treatments, nitrogen deficiency induced protein turnover.
Conclusions. Low nitrogen concentration activates alternative pathways to supply this nutrient. Protein degradation generates nitrogen, which could be used for the synthesis of new enzymes essential for cell viability.

1. WAOJ, 2020: 13(3), 100111.
2. Analytical chemistry, 2012: 84(17), 7469-7478.

PP01.064: Mass-Spectrometry Based Proteomic Based Diagnostics of Viral Meningitis and Lyme Neuroborreliosis

Drici L[1], Ottenheijm M[1,2], Christoffersen C[3], Lebech A[4], Fjordside L[4], Mens H[4], Mann M[1,5], Albrechtsen1 N[1,2,3,6]

[1]Novonordisk Foundation Center For Protein Research, [2]Department of Biomedical Sciences, Faculty of Health and Medical Sciences, [3]3Department of Clinical Biochemistry, Rigshospitalet, [4]Department of Infectious diseases, Rigshospitalet, [5]Department of Proteomics and Signal Transduction, Max Planck Institute of Biochemistry, [6]Department of Clinical Biochemistry Bispebjerg and Frederiksberg Hospital

Introduction

Meningitis is a life threating condition causing swelling of the central nervous system. Accurate and early diagnostics is important but remains challenging. This is in particular true for two common subtypes of meningitis: viral and lyme neuroborreliosis (LNB), a tick-transmitted disease. The objective of this study was to investigate if the proteomic based host-response could distinguish patients with three subtypes of meningitis: viral, LNB, bacterial. A healthy group was also included.

Methods

160 patients consisting of 50 LNB, 50 viral meningitis, 8 bacterial meningitis and 52 healthy individuals were included. CSF sample preparation was optimized based on the previously published methods (Bader et al., 2020; Geyer et al., 2016). Briefly, 20 µl CSF sample were first denatured with 30 µl PreOmics Lysis buffer (Kulak et al., 2014) and subsequently digested with LysC /trypsin enzyme mix. The resulting peptides were purified using SDB-RPS StageTips and the eluate analyzed on Evosep One liquid chromatography system coupled online to an Orbitrap Exploris 480 mass spectrometer using the 60 samples per day (SPD) gradient and 8 cm Pepsep column in data-independent analysis mode (dia). Data analysis was performed using Spectronaut software followed by the Clinical Knowledge Graph (Santos et al, Nat. Biotech, 2022) and machine learning based classification.

Results

537 proteins were quantified per CSF sample. Among these, 27 proteins showed significant differences between the LNB and the viral meningitis groups including 11 upregulated and 16 downregulated proteins in the LNB group. When comparing the LNB to the bacterial meningitis, three proteins were downregulated in the LNB. The top 20 features that predicted LNB based on the CSF proteome, exhibited an AUC of 0.95.

Conclusion

A panel of 20 proteins shows a promising potential in classifying patients with LNB and viral meningitis. We plan to validate this classifier in an independent cohort.

PP01.065: LinkedOmicsKB: A Web Portal for Exploring CPTAC Pan-Cancer Proteogenomics Data

Liao Y[1,2,3], Savage S[1,2,3], Dou Y[1,2,3], Shi Z[1,2,3], Yi X[1,2,3], Jiang W[1,2,3], Lei J[1,2,3], Zhang B[1,2,3]
[1]Lester and Sue Smith Breast Center, Baylor College of Medicine, [2]Department of Molecular and Human Genetics, Baylor College of Medicine, [3]Dan L Duncan Comprehensive Cancer Center, Baylor College of Medicine

Introduction: The Clinical Proteomic Tumor Analysis Consortium (CPTAC) has characterized over 1,000 treatment naïve primary tumors spanning 10 cancer types, many with matched normal adjacent tissues. Each sample is analyzed by whole genome sequencing, whole exome sequencing, methylation array, RNA-Seq, miRNA-Seq, proteomics, and phosphoproteomics. We performed harmonized and systematic computational analyses on these data, both within and across all 10 cancer types, and made precomputed results available in a web portal named LinkedOmicsKB for easy exploration.

Methods: CPTAC data were downloaded and reprocessed using a common pipeline to call somatic mutations and quantify copy number, methylation, mRNA, protein, and phosphosite levels. All but phosphosite quantification were aggregated to gene level. Clinical data were curated and various molecular phenotypes were computed, including chromosome instability, mutation burden, mutational signature, tumor purity, hallmark and signaling pathway activity, kinase activity, and immune infiltration and tumor microenvironment scores. Association analyses were performed using appropriate statistical tests. Meta p-values were calculated to integrate results at pan-cancer level. All precomputed results were stored in a MongoDB database.

Results: Analysis results are organized into ~40,000 gene-, protein-, mutation-, and phenotype-centric web pages, which can be browsed through LinkedOmicsKB using a single gene or phenotype of interest as the query. Information in the portal includes phosphosites coverage across all studies, cis-association across omics layers, tumor vs normal difference and mutation and phenotype associations for mRNAs, proteins, and phosphosites, respectively, and pair-wise associations between mRNAs, proteins, and phosphosites and kinases/phosphatases. Results are displayed in sortable, searchable, and filterable tables, zoomable lollipop plots, heatmaps, and various types of statistical plots, including Manhattan plots visualizing pan-cancer, multi-omics results.

Conclusions: With precomputed results of 19701 coding genes, 126547 phosphosites, and 258 genotypes and phenotypes for 10 CPATC cancers and pan-cancer analyses, LinkedOmics makes CPTAC data easily useful to the broad cancer research community.

PP01.066: A Novel Approach to Construct Variant Protein Sequences for Precision Medical Proteogenomics

Ling Y[1], Okuda S[1]
[1]Niigata Univercity

Introduction: The high-speed development of LC/MS technique made it possible to obtain large-scale proteomes of multiple cancer tissues, and promoted the application of proteogenomics approaches to be utilized in cancer diagnosis and treatment for precision medicine. However, it hasn't been unclear about the mechanism of how cancer genome-level abnormalities and protein expression changes affect proteome and cellular phenotypes. In order to more accurately predict mutated peptide sequences, we developed an algorithm to construct protein sequences that reflect genetic mutations, and then provide a basis for pathogenic mechanism research and targeted therapy selection.

Methods: Different from previous mutation annotation software that predict only one homogeneous variant in the protein sequence, we developed an algorithm to predict variant protein sequences that contain all mutations in the coding regions, combinations of all compound heterozygous mutations, as well as their effects on all isoforms at the RNA level. Reference genome sequences and CDS sequences were obtained from public databases, including different versions for human and mouse genomes.

Results: By now, we have developed an algorithm to construct variant protein sequences, not only for cancer mutations, which support the variants in VCF format and variant annotation formats from ANNOVAR or SnpEff. What's more, to explore missing protein sequences, it has been possible to create six frames for corresponding transcriptomes based on the combinations of mutant CDSs. To verify the efficiency of our algorithm, we applied it to predict actual cancer samples.

Conclusion: Our novel approach to construct variant protein sequences makes it possible to study changes in protein expression derived from genetic mutations. By acquiring various cancer genome data based on various cancer types and other meta-information, a mutant protein database corresponding to a pre-applicable cohort will be created, which can be applied to rapid proteogenomics analysis.

PP01.067: Microproteins: Translation from Small Open Reading Frames

Mnatsakanyan R[1], Eberl C[2], Bantscheff M[2], Savitski M[1]
[1]European Molecular Biology Laboratory (EMBL), [2]Cellzome GmbH, GlaxoSmithKline

The existence of nonannotated protein-coding human small open reading frames (sORFs) has been revealed through the direct detection of sORFs via ribosome profiling (Ribo-seq) and their microprotein products by liquid chromatography-mass spectrometry (LC-MS/MS). Microproteins were previously excluded from coding genome due to their short size (< 100 codons), common initiation at non-AUG start codons, and allocation at non-coding or overlap with other coding regions. Only a handful of microproteins are identified and functionally validated to date, whereas the number of likely coding sORFs reaches various thousands. The microprotein examples discovered to date demonstrate that they are ubiquitous in origin, cellular localization, and function. Differential expression of some functional microproteins has been reported under stress conditions and in disease. Microprotein discovery is hindered by a multitude of technical challenges, such as the low sensitivity and specificity of microprotein enrichment analytical strategies, and the requirement of specific data analysis pipelines.

We developed a proteogenomics strategy for microprotein analysis utilizing an optimized size exclusion chromatography (SEC) approach for enrichment of small proteins coupled to LC-MS/MS followed by data analysis using protein databases containing long-read RNA sequences (3- or 6-frame in silico translation).

Applying this strategy, we obtained a direct evidence of expression for hundreds of microproteins in human cell lines, many of which had no former protein-level evidence. Next, the functionally relevant microprotein candidates will be selected for further elucidation based on the evidence signifying their involvement in protein-protein interactions, presence of a functional domain/active site or differential expression under perturbation.

The discovery of novel microproteins will expand the size of the coding genome, and will provide insights into the molecular biology of mammalian cells, such as the ubiquitous usage of non-AUG start codons.

PP01.068: Comprehensive Proteogenomic Research on Human Pancreatic Ductal Adenocarcinoma

Nam D[1], Hyeon D[2], Han Y[3], Jang J[3], Hwang D[2], Lee S[1]
[1]Korea University, [2]Seoul National University, [3]Seoul National University College of Medicine
We report a comprehensive proteogenomic characterization of pancreatic ductal adenocarcinoma (PDAC) which is the leading cause of cancer-related deaths. Despite extensive clinical and scientific efforts, the prognosis for survival after diagnosis in patients with PDAC has not improved significantly. Integrative analysis of genomic, transcriptomic, and proteomic data in large cohort of patients with PDAC will support understanding PDAC pathogenesis and prognosis prediction. In our discovery, integrated proteogenomic analysis of mRNA, protein, and phosphorylation data from 196 patients identified six PDAC subtypes. Six subtypes were characterized as classical progenitor (TS1), squamous (TS2–4), immunogenic progenitor (IS1), and exocrine-like (IS2) subtypes using cellular pathways with mRNA and protein signatures. Especially, protein and phosphorylation data further classified the squamous subtypes (TS2–4) into activated stroma-enriched (TS2), invasive (TS3), and invasive-proliferative (TS4) compared to the mRNA data. Correlation between mutation and phosphorylation identified signaling pathways associated with somatic mutations in significantly mutated genes (SMGs) while correlation between mRNA and protein abundance revealed potential oncogene candidates correlated with patient survival. Therefore, integrated proteogenomic analysis provides potential therapeutic targets from SMGs/oncogenes, cellular pathways, and cell types to improve PDAC patient diagnosis and treatment.

PP01.069: Non-canonical Protein Identification by Protein Terminomics

Nishida H[1], Imami K[1,2], Kochi Y[3], Ishihama Y[1,4]
[1] Graduate School of Pharmaceutical Sciences, Kyoto University, [2]RIKEN Center for Integrative Medical Science, [3]Medical Research Institute, Tokyo Medical and Dental University, [4]National Institutes of Biomedical Innovation, Health and Nutrition

Introduction

Recent advances in transcriptomics and translatomics have led to the comprehensive identification of non-canonical ORFs in 'untranslated' regions. However, it is generally difficult to fully predict proteome expression levels from transcriptome expression profiles, and even with high depth global proteome analysis, the number of translated non-normal protein identities is limited, suggesting that a new approach is needed to identify non-canonical proteins with high confidence. In this study, we investigated a new workflow for the comprehensive identification of non-canonical proteins by protein terminomic technologies developed in our laboratory, in combination with customized nucleotide or amino acid sequence database.

Method and Result

First, to build a database enriched for TIS (translation initiation site), all transcribed sequences registered in GENCODE were translated into amino acids and matched with LC/MS/MS data from protein N-terminome samples. A Position Weight Matrix (PWM) of the resulting approximately 900 TIS peripheral transcribed sequences was created, and PWM scores were calculated for all AUG, CUG, and GUG codon peripheral sequences present in the GENCODE transcribed sequences. Based on these score values, TISs were predicted and a database of protein sequences translated from the start codon was constructed.

Next, deep global proteome and protein terminome analyses were performed on THP-1 cells, a human-derived monocyte cell line. In addition to the database predicted by the PWM score, we also constructed databases using various TIS prediction tools on public and experimental transcriptome datasets. As a result, we were able to identify the most peptides derived from non-canonical proteins when using the PWM-predicted DB.

Conclusion

We have developed an efficient and reliable non-canonical protein identification method using public transcriptome data and protein N-terminome data of the target samples.

PP01.070: Identification of Polycistronic Genes with the OpenProt Proteogenomics Resource

Comtois F[1], El Aissati S[1], Jacques J, Leblanc S[1], Roucou X[1]
[1]Université De Sherbrooke

Introduction

Typical protein databases are built according to genome annotations based on the assumption of a single coding sequence per transcript, usually the longest open reading frame. However, various proteogenomics strategies using custom databases relaxing these annotation rules indicate that many genes may be multi-coding and express both a reference annotated protein and a new alternative protein. Here we use the OpenProt proteogenomics resource to identify alternative proteins. Two of these genes are NUP50, encoding a component of the nuclear pore complex, and PGRMC1, encoding progesterone receptor membrane component 1. We confirm the co-expression of the corresponding reference and alternative proteins and perform their initial functional characterization.

Methods

Raw proteomics and ribosome profiling data were reanalyzed with OpenProt. Expression and quantification of endogenous reference and alternative NUP50, and PGRMC1 proteins in different cell lines and tissues were monitored by parallel reaction monitoring. The bicistronic constructs encoding NUP50 and alt-NUP50, and PGRMC1 and alt-PGRMC1 were cloned into pCDNA and transfected in cultured cells. Expression, localization, and the interactome of the alternative proteins were determined by western blot, high-resolution confocal microscopy, and affinity-based mass spectrometry.

Results

We detected endogenous expression of NUP50 and PGRMC1 and of their corresponding alternative proteins in different cell lines and tissues. The ratio between the reference and the alternative protein varies for each of these genes, indicating that NUP50 and PGRMC1 are polycistronic genes and that there is no fixed ratio between two proteins expressed from the same gene. Both reference and alternative proteins were expressed from bicistronic transcripts, confirming the ability of ribosomes to translate two coding sequences from the same transcript.

Conclusions

We have demonstrated the polycistronic nature of two human genes at the translatome and at the proteome level. We propose that many human genes are polycistronic.

PP01.071: Advancing the Functional Annotation of Non-Canonical Human Proteins With hAltKOOL: An Open Access Library for Genome-Wide CRISPR-Cas9 Screening

Lipuma D[1], Brunet M[3], MacLeod G[2], Angers S[2], Vanderperre B[1]
[1]Department of Biological Sciences, Université Du Québec À Montréal, [2]Leslie Dan Faculty of Pharmacy, University of Toronto, [3]Department of Pediatrics, Medical Genetics Service, Université de Sherbrooke

Introduction: The contribution of non-canonical open reading frames encoded polypeptides to the diversity of the proteome is increasingly recognized. Alternative open reading frames (AltORFs) can be present in lncRNAs, in mRNA's untranslated regions (UTRs) or overlapping the referenced coding sequence but in a different reading frame. Their capacity to be translated into alternative proteins, and the extent of this phenomenon in eukaryotic organisms continues to be strengthened by a wealth of Ribo-Seq and mass-spectrometry evidence. However, the functional characterization of alternative proteins is hindered by a lack of appropriate toolkits. A functional genomics tool would allow the interrogation of the function of thousands alternative proteins, helping fill this ever-increasing knowledge gap.

Methods: We have designed an open, genome-wide CRISPR-Cas9 pooled lentiviral library targeting human high-confidence AltORFs, validated by at least 2 unique peptides by MS and/or in 2 independent Ribo-seq experiments. This was done using a Python notebook that allows CHOPCHOP algorithm-based sgRNAs design and downstream filtering of AltORFs to be included in the library based on sgRNA score and target region. To prevent undesired editing of reference coding sequences, only non-overlapping regions were used for target sites selection.

Results: Our library (hAltKOOL: human Alternative proteins Knock-Out Open Library) targets >5500 AltORFs and will be validated in genome-wide pooled CRISPR-Cas9 screening in several human cells to identify AltORFs involved in cell fitness. Alternative proteins hits will be validated using an inducible KO system, and characterization by RNA-seq and TurboID will inform on the molecular mechanisms underlying their impact on cell fitness. hAltKOOL will be shared with the community via Addgene.

Conclusions: hAltKOOL will help accelerate the functional study of alternative proteins, by equipping more researchers to embrace the increasing complexity in genetic organization and proteome diversity in human.

PP01.072: NuXL in Proteome Discoverer - A Specialized Database Search Engine for the Analysis of DNA-/RNA-Protein Crosslink Data

Delanghe B[1], Sachsenberg T[2,3], Welp L[4], Sharma A[1], He Y[5], Siraj A[3], Wulf A[4], Bazso F[4], Chernev A[4], Fritzemeier K[1], Viner R[5], Kohlbacher O[2,3,7,8], Urlaub H[4,6]
[1]Thermo Fisher Scientific (Bremen) GmbH, [2]Institute of Bioinformatics and Medical Informatics, University of Tübingen, [3]Applied Bioinformatics, Dept. of Computer Science, University of Tübingen, [4]Bioanalytical Mass Spectrometry Group, Max Planck Institute for Multidisciplinary Sciences, [5]Thermo Fisher Scientific, [6]Bioanalytics Group, Institute of Clinical Chemistry, University Medical Center Göttingen, [7]Institute of Translational Bioinformatics, University Hospital Tübingen, [8]Biomolecular Interactions, Max Planck Institute for Developmental Biology

Introduction:
Technical and methodological advances enable to apply crosslinking mass spectrometry (XL-MS) for the identification of DNA and RNA binding sites within proteins in vitro. The use of chemical crosslinking agents and incorporation of photoactivatable nucleotides increases crosslinking efficiencies broadening the applicability of XL-MS to in vivo studies of DNA-/RNA-protein interactions. Hence, the demand for specialized software tools is growing that identify crosslinked peptides, including site-localizations. We introduce the new database search engine NuXL, implemented into the Proteome Discoverer software (PD), which reliably identifies DNA- and RNA-crosslinked peptides supporting a variety of crosslinking techniques.
Methods:
The NuXL node was designed to identify UV- and chemically-induced crosslinks by taking individual MS fragmentation behaviour into account. Respective presets have been assembled based on our own in vitro and in vivo datasets including human nucleosomes, HeLa cells and Escherichia coli cells as well as on published data. We calculate match-odds and subscores and employ semi-supervised score calibration using Percolator. Moreover, entrapment experiments have been employed using manually curated data ensuring proper false discovery rate (FDR) control.
Results:
NuXL robustly identifies crosslinked peptides and amino acids from XL-MS data offering optimized search settings for a broad spectrum of crosslinking agents (i.e. UV, 4SU, 6SG, formaldehyde, 1,2:3,4-diepoxybutane and mechlorethamine) for both RNA- and DNA-protein crosslink samples. Further, NuXL provides FDR control on the level of crosslinked peptide spectrum matches.
Conclusion:
We introduce the user-friendly database search engine NuXL, implemented in PD developed for the analysis of DNA-/RNA-protein UV- and chemically-induced crosslink data. Default settings for commonly used crosslinking techniques are provided and the workflow in addition allows for custom defined settings that can be individually adapted to other crosslinking agents. As NuXL is embedded in the PD environment, it can be combined with downstream analysis nodes enabling e.g. label-free quantification of crosslink sites.

PP01.073: Spatial Multi-omics Profiling of Brain Tissue through High-resolution Mass Spectrometry Imaging (HR-MSI) in Neurological Experimental Models

García-Berrocoso T[1], Chicano-Gálvez E[2], Peralbo-Molina Á[2], Melià-Sorolla M[3], Gasull T[3], Carrascal M[1], Abián J[1]
[1]CSIC/UAB Proteomics Laboratory, Institute of Biomedical Research of Barcelona, Spanish National Research Council (IIBB-CSIC/IDIBAPS), [2]IMIBIC Mass Spectrometry and Molecular Imaging Unit (IMSMI), Maimonides Biomedical Research Institute of Cordoba (IMIBIC), Reina Sofia University Hospital, University of Cordoba (UCO), [3]Cellular and Molecular Neurobiology Research Group, Department of Neurosciences, Germans Trias i Pujol Research Institute (IGTP)

Introduction:
Unraveling the spatial location of any type of molecules into the brain tissue would contribute to a better comprehension of its biology and the pathological causes of neurological diseases. Mass spectrometry imaging (MSI) has turned into a groundbreaking tool for unbiased multi-molecular histological studies in the highly complex brain structures. Seeking a broad coverage of the brain biochemical architecture through multi-omics analyses was the main goal of this study.

Methods:
Flash-frozen brains from Mus musculus and Sus scrofa were used as neurological experimental models and 10 µm tissue cryosections were collected onto ITO-coated glass slides. Different washing protocols, pre-matrix treatments (on-tissue trypsin digestion, on-tissue chemical derivatization) and matrices were employed as per the molecular analysis performed. Pre-treatments and matrices were sprayed on top of the brain sections by optimized methods using an HTX-M5 sprayer. Spatial multi-omics label-free analyses (peptidomics, lipidomics and metabolomics) were conducted by HR-MSI on a timsTOF FleX (Bruker). Compound ion distributions were visualized with SCILS™ Lab software, together with unsupervised spatial clustering and comparative analyses of detected features. Lipids and small molecules identification were performed in METASPACE. Nissl histological staining was integrated with the molecular data.

Results:
Our untargeted analyses revealed specific spatial distribution of m/z signatures in both mice and swine brains. Clusters of lipid subclasses (phospholipids, sphingolipids, sterols), neurotransmitters and both tryptic and endogenous peptides were thus associated with brain regions along all cerebral cortex layers.

Conclusions:
MSI multi-omics approaches have the potential of providing extensive molecular information in the characterization of the brain biochemical structures of typically used animal models in the context of neurological diseases. These methods could be applied to experimental models or human specimens of prominent neurological diseases, from neurodegenerative conditions such as Alzheimer's o Parkinson's diseases to more acute brain injuries including stroke and cerebral tumors.

PP01.074: Targeted Analysis of Naturally Occurring Cross-linked Collagen Peptides in Forensic Tissues

Gilbert C[1], Scanvion Q[2], Bathany K[2], Bertrand B[2], Hedouin V[2], Tokarski C[1]
[1]CBMN, UMR CNRS 5248, Proteome Platform, University of Bordeaux, [2]Univ. Lille, CHU Lille, ULR 7367 - UTML&A - Forensic Taphonomy & Anatomy

Introduction

Collagen, the most abundant extra-cellular matrix protein in the body, significantly contributes to the high mechanical strength of many tissues, due to enzymatically controlled cross-linking of the collagen network. This can be a key biomarker in many biological processes, such as cancer, gestation, and osteogenesis imperfecta. We are interested in the analysis of collagen cross-links to identify biomarkers to determine the post-mortem interval, focusing on the structural analysis of collagen cross-linking in forensic bones using optimized proteomic workflows.

Methods

We present an optimised protocol efficiently solubilising collagenous tissue, through a multi-stage extraction protocol targeting insoluble collagen using chemical digestion. Then, to isolate naturally occurring, trivalent, cross-linked peptides, a multi-chromatographic separation was used to enrich in cross-linked collagen peptides based on fluorescence activity. Finally, these fractions were analysed using nanoLC-MS/MS (Orbitrap), with targeted MS3 fragmentation methods to fully characterise isolated peptides.

Results

To characterise cross-linked collagen peptides with MS, there are significant challenges, such as the lack of bioinformatic support for the proteomic analysis of trivalent cross-links, and the heterogeneity of cross-linked collagen structures. The optimised, multi-extraction protocol proposed for collagen cross-link enrichment both increased the yield of collagen extracted, and allowed for the detection of extracellular matrix-associated proteins that were not detected using traditional GuHCl extraction methods. Additionally, posttranslational modifications associated with the collagen cross-linking mechanism were detected. We focus on the structural analysis of trivalent, mature cross-links (eg. pyridinolines); as these are more likely to persist in forensic samples, in comparison to the chemically unstable, divalent, immature cross-links (eg. ketonorleucines). We will present examples of cross-linked peptides identified through this enrichment procedure.

Conclusions

With the low concentration of naturally-occurring cross-linked peptides in biological tissues, as well as their structural heterogeneity, a multi-level enrichment procedure was necessary to confidently identify and characterise such structures using targeted MS analysis.

PP01.075: The Neuropeptide Neuroparsin-A Regulates Caretaking Behavior in Leafcutter Ants

Gilbert M[1], Glastad K[1], Fioriti M[1], Pino L[2], Kacsoh B[1], Berger S[1], Garcia B[3]
[1]University Of Pennsylvania, [2]Talus Bioscience, [3]Washington University in St. Louis

The leafcutter ant, Atta cephalotes, is the most socially complex insect known, and offers a new powerful experimental paradigm to investigate the role of gene regulation, social influence, and epigenetic mechanisms in a phenotypically plastic system at the organismal level. A. cephalotes has 8 behaviorally and morphologically distinct worker castes with nearly identical genomic DNA, but the epigenetic mechanisms underlying these differences is completely unknown. A barrier to understand chromatin-based gene regulation and sociality has been lack of a model system. Here, we find that Neuroparsin-A (NPA), a neuropeptide previously explored in the context of social behavior in locusts, regulates caretaking behavior in leafcutter ants. Brain knockdown of NPA results in acquisition of caretaking behavior, measured by a behavior assay using a 3D-printed chamber to reflect the physiology of the colony. Transcriptomic analysis of the NPA and its receptor (VKR) confirmed acquisition of nurse-biased genes. To show reversibility of the behavior, we performed absolute quantification with mass spectrometry to determine the amount of NPA in the brains of each caste. We then expressed and purified A. cephalotes NPA in Sf9 cells and injected into the brains of the gardener caste, which possess caretaking behavior at baseline. Strikingly, injection of NPA results in loss of caretaking behavior in the gardener caste. Transcriptomics following the NPA injection show and inverse correlation with the sequencing from the NPA and VKR KD. Future experiments will translate these findings in higher order eusocial species to find conserved genes and pathways.

PP01.076: Towards a Human Disease Blood Atlas for Next-Generation Precision Medicine Strategies

Edfors F[1,2], Kotol D[1,2], Bueno Alvez M[1,2], Hober A[1,2], Woessmann J[1,2], von Feilitzen K[1,2], Ponten F[3], Fagerberg L[1,2], Uhlen M[1,2,4]
[1]SciLifeLab, [2]Department of Protein Science, KTH-Royal Institute of Technology, [3]Department of Immunology, Genetics and Pathology, [4]Department of Neuroscience, Karolinska Institutet

Introduction

The tools necessary to define and differentiate between molecular patterns associated with different diseases have rapidly evolved in recent years and are now suitable for high throughput proteomics studies. Here, we present the first draft of the Human Disease Blood Atlas with the aim to provide a comprehensive map of the protein levels present in human blood in the context of disease. The effort aims to provide an open-access resource consisting of comprehensive and accurately measured protein levels in blood accompanied by minimal clinical metadata, which will be made available through the open-access Human Protein Atlas portal (www.proteinatlas.org).

Methods

Blood plasma from patient groups diagnosed with one of the included diseases has been collected and processed using state-of-the-art omics platforms that allow for the deep plasma profile to be interpreted both sensitively and quantitatively. Protein profiles have been quantified using two technology platforms; Proximity Extension Assays (PEA) and LC-SRM/MS using Stable Isotope Standard (SIS) protein fragments. More than 9,000 samples have been processed within the Human Disease Blood Atlas Consortium.

Results

The collective pan-disease dataset consists of proteome measurements of 1,463 proteins using the PEA Olink Explore platform and more than 200 proteins with LC-SRM/MS using SIS protein fragments. Preliminary results show that panels of proteins with disease-specific profiles can be defined using an AI-based section to classify different diseases accurately.

Conclusions

The open-access Human Disease Blood Atlas presents a unique knowledge resource describing variation in the plasma proteome across different diseases. It allows researchers to identify shared molecular features associated with a certain disease. This resource will allow for further in-depth exploration of various precision medicine efforts. The combined data from PEA and LC-SRM/MS cover an extensive dynamic range and will act as an important reference for future studies on disease progression, co-morbidities, and disease recovery.

PP01.077: Limited Proteolysis-mass Spectrometry as a New Strategy for Mining Oral Cancer Biomarkers

Granato D[1], Normando A[1], Trino L[1], Carnielli C[1], Lopes A[1], Yokoo S[1], Filho H[1], Patroni F[2], Domingues R[1], Pauletti B[1], Santos-Silva A[3], Lopes M[3], Brandão T[4], Prado A[4], Telles G[5], Paes Leme A[1]
[1]CNPEM, [2]Centro de Biologia Molecular e Engenharia Genética, Universidade Estadual de Campinas, [3]Departamento de Diagnóstico Oral, Faculdade de Odontologia de Piracicaba Universidade Estadual de Campinas (UNICAMP), [4]Instituto do Câncer do Estado de São Paulo, Octavio Frias de Oliveira, [5]Universidade de Campinas, Instituto de Computação

Introduction: One of the most important prognostic factors in OSCC is the presence of lymph node metastasis. The early detection of late-presenting cervical lymph node metastasis can improve the outcomes for OSCC patients, prolonging survival and providing a better quality of life. Hence, different techniques, including proteomics-based strategies in saliva, have been applied to identify prognostic biomarkers that can differentiate OSCC cases with and without lymph node metastasis. However, it is known that biological processes are mainly regulated by protein-protein interactions (PPIs), protein aggregation, enzyme chemical modifications, and post-translational modifications. In LiP-MS experiments, the use of broad-specificity proteases under controlled conditions dictates the cleavage by the structural features of the protein. Therefore, the identification of variations in LiP-MS patterns allows the recognition of protein regions implicated in structural rearrangements. Our approach pursues the proposal of novel biomarkers by LiP-MS.
Methods: We analyzed saliva samples from healthy individuals and OSCC patients (N0, without and N+, with lymph node metastasis) directly to the LiP-MS workflow which explores in parallel the protein abundance and their structural changes (LiP). We analyzed the peptide mixture by data dependent acquisition with label-free quantification and corrected data from LiP experiments for protein abundance changes to lead to structure-specific proteolytic fingerprints for every detectable protein.
Results: LiP-MS indicated that high-confidence candidates underwent structural changes in OSCC with LN metastasis, besides the protein levels observed using traditional approaches. Moreover, LIP-MS reveals potentially relevant peptides with clinical classification ($R>0.7$, $p<0.05$, $AUC>0.8$).
Conclusions: In summary, the present study has characterized and compared the saliva proteome structural conformational changes among control individuals and OSCC patients (with and without lymph node metastasis). Besides, it was capable to show that several conformotypic peptides, from saliva, are associated to clinical characteristics, showing that the conformational dynamics of saliva proteome in different conditions-related to disease.

PP01.078: Characterizing a Type III Kinase Inhibitor Using Laser-Free Flash Oxidation (Fox) Hydroxyl Radical Protein Footprinting

Hart Chea E[1], Weinberger S[1]
[1]GenNext Technologies

The Fox Protein Footprinting System is a novel hydroxyl radical protein footprinting (HRPF) method that uses a proprietary flash oxidation lamp to generate hydroxyl radicals (•OH) that irreversibly modify solvent exposed amino acid side chains. As solvent accessibility changes during inhibitor binding, the •OH modification concordantly changes. With the Fox System, a type III kinase inhibitor's interaction with Abl kinase is characterized.

Non-allosteric Abl kinase inhibitors have shown high levels of secondary resistance and severe off-target drug effects resulting in the need for allosteric inhibitors with high specificity for Abl kinase including, GNF-5. The allosteric inhibitor, GNF-5, binds specifically to Abl's myristate binding pocket inducing the active/open conformation of Abl to the inactive/closed conformation. Three conditions (1: Abl+vehicle, 2: Abl+ATP, and 3: Abl+ATP+GNF-5) were labeled using the Fox System in triplicate. Following labeling, samples were digested with trypsin and analyzed on a Thermo Orbitrap Fusion Tribrid coupled with a Dionex Ultimate 3000 liquid chromatography system. FoxWare™ Protein Footprinting Data Processing Software calculated the average peptide oxidation (APO). The APO across the three conditions were compared and peptides with a significant change in APO (p-value < 0.05) were determined.

Comparing condition 1 and 2, the ATP binding pocket and allosteric changes were identified. When the type III inhibitor was used (condition 3) it binds to an allosteric site and locks Abl into an inactive conformation. Comparing the APO between condition 2 and 3, the allosteric binding location was determined and Abl's structure change from an active to inactive kinase was characterized. All results were confirmed from various crystal structures. Taken collectively, our results illustrate that the Fox Protein Footprinting System can confidently and accurately characterize the interaction of Abl with ATP and a type III kinase inhibitor.

PP01.079: Optimization of FAIMS-XL-MS Workflow for Phospho-Enrichable Crosslinkers

Shi Y[1], Groppe B[2], Foster L[3], Bomgarden R[3], He Y[1], Viner R[1]
[1]Thermo Fisher Scientific, [2]Thermo Fisher Scientific, [3]Thermo Fisher Scientific

Introduction

Cross-linking mass spectrometry (XL-MS), a rapid and high-resolution technique, has grown dramatically into a routinely utilized strategy for characterizing protein higher-order structure and mapping protein-protein interaction networks on a proteome-wide scale. However, a major challenge for XL-MS is the relatively low identification rates of cross-linked peptides, particularly for more complex protein samples. In this work, we implemented FAIMS (high field asymmetric waveform ion mobility spectrometry) technology on Thermo Scientific™ Orbitrap™ Exploris™ 240 mass spectrometer in combination with phospho-enrichable crosslinker- PhoX to address this analytical challenge.

Methods

Amine-reactive, enrichable crosslinkers including PhoX (Disuccinimidyl Phenyl Phosphonic Acid) were used to crosslink Ecoli ribosomes. Separation of digested peptides was achieved using UltiMate 3000 RSLCnano LC system with a 75 min gradient. Following separation, the peptides were detected on the Exploris™ 240 equipped with a Thermo Scientific FAIMS Pro Duo interface. Data were analyzed using Thermo Scientific™ Proteome Discoverer™2.5 software and XlinkX 2.5 node.

Results

XL-MS continues to exhibit low peptide identification and reaction rates, even for enrichable crosslinkers, making optimization of MS operation parameters critical. Compared to conventional mass spectrometry, FAIMS increases analytical performance by incorporating additional gas-phase fractionation. Previously, we described optimized FAIMS XL-MS workflow for tribrid instruments, in this study, we focus on XL-MS methods for hybrid instruments, such as the Exploris 240. The optimal operating conditions were identified as 250°C for the ion transfer tube temperature, 1500V for the spray voltage, 0.9Arb for the total carrier gas flow, 1.5 sec for the cycle time, and 70% as the RF lens with shared dynamic exclusion list. For PhoX cross-linker, 2 CVs, -45 and -60 in standard resolution mode with HCD SCE method provides best results ribosome samples. With optimized MS conditions, the number of identified crosslinked peptides increased for un-enriched and enriched samples.

PP01.080: Systematic Identification of Conformation-specific Protein-protein Interactions by Limited Proteolysis–Mass Spectrometry

Holfeld A[1], Schuster D[1,2,3], Sesterhenn F[1], Stalder P[1], Haenseler W[1], Barrio-Hernandez I[4,5], Ghosh D[6], Khanppnavar B[2,3], de Souza N[1,7], Beltrao P[1,4], Korkhov V[2,3], Picotti P[1]
[1]Institute of Molecular Systems Biology, Department of Biology, ETH Zurich, [2]Institute of Molecular Biology and Biophysics, Department of Biology, ETH Zurich, [3]Laboratory of Biomolecular Research, Division of Biology and Chemistry, Paul Scherrer Institute, [4]European Molecular Biology Laboratory, European Bioinformatics Institute, Wellcome Genome Campus, [5]Open Targets, Wellcome Genome Campus, [6]Laboratory of Physical Chemistry, Department of Chemistry and Applied Biosciences, ETH Zurich, [7]Department of Quantitative Biomedicine, University of Zurich

Introduction: Alterations in protein-protein interactions (PPIs) are relevant to many human diseases including neurodegeneration. Parkinson's disease (PD) is characterized by intracellular proteinaceous deposits of aberrant proteins referred to as Lewy bodies. One of the major components of these inclusions is a protein called alpha-synuclein that undergoes abnormal conformational changes and protein aggregation events that modulate physiological processes and contribute to pathology. The pathogenesis of PD is not entirely described up to date. It has recently been proposed that conformational changes of alpha-synuclein can lead to alterations in protein interaction networks during diseased states. Therefore, identifying differential interactomes of alpha-synuclein driven by conformational change can vastly help to understand the underlying molecular disease mechanisms. However, current state-of-the-art techniques do not allow to map such conformation-specific interactions and their PPI interfaces directly in a complex cellular milieu.

Methods: To address this limitation, we further developed a structural proteomics approach based on limited proteolysis–mass spectrometry (LiP–MS) (1,2) to study conformation- and proteoform-specific PPIs on a proteome-wide scale by adding purified or recombinantly expressed proteins under native conditions into cellular extracts instead of small molecules.

Results: We validated the LiP–MS approach for systematic investigation of PPIs in complex proteomes. Further, we exploited the method to study conformation-specific interactors of monomeric and fibrillar alpha-synuclein. We identified a number of previously known interactors of alpha-synuclein in its monomeric and aggregated state and novel potential interactors including their protein interaction interfaces. Our data also demonstrate that identified targets are associated with common and rare variants of PD.

Conclusions: The LiP–MS technology has proven a powerful tool that can shed light on how conformational changes of proteins alter protein interaction networks and modulate cellular processes.

References:

(1) Piazza et al. Cell (2018)
(2) Piazza and Beaton et al. Nat Commun (2020)

PP01.081: Integrative Modeling of the Flexible SARS-CoV-2 NSP2-GDS1 Protein Complex Determined by Structural Proteomics and cryo-EM

Kaake R[1,2,3,4], Pulido E[4], Muralidharan M[1,2,3,4], Echeverria-Riesco I[1,2,3,4], Verba K[1,2,3], Gupta M[1,2,3], Pourmal S[1,2,3], Mehmood H[1,2,3], Diallo A[1,2,3], Trenker R[1,2,3], Merz G[1,2,3], Kalia R[1,2,3], Swaney D[1,2,3,4], QBI Coronavirus Research Group Structural Biology Consortium[1,2,3], Krogan N[1,2,3,4]
[1]University Of California San Francisco, [2]QBI Coronavirus Research Group (QCRG), UCSF, [3]Quantitative Biosciences Institute (QBI), UCSF, [4]J. David Gladstone Institutes

Introduction: As host directed therapy becomes an attractive option for treating increasingly transmissible SARS-CoV-2 variants, structural characterization of virus-host protein complexes can help identify druggable targets. The first SARS-CoV-2 protein interaction network identified an interaction between viral NSP2 and human GDS1, an interaction that is preserved across NSP2 mutants and variants. The NSP2 cryo-EM structure revealed conserved zinc-binding sites and a flexible C-terminus. To characterize NSP2-GDS1 complex stoichiometry, zinc binding, and protein interaction interfaces we use structural proteomics, cryo-EM, and integrative modeling.
Methods: Purified protein samples (NSP2, GDS1, and NSP2-GDS1) were analyzed by structural proteomics techniques: 1) cross-linking MS (XL-MS); 2) hydrogen/deuterium exchange MS (H/DX-MS); and 3) native MS (nMS). The NSP2-GDS1 protein complex was analyzed by cryo-EM, and previously reported NSP2 and GDS1 structures will be integrated with proteomics data using the Integrative Modeling Platform (IMP).
Results: nMS reveals NSP2 monomer-dimer equilibrium bound to 3 and 6 Zn2+ respectively. NSP2-GDS1 forms a stable heterodimer that is preferred over monomeric or homodimeric NSP2 and GDS1. H/DX-MS analysis identifies regions of NSP2 with EX1 kinetics, indicating multiple conformational states. When in complex with GDS1, NSP2 surprisingly has increased exchange, indicating increased flexibility. Cross-linking NSP2-GDS1 results in the formation of dimeric complexes, and XL-MS analysis revealed a high percent of intramolecular linkages that violate distance constraints of NSP2 (43%) and GDS1 (32%) structures, indicating a mixed population of hetero- and homodimers. Intermolecular linkages mapped to the flexible NSP2 regions identified by H/DX-MS. Preliminary cryo-EM results show NSP2 and GDS1 can be partially mapped to resolved domains, though remaining regions are unresolved.
Conclusions: Due to its flexibility, the NSP2-GDS1 complex presents a unique structural challenge. By integrating multiple structural proteomics, and cryo-EM datasets, we can build a more complete understanding of this dynamic complex.

PP01.082: Application of In-Cell Fast Photochemical Oxidation of Proteins for the Study of Organoids

Shortt R[1], Jones L[2]
[1]University Of Maryland Balitmore, [2]University of California San Diego

Introduction

It has been demonstrated that immortalized cell lines in cell culture function differently compared to cells in tissue. The two-dimensional models do not simulate the in vivo environment and these issues have necessitated the development of new systems that mimic in vivo conditions. Organoids are a multicellular three-dimensional model system that mimics the corresponding organ. The complexity of organoids makes them difficult for structural studies. Therefore, we have developed the in-cell fast photochemical oxidation of proteins (IC-FPOP) method for the analysis of protein interactions in organoids.

Methods

Huh-7 cells were cultured under typical two-dimensional conditions. Cells were combined with CELLINK and bio-printed in the shape of an asymmetrical oval using a Cellink BIOX. Liver organoids were cultured for five days and then subjected to FPOP. Hydrogen peroxide diffusion was assessed using fluorescence imaging of individual slices of the organoid. Post-FPOP labeling, cells were isolated from the CELLINK matrix. Cell lysis and all subsequent processing steps were performed immediately. Peptides were separated by an EvoSep LC and analyzed by an Orbitrap Fusion Lumos MS.

Results

IC-FPOP is a structural proteomics method that uses hydroxyl radicals, generated via laser photolysis of hydrogen peroxide, to footprint solvent accessible amino acids. Here, this cellular based method was further extended to study organoids. A unique bioprinting protocol for the CELLINK BIO X was curated to easily distinguish the top of the organoid from the bottom after IC-FPOP. The organoids were subjected to IC-FPOP, using one minute peroxide incubation times, frozen, and cut horizontally using a tissue slicer. Each individual layer was analyzed by LC-MS/MS and revealed modification in each organoid section. Over 100 modified proteins were detected using this novel structural biology method.

Conclusions

IC-FPOP on organoids is an innovative structural biology technique to interrogate proteins in native tumor environments.

PP01.083: Uncovering the Basement Membrane Landscape through XLMS

Stevens K[1], Jankevics A[1], Van Berkum E[1], Scheltema R[1]
[1]Universiteit Utrecht

Introduction: Basement membrane proteins contribute to the pathogenesis of a wide array of human diseases. However, resolving the structures of these proteins is challenging using common structure determination methods, such as X-ray crystallography and nuclear magnetic resonance (NMR) and Cryo-EM due to their sheer size and complexity. Chemical crosslinking-mass spectrometry (XLMS) provides an alternative route for obtaining experimental evidence of native protein structures in complex heterogenous and dynamic systems, which can be used to guide in silico modelling and docking studies.

Methods: Basement membrane proteins from commercial Engelbreth-Holm-Swarm sarcoma lysate (ECM gel) were crosslinked using the commercially available enrichable lysine-lysine crosslinker PhoX. Crosslinked peptides from tryptic digests were enriched by immobilised metal ion affinity chromatography (IMAC) using the automated AssayMAP Bravo platform (Agilent) with Fe(III)-NTA cartridges. MS/MS analysis was performed using a an Orbitrap Exploris 480 mass spectrometer (Thermo Fischer). Crosslinked peptides were identified using XlinkX for Proteome Discoverer v2.5 (Thermo Fischer).

Results: XLMS of ECM gel identified reproducible crosslinks between the laminin-111 alpha, beta and gamma chains, in addition to crosslinks with nidogen-1 and 2, were consistent with published partial crystal structures of laminin trimers and the laminin-nidogen complex. Through a combination of computational molecular modelling and docking using DisVis and HADDOCK we extended the existing model with laminin alpha-1 G-like domains. These domains are important targets for integrin-binding and modulation of these regions underlie many diseases.

Conclusions: XLMS was used to guide modelling of the laminin-111 complex in a biologically relevant model basement membrane system. This study demonstrated a workflow that can be applied for improving the accuracy of computational modelling of extracellular matrix proteins.

PP01.084: Mapping Conformational Epitopes on Ash Profilin (rFra e 2) with Cross-reactive Murine Monoclonal Antibodies IgG Anti-rubber Profilin (anti-Hev b 8)

Teran Olvera M, Mares Mejía J, Rodriguez Romero A
[1]Institute Of Chemistry, National Autonomous University of Mexico

Introduction. Fra e 2 is a profilin allergen contained in Fraxinus (ash) pollen tree, that is spread across the world, it induces 23-50% of IgE recognition causing allergic respiratory diseases, including rhinoconjunctivitis and asthma. To date, Fra e 2 IgE conformational epitopes have not been established. In the present study, we aim to map conformational epitopes of ash profilin (Fra e 2) using the murine monoclonal antibodies (mAbs) IgG 1B4 and 2D10 (anti-rHev b 8).

Methods. Recombinant rFra e 2.2 was expressed in E. coli (Rosseta DE3) and its molecular mass was verified by mass spectrometry MALDI-TOF. Cross-reactivity of IgG mAbs (anti-rHev b 8) towards rFra e 2.2, was evaluated using indirect ELISAs with the mAbs 2D10 and 1B4.

Results. Recombinant Fra e 2.2 molecular mass was 16857 Da. Binding experiments showed that mAb 2D10 recognizes rFra e 2.2, whereas 1B4 did not. This indicates ash profilin conserved amino acids along the epitope with profilin Hev b 8 shares 74% identity, which is necessary to override the recognition with IgG mAb 2D10. mAb 2D10 main epitope is localized in the N- and C- tail α-helix 1 y 3 of profilins, which is one of the most conserved areas in allergic profilins from plants. In contrast, 1B4 has variable changes along with all the epitope.

Conclusions. mAb 2D10 was useful for mapping epitopes of Fra e 2.2. The identification of profilin major epitopes will lead to improvements immunotherapy for allergic patients to this allergen.

PP01.085: Effect of APOB Polymorphism rs562338 (G/A) on Serum Proteome of Coronary Artery Disease Patients: A “Proteogenomic” Approach

Zafar M[3], Mirza M[1], Awan F[1], Malik I[1], Larsen M[1], Choudhary M[1]
[1]Dr. Panjwani Center for Molecular Medicine and Drug Research, International Center for Chemical and Biological Sciences ICCBS), University of Karachi, [2]Department of Biotechnology, University of Sargodha, [3]Health Biotechnology Division, National Institute for Biotechnology and Genetic Engineering (NIBGE)

Introduction: Cardiovascular disease is considering as complex and multifactorial diseases involving several biological and environmental risk factors. The currently available platforms for early diagnosis and for deep pathophysiology understanding, are still insufficient to provide holistic information. In the current study, APOB (rs562338) genotype-guided proteomic analysis was performed in a cohort of Pakistani population. Methods: A total of 700 study subjects, including Coronary Artery Disease (CAD) patients (n = 480) and healthy individuals (n = 220) were included as control group. Genotyping was carried out by using tetra primer-amplification refractory mutation system-based polymerase chain reaction (T-ARMS-PCR) and validated through sequencing, whereas orbitrap LC/MS was used for label free quantification of serum samples. Results: Genotypic frequency of GG genotype was found to be 90.1 %, while 6.4 % was for GA genotype and 3.5 % was for AA genotypes in CAD patients. In the control group, 87.2 % healthy subjects were found to have GG genotype, 11.8 % had GA genotype, and 0.9 % were with AA genotypes. Significant (p = 0.007) difference was observed between genotypic frequencies in the patients and the control group. The rare allele AA was found to be strongly associated with the CAD (OR: 4 (1.9 - 16.7), as compared to the control group in recessive genetic model (p = 0.04). Using label free proteomics, altered expression of 60 significant proteins was observed. Enrichment analysis of these protein showed higher number of up-regulated pathways, including phosphatidylcholine-sterol O-acyltransferase activator activity, cholesterol transfer activity, and sterol transfer activity in A allele carrier of rs562338 (G>A) as compared with the wild type G allele carriers. Conclusion: This study provides a deeper insight into CAD pathobiology with reference to proteogenomics, and proves the genotypic-phenotypic relationship of rs562338 (G>A) of APOB with CVD.

PP01.086: Human Root and Ascending Thoracic Aortic Aneurysm Have Distinct Proteomic and Phosphoproteomic Profiles: Conventional and Artificial Intelligence Analysis

Elbatarny M[1], Eliathamby D[1], Kuzmanov U[1], McFadden M[1], Alvarez J[1], Kim R[1], Chung J[1], Simmons C, Ouzounian M[1], Gramolini A[1]
[1]University Of Toronto

Introduction: Thoracic aortic aneurysm and dissection (TAAD) often requires surgical management to prevent death. Differences in affected anatomical locations have clinical implications for timing and extent of repair but biochemical signatures underpinning these differences are not well understood.

Methods: We prospectively collected 101 aortic tissue samples from 67 individuals: normal transplant donors (n=25), elective aneurysm surgery (n=65), and emergency type A dissection repair (n=6). Locations included: root (n=22), ascending (n=66), arch (n=7), and descending thoracic aorta (n=5). Samples were investigated using mass spectrometry proteomics (data independent acquisition) and phosphoproteomics (Zr, Fe, and Ti bead enrichment). Data were analyzed using conventional statistics and an AI algorithm of t-distributed stochastic neighbor embedding (tSNE). Significance values were $p<0.05$.

Results: Proteomics identified 5572 proteins, of which 622 were significantly different between root and ascending aneurysms. Of these differential proteins, were pathways related to apoptosis, ubiquitin-mediated degradation, and elastin microfibril assembly. Principal component analysis revealed two distinct clusters within aortic root aneurysm samples and 3 within ascending aortic aneurysm, driven mainly by smooth muscle contraction-related proteins. AI tSNE analysis of the entire cohort also suggested clustering of proteomic profiles by root vs ascending regions (Threshold $x<8$, $y>0$). Phosphoproteomic analysis identified 10 significantly different proteins between root and ascending aorta, primarily related to apoptosis and ubiquitin-mediated degradation.

Conclusions: Root and Ascending aortic tissues have distinct proteomic and phosphoproteomic profiles and samples cluster distinctly based on anatomical region. Significant differences related to apoptosis and microfibril assembly were identified. Further analysis may explain differences in natural history of TAAD based on anatomic location and guide need for tailored surgical management.

PP01.087: Endogenous Peptide Cluster Approach for Human Peptidome Profiling in the Context of Gender-age Variability

Elguoshy A[1,2], Yamamoto K[1], Hirao Y[1], Xu B[1], Shimada S[1], Yamamoto T[1,3]
[1]Biofluid and Biomarker Center, Kidney Research Center, Graduate School of Medical and Dental Sciences, Niigata University, [2]Department of Biotechnology, Faculty of Agriculture, Al-Azhar University, [3]Department of Clinical Laboratory, Shinrakuen Hospital

Introduction

Investigating the profile of normal urinary peptidome is extremely important as it reflects the human normal physiology. However, one of the main bottlenecks in peptidome profiling that the endogenous peptides are cascade of peptides differ from each other by few amino acids and characterizing them separately increase the variability among samples and decreasing the likelihood of detecting certain endogenous peptide repeatedly in more samples.

Thus, in this study, we developed a peptidome approach that assembles and collects all peptide cascade in one record called “peptide cluster”.

We used this approach to characterize the normal urinary peptidome as well as investigating the age- and gender-associated peptidome differences.

Material

Voided urine samples were collected from fifty five healthy volunteers (23 males, 32 females). After protein perception, the endogenous peptides were collected from supernatant using a MWCO column (30 KDa) followed by LC-MS/MS analysis and swiss-prot database search using Mascot. The peptide clusters was created using in-house R script. Wilcox and Correlation tests were used to identify the significantly gender-age associated peptide clusters.

Results

Applying our approach generated 13,163 peptide clusters from total 30,471 endogenous peptide identifications. So far, this is the highest number of endogenous peptides that have been characterized in any peptidome study. Additionally, Investigation of the differentiated peptide clusters between male and female revealed that 17 peptide clusters like Hepcidin-25 peptide cluster was enriched in males than females. Oppositely, 7 peptide clusters was enriched in female than males like Podocalyxin peptide cluster. Furthermore, Investigation of age-correlated urinary peptide returned 57 significantly ageing-associated peptide clusters ($p \leq 0.05$). Some were released from the most abundant urinary protein like uromodulin and albumin.

Conclusion

These findings might be considered as a baseline reference for biomarker discovery in different human diseases in the light of age and gender variations.

PP01.088: Proteomic Characterization of Sputum Samples from Cystic Fibrosis Patients by Mass Spectrometry

Fentker K[1,2], Völler M[3,4], Gräber S[3,4], Kirchner M[1,3], Ziehm M[1,3], Niquet S[1,3], Mall M[3,4], Mertins P[1,3]
[1]MDC Berlin, [2]Institute of Chemistry and Biochemistry, Freie Universität Berlin, [3]Berlin Institute of Health at Charité - Universitätsmedizin Berlin, [4]Department of Pediatric Respiratory Medicine, Immunology and Critical Care Medicine, Charité-Universitätsmedizin Berlin

Background
The novel small molecule treatment Elexacaftor/Tezacaftor/Ivacaftor (ETI) for cystic fibrosis showed great efficacy in clinical trials. Lung function (FEV1), sweat chloride and general respiratory symptoms such as mucus production, coughing and difficulty breathing improve significantly. Except for one report on the microbiome and metabolome of patients only little is known about the molecular alterations of the treatment on patients.

Aim
The aim of this study is to understand how the ETI treatment affects the patients on a molecular level. Because one major factor for improvement of patient's wellbeing is the improvement of lung function in patients treated with ETI we aim to understand the changes within the lungs by investigating the sputum proteome. This will help us to understand what further treatment patients might be missing.

Methods
We analyzed the sputum proteome of CF patients (n= 34) before and after 90 days of ETI therapy and compared it to the sputum proteome of healthy individuals (n= 7). To gain a deep insight into the proteome we used label free quantitative mass spectrometry.

Results
We could quantify more than 2,700 different proteins across all patients and healthy individuals. The sputum of patients and healthy individuals differs to a high extend (1,214 significantly different proteins, moderated F-test adj. $p < 0.01$). In treated patients this signature becomes more similar to healthy individuals. These changes were mainly driven by immune regulatory and inflammation processes but also other biological processes such as SRP-dependent cotranslational protein targeting to membrane and epidermis development are altered upon treatment.

Conclusions
This study gives a comprehensive overview on how ETI therapy affects the sputum proteome. It highlights how far the lung environment changes and shows possible approaches for further studies and therapies.

PP01.089: The Driving Roles of Mitochondria in Melanoma Uncovered by In-depth Molecular Profiling

Gil Valdés J[1], Kim Y[2], Doma V[3], Çakır U[3], Kuras M[1], Betancourt L[1], Pla Parada I[1], Sanchez A[1], Sugihara Y[11], Appelqvist R[11], Oskolas H[1], Lee B[1], de Siqueira Guedes J[4], Monnerat G[4], Reis Alves Carneiro G[4], CS Nogueira F[4], B. Domont G[4], Malm J[1], Baldetorp B[11], Wieslander E[11], Balázs Németh I[5], Szász A[6], Jeong Kwon H[7], Hong R[8], Pawłowski K[9], Rezeli M[10], Tímár J[12], Fenyö D[8], Kárpáti S[3], Marko-Varga G[10]
[1]Section for Clinical Chemistry, Department of Translational Medicine, Lund University, [2]Data Convergence Drug Research Center, Therapeutics and Biotechnology Division, Korea Research Institute of Chemical Technology (KRICT), [3]Department of Dermatology, Venereology and Dermatooncology, Semmelweis University, [4]Departamento de Bioquímica, Instituto de Química, Universidade Federal do Rio de Janeiro, [5]Department of Dermatology and Allergology, University of Szeged, [6]Department of Bioinformatics, Semmelweis University, [7]Chemical Genomics Global Research Lab, Department of Biotechnology, College of Life Science and Biotechnology, Yonsei University, [8]Department of Biochemistry and Pharmacology, Institute for systems genetics, New York University, Grossman School of Medicine, [9]Department of Molecular Biology, University of Texas Southwestern Medical Center, [10]Clinical Protein Science & Imaging, Biomedical Centre, Department of Biomedical Engineering, Lund University, [11]Department of Clinical Sciences Lund, Lund University, [12]Second Department of Pathology, Semmelweis University

Introduction: The most aggressive skin cancer, melanoma, develops from pigment-containing cells known as melanocytes. Melanoma shows a high genetic heterogeneity that is evident both at inter- and intra-tumor level. Skin exposure to environmental ultraviolet light, low levels of skin pigment, large numbers of pigment nevi, genetic and environmental factors, and a compromised immune system are crucial factors in the development of melanoma. Through clinical proteomics and PTM analysis on primary melanomas, we observed that pathological upregulation of mitochondrial translation and oxidative phosphorylation (OXPHOS), correlates with progression toward metastasis. We confirmed that dysregulations in mitochondrial pathways were associated with tumorigenesis, proliferation, progression, age and survival of the patients, and with the BRAF mutation status of the tumors.

Methods: 162 samples of non-tumor, tumor microenvironment, primary and metastatic melanomas were submitted to quantitative proteomics (162), metabolomics and amino acid analysis (77), PTM analysis (11), and detailed histopathological characterization (162). Melanoma cell lines were treated with OXPHOS inhibitors (VLX600, IACS-010759, and BAY 87-2243) and mitoribosome-targeting antibiotics (Doxycycline, Tigecycline and Azithromycin). Quantitative proteomics and cell proliferation assay were performed to assess the effect of treatment.

Results: Melanomas undergo metabolic reprogramming where mitochondria play a central role. Tumorigenesis is driven by a shift towards increased consumption of glutamine and reduced amino acid degradation, upregulation of the OXPHOS, TCA cycle, the mitochondrial translation, ADP/ATP transporters and regulators of the fission process, and glutathione metabolism. Upregulation of mitochondrial translation impacts tumor proliferation and, together with OXPHOS, they are overrepresented in progression and non-responder groups. Treatment with antibiotics and OXPHOS inhibitors impairs, in a dose-dependent manner, the proliferation of melanoma cells.

Conclusions: Pathological dysregulation of mitochondrial pathways plays a central role in melanoma development and progression. Melanoma vulnerabilities related to mitochondrial function has been exposed and validated in cellular models.

PP01.090: Decoding Functional High-density Lipoprotein Particle Surfaceome Interactions

Goetze S[1,2,3], Frey K[1,4], Rohrer L[4], von Eckardstein A[4], Wollscheid B[1,2]
[1]Institute of Translational Medicine, Department of Health Sciences and Technology, ETH Zurich, [2]Swiss Institute of Bioinformatics (SIB), [3]ETH PHRT Swiss Multi-Omics Center (SMOC) , [4]Institute for Clinical Chemistry University Hospital Zurich

Background: High-density lipoprotein (HDL) is a mixture of complex particles mediating reverse cholesterol transport (RCT) and several cytoprotective activities. During HDL's journey throughout the body, its function is mediated through interactions with cell surface receptors on different cell types. Despite its relevance for human health, many aspects of HDL-mediated lipid trafficking and cellular signaling remain elusive at the molecular level.

Methods: To better understand the functional interplay between HDL particles and tissue, we analyzed the surfaceome-residing receptor neighborhoods HDL potentially encounters and interacts with. We applied a combination of chemoproteomic technologies including automated Cell Surface Capturing (auto-CSC) and HATRIC-based ligand-receptor capturing (LRC) on four different cellular model systems mimicking tissues relevant for RCT.

Results: Surfaceome analysis of EA.hy926, HEPG2, foam cells, and human aortic endothelial cells (HAEC) revealed the main currently known HDL-receptor scavenger receptor B1 (SCRB1), as well as 155 shared cell surface receptors representing potential HDL interaction candidates. Since vascular endothelial growth factor A (VEGF-A) was recently found as a regulatory factor of transendothelial transport of HDL, we next analyzed the VEGF modulated surfaceome of HAEC using auto-CSC technology. VEGF-A treatment led to a remodeling of the surfaceome of HAEC including the previously reported higher surfaceome abundance of SCRB1 upon treatment. Using HATRIC-based ligand-receptor capturing (HATRIC-LRC) on human endothelial cells, we specifically aimed for the identification of other bona fide (co-)receptors of HDL beyond SCRB1. HATRIC-LRC enabled, next to SCRB1, the identification of the receptor tyrosine-protein kinase Mer (MERTK), which we show is directly contributing to endothelial HDL binding and uptake. Proximity ligation assay (PLA) further confirmed the spatial vicinity of MERTK and SCRB1 on the endothelial cell surface.

Conclusions: The data shown provide direct evidence for a complex and dynamic HDL receptome and that receptor nanoscale organization may influence functional HDL binding and uptake.

PP01.091: Prognostic Proteomic Models for Low Event Rates: A Case Study with Myocardial Infarction

Hagar Y[1]
[1]Somalogic Inc.

Introduction: Large-scale clinical proteomics provide increasing opportunities for patient risk stratification, especially with multi-marker models derived using machine learning techniques. Prognostic models can be developed as binary risk classifiers, or by using time-to-event data. Survival modeling is ubiquitous in statistical literature, but support for machine learning optimization is more limited in comparison to other regression techniques. We have developed and assessed a novel prognostic model development method combining two statistical techniques -- survival analysis and subsampling – using existing machine learning tools in R. These methods were applied to a clinical dataset to identify a highly predictive proteomic model for myocardial infarction (MI) despite a low observed event rate.

Methods: Cox elastic net with subsampling tools were developed in R. Simulations were used to demonstrate the utility and accuracy of subsampling in a survival data context, with comparisons made to logistic regression, Cox elastic net, and SVM models. Following the validation of the approach via simulations, models were developed and assessed on the HUNT3 data set (n = 756), which had 61 (8.1%) MI events within four years of blood draw. Proteomic measurements were performed using SomaScan v4.0 technology.

Results: The subsampled survival model performance was superior to competing logistic regression, Cox elastic net, and SVM models (AUC=0.82 vs. 0.75, 0.61, 0.75, respectively).

Sensitivity and specificity metrics were more balanced on hold-out test sets. Additionally, simulations showed that the proteins that were most highly correlated with MI were selected for final models, indicating that this method is a promising tool for clinical discovery and prognostic/diagnostic development.

Conclusions:

Survival analysis with subsampling can be applied to proteomic data to discover novel individual protein associations with outcome, as well as develop prognostic models with superior performance to traditional statistical models, even in the context of relatively low incidence rates.

PP01.092: Efficient Development of Certified Prognostic Laboratory Developed Tests using Proteomic Data

Hagar Y[1]
[1]Somalogic Inc.

Introduction: Proteomic technology is a powerful biological tool with established methods for identifying proteomic biomarkers, but the development of certified prognostic clinical tests based on proteomic biomarkers can be time-consuming, prone to overfitting issues, and difficult to navigate. We demonstrate the utility of combining pipeline tools, statistical learning techniques, and a knowledge base of in-silico proteomic datasets into a reproducible workflow that allows for efficient development of LDT-certifiable tests using SomaScan™ technology.

Methods: Data pipeline and analysis tools were developed using R, in conjunction with proteomic measurements obtained using the SomaLogic platform. The tools take the analyst from data processing and QC through identification of optimized models for prediction of clinical endpoints, and then through validation on a hold-out test set. The tools include an assessment of model robustness against sample handling issues, longitudinal stability, the impacts of assay noise on model performance, effects of putative interferents, and risk of failure during CLIA validation in the lab. Real-life examples of clinical applications demonstrate the effectiveness of the tool in reducing analysis time and increasing model accuracy.

Results: Analysis time for identifying the optimal clinical model to validation was reduced by at least 80%, with decreased prediction variability by up to 90%. In at least 75% of cases, application of in-silico data allows for tuning of predictive models to ensure robustness in a variety of everyday settings. This tool has led to 16 LDT certified SomaLogic tests in the last 3 years, ranging from anthropometric measurements to cardiovascular- and cancer-risk predictions.

Conclusions: Not only are powerful, proteomics-driven, prognostic tests realizable, but they can be LDT certified in an efficient manner and made to be robust to real-life variability. Efficient analysis tools allow us to leverage proteomic technology in new ways, leading to tests that can be used for precision medicine applications.

PP01.093: Setting a Threshold for the Development of Quality SRM Assays from DIA Data

Hains P[1], Williams S[1], Sykes E[1], Lucas N[1], Bucio-Noble D[1], Xavier D[1], Robinson P[1]
[1]ProCan®, Children's Medical Research Institute

Introduction

Numerous biomarkers and protein signatures have been identified in the literature; however, few have been translated to the clinic. The most common workflow for biomarker identification has been to analyse LC-MS/MS data for differentiating characteristics using an ever-increasing array of complex bioinformatic approaches. Our aim is to accelerate the successful development of diagnostic selected reaction monitoring (SRM) assays suitable for the clinic.

Methods

After DIA and bioinformatic analysis, we identified a characteristic protein signature for the datase t. Based on available data, this signature differentiated the treatment or outcome groups under study. We next carefully examined our raw MS/MS data to determine the robustness of the peptides comprising each protein in the proposed signature. Unfortunately, in a significant number of cases, the MS/MS evidence was poor. Even taking into account that SRM is more sensitive and specific than DIA, the peptides were often a poor choice for costly and time-consuming SRM assay development.

By examining our raw MS/MS data, we applied a lower intensity threshold t o our data before bioinformatic processing. This was applied solely to datasets where the ultimate aim is SRM development.

Results

Our approach advanced from typically <30% of the peptides comprising the signature showing good raw MS/MS evidence, to >95% having good raw MS/MS evidence. The next phase is to refine the approach, as a simple intensity cut-off can occasionally result in the omission of good quality peptides.

Conclusions

Once a quality threshold for developing SRM assays from DIA data is established, it is possible to move towards implementing and validating it for routine development of DIA to SRM assays. This approach is proving promising as allows us to identify a far greater proportion of high-quality peptides that can distinguish between patient treatment groups, whilst also showing clear signs of simplifying SRM development.

PP01.094: Development of Peptide-based Classification panel towards the Diagnosis and Prognosis of Meningioma using Targeted Proteomics

Halder A[1], Chauhan A, Biswas D, Srivastava S
[1]Indian Institute Of Technology,bombay

Introduction: Meningiomas, one of the prevalent primary brain tumors have been found to be the center of interest to many researchers and clinicians due to its impact. Despite many studies, the mechanistic insight behind its high-grade aggressiveness and recurrence is still unknown. Additionally, the problem of addressing early-onset diagnosis of Meningioma is still a paradox and needs thorough investigation. This study aims to develop a peptide-based assay using MRM based targeted proteomics approach for early-onset diagnosis and screening of tumors in regards to aggressiveness and recurrence which could stimulate the existing diagnostic approaches.

Methods: Around 61 serum and 70 fresh frozen tissue samples of different grades of meningioma and control were chosen for the study. A comprehensive list of protein markers has been curated from the literature and an in-house keyword search algorithm has been optimized at the peptide level for MRM experiments. The spectral library has been built using Prosit and in-house generated data in Skyline. Individual samples were run in a randomized manner with spiked-in heavy-labeled peptide as an internal standard. Processed peak intensities of peptides were taken forward to perform univariate, multivariate, and classification analyses for the identification of the potential set of peptide markers of Meningioma.

Results: The intra-day and inter-day QC based on spike-in-peptides and sample pools shows consistency throughout the sample run. Peptides from proteins like Clusterin, Vimentin, etc. have been found to have expressional differences in regards to meningioma grades. 10 peptides of potential markers show an interesting pattern in longitudinal serum samples which has further been investigated to understand its specificity using the Machine-Learning-based Classification model. Finally, a panel of peptides was taken forward to investigate the effectiveness of grade classification which could pave the way toward diagnosis.

Conclusions: This foundation study facilitated the development of a peptide-based assay for Meningioma.

PP01.095: Altered Secrteome by Diesel Exhaust Particles and Lipopolysaccharide in Primary Human Nasal Epithelium

Kim N[1], Kim H[2,3], Park M[2,3], Jeon E[2,3], Lee G[2,3], Kim S[3,4], Cheon S[2,4], Park M[5], Han D[2,3]
[1]Center for Medical Innovation, Biomedical Research Institute, Seoul National University Hospital, [2]Proteomics Core Facility, Biomedical Research Institute, Seoul National University Hospital, [3]Transdisciplinary Department of Medicine and Advanced Technology, Seoul National University Hospital, [4]School of Biological Sciences and Technology, Chonnam National University, [5]Department of Otorhinolaryngology-Head and Neck Surgery, Seoul National University Hospital

Introduction : Airway epithelial cells can actively participate in the defense against environmental pathogens to elicit local or systemic inflammation. Diesel exhaust particles (DEP), a main component of urban air pollution with particulate matter, are associated with the occurrence of acute and chronic upper airway inflammatory diseases. We sought to investigate the effect of DEP alone or in combination with lipopolysaccharide on the secretome in the primary human nasal epithelium (PHNE) and to find potential biomarkers to relate DEP exposure to upper airway inflammatory diseases.

Methods: PHNE was cultured at an air-liquid interface to create a differentiated in vivo-like model. Secreted proteins (secretome) on the bottom media of the PHNE were digested by filter-aided sample preparation. Peptide sample was analyzed by Q-Exactive mass spectrometry based on label-free quantitative approach. Statistical analysis was performed using Perseus software.

Results: Considerably more differentially expressed secreted proteins were identified in response to DEP plus lipopolysaccharide than to DEP alone. Some canonical pathways related to inflammation and cancer such as the p53, β-catenin, and extracellular signal-regulated kinase 1/2 pathways were involved. Among differentially expressed secreted proteins, leukemia inhibitory factor was also detected at a high level in the middle ear effusions of otitis media patients, and the leukemia inhibitory factor level was significantly correlated with daily mean mass concentrations of atmospheric particulate matter averaged over 8 days before sample collection.

Conclusions: Apical stimulation with DEP and lipopolysaccharide can significantly alter the basal secretome in PHNE, and this alteration can be reflected by surrounding inflammation with effusion of fluids in vivo such as middle ear effusions in otitis media patients.

PP01.096: Cross-Omics Analysis of Transcriptome, Proteome and Metabolome Dynamics during Peritoneal Dialysis

Herzog R[1], Wiesenhofer F[1], Kratochwill K[1]
[1]Medical University of Vienna

Peritoneal dialysis (PD) effluent (PDE), the waste product of the renal replacement therapy with PD, is a rich but underexplored source of markers for therapy monitoring and investigation of deregulated processes during PD. During PD hyperosmotic fluids are used to remove uremic toxins and water from the patients via the peritoneum, functioning as semi-permeable membrane. For understanding PD-pathomechanisms and transport on a systems biology level, a multi-level omics approach is particularly attractive.

Samples were obtained from stable patients at different time-points of routine check-ups. The effluent was separated into a cellular and cell-free component. Soluble proteins and metabolites in the cell-free compartment were processed using LC-MS workflows. The cellular material was subjected to RNA sequencing. A Plasma-Proteome database was used for referencing plasma proteins and estimating plasma concentration. A bioinformatic workflow conjoined information from the datasets to reveal novel insights into the “PD-effluentome”, especially unraveling the so far only speculated origin of proteins and metabolites in PDE.

Metabolomics enabled detecting of 207 unique metabolites in cell-free PDE. A mixed-effect ANOVA of all metabolites demonstrated dwell time-dependent concentration changes in 173 metabolites. Post-hoc testing revealed most metabolites (n=114) to be changed in a dwell-time dependent manner. We quantified 9,797 transcripts in PD-effluent cells and 2,729 proteins in PDE. 342 proteins were filtered from plasma, while 800 proteins were attributable to local origin or production. A quantitative analysis of the interaction proteome and cellular transcripts of ~1700 protein-transcript pairs showed clusters of proteins explained by over-expression in peritoneal cells compared to plasma concentrations.

Cross-omics profiling of PDE could be a valuable approach for revealing small molecule related changes during PD. The exploitation of PDE on multiple levels could improve the understanding of pathophysiological molecular processes and transport dynamics in the peritoneal cavity and their role in development of PD complications.

PP01.097: Proteome Characterization of Acral Melanoma

de Siqueira Guedes J[1,2], Sosa Acosta P[1,2], Quiñones Vega M[1,2], de Souza Cuconato M[1,2], Pinto De Almeida N[1,2], Santos Bernardes S[3], Abrão Possik P[4], Domont G[1], Sousa Nogueira F[1,2]
[1]Proteomics Unit, Institute of Chemistry, Federal University of Rio de Janeiro, [2]Laboratory of Proteomics, LADETEC, Institute of Chemistry, Federal University of Rio de Janeiro, [3]Institute of Biological Science, Department of General Pathology, Federal University of Minas Gerais, [4]Program of Immunology and Tumour Biology, Brazilian National Cancer Institute

Introduction: Melanoma is the deadliest form of skin cancer and one of the fastest-growing cancers in the world. It is characterized by enhanced metastatic capacity and low relative survival rates. Previous studies have demonstrated that different molecular changes facilitate the development and progression of this type of cancer. However, they are mainly based on analysis of European descents population, emphasizing the lack of clinical, morphological, and molecular data on melanoma in non-European descents. In this regard, mapping and molecular characterizing samples of acral melanoma (AM), which corresponds to the most common form of melanoma in Latin, African and Asian countries, is necessary to understand the particular pathophysiological processes of these populations. Therefore, the central goal of this study is to deeply characterize the proteome of AM in the non-European descent populations.
Methods: Cutaneous melanoma tissue samples from 44 Brazilian patients with acral melanoma at different stages were processed using the S-trap protocol and analyzed by nLC-QExactive Plus system.
Results: The shotgun proteomics analysis identified 8309 proteins. Among them, 527 were considered statistically different between the clinical stages (q-value≤0.05, One-way ANOVA). Our results emphasized that the progression of AM is associated with intense metabolic modification and increment in pathways related to supporting cell replication, including upregulation of protein transport and biosynthesis, stress response, and lipid and purine biosynthesis. Furthermore, the progression of AM appears to be connected with the decay of tumor suppressor proteins that act inhibiting the ability of migration and invasion and enhancing apoptosis, such as Ras suppressor, Galectin-7 and Cystatin-A.
Conclusions: Altogether, our results could open new insights into the biochemical changes associated with AM and its metastatic states, improving the comprehension of the pathophysiological mechanism related to this cancer, supporting the identification of new therapeutic targets, and finding evidence on the associated metabolic changes with progression.

PP01.098: The Four-protein Signature Predicts the Recurrence in the Early Lung Cancer

Hsiao Y[1,2], Lin K[1], Chen Y[1], Huang Y[2], Chen J[3,4], Chen Y[1], Chang G[5], Yu S[2]
[1]Institute of Chemistry, Academia Sinica, [2]Department of Clinical Laboratory Sciences and Medical Biotechnology, College of Medicine, National Taiwan University, [3]Department of Surgery, National Taiwan University Hospital and National Taiwan University College of Medicine, , [4]Department of Surgical Oncology, National Taiwan University Cancer Center, , [5]School of Medicine, Chung Shan Medical University,

Introduction:
Lung cancer is the leading cause of cancer mortality worldwide and stage IB patients proximately 30% still suffer from recurrence and metastasis. In this project, we aim to translate the findings of the International Cancer Moonshot Project into clinical utilities and aim to develop a high-risk early lung cancer ELISA prediction kit.
Methods:
We first evaluated 127 late-like proteins in the Moonshot cohort by using commercial ELISA. In the first-round selection, a total of 35 proteins were validated in patients' plasma and selected by the Students' t-test. These proteins were significantly high-expressed in the IA and IB late-like patients. In second-round selection, we used an independent cohort from the Chung Shan Medical University Hospital. The concentrations of 35 proteins in blood were also measured by ELISA. We focused on the protein expression in 44 patients with stage IB, and finally identified the 4-protein signature by using the Students' t-test, univariate Cox regression, receiver operating characteristic (ROC), and Kaplan-Meier survival analyses.
Results:
The 4-proteins signature had a hazard ratio of 1.302 (95% CI=1.115-1.520, p=0.001) and the score was significantly associated with progression-free survival (log-rank test, p=0.006). Furthermore, the 4-proteins signature had an area under the ROC curve of 0.74 (p=0.013).
Conclusions:
The results suggested that the 4-proteins signature is a potential prognostic marker for predicting the recurrence of early lung cancer and is able to develop a prediction ELISA kit in the future.

PP01.099: Discovery and Functional Characterization of Potential Biomarkers in Wild-Type EGFR Lung Adenocarcinoma by Quantitative Tissue Proteome Analysis

Hsiao T[1], Wang C[2,3], Wu Y[4], Wu C[5], Yu C[1,3,5,6]
[1]Graduate Institute of Biomedical Sciences, College of Medicine, [2]School of Medicine, College of Medicine, Chang Gung University, [3]Division of Pulmonary Oncology and Interventional Bronchoscopy, Department of Thoracic Medicine, Chang Gung Memorial Hospital, Linkou,, [4]Department of Thoracic Surgery, Chang Gung Memorial Hospital, Linkou, [5]Molecular Medicine Research Center, Chang Gung University, [6]Department of Cell and Molecular Biology, College of Medicine, Chang Gung University
Introduction: EGFR tyrosine kinase inhibitor (EGFR-TKI) targeted therapy has been established for the treatment of lung adenocarcinoma (LADC) patients with EGFR activating mutations (MT). However, chemotherapy is the first-line treatment for EGFR wild-type (WT) LADC, and the overall therapeutic effect is unsatisfactory. This study aims to develop potential biomarkers and therapeutic targets to benefit EGFR-WT LADC patients via proteomic approaches.

Methods: We applied a super-SILAC-based quantitative tissue proteomic approach to identify the proteins in early- and late-stage LADC tissues harboring EGFR-WT and MT. Candidates were selected by integrating the TCGA database, protein Atlas data mining, annotation of subcellular localization, and literature searches. Western blotting was used to verify protein expression levels in tissues. The second cohort of LADC tissues (n=117) was used in validation by immunohistochemistry. Serum samples from patients and healthy controls were collected for ELISA analysis. Gene knockdown coupling with cell viability and migration assay in cultured cells was applied to confirm the oncogenic roles of marker candidates.

Results: We quantified 5208 and 4437 proteins in early- and late-stage EGFR-WT tissues, respectively. For early detection niche and clinical applications, we first focused on EGFR-WT-enriched dysregulated proteins in early LADCs and their localization in the plasma membrane or extracellular space. Western blot and IHC demonstrated that LADC-001 was overexpressed in LADC tissues compared to their adjacent normal parts. ELISA showed that a high level of serum LADC-001 was positively associated with the poor prognosis of EGFR-WT LADC. Knockdown of LADC-001 reduced the viability and migration ability of EGFR-WT LADC cells.

Conclusion: Overexpression and biological activity of LADC-001 were enhanced in EGFR-WT but not EGFR-MT LADC cells. The signaling pathways and molecular mechanisms by which LADC-001 regulates cancer progression are under investigation. Our study collectively provides new prospects for the differential diagnosis and the foundation of targeted therapy of LADC.

PP01.100: Measuring Druggable Protein Mutation Abundance towards Precision Oncology by IsoPS DIA-MS

Chan H[1,2], Chen L[1,2], Hsu H[1,2], Xuan Y[3], Olsen J[4], Ishihama Y[5]
[1]Institute of Chemistry, Academia Sinica, [2]Department of Chemistry, National Taiwan University, [3]Thermo Fisher Scientific (Bremen) GmbH, [4]Novo Nordisk Foundation Center for Protein Research, University of Copenhagen, [5]Graduate School of Pharmaceutical Sciences, Kyoto University

Introduction: Somatic mutations have been used for designing targeted therapies. For example, EGFR with 40-50% mutation rate in the Asian population is the most prominent drug target in lung cancer. However, gene testing widely used to guide targeted therapy may not reflect abundances of protein variants, leading to various drug responses. The challenges of MS-based approaches remain, including detection of mutation sequences, minute amount of clinical samples, and assay robustness. Targeting lung cancer as a pilot study, we developed a new DIA assay for quantitation of mutant/wild EGFR to guide clinical decision-making toward precision oncology.

Methods: We developed a novel isotope pairs-separated data-independent acquisition (IsoPS-DIA) method for unambiguous identification and accurate quantification of oncoprotein mutations, like EGFR and KRAS. The windows resolved co-isolated light and heavy peptides to achieve better quantification accuracy. A workflow integrating membrane protein extraction and multiple protease digestion was employed to enrich drug target proteins and to enhance detectivity of mutant peptides.

Results: We first developed the pipeline on NSCLC cells harboring various EGFR mutations. The major EGFR mutants (L858R/Del19/G719A), as well as their corresponding wild type, were detected and quantified by IsoPS-DIA approach. The IsoPS-DIA enhanced 2-fold S/N and increased fragment numbers for quantification. The detection limit reached the sub-femtomolar level (0.036-0.222 fmol) with good reproducibility ($R^2>0.998$, CV<5%). The sensitivity of IsoPS-DIA was further evaluated for tissue profiling in cell line-derived xenograft models, allowing detection of L858R mutant peptides with only 1 mg tissue. Most interestingly, the quantification results showed variable EGFR mutation abundance and high abundance of wild type. The result revealed new insight in lung cancer xenograft model conventionally recognized as isogenic cell origin, and the implementation on patient tissue would be discussed.

Conclusions: The novel IsoPS-DIA pipeline offers accurate quantification of EGFR mutations and may provide complementary information to conventional DNA testing.

PP01.101: Revealing the Allergenic Composition of Palo Verde (Parkinsonia Microphylla) Pollen using Gel-Free Proteomics and Bioinformatics Tools

Hernández M[4], Morales-Amparano M[2], Álvarez-Campa D[3], Encarnación-Guevara S[4], Teran L[5], Huerta Ocampo J[1]
[1]CONACYT-CIAD, A.C., [2]Centro de Investigación en Alimentación y Desarrollo, A.C. Coordinación de Ciencia de los Alimentos, [3]Licenciatura en Ciencias Genómicas, Universidad de Sonora, [4]Centro de Ciencias Genómicas, UNAM, [5]Instituto Nacional del Enfermedades Respiratorias "Ismael Cosío Villegas"

Introduction: Palo Verde (Parkinsonia microphylla) is one of the most common trees in the deserts of northwestern Mexico and the southwestern United States. In late spring it is common to see the Palo Verde covered in yellow blossoms with a blanket of yellow at its base from dropped flowers. As a general principle, plants that produce showy, fragrant flowers do so to attract insects such as bees to distribute their pollen. Also, pollen is usually heavy and sticky, so it sticks to insects instead of being blown away in the wind. Palo Verde, although an insect pollenated tree, cause allergy problems just because of the huge volume of flowers that fall to ground, dry, and then picked up by the wind. So far, pollen allergens have not been identified.
Methods: To understand the allergenic composition of Palo Verde pollen, we isolated the total soluble proteins from mature pollen using a modified phenolic extraction method and identified proteins by a gel-free proteomics approach. Allergenic proteins were predicted using bioinformatics tools and functionally classified according to gene ontology.
Results:
A total of 4910 razor + unique peptides and 1188 proteins were identified against the Prosopis alba proteome database. Among the 809 proteins considered as successfully identified (two razor + unique peptides), 79 were identified as putative allergens using bioinformatic tools. Additional software allowed to classify putative allergenic proteins based on their sequence identity with the allergenic proteins from the WHO/IUIS Allergen Nomenclature Sub-Committee database. Strong evidence of allergenicity was established in 15 proteins, weak evidence in 21 cases, and no evidence of allergenicity in 43 cases.
Conclusions: Putative allergenic proteins may contribute to the development new diagnostic and therapeutic modalities in Palo Verde pollen allergy.

PP01.102: Immunoproteomic Approach for Identification of Allergenic Proteins in Velvet Mesquite Pollen

Huerta Ocampo J[1], Batista-Roche L[2], Morales-Amparano M[2], Robles-Burgueño M[2], Ramos-Clamont Montfort G[2], Vázquez-Moreno L[2], Ramírez-Jiménez F[3], Teran L[3]
[1]CONACYT-CIAD, A.C., [2]Centro de Investigación en Alimentación y Desarrollo, A.C. Ciencia de los Alimentos, [3]Insituto Nacional del Enfermedades Respiratorias "Ismael Cosío Villegas"
Introduction: The pollen of velvet mesquite tree (Prosopis velutina) is an aeroallergen of clinical relevance, responsible for respiratory allergies and it is abundant in desert regions of northwest Mexico and southwest United States. To date, the allergenic molecules of this pollen have not been immunodetected by two-dimensional Western blot, which is a limitation in the diagnosis and treatment of respiratory allergy.
Methods: Pollen was collected from Prosopis velutina trees. Total soluble proteins were obtained by phenolic extraction with a yield of 87.5 mg / g of pollen and separated by one-dimensional (13% SDS-PAGE) and two-dimensional (isoelectric focusing, 13% SDS-PAGE) electrophoresis. For immunodetection of the allergenic proteins of mesquite pollen by two dimensional western blot we used IgE antibodies from the serum of allergic patients. IgE recognized protein spots were identified by using tandem mass spectrometry and homology database search against the Prosopis alba proteome database.
Results: SDS-PAGE revealed 21 defined bands in a molecular mass range of 10-250 kDa, whereas one-dimensional immunodetection using individual sera from allergic patients revealed the presence of 15 IgE-reactive bands between 10 and 100 kDa. Two-dimensional gels obtained using 13 cm IPG strips pH 4-7 revealed 652 defined protein spots in a mass range of 10-100 kDa, whereas two-dimensional immunodetection using a pool of these sera showed 41 IgE-reactive protein spots between 14.5 and 75 kDa and with isoelectric points between 4.5 and 6.9. Twenty-four unique IgE reactive proteins, including proteins well-known as pollen allergens, as well as proteins widely recognized also as food, airway, or contact allergens were identified.
Conclusions: This is the first report for allergenic proteins in velvet mesquite pollen. These proteins will allow the development of diagnostic and therapeutic strategies for mesquite pollen allergy.

PP01.103: Improving the Precision of the Quantification of Apolipoprotein E Isoforms Results with a Single Point Calibrator in Cerebrospinal Fluid

Huynh H[1], Kuch K[1], Orquillas A[1], Forrest K[1], Poston K[4], Tian L[5], Lin A[5], MacCoss M[2], Emrick M[1], Hoofnagle A[1,3]

[1]Department of Laboratory Medicine and Pathology, University of Washington, [2]Department of Genome Sciences, University of Washington, [3]Department of Medicine, University of Washington, [4]Department of Neurology and Neurological Science, Stanford University School of Medicine

Introduction: The concentrations of apolipoprotein E (apoE) isoforms in cerebrospinal fluid (CSF) have been hypothesized to be associated with Alzheimer's disease and other neurodegenerative disorders. To test this hypothesis, several studies have quantified this protein and have yielded conflicting results, possibly due to variability or specificity of the assays employed.

Methods: To further address this hypothesis and to improve the accuracy and precision of quantification of apoE isoforms for precision medicine, a candidate reference method based on multiplexed targeted liquid chromatography-tandem mass spectrometry (LC-MS/MS) was developed to simultaneously quantify apoE isoforms in CSF. The addition of labeled internal standard peptide in 50% acetonitrile helped to overcome non-linear recovery observed when mixing CSF and recombinant protein-spiked CSF samples. To ensure the accuracy and precision of apoE results between laboratories and over time, primary reference materials of recombinant human apoE isoforms were carefully characterized by an amino acid analysis reference method. This primary calibrator was used to calibrate the LC-MS/MS assay and assign certified values to a matrix-matched single point calibrator.

Results: The novel assay is precise with within-day and between-day coefficients of variation ≤ 12.1%, which can be applied to large cohorts. Standard addition and the dilution of CSF with bovine CSF proved to be linear with a regression coefficient above 0.99 for all peptides. In preliminary analyses, a diagnosis of Parkinson's disease was associated with the CSF concentration of apoE4, but not with the total concentration of all apoE isoforms, supporting a possible role for the quantification of individual isoforms of apoE in the assessment of neurodegenerative disease.

Conclusions: Our method simultaneously quantifies apoE isoforms in human CSF with excellent precision and accuracy, which is the result of well-characterized calibrators. A reference material made from human CSF has been developed and is available to other laboratories to improve interlaboratory agreement.

PP01.104: Developing New Peptide Immunoaffinity LC-MS/MS Workflow to Quantify Novel Biomarkers of Collagen Turnover for Detection of Human Growth Hormone Misuse

Huynh H[1], Forrest K[1], Becker J[1], Emrick M[1], Miller G[2], Moncrieffe D[3,4], Cowan D[4], Thomas A[5], Thevis M[5], MacCoss M[6], Hoffstrom B[7], Byers P[1,8], Eichner D[2], Hoofnagle A[1,8]
[1]Department of Laboratory Medicine and Pathology, University of Washington, [2]Sports Medicine Research and Testing Laboratory, [3]Drug Control Centre, Department of Analytical, Environmental and Forensic Science, King's College London, [4]Department of Analytical, Environmental & Forensic Sciences, King's College London, [5]Center for Preventive Doping Research (ZePraeDo), Institute of Biochemistry, German Sport University, [6]Department of Genome Sciences, University of Washington, [7]Fred Hutchinson Cancer Research Center, Seattle, WA, United States; Current address: UCLA Translational Oncology Research Laboratories, University of California, [8]Department of Medicine, University of Washington

Introduction: Doping in sport has become a complex issue for fair competition and the health of athletes. Even though considerable progress has been made in the field of analytical chemistry for anti-doping activities, the detection of growth hormone (GH) misuse remains challenging. The identification of novel biomarkers of GH administration could lead to a better understanding of the physiological response to GH, more sensitive detection of GH misuse in sport, and better individualized management of patients treated for GH disorders.
Methods: We developed a multiplexed targeted liquid chromatography-tandem mass spectrometry method to simultaneously quantify new candidate biomarkers belonging to the carboxyl-terminal propeptide of type III procollagen (P-III-CP) and type III collagen degradation products in human serum. During method development, we detected an interference in serum digests that greatly reduced recovery in the immunoaffinity peptide enrichment step. After trying ultrafiltration, solid phase extraction, and delipidation, we discovered a simple acid precipitation step that reduced sample complexity and increased recovery in the immunoenrichment step.
Results: The assay was linear over an estimated concentration range of 0.3-1.0 nM and 0.1-0.4 nM for each proteotypic peptide of P-III-CP and collagen degradation products, respectively. Within-day and between-day imprecision was at or below 15 % CV. We applied the method to evaluate the concentration of each biomarker at different age ranges and after GH administration in healthy participants. Biomarker concentrations varied with age and appeared to reflect age-specific collagen turnover. Moreover, their concentrations changed after GH administration.
Conclusions: Our novel multiplexed method quantifies peptides belonging to the family of P-III-CP proteoforms and the type III collagen degradation products in human serum. This method could be applied in the routine analysis to detect the misuse of GH in sport and to better understand diseases treated with GH therapy or those caused by altered type III collagen turnover.

PP01.105: Enhancing Treatment Recommendations in Precision Oncology with Proteomics and Phosphoproteomics Data

Jensen C[1], Schneider A[1], Woortman J[1], Dormeier C[1], Resch M[1], Hamood F[1], Sakhteman A[1], Bayer F[1], Heinzlmeir S[1], The M[1], Lee C[4], Pfister S[3], Froehling S[3], Jones D[3], Blattner-Johnson M[3], Schramm K[3], Pajtler K[3], Fiesel P[3], Zuliani C[3], Jones B[3], Beck K[3], Kreutzfeldt S[3], Teleanu M[3], Horak P[3], Uhrig S[3], Huebschmann D[3], Pfuetze K[3], Kuster B[1]
[1]Chair of Proteomics and Bioanalytics, [2]Institute of General and Surgical Pathology, [3]DKFZ, [4]Young Investigator Group: Mass Spectrometry

Introduction

While DNA and RNA sequencing are increasingly applied in precision oncology to stratify tumors, the therapeutically important (phospho)proteome is rarely included. We established and integrated a clinical proteomics workflow into two molecular tumor board programs. We present a data processing pipeline for semi-automated analysis of (phospho)proteomic data that allows therapeutic suggestion with a turnaround time of two weeks.

Methods

Our pipeline processes sarcoma samples using multiple TMT-11 batches. The data is preprocessed with MS2 spectrum clustering to reduce missing values, followed by missing-value imputation and batch-effect-reducing normalizations. Phospho-peptides are functionally annotated using PhosphoSitePlus including regulation type and upstream kinase(s).

For biological interpretation, a novel approach using manually curated baskets focusing on possible druggable oncogenic pathways have been developed. Genes are weighted by taking the product of assigned weights with the Z-score giving appropriate importance to, e.g. oncogenes (high weight, possible high Z-score) and tumor suppressor genes (negative weight, possible negative Z-score). Calculating a score for each basket enables us to detect aberrant pathway activity.

Results

We developed a new approach to infer aberrant activity of druggable signalling pathways using expression data of >8000 proteins and >20000 phospho-sites per patient in a cohort of >200 sarcoma patients covering >40 different sarcoma subtypes.

Using this approach, we conducted retrospective mock-up tumor boards and found that proteomic data complemented DNA/RNA sequencing with valuable information; e.g. detection of high phosphorylation signaling downstream of normally abundant kinases, which is impossible to see at sequencing level.

We created an internal web portal for preparation for tumor boards. Here, we interactively explore the results of individual patients, sarcoma subtypes or abundance of specific proteins/basket scores across the cohort.

Conclusion

Our pipeline shows that adding a proteomic layer to cancer programs can support and add critical information for personalized therapies.

PP01.106: Comparative Sputum Proteome Analysis in Asthma Patients with Different Biologics Administration

Jeong yeon H[1]
[1]Department Of Biomedical Sciences, University Of Ulsan College Of Medicine, Seoul
Asthma is a heterogeneous disease explained by reversible airflow restriction or bronchial hyperresponsiveness. Induced sputum allows a non-invasive method for sampling protein-rich airway biofluids. Here, we analyzed the sputum proteomes of asthma patients (N=37) before and after biologics administration. Sputum samples were treated with dithiothreitol for mucus depolymerization, and proteins in the supernatant by centrifugation were digested by the suspension trap (S-trap) method. The digested peptides were quantified by LC/MS-MS analysis. The total number of proteins is 1,437. There was no statistical difference in the number of proteins in the samples before and after drug administration (P=0.388), and the median value was 587.5. In terms of the quantitative distribution of sputum protein, the saliva-elevated protein was present at high concentration, with alpha-amylase being the top-ranked protein. Eosinophil-elevation protein was significantly higher before treatment (P<0.05). In this study, four biologics drugs were used: dupilumab, mepolizumab, omalizumab and reslizumab. Differential abundant proteins were discovered before and after administration of four different drugs, and in common, four proteins were found before and three proteins after treatment. It is expected that it can be used as an indicator of drug response in the future.

PP01.107: Identification of Gene-Therapy Responsive Blood Biomarkers for Duchenne Muscular Dystrophy

Johansson C[1], Boehler J[2], Brown K[2], Spitali P[3], Niks E[4], Al-Khalili Szigyart C[1]
[1]Department of Systems biology, School of Chemistry, Biology and Health, KTH Royal Institute of Technology, [2]Solid Biosciences, [3]Department of Human Genetics, Leiden University Medical Center, [4]Department of Neurology, Leiden University Medical Center

Introduction
Duchenne muscular dystrophy (DMD) is a rare paediatric disorder characterised by progressive muscle wasting. Many blood biomarker candidates for DMD have been identified in the past decade and some have been shown to change with disease progression. In this study, a panel of blood biomarker candidates was used to monitor response to micro-dystrophin therapies. The aim of the study includes identifying biomarkers relevant for studying gene-therapy efficacy.

Methods
Plasma samples from C57BL/10ScSn- Dmdmdx/J male mice treated with increasing doses of the micro-dystrophin gene therapy STG-001 were analysed with an antibody suspension bead array consisting of 87 antibodies targeting 83 proteins. Each sample was assayed at two different plasma dilutions. Median fluorescent intensities (MFI) for each antibody were normalised and correlated to relative dystrophin expression in heart and left quadriceps muscle tissue post terminal necropsy at day 29, as measured by Western blot.

Results
10 proteins were found to be significantly elevated in untreated mdx mice compared to wild-type mice at a plasma concentration of 0.114 mg/ml, and one additional protein was significant at 0.228 mg/ml. Out of these proteins, 10 were found to correlate with rescued dystrophin expression (Spearman's correlation, FDR < 0.05). The protein with the largest fold-change and most significant correlation to dystrophin expression was a fragment of titin.

Conclusions
This study identified a panel of 10 proteins that correlate with dystrophin expression. These proteins are involved in muscle contraction, microtubule formation and protein degradation. Dystrophin deficiency is known to cause muscle cell breakage and increased protein turnover, so a restoration of wild-type levels of these proteins could be indicative of improved tissue health when micro-dystrophin expression increases.

PP01.108: Investigation of Proteomic Signatures in Healthy Aging, Mild Cognitive Impairment and Alzheimer's Disease in a Paired CSF and Plasma Study

Tognetti M[1], Feng Y[1], Bruderer R[1], Karemaker I[1], Kamber D[1], Darrow J[2], Lewis A[2], Moghekar A[2], Reiter L[1]

[1]Biognosys AG, [2]Department of Neurology, Johns Hopkins University School of Medicine

Introduction

While aging remains the primary risk factor for Alzheimer's disease (AD), the biological pathways that are altered in healthy aging vs. pathologic aging remain to be elucidated. Biomarkers in cerebrospinal fluid (CSF) and plasma can yield biological insights and support therapy development. Here, we seek to address this unmet need by applying a novel mass spectrometry-based discovery workflow.

Methods

Matched CSF and plasma samples were collected from young control subjects (n= 53), subjects with mild cognitive impairment (MCI) (n = 40), age-matched healthy control subjects (n = 40) and subjects with autopsy-proven Alzheimer's disease (n = 21, only CSF). The plasma and CSF samples were subsequently processed to tryptic peptides and analyzed using a Thermo Scientific Orbitrap Exploris 480 equipped with a FAIMS Pro device. Differential abundance testing was performed in Spectronaut and the candidate lists were filtered by an FDR <1%.

Results

Using our optimized discovery proteomics workflow, we analyzed 133 matched plasma and CSF pairs from young, old, MCI and AD specimen. This resulted in 5,727 proteins identified in CSF and 3,136 in plasma.

First, we compared young vs old. Proteins that were changed with the same directionality in both CSF and plasma included Leptin, a regulator of energy balance, Transgelin, involved in senescence, and CSPG4, which may regulate axon regeneration. Investigating AD in CSF, we found AD biomarkers e.g. BDNF and PARP-1. Proteins belonging to the memory function were down regulated and were not detected in plasma. Four candidates were shared between old vs MCI and old vs AD, indicating that some proteins indicative for AD are already altered in MCI.

Conclusions

Harnessing the power of the latest advancement in mass spectrometry-based technology, we generated a comprehensive and quantitative map of proteomes linked to healthy and pathological aging.

PP01.109: Use of DIA-MS to Determine the Effects of Storage Temperature and Time on FFPE Tissue Sections

Koh J[1], Sykes E[1], Rukhaya J[1], Anees A[1], Zhong Q[1], Balleine R[1], Hains P[1], Robinson P[1]
[1]ProCan®, Children's Medical Research Institute, The University of Sydney

Introduction:
Formalin-fixed paraffin-embedded (FFPE) tissues are invaluable resources for cancer proteomic studies and biomarker discovery. Very large numbers of them are stored in pathology laboratories and biobanks worldwide and often have associated clinical data available. FFPE tissue blocks are stable for decades when stored at room temperature (RT) and can be sectioned to produce samples for liquid chromatography-mass spectrometry (LC-MS) analysis. It is not known if the proteome of FFPE tissue after sectioning may be affected by storage temperature or time. To address this, we stored FFPE tissue sections at RT and -80°C for up to 336 days and analysed them at different timepoints to determine the proteome stability.

Methods:
A total of 297 FFPE 10 µm sections (triplicates of rat brain, kidney and liver) were cut from tissue blocks and stored at either RT or -80°C. Control samples were freshly cut sections from FFPE blocks. Samples were prepared for LC-MS analysis by digestion at 11 timepoints after storage (up to 336 days) and analysed by microflow HPLC on Triple TOF 6600 mass spectrometers (SCIEX) using data-dependent acquisition (DDA) mode. Kidney and liver digests were further analysed in data-independent acquisition (DIA) mode for quantitative analysis.

Results:
Nine post-translational modifications (PTMs) that are specific for FFPE samples were identified from the DDA data analysis. These PTMs were monitored for quantitative changes in samples that were stored at different temperatures and for different time periods using DIA data. Overall, the storage temperature and time did not have significant effects on the proteomic analysis of FFPE sections.

Conclusions:
After FFPE blocks are cut, the tissue sections can be safely stored at either RT or -80°C for at least 1 year without significant impacts on the identified proteome. Such samples are suitable alternatives for fresh frozen tissues in proteomic studies.

PP01.110: Proteogenomics Approach to Advance Diagnosis of Von Willebrand Disease

Kreft I[1], van Kwawegen C[2], van Duijl T[1], Atiq F[2], van der zwaan C[1], meijer A[1], Hoogendijk A[1], Bierings R[2], Leebeek F[2], van den Biggelaar M[1]
[1]Sanquin, [2] Erasmus MC, University Medical Center Rotterdam

Introduction

Von Willebrand disease (VWD) is the most common inherited bleeding disorder. VWD is caused by either reduced plasma levels of Von Willebrand Factor (VWF) (type I), qualitative defects in VWF (type II) or a complete absence of VWF leading to a life-threating bleeding phenotype (type III). Diagnosis of VWD is complex due to heterogeneity of genetic variants in VWF and diversity in bleeding phenotypes. Moreover, for a subgroup of patients with low levels of VWF no mutation can be detected in the coding region of VWF. Here, we used mass spectrometry (MS) analysis to (1) profile plasma proteomes in VWD, (2) identify VWF associating plasma proteins (3) phenotype VWD pathogenic variants on peptide level.

Methods

Plasma samples from 39 VWD patients (typeI n=19, typeII n=16, typeIII, n=4) included in the von Willebrand in the Netherlands study [24800796] and healthy donors were analysed by unbiased data dependent proteomics. To identify the VWF associated plasma proteins, VWF was immunocaptured prior to MS analysis. Finally, targeted MS in parallel monitoring reaction acquisition combined with proteogenomics was performed to enable peptide variant identification and quantitation of the propeptide domain relative to mature VWF.

Results

Unbiased plasma proteomics enabled detection of alterations in VWF plasma levels and in concordance with VWF:Antigen diagnostic test results (r=0.8). Plasma VWF associated with coagulation factor FVIII and platelet proteins TLN1 and TMSB4X. Sequence coverage was >70% in mature VWF and >50% in propeptide VWF, allowing to distinguish VWD subtypes. 16 pathogenic peptide variants corresponding to 17 VWD patients were identified by proteogenomics. Both wild type and variant peptides were detectable in patient harboring heterozygous mutations, whereas only variant peptides was detectable in case of homozygous Cys1190Arg mutation.

Conclusions

Plasma proteogenomics can successfully phenotype VWD and thus represents a promising strategy to address the medical need for precision diagnosis

PP01.111: Proteogenomics Linked to Histopathology Identifies Subtypes and Progression Biomarkers in Malignant Melanoma

Kuras M[1], Betancourt L[2], Horvatovich P[3], Rezeli M[4], Appelqvist R[4], Gil Valdés J[1], Malm J[1], Hong R[6], Szadai L[5], Deszcz B[9], Pla I[1], Szeitz B[8], Sugihara Y[10], Sanchez A[1], Szasz M[11], Fenyö D[6], Pawłowski K[7], Wieslander E[1], Nemeth I[5], Marko-Varga G[4]
[1]Section for Clinical Chemistry, Department of Translational Medicine, Lund University, [2]Division of Oncology, Department of Clinical Sciences Lund, Lund University, [3]Department of Analytical Biochemistry, Faculty of Science and Engineering, University of Groningen, [4]Clinical Protein Science & Imaging, Biomedical Centre, Department of Biomedical Engineering, Lund University, [5]Department of Dermatology and Allergology, University of Szeged, [6]Institute for Systems Genetics, NYU Grossman School of Medicine, [7]Department of Molecular Biology, University of Texas Southwestern Medical Center, [8]Division of Oncology, Department of Internal Medicine and Oncology, Semmelweis University, [9]Department of Biochemistry and Microbiology, Warsaw University of Life Sciences, [10]Clinical Protein Science & Imaging, Biomedical Centre, Department of Biomedical Engineering, Lund University, [11]Department of Bioinformatics, Semmelweis University

Introduction: Malignant melanoma is the most aggressive type of skin cancer with high metastatic potential, but the metastatic process is still not well understood. In the past decades, genomic studies revealed the importance of oncogenic driver mutations of the BRAF, NRAS, NF1 and KIT genes in tumor development. However, predictive biomarkers are still missing. In this study, proteogenomic analysis coupled with clinical histopathology revealed proteomic subtypes and identified candidate markers, which uncovered tumor biology related to patient survival.
Method: Treatment-naïve frozen lymph node metastases from 142 patients with malignant melanoma were sectioned and histopathologically examined. Global proteomics was performed by isobaric labeling with TMT 11-plex. Phosphoproteomic analysis was performed using DIA-MS following Fe(III)-NTA enrichment. The peptides were analyzed by nanoLC-MS/MS using an Ultimate 3000 UPLC coupled to a QExactive HF-X.
Results: A total of 12,695 proteins and 45,356 phosphosites were quantified. This newly generated proteomic data was integrated with a previously published transcriptomic dataset from matched tumor specimens (Cirenajwis et al., 2015). Using consensus clustering, we could identify five melanoma subtypes, which were classified into extracellular, extracellular-immune, mitochondrial, mitochondrial-immune, and extracellular-mitochondrial categories based on the annotations of their highly expressed proteins. The proteomic subtypes displayed differences in phenotype switching, immune surveillance, the levels of known melanoma markers, and were associated with survival. In addition, clinical parameters such as the expression of mutated BRAF V600E protein and the composition of the surrounding tumor microenvironment were found to be good independent indicators of prognosis. Lastly, validation of survival-related proteins in an independent cohort identified five candidate markers for predicting disease progression and patient survival (ADAM10, SCAI, CTNND1, CDK4, FGA).
Conclusions: This study provides insights into the complex biology of melanoma while highlighting the importance of the tumor microenvironment. It also offers the opportunity to identify novel drug target candidates.

PP01.112: Ultra-deep Brain Proteomic Analysis Identifies Pathogenic Drivers in Alzheimer's Disease

Lee D[1], Li Y[2], Liu D[1], Peng J[1,2]
[1]Departments of Structural Biology and Developmental Neurobiology, St. Jude Children's Research Hospital, Memphis, TN, USA, [2]Center for Proteomics and Metabolomics, St. Jude Children's Research Hospital, Memphis, TN

Introduction

Alzheimer's disease (AD) is an irreversible aging-associated neurodegenerative disorder that estimated 50 million people live with Alzheimer's disease and dementia in 2022. The hallmark of AD is associated with the amyloid beta (Aβ) plaque accumulation and hyperphosphorylated tau. However, the precise cause of AD is not fully understood. To understand the molecular mechanism of AD, proteome and phosphoproteome were analyzed using the ultra-deep brain proteome analysis with tandem mass tag (TMT) multiplexed labeling strategy.

Methods

500 human brain tissues were used for protein quality control analysis, 465 of which were finally chosen for the explorative proteomics study. Brain samples were weighed and homogenized in the lysis buffer (50 mM HEPES, pH 8.5, 8 M urea, and 0.5% sodium deoxycholate, protein:buffer = 1:10, v/v). The lysate was proteolyzed with Lys-C and trypsin. After acidification with TFA, the resulting peptides were labeled with 16-plex TMTpro reagents and quenched with 0.5% NH3OH. The labeled peptides were pooled equally, followed by further desalting and fractionated by an offline basic pH reverse phase LC into 40 concatenated fractions. The fractions were further analyzed by the acidic pH reverse phase LC-MS/MS (Q Exactive HF Orbitrap MS). To identify and quantify protein and peptide with high sensitivity, JUMP software suite was used.

Results

More than 13,000 unique proteins (derived from 10,000+ unique genes) were quantified from the 33 batches of human brain samples, with a protein false discovery rate (FDR) less than 5%. The proteins were filtered with a previously reported statistical method. Interestingly, hundreds of AD pathology correlated proteins were detected. Amyloid beta-associated proteins, phosphorylated tau-associated proteins, and proteins associated with both were clustered differently.

Conclusions

The ultra-deep brain proteome analysis can help a comprehensive understanding of AD molecular mechanism. It can help provide potential diagnostic and therapeutic biomarkers for AD and subtypes of AD.

PP01.113: New insights into Cancer Development in Non-alcoholic Steatohepatitis: Metabolomic Screening Study

Ahmed E[1], O. El-Derany M[2], Mostafa Anwar A[3], M. Saied E[4], Magdeldin S[5]
[1]Proteomics and Metabolomics research program, Department of Basic Research, Children's Cancer Hospital 57357, 11441, Cairo, Egypt ; Department of Pharmacology, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, 41522, Egypt; , [2]Department of Biochemistry, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt, [3]Proteomics and Metabolomics research program, Department of Basic Research, Children's Cancer Hospital 57357, 11441, Cairo, Egypt , [4]Institute for Chemistry, Humboldt Universität zu Berlin, Brook-Taylor-Str. 2, 12489 Berlin, Germany, [5]Proteomics and Metabolomics research program, Department of Basic Research, Children's Cancer Hospital 57357, 11441, Cairo, Egypt

Introduction: With the rising incidence of hepatocellular carcinoma (HCC) from non-alcoholic steatohepatitis (NASH), identifying new metabolic readout that functions in metabolic pathway perpetuation is still a demand.

Aim:The study aimed to compare the metabolic signature between NASH and HCC-NASH patients to explore novel reprogrammed metabolic pathways that might modulate cancer progression in NASH patients.

Methods: NASH and HCC-NASH patients were recruited and screened for metabolomics and targeting isotope-labeled lipidomics profiling using the EXION-LCTM system equipped with Triple-TOFTM 5600+ system.
Results: Our results demonstrated higher levels of triacylglycerol, AFP, AST, and cancer antigen 19-9 in HCC-NASH than in NASH patients, while prothrombin time, platelet count, and total leukocyte count were decreased. Metabolic profiling showed a panel of twenty metabolites in both targeted and non-target analysis that could segregate HCC-NASH from NASH patients. These metabolites have been implicated in the down-regulation of necroptosis, amino acid metabolism, and regulation of lipid metabolism by PPAR-α, biogenic amine synthesis, fatty acid metabolism, and mTOR signaling pathway.
On the other hand, cholesterol metabolism, DNA repair, methylation pathway, bile acid, and salts metabolism were significantly upregulated in HCC-NASH compared to the NASH group. Well-known proteins encoding genes that play crucial roles in cancer, including PEMT, IL4I1, BAAT, TAT, CDKAL1, NNMT, PNP, NOS1, and AHCYL were identified via the metabolite–protein interactions network.

Conclusion: Taken together, reliable predictive fingerprint metabolites are presented and illustrated in a detailed map for the most predominant reprogrammed metabolic pathways that target HCC development from NASH.

PP01.114: Identification of Allergens of Ligustrum Lucidum Pollen cause Respiratory Allergies by Immunoproteomics Approach

Montero Vargas J[1], Porras Gutiérrez de Velasco R[1], Vizuet de Rueda J[1], Teran L[1]
[1]Instituto Nacional De Enfermedades Respiratorias Ismael Cosío Villegas

Introduction: Respiratory allergies are a significant health concern increasing in recent years, directly impacting the patient's quality of life. Until 20% of the world's population is affected by allergic rhinitis, asthma, and pollen food allergy syndrome, among others. Inhalation of pollen cause inducing an IgE-mediated hypersensitivity reaction in sensitized patients. Therefore, the characterization of allergens is essential for accurately diagnosing and treating respiratory allergies. Earlier, we identified six protein allergens in Ligustrum lucidum pollen, a highly allergenic tree widely distributed worldwide. The aim of this study was to identify other potentially allergenic proteins employing sera from polysensitized patients.

Methods: Pollen proteins of L. lucidum collected in Mexico City were extracted and separated by two-dimensional gel electrophoresis (2-DE). Then, sera from polysensitized patients for L. lucidum were used for western blot differential analysis. Finally, the identification of immunoreactive proteins was made by mass spectrometry.

Results: In this work, we report novel IgE-binding proteins from L. lucidum pollen associated with respiratory allergies in polysensitized patients. The 2-DE profile show more than 200 spots, of which 18 immunoreactive spots had not been detected using sera from monosensitive patients. The proteins identified include pectinesterase, UDP-arabinopyranose mutase, glucan endo-1,3-beta-glucosidase, malate dehydrogenase, glyceraldehyde 3-phosphate dehydrogenase, flavanone 3-hydroxylase, fructokinase, and among others. A major pollen allergen Lig v 1 and Fra e 1 that had not previously been found in L. lucidum employing monosensitized sera were also identified.

Conclusions: Identification of allergens from L. lucidum pollen, an underestimated sensitization aeroallergen, is essential for accurate allergy diagnosis. The IgE-mediated response in polysensitized patients is different from the monosensitized, hence the necessity of personalized medicine.

PP01.115: Thermal Proteome Profiling of Diamond-Blackfan Anemia Patient-derived Cell Lines Identifies Thermal Stability Changes Related to Ribosome Homeostasis

Mosley A[1]
[1]Indiana University School Of Medicine

Introduction

Diamond-Blackfan Anemia (DBA) is a bone marrow disorder that prevents the development of red blood cells caused by haploinsufficiency of ribosomal protein subunits. It is unclear if haploinsufficiency can be effectively studied using thermal proteome profiling (TPP). To this end, we utilized TPP to determine changes in thermal stability of the proteome as a consequence of expression of DBA-associated variants.

Methods

Lymphoblastic cell lines were derived from patients with pathological variants in RPS19, RPL5, RPL35A, and RPS24 versus healthy donor controls. TMT-based global proteomics was performed on an Orbitrap Eclipse following off-line high pH reversed phase fractionation. Differential abundance was determined using MSStatsTMT with an adjusted p-value ≤ 0.05. TPP was performed for eight temperature treatments. Protein melt curves / temperatures were determined using R package Inflect-SSP. Statistically significant thermal shifts were set at a cutoff of $p \leq 0.05$.

Results

TPP of the DBA cell lines was conducted and analyzed using Inflect-SSP, resulting in 236 proteins with statistically significant thermal shifts. Enrichment analysis of these proteins using gprofiler2 found terms associated with ribosomal biogenesis for small and large subunits, recapitulating the known biology of pathogenesis of DBA. We also discovered enrichment of proteins suggesting a role of the ubiquitin-proteasome system UPS in DBA. Proteins associated with microcephaly, a clinical feature of DBA that affects a small percentage of patients, were also enriched. These findings reveal novel insights into the cellular dysfunction associated with DBA-causing protein variants including precise targets within the extensive UPS – with specific E3 ligases and regulatory proteins identified with thermal stability changes.

Conclusions

We have shown that TPP is an effective approach for profiling various types of protein functional alleles. Long term, we are developing tools to apply TPP to the study of rare disease sequence variants that lack prior functional characterization.

PP01.116: Identification of Prognostic Candidates using Comprehensive Kinase Activity Assay in Non-small Cell Lung Cancer Biopsy Samples

Noguchi R[1], Yoshimura A[2], Uchino J[2,3], Takeda T[4], Chihara Y[5], Ota T[6], Hiranuma O[7], Gyotoku H[8], Takayama K[2], Masuda M[9], Kondo T[1]
[1]Division of Rare Cancer Research, National Cancer Center Research Institute, [2]Department of Pulmonary Medicine, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, [3]Bannan Central Hospital, [4]Department of Respiratory Medicine, Japanese Red Cross Kyoto Daini Hospital, [5]Department of Respiratory Medicine, Uji-Tokushukai Medical Center, [6]Department of Medical Oncology, Izumi City General Hospital, [7]Department of Respiratory Medicine, Otsu City Hospital, [8]Department of Respiratory Medicine, Graduate School of Biomedical Sciences, Nagasaki University, [9] Department of Cancer Proteogenomics, National Cancer Center Research Institute
Introduction: EGFR mutations are strong predictive markers for EGFR-tyrosine kinase inhibitor (EGFR-TKI) therapy in patients with non-small-cell lung cancer (NSCLC). NSCLC patients with sensitizing EGFR mutations spend better prognoses, some patients exhibit worse prognosis. We hypothesized that various activities of kinases could be potential predictive biomarkers for EGFR-TKI treatment in NSCLC patients harboring the sensitizing EGFR mutations.

Methods: In 18 patients with stage IV NSCLC, EGFR mutations were detected using panel sequencing and comprehensive kinase activity assay was performed using the peptide array Pamstation12 for 100 tyrosine kinases. For the kinase activity assay, extracted protein from biopsied tumor samples were used. Prognosis was observed prospectively after the administration of EGFR-TKIs. Finally, the kinase profiles were analyzed in combination with the prognoses of the patients.

Results: The kinase activity analysis identified specific kinase features consisting of 102 peptides and 35 kinases in NSCLC patients with sensitizing EGFR mutations. Network analysis and pathway analysis revealed several kinases and pathways commonly activated. Highly activation of several kinases was also elucidated in patients with poor prognosis.

Conclusion: Comprehensive kinase activity profiles may provide predictive biomarkers for screening patients with advanced NSCLC harboring sensitizing EGFR mutations.

PP01.117: Exploring Deubiquitinase USP19 as a Target for Small-Molecule Inhibition in Sarcopenic Obesity

O'Brien D[1], Pinto Fernández A[1,2], Leuvenink R[1], Philpott M[3], Wittemans L[4], Fischer R[1,2], Rathjen T[5], Fritsch M[5], Bothe U[5], Zollner T[5], Lindgren C[4], Oppermann U[3], De Leo B[5], Mueller J[5], Kessler B[1,2]
[1]Target Discovery Institute, Centre for Medicines Discovery, Nuffield Department of Medicine, University of Oxford, [2]Chinese Academy of Medical Sciences Oxford Institute, University of Oxford, [3]Botnar Research Centre, Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences, University of Oxford, [4]Big Data Institute, Nuffield Department of Medicine, University of Oxford, [5]Research & Early Development, Bayer AG

Introduction: Ubiquitin specific protease 19 (USP19) is a membrane bound deubiquitinase which has caretaker properties by eliminating misfolded proteins and cellular debris. Recent studies have implicated the protein in glucocorticoid and insulin signalling; USP19-deficient mice show a lean phenotype and are less prone to obesity and insulin resistance. Using integrative Omics, we sought to elucidate the molecular and functional mechanisms underlying these observations.

Methods: A panel of small molecule compounds targeting USP19 and based on a patent by Almac (WO2018020242A1, 2019) were synthesized in-house. Inhibitor potency and target cellular engagement was assessed by activity-based protein profiling. USP19 substrate discovery experiments were performed in an MCF7 breast cancer cell line using advanced proteomics and ubiquitomics pipelines. In support of our pre-clinical studies, an in-house knockout mouse was generated and wildtype and USP19 -/- mice (n = 11 females per group) were exposed to a high fat diet (HFD) regime. Analogous discovery experiments were performed on adipose and muscle tissues extracted from these animals.

Results: USP19 inhibitor capacity was determined for three compounds in vitro and in cells. Analysis of the body weight of the wildtype and USP19 KO mice revealed a clear reduction in overall body weight in the absence of USP19 when fed a HFD. Our exploratory Omics identified several pathways involved in adipogenesis, thermogenesis, and insulin and glucocorticoid signalling in both the cell lines and HFD rodent model. USP19 compounds attenuated adipocyte differentiation and reduced muscle wasting.

Conclusions: Pharmacologically dampening USP19 leads to reduced body weight and attenuated adipocyte differentiation and muscle wasting. Our results highlight the therapeutic potential of USP19 inhibition in obesity-related sarcopenia and associated disorders.

PP01.118: Mass Spectrometry-based Clinical Proteomics Indicates a Prognostic Signature for Head and Neck Cancer

Busso-Lopes A[1], Camara G[1], Granato D[1], Neves L[1], Sá J[1], Yokoo S[1], Rivera C[1,2,3], Patroni F[4], de Rossi T[1], Normando A[1,2], Domingues R[1], Heberle H[5], Pretti M[6], Mello B[7], Rodrigues A[8], Lacerda P[8], Galdino N[9], Gollob K[9], Medina T[9], Cervigne N[8], Prado-Ribeiro A[2,10], Brandão T[10], Villa L[7,10], Uno M[10], Boroni M[6], Kowalski L[11,12], González-Arriagada W[13,14], Paes Leme A[1]

[1]Laboratório Nacional de Biociências - LNBio, Centro Nacional de Pesquisa em Energia e Materiais - CNPEM, Campinas, SP, Brazil, [2]Departamento de Diagnóstico Oral, Faculdade de Odontologia de Piracicaba, Universidade Estadual de Campinas - UNICAMP, [3]Departamento de Ciencias Básicas Biomédicas, Facultad de Ciencias de la Salud, Universidad de Talca - UTALCA, [4]Centro de Biologia Molecular e Engenharia Genética – CBMEG, Universidade Estadual de Campinas - UNICAMP, [5]Instituto de Ciências Matemáticas e de Computação, Universidade de São Paulo - USP, [6]Laboratório de Bioinformática e Biologia Computacional, Divisão de Pesquisa Experimental e Translacional, Instituto Nacional do Câncer - INCA, [7]Departamento de Radiologia e Oncologia, Faculdade de Medicina, Universidade de São Paulo - USP, [8]Departamento de Morfologia e Patologia Básica, Faculdade de Medicina de Jundiaí - FMJ, Jundiaí, SP, Brazil, [9]Centro Internacional de Pesquisa - CIPE, A.C. Camargo Cancer Center, [10]Instituto do Câncer do Estado de São Paulo - ICESP, Faculdade de Medicina, Universidade de São Paulo - USP, [11]Departamento de Cirurgia de Cabeça e Pescoço e Otorrinolaringologia, A.C. Camargo Cancer Center, [12]Departamento de Cirurgia de Cabeça e Pescoço, Faculdade de Medicina, Universidade de São Paulo - USP, [13]Facultad de Odontología, Patología Oral y Maxilofacial, Universidad de Los Andes, [14]Centro de Investigación e Innovación Biomédica, Universidad de Los Andes

Introduction: Head and neck cancer (HNC) is ranked the eighth leading cause of cancer worldwide and exhibits high prevalence and morbidity. Despite the efforts to improve diagnosis, prognosis, and therapeutic modalities, lymph node (LN) metastasis, local relapses, and poor survival rates represent a clinical challenge for the disease. Therefore, there is an urgent need for prognosis markers to guide clinical decisions.

Methods: We investigate the HNC landscape from multiple sites, including primary and matched lymph node tissues (malignant and non-malignant cells), saliva, and blood samples, in a discovery phase using data-dependent acquisition. The prioritized candidates were verified in fluids by SRM, PRM, and RT-qPCR. Data were submitted to machine learning models to indicate a signature of metastasis, followed by flow cytometry of the metastasis-signature using blood samples.

Results: The protein groups are strongly associated with immune modulation across multiple sites, with 106 differentially abundant proteins comparing patients with (pN+) and without metastasis (pN0) in tissue and fluid samples. The integrated proteome highlights potential candidates as prognostic markers verified in fluids, and generated a high-performance metastasis-dependent signature. Remarkably, the altered levels of the metastasis-signature targets in immune populations strengthen the relevance of specific subpopulations in the LN metastasis.

Conclusions: These approaches provide a deeper understanding of HNC biology and indicate a robust signature of lymph node metastasis that may assist in the clinical decisions to improve the prognosis of patients with head and neck cancer.

Financial support: FAPESP and CNPq.

PP01.119: Clinical Serum Proteomics of Severe Fever with Thrombocytopenia Syndrome Patients

Lee S[1], Yun S[1], Lee H[1], Lee C[2], Kim S[1], Park E[1,3]
[1]Korea Basic Science Insititute, [2]Chonbuk National University Medical School, [3]Korea Research Institute of Bioscience and Biotechnology

INTRODUCTION: Dabie bandavirus, also termed as severe fever with thrombocytopenia syndrome virus (SFTSV), was first isolated in China in 2010. At this time, the virus was found to have spread to South Korea, Japan, and other countries. A high case fatality rate is reported for SFTS, ranging from 12–50% within various sources. Several omics for clinical studies among SFTS patients as well as studies of cultured SFTSV have attempted to characterize the relevant molecular biology and epidemiology of the disease. However, a global serum proteomics analysis among SFTS patients has not yet been reported to date.
METHODS: In the current study, we evaluated comparative serum proteomics among SFTS patients (eight recovered patients and three deceased patients) with the goal of identifying the protein expression patterns associated with the clinical manifestations of SFTS.
RESULTS: The proteomic results in the current study showed that the coagulation factor proteins, protein S and protein C, were statistically significantly downregulated among the deceased patients. Downregulation of the complement system as well as prolonged neutrophil activation were also observed. Additionally, the downstream proteins of tumour necrosis factor alpha, neutrophil-activating cytokine, and interleukin-1β, an inflammatory cytokine, were overexpressed.
CONCLUSIONS: Thrombocytopenia and multiple organ failure are the major immediate causes of death among SFTS patients. In this study, serum proteomic changes related to thrombocytopenia, abnormal immune response, and inflammatory activation were documented in SFTS patients. These findings provide useful information for understanding the clinical manifestations of SFTS.

PP01.121: Immunoproteomics of Privet Tree (Ligustrum lucidum) Pollen Allergens

Porras Gutiérrez De Velasco R[1], Neftalí Bravo R[1], Montero Vargas J[1], Vizuet de Rueda J[1], Teran L[1]
[1]Instituto Nacional de Enfermedades Respiratorias.

Introduction: The Ligustrum lucidum is one of the main ornamental species cultivated in Mexico City. Inhalation of its pollen is a significant cause of respiratory allergies in Mexico and other countries[1]. The allergenicity of pollen is related to the protein composition influenced genetically and environmentally[2,3]. To effectively diagnose and treat allergic respiratory diseases, it is crucial to identify the allergenic proteins. This work aims to characterize the L. lucidum pollen proteins that cause respiratory allergies in polysensitized patients. Additionally, we analyze the variability in the allergenicity of Ligustrum lucidum pollen in different sites in Mexico.

Methods: We collected inflorescences of L. lucidum in 37 different sites in Mexico City. Then, the pollen proteins were extracted with a phenol-modified method and separated by two-dimensional gel electrophoresis (2-DE). For western and dot blot differential analysis, sera from polysensitized patients for L. lucidum were used as a source of the Ig-E antibodies. At the same time, allergenicity was analyzed by colorimetry using the ImageJ software. Finally, the identification of immunoreactive proteins was made by LC-MS/MS.

Results: L. lucidum pollen proteins characterized by 2-DE showed more than 200 spots in a pI 3-10, of which 24 showed immunoreactivities with a pool of sera from patients. We identified 22 novel proteins not previously associated with allergic reactions in L. lucidum, such as pectinesterase, UDP-arabinopyranose mutase, glucan endo-1,3-beta-glucosidase, malate dehydrogenase, glyceraldehyde 3-phosphate dehydrogenase, flavanone 3-hydroxylase, fructokinase, and among others. Also, we found that samples collected at sites with high pollution levels and under increased stress for trees showed increased allergenicity.

Conclusions: Knowledge of allergenic proteins of pollen is essential for accurate diagnosis and treatment of respiratory allergies. Here we showed novel potential allergenic proteins of L. lucidum and how the ambient factors are associated with the allergenicity.

PP01.123: Using Covaris AFA Technology to Investigate Cardiac Perturbations in a HTP Cell-based Assay System

Seyedmohammad S, Rivas A, Zhgamadze M, Haghani A, Kreimer S, Vasantgadkar S, Daviso E, Pal K, Van Eyk J

Introduction: Precision medicine via molecular phenotyping enables the quantification of an individual's proteome, where a disease risk can be assessed. Having an established pipeline such as a High Throughput (HTP) screening workflow can allow the accurate quantification for discovery, mechanism, and clinical data generation, resulting in the identification of potential biomarkers for morbidities. We developed a bottom-up proteomics approach based on HTP cell-based assay.

Methods: AC16 human cardiomyocytes (Sigma) were grown in 96-well plates and optimized density of cells was lysed in a detergent-free buffer on an LE220 Plus sonication system COVARIS. Proteins were reduced, alkylated, and digested on a Biomek i7 (Beckman) automated workstation, in a temperature regulated on-deck incubator. Protein digestion was optimized, and desalted peptides were loaded on an Ultimate 3000 (Thermo) liquid chromatography system coupled to an Exploris 480 Orbitrap (Thermo) mass spectrometer. Data-Independent Acquisition (DIA) was performed over a 45-minute gradient and searched using the Pan human library (PXD000954).

Results: Data analysis allowed the quantification of 2,225 proteins over a dynamic range of 6 orders of magnitude (median over 4 replicates) for the optimized classic digestion. Addition of AFA sonication during digestion significantly increased the number of differentially detected proteins, where 450 of the 546 proteins were associated with AFA-assisted digestion and displayed an increase in intensity over the entire proteome dynamic range that also allowed the detection of lower abundant proteins. Protein interaction analysis revealed the detection of 82 additional membrane-associated proteins, of which 63 of the differentially detected proteins were associated with mitochondrial organization and transport. We then performed our optimized sonication-assisted sample preparation to highlight protein regulations involved in the response of cardiac cell perturbations.

Conclusions: We identified important cardiac modulators in ischemic AC16 cells from hypoxic assays and hope to apply our method to the analysis of proteomes using stable isotope-labeling.

PP01.124: Evaluation of Cysteamine Nanoemulsions and the Cellular Pathways Associated with Cigarette Smoke-induced Chronic Obstructive Pulmonary Disease

Sharma G[1], Pund S, Govindan R, Nissa M, Biswas D, Ganguly K, P.A. M, Banerjee L, Srivastava S, Midha S

[1]Indian Institute Of Technology Bombay, India

Introduction

As per the Global Initiative for Chronic Obstructive Lung Disease (GOLD), a person having a forced expiratory volume 1% (FEV1%) < 0.7 is regarded as COPD. COPD is closely associated with emphysema and chronic bronchitis (CB). Emphysema deals with structural issues, whereas CB deals with mucus issues. Here in this work, a wide range of inflammatory and anti-inflammatory markers was evaluated both in vitro and in vivo, along with histopathology. Furthermore, proteomics analysis of the lung tissue was carried out to assess proteome alteration in the normal, COPD, and treated rats, using potential pathways and proteomics validation studies.

Methods

Cysteamine nanoemulsions were prepared using the high-speed homogenization technique and tested both in vitro and in vivo. In vitro, COPD model was created in human bronchial epithelial cells lines (BEAS2B) using the 5% cigarette smoke extract (CSE), and the cell culture supernatant was analyzed using the ELISA. In vivo, COPD rat model was created using the 1R6F research-grade cigarettes. Various inflammatory and anti-inflammatory markers were evaluated in the rat BALF, whereas rat lung proteomics was carried out to study the differentially expressed proteins (DEPs) and the molecular pathways involved in cigarette smoke-induced COPD.

Results

TNF-α, IL-6, IL-8, E-cadherin, vimentin, sphingolipids, and many others showed the up-regulation trend in the disease condition and downregulation trend in the normal and the treated conditions both ,in vitro and in vivo. Regulation of cellular growth, regulation of synaptic plasticity, and cellular response to stress were the major pathways found in the rat lungs through proteomics. COX2 and PICALM, and SERPINA3L were the proteins that were validated using the MRM assay.

Conclusion

We found that the cysteamine nanoemulsions have the potent anti-oxidant capability to normalize cigarette smoke-induced COPD both in vitro and in vivo and the same has been validated through proteomics studies.

PP01.125: Autoantibody Profiling for Prediction of Disease Progression and Treatment Outcome in Rheumatoid Arthritis

Skallerup J[1,2], Just S[3], Aboo C[1,2], Poulsen T[1], Stensballe A[1]
[1]Department of Health Science and Technology, Aalborg University , [2]Sino-Danish Center for Education and Research, University of Chinese Academy of Sciences, [3]Section of Rheumatology, Department of Medicine, Svendborg Hospital - Odense University Hospital

Introduction: Rheumatoid arthritis (RA) is a common systemic autoimmune disease. RA primarily affects the joints by causing inflammation of the synovial membrane. Treatment of RA is focused on pain relief, maintenance of function, and preventing permanent joint damage. However, approximately one-third of patients do not achieve remission in response to treatment, and patients commonly experience adverse events (AEs) following treatment, thus, highlighting the need for tools able to predict treatment outcome. Furthermore, one of the most common extra-articular manifestations of RA is lung involvement. Ten to twenty percent of RA patients develop interstitial lung disease (ILD). ILD is a leading cause of death in RA patients with a median survival time after diagnosis reported to be 5 to 8 years. We aim to identify autoantibody markers of treatment outcome and development of ILD before initiation of treatment, potentially allowing for personalization of the treatment approach in RA.

Methods: The autoantibody profile of the plasma (collected at 0, 3, and 6 months) of 20 treatment-naïve RA patients and 20 patients with long-standing RA, along with 16 samples from healthy donors was investigated by protein microarrays developed using the KREX® protein-folding technology ensuring correctly folded and functional proteins on the microarrays. One patient sample was analyzed per microarray to ensure that identified autoantibody reactivities reflect the individual patients.

Results: We identified novel autoantibody markers whose presence and concentrations before initiation of treatment were correlated to the treatment outcome of the RA patients reflected by improvement in several clinical measures such as DAS28-CRP and lung involvement.

Conclusions: Protein array-based autoantibody profiling shows great value in its ability to differentiate patients based on their treatment outcomes before treatment initiation. Thus, autoantibody profiling of newly diagnosed RA could act as a companion diagnostics tool to aid decision-making in the treatment approach taken for each patient.

PP01.126: Companion Diagnostics and Precision Proteomics-based Translational Biomarker Analysis for Improved Disease Diagnostics and Prognostics

Aboo C[1], Poulsen T[1], Andersen J[1], Arendt-Nielsen L[1], Ladekarl M[2], Dybkær K[2], Sangild P[3], Rasmussen S[2], Blackburn J[4], Stensballe A[1]
[1]Aalborg University, [2]Aalborg University hospial, [3]Copenhagen University, [4]University of Capetown
Introduction: Researchers and clinicians are striving to investigate early diagnosis and apply advanced biomarker assisted treatment by precision proteomics strategies including companion diagnostics (CDx). Comprehensive personalized profiling of autoautobodies serve as key to early diagnostics while deep proteomic profiling and clinical correlation analysis have proven pivotal to novel translational biomarker discovery. We present recent concepts and clinical studies investigating inflammatory diseases accessing causalities leading to inflammation and pain as well as translational biomarkers enabling precision medicine.
Methods: Biobank samples (plasma, serum, synovial fluid, CSF, tissue microbiopsies, isolated b-cells) were analyzed by advanced 4D_MS based discovery and targeted clinical proteomics (timsTOFPRO2, EvosepOne). Biomarker discovery and clinical biomarker correlation analysis were accomplished by quantitative proteomics (Biognosys; BSI_PEAKS, MaxDIA platforms) followed by repeated measures driven cohort investigations (MixOmics). Detectable inflammatory markers and global/targeted autoantibodies were analyzed by protein array technology (Sengenics Immunome array) and multiplex analysis (Olink).
Results: Protein arrays (1634panel) enabled profiling of native and modified autoantigens (AAg) from patient biofluids enabling comprehensive subtyping of rheumatic patients (ACPA; >140AAgs) and stratification of lung cancer patients affected by adverse effects (irAEs; >60AAgs). Prediction of treatment outcome and pain in low-grade inflammatory diseases and cancers were accomplished by proteomic (biofluid >600-2100DEPs; immune cell/tissue 4500-7500 DEPs). Selected results include correlation coefficient analysis enabled prediction of treatment outcome and pain in rheumatic diseases (R^2 0.75-0.93) and early neonate sepsis (7protein biomarker panel; >93% accuracy). Label-free quantitative shotgun and targeted proteomics approaches enabled profiling and correlation of patient cohorts and cell line libraries subjects providing biomarkers for diagnosis and treatment efficacy for immune cell driven pathologies including hematological disorders.
Conclusions: Companion diagnostics and proteomics strategies enable new opportunities for personalized tracking of treatment outcomes by profiling between systemic and local sites of inflammation e.g. plasma and synovial fluid enables biomarker analysis and in-depth insight into disease pathology.

PP01.127: A Mass Spectrometry Quality Control Pipeline to Enable Clinical Proteomics

Sykes E[1], Lucas N[1], Xavier D[1], Bucio-Noble D[1], Williams S[1], Koh J[1], Humphries E[1], Smith D[1], Ashman K[2], Robinson P[1], Hains P[1]
[1]Children's' Medical Research Institute, [2]SCIEX
Introduction:

A major impediment in introducing mass spectrometry (MS)-based proteomics into a clinical setting is the lack of validation studies demonstrating reproducibility on a high-throughput scale. Standardisation through defined quality control measures and use of analytical standards are required for MS-based proteomics to be implemented in the clinic. Here we aim to develop a quality control pipeline (QCP) suitable for managing future clinical grade workflows.

Method:

We evaluated the effectiveness of quality control measures used in generating reproducible high quality MS data. Over a 4-year span >85,000 MS files (~43,000 samples, ~31,000 BSA standards and ~7,000 HEK293 MS standards) were acquired in a single laboratory across 6 instruments. The quality control measures encompassed sample preparation standards, both simple and complex MS standards, instrument-specific MS1 and MS2 thresholds, LC stability tolerances and an automated search result pipeline. All complex samples were acquired on six SCIEX 6600 Q-TOFs with 90 min runs in data independent acquisition (DIA) mode with microflow LC gradients.

Results:

The effectiveness of the QCP measures is shown by an average of 5,500 protein IDs per run obtained from ~10,000 cancer patient MS acquisitions over a 6-month period across six instruments. High reproducibility was demonstrated in a series of longitudinal replicates comprised of 46 cancer samples run in technical replicates (n=3 to 6) from 1 week up to 3 years apart. The resulting replicates maintained a per sample correlation of >0.85 and remained clustered with unsupervised hierarchical clustering.

Conclusion:

Our QC standards and QCP enables high-throughput collection of MS data to a standard that will facilitate translation of this data into a clinical setting. The data demonstrates the quality of the results achievable with the real-world implementation of such a QCP over a period of 4-years and across more than 85,000 MS acquisitions.

PP01.128: Proteomic Characterization of the Axis Prolactin/Vasoinhibins/Cathepsin D in Murine Models of Renal Ischemia-Reperfusion

Verdugo Molinares M[1], Cardenas A[2], Franco A[2], Rojas Cervantes K[1], Velazquez J[1], Rodriguez J[1], Beltran M[3], Melo Z[2], Vallejo A[2]
[1]Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco, [2]CONACYT-Centro de Investigación Biomédica de Occidente, IMSS, [3]Universidad Autonoma de Guadalajara
Introduction: Ischemia-reperfusion injury (IRI) is a phenomenon that aggravates acute kidney damage. The therapeutic options and biomarkers for early detection and prevention are still limited. Thus, it is fundamental to continue the study of proteins involved in IRI to develop better options for patients. Evidence suggests female sex hormones as protective agents against this damage. Prolactin (PRL) promotes angiogenesis, vasodilation, and vasopermeability. However, it is known that PRL loses its functions by the cleavage by cathepsin D (CTD) resulting in small peptides called Vasoinhibins (Vi). This axis is not well studied in renal IRI; therefore, the use of proteomics will be a powerful tool to deduce cell mechanisms involved in this axis interaction.

Methods: Wild type and CTD heterozygous C57/BL6 mice were inoculated with PRL expressing lentiviral vectors. Ischemia was performed by clamping the left renal hilum for 45 minutes with subsequent 24-hour reperfusion. Serum creatinine was measured by dry chemistry. Kidney proteome of murine models was analyzed using UHPLC ESI Q-TOF Waters Xivo with a label-free top down method.

Results: We first confirmed that PRL can be cleaved by CTD in the kidney. Then, we observed that PRL expression via lentiviral vector transduction in CTD mice provides renoprotection after kidney injury, as observed by the lower levels of serum creatinine and KIM-1 expression. Kidney proteins were satisfactorily extracted and digested with trypsin. Peptides were analyzed in the spectrometer and compared with database to realize the changes in proteome. Statistical differences in abundance were analyzed taking $P<0.05$ by ANOVA using Progenesis software.

Conclusions: Expression of PRL and a decrease of CTD are associated with less kidney damage. The extraction of protein, digestion, and analysis allowed us to determine the kidney proteome in each group and observed the changes that induced the manipulation of the axis.

PP01.129: Comparative Heterologous Protein Expression of the Major Ash Pollen Allergen Fra e 1

Vizuet de Rueda J[1], Montero Vargas J[1], Jimenez Rios J[1], Nicolás Salazar C[1], Marsch Martínez N[2], Teran L[1]

[1]Instituto Nacional de Enfermedades Respiratorias Ismael Cosío Villegas, [2]Centro de Investigación y Estudios Avanzados del Instituto Politécnico Nacional. Campus Irapuato

Oleaceae trees are distributed worldwide, and there is a high rate of allergic respiratory diseases caused by the inhalation of their pollen. Fraxinus (ash) is one the most allergenic genera of the Oleaceae family, whose major allergen is Fra e 1. Whole pollen protein extracts are commonly used to diagnose and treat respiratory allergies. However, these extracts comprise thousands of non-well characterized proteins, making the diagnosis inaccurate. One way to solve this problem is to use allergens expressed from heterologous organisms, such as bacteria. Unfortunately, these organisms cannot add all the post-translational modifications (PTMs) found in plants. These PTMs sometimes can affect recognition mediated by IgE. Therefore, we expressed Fra e 1 in plants and bacteria to determine if PTMs are essential for IgE patient recognition.

Fra e 1 gene was synthesized and cloned in E. coli BL21. Furthermore, the Fra e 1 gene was amplified by PCR from ash pollen, sequenced, and cloned into a plant expression vector for Nicotiana benthamiana agroinfiltration. Then, protein extraction was done from bacteria and plant leaves, and purification of recombinant Fra e 1 was performed with an IMAC column. Finally, the recombinant allergens were TEV-digested and will be used in Western-blot using sera from allergic patients to validate whether PTMs are necessary for correct IgE recognition.

Until now, we have amplified Fra e 1 directly from native ash pollen; the sequence does not coincide with any of the isoforms reported, suggesting that it could be a new isoform. Fra e 1 was expressed in bacteria and plants, and the purification and TEV digestion are under process for use in immunoblot. The heterologous expression of Fra e 1 represents an alternative production of this allergen that can be used in diagnosing and treating allergies after validation with immunoblots assays

PP01.130: Ultra-high Sensitivity for Targeted Clinical Proteomics using Evosep-MRM

Mc Ardle A[1], Kumar Raghuraman B[1], Bekker-Jensen D[1], Bache N[1]
[1]Evosep

Introduction:
The Evosep One represents a powerful platform for high sensitivity work including single cell proteomics. With the recent introduction of Whisper Flow Technology, the Evosep One supports robust nanoflow chromatography (100nl/min) providing ultra-high sensitivity, which is required when starting material is limited. Here we showcase the robustness, sensitivity, and throughput of the Whisper methods on the Evosep One in combination with the Agilent 6495C triple quad mass spectrometer. We demonstrate that this approach enables targeted proteomics of clinically relevant proteins in single cells.

Methods:
To benchmark the sensitivity of our workflow in a complex sample, we developed a multiple reaction monitoring (MRM) assay and tested this with a dilution curve from a HeLa digest in triplicates using the Whisper 40 SPD method on the Evosep One coupled to an Agilent 6495C. The MRM assay was then applied to single HeLa cells, which were sorted, harvested and digested using the cellenONE in separate proteochip wells.

Results:
We were able to generate excellent linear curves ($r2 \leq 0.99$) for all HeLa peptides across a concentration range spanning more than 4 orders of magnitude. Selected peptides could be quantified with CVs < 20 % in sub ng of material. We challenged the sensitivity of our MRM assay further by measuring a large number of individually sorted and digested HeLa cells. Thereby demonstrating the ability to measure low abundant targets in our assay and dissect clinical relevant heterogeneity of single cells as our targets represent epigenetic markers, cancer biomarker proteins and cell cycle markers.

Conclusion:
Our data demonstrates that the Evosep One in combination with the Agilent 6495C represents a powerful platform for targeted analyses that require ultra-high sensitivity, reproducibility and throughput.

PP01.131: Multi-omic Landscapes of Nasopharyngeal Carcinoma Reveals Patterns Associated with Induction Chemotherapy Response

Wang Y[1], Li Y, Shao X, Sun A, Ma J, He F
[1]National Center For Protein Sciences(Beijing)

Introduction:
Our multicenter, randomized trial reported significantly improved survival using gemcitabine plus cisplatin (GP) induction chemotherapy (IC) in locoregionally advanced nasopharyngeal carcinoma (LA-NPC), which is recommended as the standard treatment by the National Comprehensive Cancer Network guidelines. However, 15% of patients gain little benefit. Using multi-omic approaches, we aim to discover molecular patterns that are associated with IC response and novel drug targets for non-response patients.

Methods :
We recruited 20 patients with chronic nasopharyngitis and 240 patients with LA-NPC, including 120 received GP IC combined with concurrent chemoradiotherapy and 120 received the concurrent chemoradiotherapy alone. Fresh frozen biopsy samples were lysed and homogenized in Buffer RLT from Qiagen Allpre kit. After eluting DNA and RNA from the spin column, the flow-through was precipitated for Protein extraction. For full proteome, 50 μg digest was desalted and analyzed with data independent acquisition. For phosphoproteome, 200 μg digest was used for Fe-IMAC enrichment and analyzed with data dependent acquisition. The data files were searched against human Uniprot database for identification and quantification of peptides, proteins and phosphosites in Spectronaut and MaxQuant. The data analyses are performed in R and Python.

Results:
We utilized a single sample to simultaneously extract high quality total DNA, RNA and Proteins to avoid the heterogeneities among omic-levels. Multi-omics of genomic, transcriptomic, proteomic and phosphoproteomic were performed across 260 patients. The proteomic and phosphoproteomic methods were validated in pairs of co-extracted and direct-extracted samples were from a single tissue. No significant differences were found either in identification and quantitation. On proteome level, we generated a library of 13,500 protein groups, identified 11,000 protein groups in total, and on average 8,000 protein groups per sample. The integrated multi-omic analyses are on-going.

Conclusions:
We generated the first multi-omic landscapes of nasopharyngeal carcinoma to improve the precision therapy.

PP01.132: Ultra-high Throughput Peptide Quantification of Inflammation Panel using Acoustic Ejection Mass Apectrometry (AEMS)

Wang Y[1], Hoedt E[1], Fu Q[1], Hunter C[2], Van Eyk J[1]
[1]Cedars-sinai Medical Center, [2]SCIEX

Introduction: Ultra-high-throughput strategies that allow for the precise and robust quantification of target proteins on 10,000s of samples could transform biomarker validation, population science, and precision medicine research. Specific enrichment of plasma proteolytic and proteotypic peptides that represent target proteins using the SISCAPA (aka. iMRM) approach has greatly simplified sample complexity and improved the assay sensitivity however, LC-MS data acquisition time is still on the order of minutes per sample. Here, we explore the use of the AEMS on the Echo® MS system for ultra-high-throughput quantification. Optimization was carried out using ten stable isotope-labeled peptides of the acute phase protein multiplex SISCAPA assay (Alb, A1AG, C3, CRP, Hx, IgM, LPSBP, MBL, MPO, SAA) following immunocapture from plasma with the goal for 1 sample/second acquisition.

Methods: The mixture of 10 stable isotope-labeled peptides and 10 corresponding light peptides was analyzed on the Echo® MS system on the SCIEX 6500+ system. SISCAPA workflow was applied to enrich the peptides in the acute phase inflammation panel from human plasma, the captures were run by AEMS.

Results: Multiple 384-well plates containing the mixture of heavy and light SIS peptides were run by various MS methods of different numbers of MRMs and peptides in multiple days and weeks, which confirmed high reproducibility and repeatability. Calibration curves in CHAPS (main matrix in SISCAPA) with constant heavy peptides and varying light peptides indicated the high sensitivity. Automated SISCAPA captures of healthy human plasma and chicken plasma (surrogate matrix) mixed at different ratios then measured by AEMS showed high quantitative fidelity. In comparison to LC-MS, a 384-well plate scale of SISCAPA sample cohort ran with EchoTM-6500+ MS provided faster time to results (~10,000 replicate samples were run in <6 hrs).

Conclusions: Ultra-high throughput peptide quantification using the AEMS at 1 sample/second is robust and precise.

PP01.133: Deciphering Protein Movement from Brain to CSF to Facilitate Dementia Biomarker Discovery

Waury K[1], de Wit R[1], Teunissen C[2], Abeln S[1]
[1]Vrije Universiteit Amsterdam, [2]VU University Medical Center

Introduction

Cerebrospinal fluid (CSF) is an important source for biomarkers of neurological diseases. While exploratory mass spectrometry is the optimal approach to discover the CSF proteome, low abundant proteins can be difficult to detect in complex matrices. To identify novel CSF biomarker candidates, it is beneficial to gain a deeper understanding of the processes within the brain that lead to the release of proteins to CSF. Here, we aimed to explore if protein movement from the brain to the CSF can be predicted from protein sequence and which factors determine this process.

Methods

Six previously published mass spectrometry studies of healthy human CSF samples were combined to create a large CSF proteome of 5297 proteins. This dataset was overlapped with the Human Protein Atlas elevated brain proteome to identify CSF-secreted and retained brain proteins. A logistic classifier was trained to differentiate between the two protein classes utilizing sequence-based features.

Results

Prediction of CSF protein secretion achieved a balanced accuracy of 72.44%. The model accuracy increased further to 80.70% if only including CSF proteins that have been found in at least half of the mass spectrometry studies. Highly important for correct classification are signal peptides and subcellular localization. We provide an illustrative example for the model's application to biomarker candidate identification within Alzheimer's Disease proteomics studies.

Conclusions

A comprehensive CSF proteome was collected, however, our results show that a minimum study criterion is necessary to exclude likely false positively identified proteins. The trained classifier performs well, and feature analysis elucidates the underlying mechanisms of brain protein secretion and leakage processes. The model can be utilized to identify potential CSF biomarkers difficult to detect in discovery proteomics studies and guide candidate selection for biomarker development.

PP01.134: Subtypes of Intrahepatic Cholangiocarcinoma Show Distinct Biological Motifs that are Linked to Patient's time to Recurrence

Werner T[1,2,3], Budau K[1], Kurowski K[1], Cosenza-Contreras M[1,2,4], Pinter N[1], Werner M[1], Sigel C[5], Tang L[5], Bronsert P[1], Schilling O[1]
[1]Institute for Surgical Pathology, Faculty of Medicine, University Medical Center Freiburg, [2]Faculty of Biology, University of Freiburg, [3]Spemann Graduate School of Biology and Medicine (SGBM), University of Freiburg, [4]MeInBio Graduate School, University of Freiburg, [5]Department of Pathology, Memorial Sloan Kettering Cancer Center

Introduction

Pathological classification of intrahepatic cholangiocarcinoma (iCCA) remains challenging, given this cancer's inaccessibility to diagnostic probing and insufficient molecular characterization. Although iCCA frequently reappear after initial surgery, only few and imprecise tools are available to predict highly individual times-to-recurrence (TTR). In our study, we investigated a cohort of iCCA patients to characterize proteomic profiles, which could be used as predictive markers for the TTR.

Methods

Macrodissected tumor samples from 80 patients were measured via liquid-chromatography mass-spectrometry (LC-MS/MS) based proteomics in data independent acquisition mode (DIA).

Results

In a hierarchical clustering analysis, we could define two patient subgroups, which showed significantly diverging TTR distributions. Enrichment analysis revealed extracellular matrix (ECM) constituents to be upregulated in cluster 1, which is associated with a beneficial prognosis, and increased RNA and protein synthesis and processing in cluster 2. In a second, independent analysis, we iteratively applied Cox' proportional hazards model (CPHM) to identify single proteins whose expression correlates with TTR distribution. Gene set enrichment analysis of protein hits with a positive hazard ratio, which indicates increased risk for early recurrence, revealed an involvement in cell cycle advancement, and RNA and protein biosynthesis. Conversely, proteins associated with low hazard ratios and low risk were mostly linked to the ECM.

Conclusion

A comparison of significant protein hits from both approaches revealed largely identical proteins as determinants of the TTR, with ECM proteins apparently playing a special role in disease progression. Six of these proteins were selected as a marker candidate panel to estimate the TTR. These include cluster1 / low risk associated proteins Vitronectin, Biglycan, and Histone 1.0, and cluster 2 / high risk related proteins SKI, Fructose-bisphosphate aldolase A, and L-lactate dehydrogenase, all of which will be further validated via immunohistochemistry and against a second, independent iCCA cohort.

PP01.135: Expanding Individualized Therapeutic Options via Genoproteomics

Zhan D[1,2], Zheng N[3], Zhao B[1,4], Cheng F[1,2], Tang Q[1], Tang X[1], Wang J[1], Liu H[1,2], Li X[1,2], Su J[1], Zhong X[1], Bu Q[5], Cheng Y[1,4], Wang Y[1,3], Qin J[1,3]

[1]KingMed-Pineal Joint Innovation Laboratory of Clinical Proteomics, Guangzhou KingMed Center for Clinical Laboratory Co., Ltd., [2]Beijing Pineal Diagnostics Co., Ltd., [3]State Key Laboratory of Proteomics, Beijing Proteome Research Center, National Center for Protein Sciences (Beijing),Beijing Institute of Lifeomics, [4]KingMed College of Laboratory Medical of Guangzhou Medical University, [5]Department of Medical Oncology, the Fisrst affiliated Hospital of Guangxi Medical University

Abstract

Introduction: Clinical NGS testing has enabled the treatment recommendations for cancer patients detected with driver gene mutations. For patients without driver gene mutations, targeted therapy options are lacking.

Methods: we carried out NGS and proteomics testing on 169 formalin-fixed paraffin-embedded (FFPE) samples, including non-small cell lung cancers (N=65), colorectal cancers (N=61), thyroid carcinomas (N=14), gastric cancers (N=12), gastrointestinal stromal tumors (N=11) and malignant melanomas (N=6).

Results: NGS detected 14 mutated genes with actionable mutations in 73 samples, providing treatment options for 43% of the patients. Proteomics identified 62 clinical drug targets in 136 samples, providing treatment options for 80% of the patients. In one sample, proteomics detected 3 immune therapy targets (PDL1, PDL2, and LAG3), making combinatorial immune checkpoint blockade therapy a preferred option for the patient.

Conclusions: Our analysis suggests that genoproteomics, which combines NGS and proteomics, could expand the targeted treatment options to 90% (152/169) of the cancer patients in this study.

Keywords: NGS; proteomics; real-world; targeted therapy; actionable mutation; clinical drug target, precision medicine

PP01.136: Fragterminomics: Extracting Information on Proteolytic Processing from Shotgun Proteomics Data

Cosenza-Contreras M[1], Seredynska A[2], Pinter N[2], Brombacher E[1,4,5,6], Dinh T[1,2], Bernhard P[1,2], Rogg M[2], Liu J[7], Willems P[8,9], Stael S[8,9], Huesgen P[10,11], Kuehn W[7], Schell C[2], Kreutz C[5], Schilling O[2,3]
[1]Institute for Surgical Pathology, University Medical Center Freiburg, [2]Faculty of Biology, University of Freiburg, [3]German Cancer Consortium (DKTK) and German Cancer Research Center (DKFZ), [4]Centre for Integrative Biological Signaling Studies (CIBSS), [5]Institute of Medical Biometry and Statistics, Faculty of Medicine and Medical Center, University of Freiburg, [6]Spemann Graduate School of Biology and Medicine (SGBM), University of Freiburg, [7]Renal Department, University Medical Center Freiburg , [8]Department of Plant Biotechnology and Bioinformatics, Ghent University, [9]VIB-UGent Center for Plant Systems Biology, [10]Central Institute for Engineering, Electronics and Analytics, ZEA3, Forschungszentrum Jülich, [11]CECAD, University of Cologne

Introduction: The use of modern bioinformatics algorithms for peptide-to-spectrum matching (PSM) with robust tools for their statistical scoring allows us to increase the range of variable features that can be reliably obtained from the MS data without the need for biochemical enrichment. Semi-specific searches offer a powerful option to identify peptides that arise from endogenous proteolytic activity (referred also as 'neo-N-termini' analysis or 'N-terminomics'). Nevertheless, the extraction of biological meaning from these kinds of search outputs can be a challenging task in terms of data mining and analysis.
Methods: We describe an R-based data analysis approach for the (1) annotation of peptides according to their representative feature type (2) explorative analysis of neo-N-termini location, (3) mapping neo-N-termini to known protein processing features, and (4) differential abundance and enrichment analysis of N-terminal sequence motifs and their association with protease specificity. We used a mouse model of polycystic kidney disease and isobaric labeling at the protein level as a use case. In brief, protein was extracted from 11 FFPE tissue samples and directly labeled before trypsin digestion using TMT 11plex.
Results: 1436 peptides fulfilled the conditions to be considered proteolytic products (among a total of 30879 identified peptides). We observe a differential behavior in terms of proteolytic processing between polycystic and control mice, both in general terms of proportional abundance and differential abundance of proteolytic products. The cleavage sequence motif analysis revealed specifically enriched patterns for either up- or downregulated proteolytic products.
Conclusion: The combination of modern tools for peptide identification and an integrative data analysis approach, allows us to extract biologically relevant information related to proteolytic processing from shotgun proteomics data without the need for biochemical enrichment. We suggest this as a guideline that can be used for deeper exploration of MS proteomics data, including new alternatives for data re-analysis.

PP01.137: AMP® PD: Correlations Between Protein Expression and APOE Genotype

Dardov V[1], Babcock D, Kirsch L, Sardi P, AMP PD Consortium
[1]Technome

Introduction: Apolipoprotein E (APOE) has been shown to directly influence the development of α-synuclein pathology in Parkinson's disease (PD) dementia, showing the importance of APOE as a potential therapeutic target. Utilizing publicly available data from AMP-PD, we combined targeted proteomics data from cerebrospinal fluid (CSF) and plasma, demographics and APOE genotype to understand the relationship between APOE genotype and measured proteins.

Method: As of July 2022, The Accelerating Medicines Partnership® (AMP®) PD FAIR resource includes data from 10,418 participants. Modalities available include longitudinal and cross-sectional clinical data, longitudinal blood and CSF based transcriptomics, genomics and targeted proteomics data. In this use case, we integrated targeted proteomics data with clinical, demographics data and APOE genotype. Targeted proteomics data was acquired on CSF and plasma samples utilizing the Olink platform. WGS data from blood was acquired using the Illumina HiSeq-Xten platform and APOE genotype was extracted. The open source Terra platform was utilized to generate R scripts to pull data from GCS, combine it and perform analyses.

Results: We combined targeted proteomics data with available AMP-PD data, including demographics and clinical data and APOE genotype for 205 participants, each with multiple collection timepoints. 42 participants were either APOE4 homozygous or heterozygous. PCA analysis showed no separation of samples based on clinical diagnosis, sex or APOE genotype. Hierarchical clustering analyses and correlation analyses were also performed.

Conclusion: APOE as a clinical target for Parkinson's Disease is relatively recent. APOE genotype is most widely associated with Alzheimer's Disease risk, however, recent studies show the importance of APOE in Parkinson's Disease. In this case study, we utilized public AMP PD data and combined proteomics data with genotype for exploratory analyses. The analysis notebooks are available as a resource and accessible via Terra to anyone who signs a data use agreement for AMP PD data.

PP01.138: Elucidating Molecular Mechanisms of IRF4-Steered Effector Functions in Th17 and Treg Cells

Gabele A[1], Sprang M[2], Dietzen S[1], Klein M[1], Pape K[3], Tenzer S[1], Bopp T[1], Distler U[1]
[1]Institute of Immunology, University Medical Center of the Johannes-Gutenberg University Mainz, [2]Institute of Organismic and Molecular Evolution (iOME), Faculty of Biology, Johannes-Gutenberg University Mainz, [3]Department of Neurology, University Medical Center of the Johannes-Gutenberg University Mainz, Mainz

Introduction: Interferon regulatory factor 4 (IRF4) promotes the maturation of T helper (Th) cell populations into effector cells. Despite its central role in Th lineage determination, molecular mechanisms of IRF4-mediated gene expression are still poorly understood and the question of how IRF4 interacting proteins steer IRF4-mediated target gene transcription in different Th cell subtypes remains unresolved to date. We established a mouse model for the ex vivo characterization of IRF4 interactomes in functionally opposing T cell subtypes, such as Th17 and T-regulatory (Treg) cells. To identify novel players in IRF4-mediated gene regulation, we integrated the IRF4 interactome with IRF4-ChIP-Seq and Th17/Treg proteome data.
Methods: To generate in vivo biotin-tagged IRF4, mice expressing IRF4 fused to a BirA recognition site were crossed with the ROSA26BirA strain [1]. Proteins from pulldown experiments and full Th17/Treg proteomes were processed as described before [2] followed by LC-MS analysis on an Exploris 480. ChiP-Seq analyses were performed on an Illumina NovaSeq6000 instrument.
Results: We focused on the characterization of the transcription factor IRF4 and its interplayers in ex vivo propagated CD4+ T cell subsets developing a robust and highly reproducible affinity purification protocol. We could describe a “core IRF4 interactome” preserved even in functionally opposed T cell subsets. Additionally, we detected lineage-specific interactors involved in IRF4-steered gene expression including novel, yet uncharted interplayers in Th17 as well as Treg cells. IRF4-ChIP-Seq as well as proteome analyses of IRF4 KO mice revealed pathways controlled by the IRF4-complex crucial for the regulation of classical Th17 but not for Treg-specific signature genes. Over 90% of the identified IRF4 interactors could be also detected in Th17 cells isolated from multiple sclerosis patients.
Conclusions: Affinity purification mass spectrometry facilitated the identification of novel players in IRF4-mediated gene regulation in CD4+ T cells.

1. Driegen. TransgenicRes. (2005).
2. Hughes. MolSystBiol. (2014).

PP01.139: Sequential Participation of Peptidases in the Digestion Process of the Whiteleg Shrimp Litopenaeus vannamei

Estrella Soliz C, Huerta Ocampo J[1,2], Bojórquez-Velázquez E[3], Ruiz-May E[3], Rojo Arreola L[4], González Ríos H[1], Muhlia Almazán A[1]

[1]Centro De Investigación En Alimentación Y Desarrollo A. C., [2]Consejo Nacional de Ciencia y Tecnología, [3]Instituto de Ecología, A.C. (INECOL), [4] Centro de Investigaciones Biológicas del Noroeste S.C.

Introduction: Although there have been multiple advances in the study of the digestive process of L. vannamei, the specific sequence of enzyme´s participation in the protein digestion process is still unknown. Since the whiteleg shrimp is a major aquaculture product in Mexico, this knowledge becomes important to improve the culture process. To accomplish this, we analyzed the digestive peptidases abundance and activity in the shrimp digestive gland at three different times, each representing an essential stage in the digestion process. We used zymograms and Tandem Mass Tags (TMT) for relative quantitative proteomic analysis.

Methods: Midgut glands were sampled during the digestion process at three times: preprandial, postprandial (1 h), and postprandial (3 h). Samples were examined by activity tests (zymograms) and proteomics. Briefly: Proteins were digested with MS-grade trypsin, peptides labeled with Amine-reactive TMT-10plex and fractionated by SCX. Protein identification and relative quantitation analysis was performed by SPS-MS3 using an Orbitrap and raw-data were processed with Proteome Discoverer 2.1 using Amanda, Sequest-HT, and Mascot search engines against the L. vannamei proteome database (UP000283509).

Results: Serine peptidases (trypsin and chymotrypsin isoforms) were active overall digestion process, but differences in abundances were detected in only two trypsins at postprandia (3 h), trypsin A0A3R7M136 decreased, and trypsin A0A3T7NWS2 increased. Digestive cathepsins D and L both showed significant changes in activity and abundance at preprandia (cathepsin D) and postprandia 3 h (cathepsin L). Three digestive metallopeptidases showed a similar change with their maximal abundances during postprandia 1 h, and no-activity during postprandia (3 h).

Conclusions: Even though trypsin and chymotrypsins are the most important peptidases in digestive process, regulated peptidases were cathepsins, being cathepsin D most abundant at preprandia, whereas cathepsin L during postprandia. Metallopeptidases had similar abundance in the three times but showed no-activity at postprandia.

PP01.140: Longitudinal Multiomics Integration of Mitochondria to Identify Key Regulators During Development of Hepatocellular Carcinoma in Rat

García-Chávez J[1], Montiel R[1], Villa Treviño S[2]
[1]Advanced Genomics Unit, CINVESTAV, [2]Cell Biology Department CINVESTAV

Mitochondria participate in multiple functions in eukaryotic cells. Although disruption of mitochondrial function has been associated with energetic deregulation in cancer, the chronological changes in mitochondria during cancer development remain unclear. With the aim to assess the role of mitochondria throughout cancer development, we analyzed samples chronologically obtained from induced hepatocellular carcinoma (HCC) in rats. In our analyses, we integrated mitochondrial proteomic data, mitochondrial metabolomic data and nuclear genome transcriptomic data. We used pathway over-representation and weighted gene co-expression network analysis (WGCNA) to integrate expression profiles of genes, miRNAs, proteins, and metabolite levels throughout HCC development. Our results show that mitochondria are dynamic organelles presenting specific modifications in different stages of HCC development. We also found that mitochondrial proteomic profiles from tissues adjacent to nodules or tumor are determined more by the stage of HCC development than by tissue type, and we evaluated three models to predict HCC stage of the samples using proteomic profiles. Finally, we propose an omics integration pipeline to massively identify molecular features that could be further evaluated as key regulators, biomarkers, or therapeutic targets. As an example, we show a group of miRNAs and transcription factors as candidates, responsible for mitochondrial metabolic modification in HCC.

PP01.141: Reactome Pathway Analysis - Disease Association Overlay

Ragueneau E, Tiwari K, Gong C, Chuan D, Viteri G, Hermjakob H[1]
[1]EMBL-EBI

Introduction:
The Reactome Pathway Knowledgebase [https://reactome.org], an Elixir core resource, provides manually curated molecular details across a broad range of physiological and pathological biological processes in humans, including both hereditary and acquired disease processes. The processes are annotated as an ordered network of molecular transformations in a single consistent data model. Those processes are visually represented as pathway diagrams.

Methods:
We are providing an interface which allows pathway diagrams to be enriched by overlaying pairwise associations from other resources associated with their participant molecules. These associations can optionally be characterised by a score which can be used as a filter within the user interface. This functionality is currently used for Protein-Protein Interactions from IntAct[1] and Gene-Disease associations from DisGeNET[2], but can be extended to any type of binary associations involving biological molecules in Reactome, for example pathway modulators like antibodies or drug-like molecules. Sets of pairwise associations like those provided by DisGeNET can also be used as direct input to Reactome's pathway over-representation analysis, for example providing one-click enrichment analysis of the genes found to be associated with one disease:
https://reactome.org/overlays/disgenet

Conclusions:
The Reactome overlay of binary associations provides a powerful tool to analyse user data in the context of additional external data, for example gene-disease associations, or pathway modulators like drugs, and opens up new avenues to move from raw data to biological insights.

References:
[1] Gillespie M, Jassal B, Stephan R, et al. The reactome pathway knowledgebase 2022. Nucleic Acids Research. 2022 Jan;50(D1):D687-D692.
[2] Piñero J, Ramírez-Anguita JM, Saüch-Pitarch J, Ronzano F, Centeno E, Sanz F, Furlong LI. The DisGeNET knowledge platform for disease genomics: 2019 update. Nucleic Acids Res. 2020 Jan 8;48(D1):D845-D855.

PP01.142: Effective Derivation of Ventricular Cardiomyocytes from hPSCs using Maturation Medium

Kim K[1], Kim E[1], Kim J[2], Lee H[1]
[1]Korea Institute Of Toxicology, [2]Dongguk university

-Introduction

Cardiomyocytes derived from human pluripotent stem cells (hPSCs) could be used in applications including disease modeling, drug safety screening, and novel cell-based cardiac therapies. Here, we report an optimized selection and maturation method for inducing maturation of cardiomyocytes into a specific subtype after differentiation driven by the regulation of Wnt signaling.

-Methods

The medium used to optimize selection and maturation was a combination of glucose starvation conditions with either a nutrition complex or ascorbic acid. Methods were used to analyze maturation of cardiomyocytes derived from hPSCs as follows

1. Immunostaining
2. Flow cytometry
3. Electron microscopy
4. Whole-cell patch-clamp recording for action potential analysis
5. Next-generation sequencing (NGS) analysis

-Results

Following selection and maturation using albumin and ascorbic acid, the population of the cTnT-positive cardiomyocytes was higher than when using B27. In addition, ascorbic acid in selection and maturation induced maturation of the ventricular type of cardiomyocytes. We compared gene expression under selection and maturation conditions by NGS analysis.

-Conclusions

Our optimized conditions will enable simple and efficient maturation and specification of the desired cardiomyocyte subtype, facilitating both biomedical research and clinical applications.

-Acknowledgements

This research was funded by the Ministry of Trade, Industry and Energy (20009748) and the Korea Institute of Toxicology, Republic of Korea (1711159826)

PP01.143: Identifying the Genes Impacted by Cell Proliferation in Proteomics and Transcriptomics Studies

Locard-paulet M[1], Palasca O[1], Jensen L[1]
[1]Novo Nordisk Foundation Center for Protein Research, University of Copenhagen, Denmark
Hypothesis-free high-throughput protein profiling allows relative quantification of thousands of proteins across samples and thereby identification of differentially expressed proteins. It is used in many biological contexts to characterize differences between cell lines and tissues, identify drug mode of action or drivers of drug resistance, among others. This type of analysis remains challenging. It often results in hundreds of regulated hits, among which it is difficult to identify what protein is specifically interesting to then select targets for functional validation or clinical trials. This can be facilitated by identifying hits that are not regulated because of the specific treatment or disease of interest, but because of confounding factors that were not accounted for in the experimental plan, such as change in cell proliferation.
Here, we combined five proteomics and three transcriptomics data sets containing the proteomes of 449 and the transcriptomes of 1,140 cancer cells growing at different speed. For each data set, we calculated the correlation of all their genes/proteins to relative cell proliferation to identify 223 genes that reproducibly correlate with proliferation rates at protein and transcript level. These include many actors in DNA replication and mitosis, and genes periodically expressed during the cell cycle, but also genes not directly known to be functionally associated with cell cycle.
This resource can be used to ease and speed up target selection in discovery studies where cell proliferation changes between conditions and/or samples. We reanalyzed in vitro drug screens and tumor samples to show how disregarding these proliferation confounders allows to focus on experiment-specific regulation events otherwise buried in the statistical analysis and easily dig deeper in the wealth of available data.
The list of proliferation confounders can be found alongside our preprint (https://doi.org/10.1101/2022.03.15.483931), and can be included in the routine analysis of any high-throughput dataset.

PP01.144: Enhancing Aquaculture: Biomarker Discovery in Shrimp and Salmon using DIA, Targeted Proteomics and Metabolomics

Mendoza-Porras O[1], Beale D[2], Broadbent J[1], Osborne S[1], Simon C[1], Wade N[1]
[1]CSIRO Agriculture And Food, [2]CSIRO Land And Water

Introduction: Aquaculture production of shrimp and salmon was valued at USD$50 billion in 2017 (FAO, 2019). To future-proof the aquaculture industry, animal welfare, diet performance, and sustainability must be improved while enhancing host resilience against disease and environmental stress. In shrimp, the bacterial biomass NovacqTM promotes health and growth in Penaeus monodon when supplemented in feeds. In Atlantic salmon, dietary strategies that mitigate the effects of climate change are being developed to help secure the future of the salmon industry.

Methods: DIA proteomics and metabolomics were used to elucidate metabolic pathways in shrimp hepatopancreas, and plasma haemolymph activated by NovacqTM and, krillmeal compared with a control group fed a fishmeal diet. In salmon, a 45-peptide targeted proteomics method was used to identify biomarkers for heat stress in liver of salmon exposed to gradual temperature increases from 15°C to 20°C.

Results: A strong signature of glycoconjugate metabolism, driven by hexosaminidases and arylsulfatases, was observed in DIA results for NovacqTM fed shrimp. Joint-pathway analysis indicated that NovacqTM fed shrimp preferred using energy from carbohydrates through activation of the amino- and nucleotide sugar metabolic pathway while krillmeal fed shrimp activated pathways for lipid metabolism. In salmon, the abundance of two different protease inhibitor peptides significantly increased as the temperature increased to 17°C, 19°C and 20°C compared with a control group maintained at 15°C.

Conclusions: Combined DIA proteomics and metabolomics revealed heterogeneity in shrimp metabolic pathway activation in response to specific feed ingredients. In salmon, two protease inhibitor peptides showed the greatest potential as heat stress biomarkers that could help monitor the effectiveness of feed additives for maintaining salmon welfare at high temperature

PP01.145: Enhancing Aquaculture: Biomarker Discovery in Shrimp and Salmon Using DIA, Targeted Proteomics and Metabolomics

Mendoza-Porras O[1], Beale D[2], Broadbent J[1], Osborne S[1], Simon C[1], Wade N[1]
[1]CSIRO Agriculture And Food, Queensland Bioscience Precinct, [2]CSIRO Land and Water, Ecosciences Precinct

Introduction: Aquaculture production of shrimp and salmon was valued at USD$50 billion in 2017 (FAO, 2019). To future-proof the aquaculture industry, animal welfare, diet performance, and sustainability must be improved while enhancing host resilience against disease and environmental stress. In shrimp, the bacterial biomass NovacqTM promotes health and growth in Penaeus monodon when supplemented in feeds. In Atlantic salmon, dietary strategies that mitigate the effects of climate change are being developed to help secure the future of the salmon industry.
Methods: DIA proteomics and metabolomics were used to elucidate metabolic pathways in shrimp hepatopancreas, and plasma haemolymph activated by NovacqTM and, krillmeal compared with a control group fed a fishmeal diet. In salmon, a 45-peptide targeted proteomics method was used to identify biomarkers for heat stress in liver of salmon exposed to gradual temperature increases from 15°C to 20°C.
Results: A strong signature of glycoconjugate metabolism, driven by hexosaminidases and arylsulfatases, was observed in DIA results for NovacqTM fed shrimp. Joint-pathway analysis indicated that NovacqTM fed shrimp preferred using energy from carbohydrates through activation of the amino- and nucleotide sugar metabolic pathway while krillmeal fed shrimp activated pathways for lipid metabolism. In salmon, the abundance of two different protease inhibitor peptides significantly increased as the temperature increased to 17°C, 19°C and 20°C compared with a control group maintained at 15°C.
Conclusions: Combined DIA proteomics and metabolomics revealed heterogeneity in shrimp metabolic pathway activation in response to specific feed ingredients. In salmon, two protease inhibitor peptides showed the greatest potential as heat stress biomarkers that could help monitor the effectiveness of feed additives for maintaining salmon welfare at high temperature.

PP01.146: The Labeo Rohita PeptideAtlas Facilitates the use of Peptide Data for Improved Aquacultural Research

Srivastava S[1], Un Nissa M
[1]Indian Institute Of Technology Bombay

Introduction

Labeo rohita (Rohu), a significant food fish holds a prominent position in global aquaculture. Advancements in mass spectrometry-based proteomics has helped us comprehend biology in a new way. Data repositories like PeptideAtlas, PRIDE and Global Proteome Machine Database enable successful planning of MS-based experiments. Very few proteomics studies have been done on Rohu or other food fishes limiting such resources for the aquaculture community. We developed PeptideAtlas resource for this aquaculture species.

Methods:

Extensive proteomic profiling of 19 histologically normal sample types including 17 tissues, blood plasma and embryo was performed using high resolution Orbitrap Fusion Mass spectrometry. The Trans proteomic workflow was used to process all the mass spectrometric data from various tissues to create the PeptideAtlas resource for Rohu.

Results:

The Rohu peptideAtlas build developed in this study comprised information for ~150 thousand peptides with ~2.9 million peptide spectral matches (PSMs). It also includes information on PTMs, including 826 phosphopeptides, 6870 acetylated peptides and 3679 methylated peptides. The data corresponds to 6015 canonical proteins, 667 indistinguishable representative proteins, 671 marginally distinguished proteins, 768 representative proteins, and 1165 other proteins. The build is made available at www.peptideatlas.org/builds/rohu/

Conclusions

This is the first open-source peptide repository along with PTM information for an important aquaculture species. It would serve as valuable resource for the scientific community to complement other ‘omics’ information for pursuing future research. The information provided on proteins, peptides and PTMs would promote basic and applied aquaculture research to address the most critical challenge of ensuring nutritional security for a growing population.

PP01.147: From Renewables to Value-added Products: Applying Discovery-based Proteomics of Natural Lignocellulolytic Systems

Squina F[1]
[1]Universidade De Sorocaba

Introduction.
Plant biomass conversion technologies can promote economic, social, and environmental development worldwide through sustainable energy production scenarios and replace petroleum-based materials. Understanding biomass conversion in natural utilization systems, can support the development of enzyme combinations or microbial communities and harness the potential of biomass conversion technologies into biofuels and bioproducts.

Methods. Our group has conducted genomics, transcriptomics, and proteomics analyses from natural plant biomass-degrading systems, such as fungi (1), termites (2), hyperthermophilic bacteria (3), soil-derived microbial communities, and soil metagenome (4).

Results and Conclusions. These studies have assigned novel biocatalysts to biotechnological applications and proposed new routes to produce high-value chemicals. For instance, the Aldo-keto reductase (AKR) from the lower termite Coptotermes gestroi has been applied to develop synthetic routes based on the reduction of organic aldehydes, along with the carboxylic acid reductases (CAR). Based on the CAR/ AKR system using E. coli as a host, along with the expression of auxiliary activities, we have promoted the production of coniferol, which is a high-value chemical, from lignocellulosic biomass such as wheat straw. Furthermore, we demonstrated that an ancient redox active enzyme encoded by C. gestroi, a Cu/Zn superoxide dismutase (CgSOD1), plays a previously unknown role in plant biomass degradation. We discovered that this CgSOD1 degrades polysaccharides through an oxidative mode of action, thereby boosting the action of canonical GH enzymes and adding a new member to the group of 'Auxiliary Activity' enzymes used by Nature in biomass utilization, opening opportunities for scouting other redox enzymes related for applications on biomass into bioproducts conversion.

References. (1) L. B. Brenelli et la., Scientific Reports. 9 (2019). (2) J. P. L. Franco Cairo, et al. Frontiers in Microbiology. 7 (2016). (3) F. Mandelli, et el. Extremophiles. 21, 775–788 (2017). (4) E. C. Moraes, et el. Biotechnology for Biofuels. 11 (2018),

PP01.148: Understanding all of Biology: SimpliFi the Analysis and Accessibility of Multiomics Data

Palmeri J[1], Pappin D[2], Wilson J[1]
[1] ProtiFi, LLC, [2]Cold Spring Harbor Laboratory
Huge quantities of omics data limit our ability to understand what that information means. Additionally, our collaborators often have specialties far outside of omics. To address this, we created SimpliFi, the world's first cloud-based, GPU-driven, streamlined and browser-accessible data-to-meaning engine for integrated omics analysis of all kinds: proteomics, lipidomics, metabolomics, transcriptomics, glycomics and genomics. SimpliFi obligates QC including batch and run order effects and results. Results are easily shared, explored or published simply by sending a URL.

SimpliFi models biology using exclusively nonparametric statistics wherein biological replicates to define their own distributions: p-values and fold-changes are determined as a function of biological variation, number of samples and observations and measurement error. Optimized GPU CUDA routines generate confidence intervals via resampling for all values, including p-values. Crucially, SimpliFi does not transform data and accounts for increased data variance at low or high intensities. SimpliFi's user interface is intuitive and user-friendly even for new-to-omics users. All computation is done on external servers so users can view results on any browser; results are shared by simply sending a URL. To our knowledge, SimpliFi is the first platform to combine unbiased statistics, GPU computation and an interactive and intuitive user interface.

Non-parametric p values often differ by several orders of magnitude compared to T-tests. This is due to the non-Gaussian nature of biology and omics data, combined with either oversampling of biological variability (for example with the presence of an outlier), yielding false negatives, or undersampling of variability (for example when replicates are by chance tightly clustered), which yield false positives of differential expression. Non-parametric SimpliFi analyses are always presented with their confidence intervals, informing end-user decisions of the potential risks of the next experimental choices. As SimpliFi helps data sharing, understanding and analysis to help bring meaning to omics data.

PP01.149: Daily Regulation of Mammalian Physiology by mTORC1/2

Zeng A[1], Peak-Chew S[1], O'Neill J[1]
[1]MRC Laboratory of Molecular Biology

Introduction:
Endogenous clocks in every cell drive circadian (~24h) rhythms that underpin the daily organisation of behaviour, physiology, and cellular function in mammals. Circadian regulation of biological function is usually attributed to rhythms in the synthesis and abundance of “clock-controlled proteins”, but there is little direct evidence to support this. In contrast, recent observations have suggested that post-translational regulation of protein activity, via daily rhythms in mTORC1/2 signalling, is the main driver of daily rhythms in physiology, both in vivo and in cultured cells. To distinguish between these two hypotheses, we used quantitative mass spectrometry to assess whether mTORC activity is required for daily rhythms in (phospho)proteome composition and solubility in mouse liver and fibroblasts.

Methods:
Mice were given either INK128 (inhibitor of mTORC1/2) or vehicle in drinking water for 3 days under 12h:12h light:dark cycles, with simultaneous measurement of locomotor activity. Mice were euthanised and livers extracted every 6 hours across 24 hours (N=4). Mouse lung fibroblasts were grown until confluent and synchronised by daily temperature cycles (12h:12h 32°C:37°C) before sampling across a circadian cycle ± INK128 (N=4). Liver and fibroblast time courses were lysed by 8M urea (total protein) or digitonin (soluble protein), then total and soluble proteomes and TiO2-enriched phosphoproteomes were quantified using TMTpro 16-plex on an Orbitrap mass spectrometer. Statistical analysis was performed in R.

Results:
mTORC inhibition profoundly reduced daily variation in protein and phosphoprotein abundance and solubility in mouse cells and tissues, with minimal effect on circadian timekeeping, whilst severely attenuating rhythms in cellular and organismal function.

Conclusions:
Daily rhythms in mTORC activity are one of the principle means by which mammalian biology is co-ordinated to anticipate the different challenges of day and night.

PP01.150: Advanced Assesement through N-glycopeptide Analysis of Infliximab's Originator and Biosimilar Therapeutic Monoclonal Antibodies using LC-MS/MS

Bang G[1], Kim H[1], Oh M[2,3], An H[2,3], Yoo J[1], Kim J[1], Hwang H[1]
[1]Research Center for Bioconvergence Analysis, Korea Basic Science Institute, [2]Graduate School of Analytical Science and Technology, Chungnam National University, [3]Asia-Pacific Glycomics Reference Site

Introduction: Characterizing therapeutic monoclonal antibodies (mAbs) represents a major challenge for analytical sciences due to their heterogeneity associated with post-translational modifications (PTMs). The protein glycosylation requires comprehensive identification because of its major influence on protein structure and effector functions of mAbs. Herein, we demonstrated an ultra-high-performance liquid chromatography in characterizing and comparing baseline and biosimilar mAbs at an advanced level. Specifically, we focused on infliximab and compare the N-based glycopeptide profiles for biologics and their corresponding biosimilar products with the brand names Remicade® and Remsima®, respectively.

Methods: Five batches each of Remicade® and Remsima® were prepared to evaluate the similarity. N-glycopeptides were prepared through trypsin digestion and HILIC enrichment, and then a LC-MS/MS analysis was performed. N-glycopeptides were identified using the GPA 2.0 processing program.

Results: Total of 54 and 63 N-glycopeptides in Remicade® and Remsima® were identified. Based on the number of identified glycopeptide-spectrum matches (GSMs), the originator and biosimilar of Infliximab were quantitatively compared. As a result, the similarities between batches in same mAbs (0.88-0.97) were higher than similarities between batches of originator and biosimilar (0.64-0.90).

Conclusions: Since major glycan types differed quantitatively for each glycosylation site, it would be very appropriate to evaluate glycosylation through glycopeptide for more complex glycoproteins or mAbs with multiple glycosylated sites. This study showed that these properties may be useful for biopharmaceutical evaluations based on glycosylation profiling.

PP01.151: Bridging Metabolic Oligosaccharide Engineering to BioID: Meeting of two Worlds to Carry Large-Scale Glycoprotein-Based Interactomics

Cavdarli S[1], Coyaud E[1], Wisztorski M[1], Franck J[1], Fournier I[1]
[1]PRISM U1192-Inserm; Centre Oscart Lambret; Lille University

Introduction

Protein-protein interactions are at the core of cellular pathways and drive cell signaling through a fine orchestration of stoichiometry, affinity, and post-translational modification (PTM) which all together drive different physiological outcomes. It has been demonstrated that transcriptional and metabolic reprogramming of cancer cells has a huge impact on the glycoproteome. Therefore, cancer-specific alterations in protein glycosylation associated with specific protein networks provide a unique opportunity for clinical intervention. Interactomic by Proximity-dependent biotin identification (BioID) allows the mapping of proteins in the close vicinity of a bait protein in living cells, but to date no PTMs especially glycosylation have been identified. Integration of Metabolic oligosaccharide engineering (MOE) strategy in the BioID workflow was developed to reach large-scale glycoprotein-based interactomics.

Methods

HEK293-Turbo-Flag-BirA* cells were used to perform MOE and BioID sequentially. Incorporation of modified sialic acids containing a clickable moiety was achieved. The interacting partners of the bait protein were then labeled by a cleavable biotin through BioID allowing for their enrichment on beads. Intact interacting proteins were subsequently eluted under reducing conditions and the modified sialic acids containing glycoproteins enriched by click-chemistry-based approach. Interacting glycoproteins were finally identified after trypsin digestion and nanoLC-HR-MS/MS analysis.

Results

Using the developed strategy, thousands of proteins were found by BioID as proximal interactome. For example, for PIN1 protein, a network of thousand proteins and hundreds of glycoproteins, especially glycosyltransferases were identified. Based on this identification, colocalization between glycoproteins and proteins identified respectively by MOE and BioID was observed using immunofluorescence.

Conclusion

Coupling BioID to MOE demonstrates to show strong advantages to identify glycosylation inside an interaction network through in cellulo modifications. The applications of this strategy may be explored to selectively target tumor cells, provide non-invasive biomarkers, and shed into body fluids from a tumor.

PP01.152: Detailed N-Glycome Profiling of Resting and Thrombin-Activated Platelets

Chau T[1], Chatterjee S[1], Houlahan C[2], Passam F[2,3,4], Kawahara R[1], van der Wal D[5], Larance M[6], Thaysen-Andersen M[1,7]
[1]School of Natural Sciences, Faculty of Science and Engineering, Macquarie University, [2]Faculty of Medicine and Health, University of Sydney, [3]Royal Prince Alfred Hospital, [4]Heart Research Institute, [5]Australian Red Cross Lifeblood, [6]Charles Perkins Centre, School of Life and Environmental Sciences, Faculty of Science, University of Sydney, [7]Biomolecular Discovery Research Centre, Macquarie University

Introduction

Platelets play central roles in the vascular and immune systems, including in haemostasis, thrombosis, inflammation, host defence, and carcinogenesis. Tissue injury promptly activates resting platelets, triggering profound morphological changes and granule exocytosis resulting in the release of granular proteins (releasate) that mediate injury-related response processes. Whilst previous studies have documented the importance of protein glycosylation in platelet biology, the platelet glycome remains poorly defined. This study employs quantitative glycomics to map the dynamic and heterogeneous N-glycome of the resting and thrombin-activated platelet releasate.

Methods

Primary platelets were isolated in their resting condition from blood of healthy donors. Platelets were left unstimulated (n=10) or were either partially (n=6) or fully (n=5) activated with α-thrombin, a potent platelet agonist. Thrombin-mediated platelet activation was confirmed using PAC-1- and CD62P-centric flow cytometry. The releasate fractions were collected and separated from platelet cellular fractions (lysate). The N-glycome of the platelet releasate was mapped and compared using quantitative PGC-LC-MS/MS-based glycomics.

Results

In total, 40 N-glycan isomers spanning 28 glycan compositions were identified in the platelet releasate fractions that were consistently rich in sialylated (>90%) and core fucosylated (>50%) complex-type N-glycans across all samples. Oligomannose, paucimannose, and bisecting GlcNAcylation were detected in lower abundance. Excitingly, a thrombin dose-dependent elevation of sialylated and fucosylated complex-type N-glycans displaying a higher degree of branching and a higher global protein occupancy as well as a concomitant reduction in bisecting GlcNAcylation were observed under activated conditions. Interestingly, the activation-induced N-glycome remodelling of the platelet releasate was found to accurately reflect the glycosylation of proteins residing in the platelet α-granules supporting, in line with literature, that these compartments are readily degranulated upon thrombin activation.

Conclusions

This is the first unbiased high-definition map of the heterogenous N-glycome of platelet releasate. Our glycome map represents a useful resource for future studies of platelet glycobiology.

PP01.153: Position-specific Glycans of the Reactive Centre Loop Impact Neutrophil Elastase-Mediated Proteolysis of Corticosteroid-Binding Globulin

Chernykh A[1], Abrahams J[1,2], Grant O[3], Kambanis L[4], Sumer-Bayraktar Z[1,5], Kawahara R[1,6], Corcilius L[4], Payne R[4], Woods R[3], Thaysen-Andersen M[1,6]
[1]School of Natural Sciences, Macquarie University, [2]Glycosciences Laboratory, Department of Metabolism, Digestion and Reproduction, Imperial College London, [3]Complex Carbohydrate Research Center, University of Georgia, [4]School of Chemistry, The University of Sydney, [5]Glycometabolic Biochemistry Team, Cluster of Pioneering Research, RIKEN, [6]Biomolecular Discovery Research Centre, Macquarie University

Human corticosteroid-binding globulin (CBG) is a key glycoprotein that transports and delivers anti-inflammatory cortisol to inflamed tissues upon site-specific proteolytic cleavage of an exposed reactive centre loop (RCL) by neutrophil elastase (NE). We have previously indicated that RCL-localised Asn347-glycans impact NE-mediated proteolysis, but a comprehensive structure-function characterisation of the RCL glycosylation is required to better understand the CBG glycobiology. To this end, we performed a deep RCL-centric glycan and glycopeptide profiling of CBG from healthy sera using LC-MS/MS. We first determined the fine structures of the heterogeneous Asn347-glycans and confirmed their impact on NE proteolysis using molecular dynamics (MD). Importantly, we also identified a hitherto unknown presence of O-glycosylation across four discrete RCL sites spanning Thr338, Thr342, Thr345 and Ser350. Notably, Thr345 decorated mostly by sialylT and disialylT O-glycans was strategically-positioned proximal to the NE-targeted Val344-Thr345 cleavage site. While no multi-O-glycosylated CBG molecules were observed, some CBG glycoforms displayed an intriguing N-/O-glycan co-occurrence involving exclusively Asn347 and Thr338, suggesting an element of glycan crosstalk on the RCL, which was supported by MD simulations of a GalNAcT-CBG complex. Glycoprofiling of HEK293-derived recombinant CBG (rCBG), in contrast, revealed high levels of homogenous Thr345-glycosylation. Longitudinal cleavage experiments demonstrated that both sialo- and asialo-glycans decorating Thr345 of rCBG strongly protect against NE proteolysis. Synthetic RCL O-glycopeptides were used to expand on these findings by showing that Thr345-GalNAc (Tn) and Thr342-GalNAc confer strong and moderate protection against NE cleavage, respectively. MD simulations substantiated that a minimal Thr345-Tn structure limits interactions with NE and, in turn, blocks cortisol release from CBG. In conclusion, we report on strategically-positioned and functionally-relevant glycans occupying the RCL of CBG, which improve our understanding of the molecular mechanisms governing the timely delivery of cortisol to inflamed tissues.

PP01.154: CellSurfer Reveals the First Surfaceome Map of Primary Human Cardiac Cells and Insights into Surfaceome Remodeling in Advanced Heart Failure

Berg Luecke L[1,2], Waas M[1,3], Littrell J[1], Gundry R[1]
[1]University Of Nebraska Medical Center, [2]Medical College of Wisconsin, [3]Princess Margaret Cancer Centre

Introduction

In the heart, cell surface glycoproteins are essential for sustaining normal organ function and play critical roles in cardiac development, disease, and drug uptake. However, the lack of a detailed cell type- or chamber-resolved view of the cell surface proteome of the adult human heart currently limits discovery of new targets for precision drug delivery and the development of practical approaches for studying how different cell types contribute to the development of cardiac disease.

Methods

CellSurfer, a new analytical platform, was applied to cardiac cells isolated from five regions (LA, LV, RA, RV, IVS) of human hearts. Briefly, cell surface N-glycoproteins on ~1 million cells were selectively enriched using streptavidin magnetic beads with an automated liquid handling robot and analyzed by mass spectrometry (MS). MS data were analyzed with Proteome Discoverer, Spectronaut, MSstats, R and Veneer, a novel tool for surfaceome data curation and annotation.

Results

Application of CellSurfer to primary cardiomyocytes, cardiac fibroblasts, endothelial cells, and smooth muscle cells resulted in the generation of the first chamber-, cell-type-, and patient-specific map of the cell surface N-glycoproteome in the adult heart. Experimental evidence of surface localization and transmembrane orientation for >1100 cell surface N-glycoproteins were detected. Data reveal >50 GPCRs and 48 and 21 putative selective markers for cardiomyocytes and cardiac fibroblasts, respectively. Novel monoclonal antibodies generated for one cardiomyocyte marker uniquely localize to cardiomyocytes within human heart tissue and stem cell derivatives, suggesting its value for cell-type specific targeting and immunophenotyping. Analysis of cardiomyocytes reveals previously undescribed cell surface protein dynamics between failing and control hearts.

Conclusions

These data represent the first major step towards a donor, cell-type, subtype, and chamber-resolved reference map of cell surface phenotypes in the adult human heart and reveal new targets for immunophenotyping, drug delivery, and benchmarking explanted cells and stem cell derivatives.

PP01.155: Site Specific Characterization of N- and O-Glycosylation in Etanercept by TMT-labeling and LC-MS/MS

Hwang H[1], Kim H[2], An H[3], Kim J[1], Yoo J[1]
[1]Korea Basic Science Institute, [2]Korea Advanced Institute of Science and Technology, [3]Chungnam National University

Introduction: Improved methods are required for glyco-characterization of biopharmaceuticals and comparison between their corresponding biologics and biosimilar, where they have multiple glycosylation sites such as Etanercept of a fusion protein.

Methods: In order to enhance sensitivity of discovery and minimize the sample loss, the combination of a TMT labeling method and fractionation using high flow HPLC has contributed to detect low amount of peptide in data dependent mode of LC-MS/MS. Here, we report a new method combined with TMT labeling, fractionation using ZIC-HILIC HPLC, LC-MS/MS using HCD triggered CID/EThcD, and computational software for identification of site-specific N- and O-glycopeptide and comparison of their corresponding biologics and biosimilar.

Results: Of three different Etanercept, such as Enbrel (biologics), Etoloce and Eucept biosimilars), a total of 115 N- and O- glycopeptides were identified and quantified using TMT-11plex labeling including triplicates and 22 ZIC-HILIC fractionation in three and 15 of N- and O- glycosylation sites, respectively. Two O-glycopeptides in different O-glycosylation site and seven core 2 type of O-glycopeptides were first reported. In particular, three kinds of O-glycopeptides with NeuGC which could be a foreign agent of immune response.

Conclusions: In depth of quantification, our strategy shows site-specific similarity is quite not similar between biologics and biosimilar.

PP01.156: Regulation of Protein N-linked Glycosylation Site Occupancy

Khaleque M[1], Nouwens A[1], Schulz B[1]
[1]The University Of Queensland

Introduction: N-glycosylation is an essential co/post-translational modification of proteins in eukaryotes, which involves the transfer of glycan from lipid-linked oligosaccharide (LLO) to select asparagine side chains in Asn-Xaa-Thr/Ser (Xaa≠Pro) sequons of newly synthesized polypeptides in the lumen of the endoplasmic reticulum. N-glycosylation directly affects protein folding and plays important roles in protein function, stability, solubility, secretion, resistance to proteases and temperature, and enhancing half-life. Interestingly, the importance of N-glycosylation is highly variable between different glycosylation sites. However, the factors that determine the efficiency of site-specific N-glycosylation are not well understood due to the lack of tools to quantify site-specific glycosylation occupancy. Here, we aimed to understand how control of LLO biosynthesis affected site-specific N-glycosylation occupancy.

Method: We developed and optimized a targeted DIA LC-MS/MS MRM-HR method for quantifying site-specific occupancy at diverse N-glycosylation sequons in yeast cell wall glycoproteins and used this method to compare global site-specific glycosylation under two LLO stress conditions: deficiency of Alg6p or Alg7p, enzymes which catalyse distinct key steps in LLO biosynthesis.

Results: We found that a subset of N-glycosylation sites was differentially occupied in these different LLO stress conditions, consistent with active regulation of site-specific N-glycosylation depending on distinct amino acid sequence features surrounding the glycosylation sequons.

Conclusion: Our results are consistent with a model in which cells under glycosylation stress maintain efficient glycosylation at critical sites in glycoproteins through regulated recognition of specific extended N-glycosylation sequons.

PP01.157: Glycoproteomics Analysis of Human Brains of Individuals Spanning from Normal Cognition to Severe Dementia

Levin Y[1], Tickotsky-Moskovitz N[2], Morgenstern D[1], Levy H[1], Bennet D[3], Buchman A[3], Beeri M[2]
[1]The Nancy and Stephen Grand Israel National Center for Personalised Medicine, Weizmann Institute of Science, [2]The Jospeh Sagol Neuroscience Center, Sheba, Medical Center, [3]Rush Alzheimer's Research Center, Rush University

Introduction

Alzheimer's disease and related dementia (ADRD) pathologies and degeneration only partially explain cognitive decline. Amyloid, tau, and others are primary causes of ADRD. Yet, over 30% of adults do not manifest cognitive deficits prior to death despite the presence of these pathologies, suggesting unidentified factors providing resilience to mitigate the effects of brain pathologies. Therefore, there must be other proteins and posttranslational modifications involved in cognitive decline which have not been discovered as yet.

Protein glycosylation, the enzyme-directed site-specific process that attaches glycans to proteins, is a ubiquitous posttranslational modification that regulates the folding and function of most proteins, including cell signaling, immune recognition, and energy metabolism. Our aim was to investigate the glycorpteomic profiles of human brains with relation to cognitive decline.

Methods

We analyzed 392 postmortem, human brain samples from individuals with varying levels of cognitive decline, through to fully demented patients and matched non-demented controls. These were subjected to our novel glycoproteomic workflow1 which consisted of protein extraction, tryptic digestion a novel boronic acid-based glycopeptide enrichment. The glycopeptide samples are then analyzed on a Tribrid in EThcD mode. Data processing includes Byonic for generating the glycopeptide identifications and FlashLFQ for generating the quantitative information from the raw data.

Conclusions

17,299 glycopeptide forms, from 1,577 proteins were quantified. Preliminary analysis of a sub set of samples revealed the top biological process to be 'peripheral demyelinating neuropathy' and the top KEGG pathway to be related to muscle contraction.

The complete results from the full data set will be presented in detail.

This is by far the most comprehensive glycoproteomic analysis of the human brain and the first to relate changes in glycoproteomic profiles with decline in cognition.

Reference

1. Optimized Glycopeptide Enrichment Method–It Is All About the Sauce. Morgenstern, et al. Analytical Chemistry Article DOI: 10.1021/acs.analchem.2c00524

PP01.158: Glycoproteomic Characterization of Clear Cell Renal Cell Carcinoma

Lih T[1], Cho K[1], Schnaubelt M[1], Hu Y[1], Zhang H[1]
[1]Department of Pathology, School of Medicine, Johns Hopkins University

Introduction: Clear cell renal cell carcinoma (ccRCC) represents the most common form of RCC with high mortality rate. Glycosylation is one of the most prevalent post-translational modifications of proteins. Dysregulation of glycoproteins have been shown to affect biological functions or disease development. In this study, comprehensive glycoproteomic analysis was conducted to characterize ccRCC using 103 tumors and 80 paired normal adjacent tissues (NATs).

Methods: Intact glycopeptides were enriched from 183 tissue samples (103 tumors and 80 NATs) using mixed anion exchange (MAX) cartridge. The differential analysis was carried out by calculating the median log2 fold changes between two groups (e.g., tumors and NATs). The p-values were computed using Wilcoxon rank sum test and adjusted via Benjamini-Hochberg method when applicable. To analyze the association between glycosylation and phosphorylation, we utilized non-negative matrix factorization (NMF)-based multi-omics clustering.

Results: Alteration in glycosylation was observed in ccRCC compared to NATs as well as in BAP1-mutant and PBRM1-mutant tumors. Furthermore, the inter-tumor heterogeneity of ccRCC was detected by subtyping the tumors into three distinct Glyco subtypes. In addition, a cross correlation was observed between glycosylation and phosphorylation in ccRCC.

Conclusions: This study reports a large-scale glycoproteomic analysis of ccRCC and the relation to genomic, transcriptomic, proteomic, and phosphoproteomic changes, suggesting the role of glycosylation in ccRCC to develop potential therapeutic interventions for treating the cancer.

PP01.159: Integrated Proteomic and Glycoproteomic Investigation Reveals Alterations in N-Glycoproteomic Network Induced by 2-Deoxy-D-Glucose in Colorectal Cancer Cells

Ma C[1], Tsai H[2], Zhang Q[3], Li L[3], Chin L[3], Chen R[2], Pan S[1]
[1]UT Health Science Center at Houston, [2]Baylor College of Medicine , [3]Emory University
Introduction: As a well-known glycolysis inhibitor for anticancer treatment, 2-Deoxy-D-glucose (2DG) inhibits the growth and survival of cancer cells by interfering with the ATP produced by the metabolism of D-glucose. In addition, 2DG inhibits protein glycosylation in vivo by competing with D-mannose, leading to endoplasmic reticulum (ER) stress and unfolded protein responses in cancer cells. However, the molecular details underlying the impact of 2DG on protein glycosylation remain largely elusive.
Methods: With an integrated approach of glycoproteomics and proteomics, we characterized the 2DG-induced alterations in N-glycosylation, as well as the cascading impacts on the whole proteome using H29 colorectal cancer cell line as a model system.
Results: More than 1700 site-specific glycoforms, representing by unique intact glycopeptides (IGPs), were identified. The treatment of 2DG had a broad effect on the N-glycoproteome, especially the high-mannose types. The glycosite occupancy of the high-mannose N-glycans had the most decreases compared to the sialic acid and fucose-containing N-glycans. Many of the proteins with down-regulated high-mannose were implicated in the functional networks related to response to topologically incorrect protein, integrin-mediated signaling, lysosomal transport, protein hydroxylation, vacuole and protein N-glycosylation. The treatment of 2DG also functionally disrupted the global cellular proteome, evidenced by significant up-regulation of proteins implicated in protein folding, endoplasmic reticulum, mitochondrial function, cellular respiration, oxidative phosphorylation and translation termination.
Conclusions: Our findings revealed the complex changes in protein glycosylation and expression underlying the various effects of 2DG on cancer cells, and may provide insightful clues to inform development of therapeutics targeting protein glycosylation.

PP01.160: Lower CSF ApoE Glycosylation Associates with Measures of Alzheimer's Disease Biomarkers

Nedelkov D[1], Meuret C[2], Hu Y[1], Martinez A[2], Smadi S[2], Yassine H[2]
[1]Isoformix, [2]University of Southern California

INTRODUCTION

The mechanisms of how the APOE4 allele (APOE4) increases the risk of Alzheimer's disease (AD) pathology have not been fully elucidated. In cerebrospinal fluid (CSF), apoE is heavily glycosylated but its contribution to AD pathology is not known. The objective of this study was to determine the impact of APOE genotype and cognitive status on the relative abundance of apoE protein isoforms and their specific glycosylation patterns in CSF and plasma, using a mass spectrometric immunoassay (MSIA) assay.

METHODS

Total glycosylation and ApoE isoform-specific glycosylation were analyzed in plasma and CSF from a group of older individuals (n = 106) from the USC ADRC cohort, grouped into cognitively normal, participants with mild cognitive impairment, and with AD dementia. We used a new mass spectrometric immunoassay that simultaneously detects the apoE isoforms and glycoforms (O-linked GalNAc(-Sia)-Gal-Sia, and various combinations thereof).

RESULTS

For all individuals, a single O-linked glycan was observed in plasma, while two glycans (of the same type) per apoE were observed in CSF. The ratio of glycosylated to total apoE was greater in CSF compared to plasma for all apoE isoforms. We identified distinct total and secondary isoform-specific cerebral spinal fluid (CSF) apoE glycosylation and sialylation profiles, with the apoE4 isoform having the lowest glycosylation percentage (E2>E3>E4). In CSF, apoE4 was 35% and 25% less glycosylated ($P<0.001$) than ApoE2 and ApoE3 respectively. The % of secondary glycosylation was positively correlated with CSF Aβ42 levels ($R=0.55$, $p<0.001$) and negatively correlated with CSF total Tau ($R=-0.38$, $p=0.001$).

CONCLUSION

CSF glycosylation is lower in the apoE4 isoform and in patients with cerebral amyloidosis, and it correlates with markers of AD pathology. Ongoing experiments are delineating the effects of ApoE glycosylation on its functions. ApoE4 glycosylation may represent a new target of treatment in AD.

PP01.161: Glycoprofile of Clinically Important Herpesviral Protein UL144 and its Receptor Bindings Involved in Viral Immune Evasion

Nemčovičová I[1], Bitala A[1], Lenhartová S[1], Benko M[1], Nemčovič M[2]
[1]Biomedical Research Center SAS, [2]Institute of Chemistry SAS

Introduction: Viruses have long been studied not only for their pathology and associated disease but also as model systems for molecular processes and as tools for identifying important cellular regulatory proteins and pathways. Human cytomegalovirus (HCMV) is a ubiquitous herpesvirus that persistently infects the majority of the world's population. The virus goes through three distinct infection phases to establish lifelong détente with its host. The virus UL144 gene blocks apoptosis by interfering with the expression of immune recognition molecules on the cell surface to avoid lysis by cytotoxic T cells. Sequence of UL144 shows 10 N-linked glycosylation sites located in extracellular part, many of them are not present in other viral species that suggest their importance only in humans and may play a role in ligand-binding recognition.

Methods: Newly synthesized variants of recombinant viral glycoprotein UL144 isolated from baculovirus-insect system with altered glycosylation were characterized by liquid chromatography-tandem mass spectrometry (LC-MS/MS) and X-ray crystallography methods. The surface plasmon resonance (SPR) binding assay was performed with unpurified ligands (crude cell extracts) to highly-purified UL144 to reveal the functional potential of its glycans.

Results: By LC-MS/MS we have identified up to 6 peptides of HCMV UL144 genes that have covered >45 %. Intact protein analysis using a combination of LC/MS determined the accurate masses of the glycoprotein variants and the relative abundance of their isoforms. We have analyzed the glycan' profiles and identified the most glycosylated UL144 species. The SPR studies together with crystallographic analysis of molecular complexes have revealed binding characteristics of UL144 to known cellular ligands. Altogether, the results provide the evidence of UL144' glycans to be involved in HCMV immune recognition.

Conclusions: The development of proteomic methods has revolutionized our ability to assess protein interactions and cellular changes on a global scale, allowing the discovery of previously unknown connections.

PP01.162: A workflow for Large Scale Quantitative Proteome and N-Glycoproteome Analysis of different Cancer Cells

Peng C[1,2], Tian X[1], Wu P[1], Liao K[3], Li B[3]
[1]BaizhenBio Inc., [2]National Facility for Protein Science,CAS, [3]School of Medicine, Sun Yet-Sen Univeristy

As one of the most prevalent protein modification, N-linked glycosylation plays a vital role in many biological processes, especially in the pathogenic development and progression of cancer. Apoptosis-linked gene-2 (ALG-2), also considered as programmed cell death 6 (PDCD6), is related with cell proliferation and death. Many tumor studies indicated that the expression of ALG2 in tumor tissues or tumor cell lines is aberrantly expressed. In this study, we aimed to investigate the effect of ALG2 on protein N-glycosylation to clarify the possible functions of ALG2 in different tumors. To address this question, we established an efficient workflow for large-scale intact N-glycoproteomics and proteomics. Finally, we identified a total of 13676 and 15278 intact glycopeptides from 1190 and 1280 glycoprotein in ALG2-knockout and wild type cell lines, respectively. Our data suggest that N-glycosylation of ITGA3(Recombinant Integrin Alpha 3) is prominently down regulated in four different cancer cells, indicating knocking out of ALG2 may decrease expression of the enzyme involved in the glycosylation progress of N-linked glycoproteins.

PP01.163: A Novel Pipeline for Neoantigen Discovery by MS-based Denovo-Sequencing with DeepNovo and DeepImmu Platform

Peng C[1,2], Wu P[1], Miao H[1]
[1]Baizhenbio, [2]National Facility for Protein Science,CAS

Tumor-specific antigen (TSA) are the peptides expressed and presented by human leukocyte antigen (HLA) only on the surface of cancer cells and hence represent ideal targets for the immune system to distinguish cancer cells from normal cells. A small fraction of those peptides can be recognized by T cells to trigger immune responses and defined as neoantigens. Recently, neoantigens are playing more and more important roles in cancer immunotherapy, either in vaccine development or combination therapy with PD-1 and etc.. To increase the rate of success, we developed a novel pipeline for neoantigen discovery by MS-based Denovo Sequencing with DeepNovo and DeepImmu Platform, including antibody generation, extraction and enrichment of the HLA-peptides complex, separation of the immunopeptides from the HLA complex, optimized MS methods for the immunopeptides, denovo-sequencing of TSA peptides by DeepNovo and immunogenicity prediction by DeepImmu platform. The peptide candidates can be further validated by Elispot and other methods. Briefly speaking, we established the large scale of antibody production and optimized the protein extraction methods for tissues and cell lines to reduce the requirement of the starting materials as low as 10mg for neoantigen discovery. Besides this, different separation methods， such as HPLC or filters， were also tested for different amounts of starting materials. And combinated with optimized MS methods and deepnovo model for denovo-sequencing, we can identify around 10% more immunopeptides than other methods. Furthermore, newly developed DeepImmu platform can boost the accuracy of the immunogenicity prediction. Taken together, we optimized standard operation protocol of the immunopeptides discovery and establish a novel pipeline for neoantigen discovery by MS-based Denovo-Sequencing with DeepNovo and DeepImmu Platform. And using this pipeline, we can identify thousands of immunopeptides in different cell lines and tumor tissues.

PP01.164: A Stream-lined Workflow for the Comprehensive Analysis of N-linked and O-linked Glycopeptides

Sun W[1], Zhang X[1], Zhang Q[2], Xin L[1], Shan B[1]
[1]Bioinformation Solutions Inc, [2]University of Waterloo

Introduction: LC-MS is an effective technique for the analysis of glycosylation. In general, there are two different identification strategies, either first identify the glycan candidates by matching glycan Y-ions, or first search for the peptide sequences by treating the attached glycan as a mass offset. Glycan-search-first approach mainly requires abundant Y-ions, in the meanwhile peptide-search-first method relies on the quality low-mass end peaks and precise precursor mass. In this work, we have developed a stream-lined workflow to integrate glycan-first-search, peptide-first-search and glycan de novo sequencing for automated N-linked and O-linked glycopeptide analysis. Our proposed workflow provides deep profiling of glycopeptides and is less dependent to glycan databases.

Methods: A peptide search is performed first to identify the peptide with glycan mass from a given glycopeptide spectrum. Then, a glycan search is applied to find a confident glycopeptide. In case that the confident peptide cannot be found from the spectrum, an open glycan search will be performed in order to provide a complementary identification of glycan moiety. Finally, if there is a good peptide sequence derived from the spectrum but no reliable glycan in the glycan database is found, glycan de novo sequencing will be conducted to boost the sensitivity of glycan identification.

Results: The proposed workflow was implemented into software package GlycanFinder and compared with two well-known software packages pGlyco3 and MSFragger-Glyco with two datasets. Testing on a fission yeast data show that the stream-lined workflow could identify 20% more spectra than pGlyco3 (glycan-first-search). Searches of the mouse brain data were performed with one ammonium adduct. GlycanFinder also reported 10% more glycopeptide PSMs than pGlyco3. The overlap with pGlyco3 is 10% more than that of pGlyco3 with MS-Fragger.

Conclusions: A stream-lined workflow with algorithms of glycan-base search, peptide-based and glycan de novo sequencing provided deep glycopeptide profiling with LC-MS.

PP01.165: Novel MS Fragmentation Technology allows Enhanced Glycopeptide Characterization in Glycoproteomics

Tran K[1], Albanese J[1], Zhang Z[1]
[1]Sciex

Introduction:
A newly developed electron activated dissociation (EAD) approach allows tunability of electron kinetic energy to produce different fragmentation patterns of the electron-based dissociation (ExD) family.
Although collision induced dissociation (CID) is used traditionally for peptide mapping, these traditional approaches do not allow consistent identification and localization of glycans on peptides. By combining a Zeno trap with EAD technology on a QTOF instrument, higher confidence in data analysis is enabled, suggesting the Zeno-EAD combination ideal for in-depth glycopeptide analysis.

Methods:
Therapeutic monoclonal antibodies (adalimumab and trastuzumab) were denatured with 7.2M guanidine hydrochloride, 100 mM Tris buffer pH 7.2, followed by reduction with 10mM DL-dithiothreitol and alkylation with 30 mM iodoacetamide. Digestion was performed with trypsin/Lys-C at 37°C for 16h. 10 µl (4 µg) of the trypsin/Lys-C digest was separated with a CSH C18 column (1.7 µm particle size, 130Å, 2.1×100 mm). The column temperature was maintained at 50°C. Data was acquired in DDA mode with Zeno trapping using a ZenoTOF 7600 system and either CID or EAD MS/MS fragmentation. Data was processed using commercially available software.

Results:
Data interpretation from several digests and sample sets resulted in the identification of peptide and peptide glycan fragments, oxonium ions, and overall sequence coverage. Comparing CID and EAD sequence coverages resulted in identification of major differences for glycosylated species. While CID MS/MS spectra were dominated by oxonium ions, they showed low abundant b- and y-ions. However, EAD MS/MS spectra showed a rich abundance of c- and z-ions with higher intensity and more coverage of the peptide backbone for various glycosylated peptides. The EAD data provided fragment ions with the intact glycosylation attached, allowing for unambiguous localization of the glycan portion.

Conclusion:
EAD technology with Zeno trapping enabled allows for more accurate and in-depth characterization of glycopeptides, including those of low abundance.

PP01.166: Glycoprotein Characterization Combining PTCR, Native and Charge Detection Mass Spectrometry

Liu W[1], Yip P[1], Viner R[1]
[1]Thermo Fisher Scientific

Introduction

Glycoproteins is gaining attention as they are actively involved in physiological functions as well as progression of certain diseases. However, glycoprotein characterization using intact and top-down mass spectrometry remains challenging as its heterogeneity leading to complex spectrum. Human Fetuin A. is a heterodimeric protein composed of A chain and B chain connected by an interchain disulfide bond. Heterogeneity of intact Fetuin results from its two N-glycosylation sites, seven O-glycosylation sites, and seven phosphorylation sites. Additionally, six disulfide bonds and sequence variants further contribute to the hurdle of top-down analysis. In this study, we evaluated a few novel techniques including Direct Mass Technology and proton transfer charge reduction (PTCR) to unravel Fetuin proteoforms comprehensively under native condition.

Methods

Human Fetuin A (~40 kDa) from human plasma was purchased from Sigma-Aldrich. Native MS and Direct Mass Technology were performed by direct infusion with Nanoflex ion source coupled to Thermo Scientific™ Q Exactive™ UHMR. PTCR (in 20 Th isolated windows) and EThcD analyses were performed on Thermo Scientific™ Orbitrap Eclipse™. Data were analyzed using Thermo Scientific™ BioPharma Finder™ 5.0 Software and STORIboard software (Proteinaceous).

Results

Initial native MS analysis of Fetuin shows a compact charge envelope across m/z 3000- 4000 from charge state +14 to +11. PTCR could reduce the charge state to +6 and extend the charge envelope to m/z 7500. PTCR with 20 Th isolation width separates proteoforms which were previously overlapped in the full scan and increased further number of identified glycoforms. Recently, Direct Mass Technology emerged as another technique with high potential for heterogenous species characterization. Direct Mass Technology analysis, without any charge reduction, was extremely sensitive and can detect all forms in each preparation vs native MS analysis.

By combining different MS methods such as CDMS, PTCR and EThcD, we could successfully characterize heavily glycosylated proteins

PP01.167: N-glycosylation Analysis of Homogenized Oral Squamous Cell Carcinoma Soft Tissue Samples

Demianova Z[1], Horompoly K[2], Szabo A[3], Jarvas G[2], Demianova Z[4], Guttman A[2,5]
[1]Department of Dentoalveolar Surgery and Dental Outpatient Care, Faculty of Dentistry, University of Debrecen, [2]Translational Glycomics Group, Research Institute of Biomolecular and Chemical Engineering, University of Pannonia, [3]Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, University of Debrecen, [4]PreOmics, Am Klopferspitz 19, 82152 , [5]Horváth Csaba Memorial Laboratory of Bioseparation Sciences, Research Center for Molecular Medicine, Faculty of Medicine, Doctoral School of Molecular Medicine, University of Debrecen

Introduction: The number of malignant diseases proliferates, and more than 500,000 new oral cancer cases are diagnosed worldwide annually. In addition, the mortality rates of oral cancers have increased six-fold in the last 50 years. Besides the most commonly known risk factors of alcohol consumption, tobacco, poor oral hygiene, and HPV infection, long-term immunosuppressant therapies can also increase the risk of secondary malignancies. N-glycosylation modifications of proteins have a pivotal role in tumorigenesis and metastasis formation. The aim of this study was to develop a new method capable to identify novel glycobiomarkers to assist in early diagnosis, predict more exact prognosis and help to suggest efficient therapeutic alternatives for oral cancers.
Methods: Oral mucosal soft tissue samples were obtained from seven patients with oral squamous cell carcinoma, both from the malignant and the healthy sides, as well as from seven healthy control volunteers with the appropriate Ethical Permissions and Informed Patient Consents. The collected tissues were properly homogenized (BeatBox, PreOmics, Munich, Germany), followed by N-glycan profiling of endoglycosidase released and fluorophore-labeled carbohydrates using capillary electrophoresis coupled with ultra-sensitive laser-induced fluorescent detection (CE-LIF, Beckman Coulter, Brea, CA).
Results: The total N-glycan profiles obtained by analyzing the samples collected from the malignant and healthy sides of oral squamous cell carcinoma patients and the healthy control volunteers revealed differences at various levels. All carbohydrate structures showing significant alterations were elucidated by automated exoglycosidase array-based oligosaccharide sequencing by CE-LIF, with particular attention to labile sugar residues such as sialylation and fucosylation.
Conclusions: The high-resolution CE-LIF-based glyocoanalytical method reported in this paper combined improved tissue homogenization with CE-LIF analysis of the released N-glycans and proved to be an efficient and sensitive workflow for glycobiomarker-based molecular diagnostics of oral malignant lesions.

PP01.168: Effective Mass Spectrometry-Based Methods to Comprehensively Study Glycoproteins on the Cell Surface and Their Dynamics

Wu R[1]
[1]Georgia Institute Of Technology

Introduction

The surface of mammalian cells is covered with glycans, which are bound to proteins and lipids. Surface glycoproteins are essential for cells to interact with other cells and the extracellular matrix. Aberrant protein glycosylation on the cell surface is directly related to human diseases such as cancer and infectious diseases. Considering that surface glycoproteins are involved in nearly every extracellular activity, comprehensive analysis of glycoproteins on the cell surface and their dynamics will help identify surface glycoproteins as effective biomarkers and drug targets.

Methods and Results

In our lab, we have worked on the development of effective mass spectrometry (MS)-based methods to target surface glycoproteins. Through integrating metabolic labeling and bioorthogonal chemistry, we specifically tagged surface glycoproteins and then selectively enriched them. In combination with MS-based proteomics, we performed global identification and quantification of surface N-glycoproteins site-specifically. The copper-free click reaction is ideal to tag surface glycoproteins because it is quick and specific, and does not need cytotoxic metal ions. After cell lysis and protein extraction, the tagged surface glycoproteins were selectively enriched, and then released for MS analysis. One main advantage of this method is that the conditions are mild, which allows us to study the dynamics of surface glycoproteins. Therefore, this method was applied to quantify the dynamics of surface glycoproteins in monocytes and macrophages in response to the infection. Besides well-known surface proteins changed during the infection, we identified novel surface glycoproteins in response to the infection. Systematic investigation of surface glycoproteins and their dynamics results in a better understanding of glycoprotein functions and cellular activities.

Conclusion

Combining selective enrichment of surface glycoproteins with MS-based proteomics, we systematically studied surface glycoproteins and their dynamics. Considering the importance of surface glycoproteins, effective methods to study surface glycoproteins are extensively applied in the biochemical and biomedical research fields.

PP02.001: Development of a Highly Specific and Sensitive Multiplex Multi-disease Serology Assay

Hellström C, Nilsson P[1], Andersson E, Olofsson J, Skoglund L, Bayati S, Mravinacova S, Jernbom Falk A, Tegel H, Hober S, Bergström S, Månberg A, Pin E, Sjöberg R, Anggraeni Andersson M[1]
[1]SciLifeLab & KTH Royal Institute Of Technology

Objective
The COVID-19 pandemic imposed an immense and immediate need for accurate, sensitive and high-throughput serological assays. Here, the development and thorough validation of a high-throughput multiplex bead-based serological assay is presented together with an extensive utilization with 200.000 samples analyzed. Furthermore, a subsequent focus on a multi-disease antigen panel is being implemented and thereby enabling future broad seroprevalence studies.

Methods
More than 100 representations of SARS-CoV-2 proteins were designed and included for initial evaluation, including antigens produced in bacterial and mammalian hosts as well as synthetic peptides. The five best-performing antigens, three representing the spike glycoprotein and two representing the nucleocapsid protein, were further evaluated for detection of IgG antibodies in 2400 negative and positive control samples. A long range of new variants of concern and variants of interest of the spike protein have subsequently been incorporated and evaluated.

Results
The finally selected antigens, represented by a soluble trimeric form and the S1-domain of the spike glycoprotein as well as by the C-terminal domain of the nucleocapsid. The best assay performance was although achieved when utilizing two antigens in combination, enabling a sensitivity of up to 99.7% combined with a specificity of 100%. Requiring any two of the three antigens resulted in a sensitivity of 99.7% and a specificity of 99.4% (1). The finalized serology assay has been used extensively for a long range of studies (2,3). To complement the serology, we are also investigating the association of proteomic sized autoantibody profiles with long-covid.

Conclusion
These observations demonstrate that a serological test based on a combination of several SARS-CoV-2 antigens enables a highly specific and sensitive multiplex serological COVID-19 assay.

References
(1) Hober … Nilsson (2021) Systematic evaluation of SARS-CoV-2 antigens enables a highly specific and sensitive multiplex serological COVID-19 assay. Clinical & Translational Immunology
(2) https://covid19dataportal.se/data_types/health_data/serology-statistics/
(3) http://www.publicationslist.org/nipe

PP02.002: Multi-disease Serology for Pandemic Preparedness

Bergström S[1], Sivertsson Å[1], Olofsson J[1], Andersson E[1], Hellström C[1], Skoglund L[1], Sjöberg R[1], Månberg A[1], Tegel H[1], Hober S[1], Pin E[1], Nilsson P[1]
[1]Dept Protein Science, SciLifeLab, KTH Royal Institute of Technology

Introduction

The initial phase of new pandemics is accompanied by uncertainty. A key point of pandemic preparedness is therefore to be able to learn from the past by collecting as much data as possible on viruses causing diseases with pandemic potential. Comparative serological studies are well needed to understand the extent and duration of the immune response in future pandemics.

Method

During the COVID-19 pandemic we developed a high-throughput multiplex bead-based serological assay (1). More than hundred representations of SARS-CoV-2 proteins were evaluated, before the final set of antigens was selected with a sensitivity of 99.7% and a specificity of 100% and the assay has now been used to analyze over 200,000 samples. We are currently extending our serological assay to include additional diseases. The flexible bead-based setup enables a step wise addition of new proteins which allow us to continuously increase the number of analyzed antigens. Proteins are designed and produced in-house to enable rapid adaptations for new proteins of interest.

Results

We have continuously extended our serological assay with new SARS-CoV-2 spike variants as they appear. The first additional viruses we will include in the assay are the other six human coronaviruses: SARS, MERS and four causing common cold. Furthermore, a range of selected antigens from the various strains of influenza viruses as well as respiratory syncytial virus are under production. Several antigen representations are under design, for example from herpesvirus, parainfluenza virus, adenovirus, enterovirus, and the recently included orthopoxvirus, as well as the viral infections included in the Swedish vaccination program.

Conclusion

To enable broad and high-throughput studies on seroprevalence we are extending our highly specific and sensitive multiplex COVID-19 serological assay to also include a broad range of other viral infections with pandemic potential or association.

References

(1) Hober … Nilsson, Clin Transl Immunology. 2021

PP02.003: Molecular Map of SARS-CoV-2 Variants of Concern: Quantitative Proteomics, Mechanism, and Therapy

Bouhaddou M[1,5], Zuliani-Alvarez L[1], Reuschl A[2], Thorne L[2], Polacco B[1], Ummadi M[1], Batra J[1], Jang G[1], Xu J[1], Moen J[1], Zhou Y[1], Harjai B[1], Stevenson E[1], Rojc A[1], Pelin A[1], White K[3], Rosales R[3], Abdullah S[7], Richards A[1], Obernier K[1], Fabius J[1], Soucheray M[1], Martinez-Sobrido L[4], Ye C[4], Palmarini M[6], Miorin L[3], Doudna J[7], Garcia-Sastre A[3], Jolly C[3], Towers G[3], Swaney D[3], Krogan N[1]
[1]University of California, San Francisco (UCSF), [2]University College London, [3]Icahn School of Medicine at Mount Sinai, [4]Texas Biomedical Research Institute, [5]University of California, Los Angeles, [6]University of Glasgow, [7]Gladstone Institutes

Introduction

Five variants of concern (VOCs) have dominated COVID-19 disease etiology since 2020—Alpha, Beta, Gamma, Delta, and Omicron. Relative to early-lineage virus isolated from Wuhan, the VOCs have over 150 defining genomic alterations, which can impact diverse aspects of the viral life cycle including viral gene and protein expression, virus-host protein interactions, and host cellular pathway responses.

Methods

Here, we infect Calu-3 human lung epithelial cells with each of the 5 VOCs and 2 early-lineage controls, performing mass spectrometry abundance proteomics and phosphoproteomics, as well as bulk RNAseq at 10 and 24 hours post infection. Next, we performed affinity purification mass spectrometry (APMS) comprehensively on all VOC mutant viral proteins (52), individually expressing mutant and “wild-type” forms in human cells, scoring high-confidence interactions, and quantitating differential interactions. Lastly, we engineered mutant viruses to study the impact of sequence alterations on pathogenesis.

Results

We observed variant-specific differences in viral RNA and protein expression, most notably for N (nucleocapsid), Orf9b, and Orf6. Phosphorylation of viral proteins also differed between the VOCs, most notably on N. Variants uniformly antagonized host innate immune activation, except for Omicron. Interestingly, Omicron strongly activated interferon stimulated genes (ISGs), but not pro-inflammatory genes, which may explain its decreased disease severity; we hypothesize this is caused by selective Orf6 downregulation by Omicron, which we studied using a recombinant Orf6 deletion virus. Strep-tag affinity purification of 52 mutant viral proteins revealed 233/1668 differential virus-host interactions including host proteins of known functional relevance to SARS-CoV-2 infection. Lastly, treatment of VOC infected cells in culture or live mice with translation inhibitor plitidepsin (eEF1A) confirmed it as a pan-variant therapeutic candidate.

Conclusions

Using proteomics to understand how VOCs differ can help us understand SARS-CoV-2 evolution and how to prepare for future variants. Understanding how VOCs are similar may help us develop multi-variant therapies.

PP02.004: Mapping Human Targets of the SARS-CoV-2 Main Protease, 3CLpro/nsp5, using Inactive Catalytic Domain Capture Interactomics.

Butler G[1], Vlok M[1], Machado Y[1], de Jesus H[1], Pablos I[1], Solis N[1], Bell P[1], Grin P[1], Nguyen J[2], Cao Q[2], Nore L[3], Hirota J[2], Lindskog Bergstrom C[3], Overall C[1]
[1]University Of British Columbia, [2]McMaster University, [3]Uppsala University

Introduction.

The coronavirus, SARS-CoV-2, has infected >550 million and killed >6.3 million people since the start of the COVID-19 pandemic. The recent approval of PAXLOVID to treat COVID-19, which inhibits the SARS-CoV-2 main protease 3CLpro, emphasizes the importance of this viral protease in SARS-CoV-2 pathology. However, reports of viral rebound after cessation of treatment and the potential for the emergence of resistant viral strains means that new therapeutic targets to combat COVID-19 are still required. We sought to identify potential intervention points and to elucidate how 3CLpro modifies the human proteome to promote infection and viral replication.

Methods

Inactive Catalytic Domain Capture (ICDC): Using epitope-tagged catalytically inactive 3CLpro-C145A, we captured interactors of SARS-CoV-2 3CLpro from human cell lines on magnetic beads and isotopically labelled them before LC-MS/MS. We screened interactors for potential 3CLpro cleavage sites using a MALDI peptide cleavage assay. Cleavage of human proteins was validated by incubating 3CLpro with recombinant proteins, lung epithelial cell lysates, or by examining SARS-CoV-2-infected cell lysates by Western blotting. Substrates were imaged by immunofluorescence in SARS-CoV-2-infected cells and by immunohistochemistry in post-mortem COVID-19 patient lung.

Results

From 12 ICDC experiments, 24 data sets were analyzed using Maxquant and Mascot/X!Tandem/Skyline. Of 259 proteins in the 3CLpro interactome, 145 contained at least one canonical cleavage motif, and 20 proteins had >5 sites. We validated cleavage of 10 human proteins by Western blotting of 3CLpro-treated lung cell lysates and demonstrated cleavage of 11 proteins in SARS-CoV-2-infected cells that was prevented by treatment with the 3CLpro inhibitor GC376. Five of these proteins were decreased in post-mortem COVID-19 human lung.

Conclusions

We validated >12 novel human 3CLpro substrates. Network and pathway analysis of this interactome reveals pathways that are critical for SARS-CoV-2 that help explain the disease's complex pathogenesis and may help design new therapeutics to combat COVID-19.

PP02.005: Site-specific N-glycosylation Alterations of SARS-CoV-2 Spike Protein using Cyclic Ion Mobility Mass Spectrometry System

Chatterjee S[1], Klein J[2], Zaia J[1,2]
[1]Department of Biochemistry, Center for Biomedical Mass Spectrometry, Boston University School of Medicine, [2]Bioinformatics Program, Boston University School of Medicine
Introduction: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the cause of the coronavirus disease 2019 pandemic that continues to pose a significant threat to public health worldwide. SARS-CoV-2 encodes four structural proteins namely membrane, nucleocapsid, spike and envelope proteins that play essential roles in viral entry, fusion, and attachment to the host cell. The extensively glycosylated spike protein binds to the host cell surface angiotensin-converting enzyme 2 initiating viral entry and pathogenesis. Spike protein contains 22 N-glycosylation sites and several O-glycosylation sites. SARS-CoV-2 has rapidly evolved by generating mutations leading to a number of variants circulating among the human population across the world. Although reverse transcriptase polymerase chain reaction identifies the presence of SARS-CoV-2, it alone cannot differentiate between the mutants. Alternatively, mass spectrometry-based proteomics and glycoproteomics enable a more detailed and holistic view of the viral proteins and provide information necessary to model host-pathogen interactions.
Method: Recombinantly expressed in HEK293, spike protein from a set of SARS-CoV-2 variants of concern were investigated using a cyclic ion mobility mass spectrometer. Alpha-1-acid glycoprotein was used as a control. Parameters including collision energy ramps and wave velocity/height measures were tested to determine the best approach for confident identification of glycopeptides. Data-independent acquisition strategy was used and proteomics and glycoproteomics analyses were performed on PEAKS and GlycReSoft, respectively.
Result: Distinct site-specific N-glycosylation on the spike protein including sialylation and fucosylation were observed among the variants of concern. The micro-heterogeneity and glycosylation similarities for each site was calculated using the LC-cyclic ion mobility-mass spectrometry data and advanced bioinformatics tools.
Conclusion: This study generated a comprehensive mapping of the N-glycoproteome of spike protein from SARS-CoV-2 variants of concern. These observations can be further expanded into functional investigations that will help in assessing the biological significance of the altered glycosylation found on SARS-CoV-2 variants.

PP02.006: Proteomic Profiling of Host Proteins Incorporated in SARS-CoV-2 Viral Particles

Andersson S[1], Boulouednine P[1], Sahi M[1], Mattson C[1], Kagioglou S[1], Dale M[1], Hofström C[1], Persson H[1], Pernemalm M[2], Klingström J[3], Chiodi F[4], Fredolini C[1]
[1]Science for Life Laboratory, School of Engineering Sciences in Chemistry, Biotechnology and Health, Kth Royal Institute Of Technology, [2]Science for Life Laboratory, Department of Oncology and Pathology, Karolinska Institutet, [3]Center for Infectious Medicine, Department of Medicine Huddinge, Karolinska Institutet, [4]Department of Microbiology, Tumor and Cell Biology, Biomedicum, Karolinska Institutet

Introduction: Proteomic analysis of enveloped viruses revealed that host proteins are incorporated in newly formed extracellular viral particles. The process is suggested to be selective and conservative and to affect viral replication, infectivity, and pathogenesis [PMID30669528;PMID30351952]. In this study, we performed a proteomics profiling of host proteins incorporated in Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) with the aim to identify key proteins involved in virus-host interaction. Methods: SARS-CoV-2 viral particles were purified using magnetic beads coupled to angiotensin-converting enzyme-2 (ACE2) from the cell supernatant of human Calu-3 cells infected by SARS-CoV-2 strains B (Wild Type Wuhan reference strain) (WT) and B.1.1.7 (Alpha, first documented in UK) (UK). UV inactivated virus was eluted from the beads and (i) lysed prior to targeted profiling of 366 proteins by proximity extension assays (PEA) or (ii) digested by trypsin to perform LC-ESI-MS/MS analysis using a Dionex Ultimate™ 3000 RSLC nano System coupled to a Q-Exactive mass spectrometer (Thermo Scientific).
Results: Combined PEA and MS data analysis revealed 87 putative human host proteins in SARS-CoV-2 virions. Twenty-three proteins were identified in the viral particles of both strains, while 49 and 15 were found specific respectively for WT and UK. Among the identified proteins, there were transmembrane, intracellular and secreted proteins, including enzymes and receptors already found in other enveloped viruses. Proteins identified are known for their role in adaptative immune response, cellular homing, cardiovascular and neurological diseases.
Conclusions: We assembled a data set of putative host proteins incorporated into SARS-CoV-2 viral particles. The proteins identified suggest novel insights into molecular mechanism of infection and propagation and could be investigated as targets for broad-spectrum antiviral inhibitors. A multiple detection sandwich assay was developed using the Luminex xMAP Technology to verify the detectability of the identified host proteins on the surface of intact viral particles.

PP02.007: Serum Proteomics of COVID 19 Samples Analysed by Liquid Chromatography and a Cyclic Ion Mobility-Enabled Mass Spectrometer

Hughes C[1], Shareef S[2], Matthews E[2], Gethings L[1,2], Plumb R[3], Simpson A[2], Fowler S[2], Felton T[2], Mills C[2]
[1]Waters, [2]University of Manchester, [3]Waters

Over the last couple of years, COVID-19 has caused a major public health crisis across the globe. Using a proteomics approach to profile serum from COVID-19 inpatients over the course of their hospital stay, we identify and characterise prognostic biomarkers to support the development of mass spectrometry clinical screening tools. However, in order to provide a comprehensive and statistically valid data set, samples from a large cohort of individuals are required. In this study samples from a large cohort were used for evaluating the Cyclic IMS for high throughput proteomic profiling of serum from COVID-19 infected individuals.

Samples corresponding to different disease states or severity were created from COVID-19 patients and these samples were subjected to reduction, alkylation and trypsin digestion. Samples were randomised (with QC's interspersed) and separated using 2.1mm scale chromatography over a 15 min gradient. The liquid chromatography system was coupled to an IMS oa-QTof mass spectrometer and data were obtained using the ion mobility enabled DIA method. Data were processed using a variety of informatic tools and searched with respective databases.

The acquired dataset was imported and processed using both ProteinLynx Global Server and Progenesis QI for Proteomics and searched against a Uniprot Homo sapien database (containing only reviewed sequences) limited to 1% FDR. The samples were then assigned to their groups, revealing a significant number of proteins with differential regulation between the sample groups. Proteins occurring in a minimum of two of the biological replicates and with ANOVA $p < 0.05$ were considered as significant and peptides associated with these were further analysed where multivariate analysis showed clear separation between the different groups. Analysis of the QC's demonstrated high reproducibility and robustness of the methodology, thereby highlighting its utility for large cohort proteomic studies. For example, chromatographic retention times were typically within 1% coefficient of variation.

PP02.008: Comparative Analysis of SARS-CoV-2 Vector Vaccines

Hentschker C[1], Michalik S[1], Siegerist F[2], Palankar R[3], Franzke K[4], Schindler M[2], Reder A[1], Seifert U[5], Cammann C[5], Wesche J[3], Steil L[1], Gesell Salazar M[1], Beer M[6], Endlich N[2], Greinacher A[3], Völker U[1]
[1]Interfaculty Institute of Genetics and Functional Genomics, University Medicine Greifswald, [2]Institute for Anatomy and Cell Biology, University Medicine Greifswald, [3]Institute of Immunology and Transfusion Medicine, University Medicine Greifswald, [4]Institute of Infectiology, Friedrich-Loeffler-Institute, [5]Friedrich Loeffler-Institute of Medical Microbiology-Virology, University Medicine Greifswald, [6]Institute of Diagnostic Virology, Friedrich-Loeffler-Institute

Vaccination is a key factor for the control of the SARS-CoV-2 pandemic. Adenoviral vector-, mRNA-, and antigen-based vaccines are currently in use worldwide. Since February 2021, the rare but severe adverse reaction of vaccine-induced immune thrombotic thrombocytopenia (VITT) has been observed after application of vector-based SARS-CoV-2 vaccines. The adenoviruses used for vaccination are produced in human host cell lines and previous studies have shown that the vaccine ChAdOx1 nCoV-19 still contains a large number of host cell proteins (HCP). In epidemiological studies, the ChAdOx1 nCoV-19 vaccine showed a higher incidence rate of VITT compared to Ad26.COV2.S. Therefore, we comparatively analyzed ChAdOx1 nCov-19 (AstraZeneca) and Ad26.COV2.S (Johnson & Johnson) to reveal differences in the vaccines that might explain the different incidence of VITT.

Methods:
We comprehensively studied ChAdOx1 nCov-19 and Ad26.COV2.S. Both vaccines were comparatively profiled using a DIA-MS approach. Since vector-based SARS-CoV-2 vaccines have been linked to VITT, we also applied transmission electron microscopy, dynamic light-scattering (DLS), single-molecule light microscopy, and a capillary leakage assay to analyse PF4 complex formation or clustering of proteins.

Results:
Our analyses revealed major differences between ChAdOx1 nCoV-19 and Ad26.COV2.S vaccines. ChAdOx1 nCoV-19 vaccine contained a high proportion of HCP (54%), but only a very low level of HCP was detected in Ad26.COV2.S (1.5%). Furthermore, ChAdOx1 nCoV-19 exhibited a high Chymotrypsin-like activity of the proteasome, induced vascular hyperpermeability and formed clusters with PF4.

Conclusion:
In summary, we show that process-related impurities in the form of HCP and active proteases are not a general feature of vector-based SARS-CoV-2-vaccines. Impurities and the resulting PF4-complex formation might explain the higher incidence rate of VITT for ChAdOx1 nCoV-19. More stringent purification strategy will likely reduce the complex formation of PF4 with vaccine constituents and should be considered in the future.

PP02.009: Rapid and Sensitive Detection of SARS-CoV-2 Infection using Quantitative Peptide Enrichment LC-MS Analysis

Hober A[1,2], Tran-Minh K[1,2], Foley D[3], McDonald T[3], Vissers J[3], Pattison R[3], Ferries S[3], Hermansson S[3], Betner I[3], Uhlen M[1,2], Razavi M[4], Yip R[4], Pope M[4], Pearson T[4], Andersson L[4], Bartlett A[3], Calton L[3], Alm J[5], Engstrand L[5], Edfors F[1,2]
[1]KTH - Royal Institute Of Technology, [2]Science for Life Laboratory, [3]Waters Corporation, [4]SISCAPA Assay Technologies, Inc., [5]Karolinska Institutet

Introduction

Establishing robust and reliable methods for large-scale molecular testing for SARS-CoV-2 has been essential to monitoring the ongoing COVID-19 pandemic. Here we present a scalable analytical approach to detect proteins from the viral pathogen utilizing peptide immunoaffinity enrichment combined with liquid chromatography mass spectrometry (LC-MS). The established SISCAPA assay improves the sensitivity and selectivity while allowing for rapid sample turnaround times. The subsequent targeted proteomics analysis, relying on LC-MS readout, allows for precise quantification and detection of SARS-CoV-2 specific peptides directly in throat, nasopharyngeal and saliva samples from patients.

Method

Patient samples were denatured in 1% RapiGest, 50mM DTT for 15 min and subjected to trypsin digestion for 30 min. The samples were subsequently spiked with SIL peptides towards the viral NCAP protein and incubated with bead-coupled anti-peptide antibodies for 1 hour. The beads were collected and washed 3x30 s before elution. The eluates were thereafter analyzed by LC-MS on a 5 min method.

Results

Our findings show that the LC-MS-based detection of SARS-CoV-2 correlates well with the RT-PCR-based detection (r=0.79). Additionally, the workflow shows sample turnaround times comparable to the standardized RT-PCR diagnostic workflow. The LC-MS method is suitable for quantitative viral protein readout and successfully classified samples with cycle thresholds (Ct) equivalents ranging from 21 to 34.
The novel method shows a 100% negative percentage agreement and a 95% positive percentage agreement when analyzing 346 clinical samples collected from asymptomatic individuals with a Ct within the limit of quantification of the mass spectrometer (Ct≤30).

Conclusion

Our findings suggest that the developed method can be successfully deployed in a low prevalence setting without compromising the positive predictive value of large-scale screenings. Further, this scalable analytical LC-MS-based workflow has an indisputable place in future pandemic preparedness centers as a complement to current technologies for viral detection and diagnostics.

PP02.010: Functional Interactions between p38 Kinases and SARS-CoV-2

Higgins C[1,2], Nilsson-Payant B[1], Kurland A[1], Ye C[3], Adhikary P[1], Golynker I[1,2], Panis M[1,2], Martinez-Sobrido L[3], tenOever B[2], Johnson J[1]
[1]Icahn School Of Medicine At Mount Sinai, [2]New York University Langone Health, [3]Texas Biomedical Research Institute

Introduction: Phosphoproteome studies have revealed that SARS-CoV-2 infection leads to activation of the p38 mitogen-activated protein kinase (MAPK) pathway, which plays a major role in regulating pro-inflammatory cytokines that characterize the immune profile of severe COVID-19 cases. Inhibition of the p38/MAPK pathway in SARS-CoV-2-infected cells reduces both cytokine production and viral replication, but the mechanism by which this occurs is unknown. Here, we combined genetic screening, quantitative proteomics, and molecular virology to better understand functional interactions between the p38/MAPK pathway and SARS-CoV-2 in human lung epithelial cells.

Methods: An siRNA screen of the p38 pathway was performed in SARS-CoV-2-infected ACE2-A549 lung epithelial cells. Infection rates were read out by immunostaining for viral nucleoprotein and imaging on a Celigo mass cytometer. Quantitative proteome/phosphoproteome analysis was performed on ACE2-A549 cells infected with SARS-CoV-2 with siRNA depletion or chemical inhibition of p38 kinases using a data-independent acquisition mass spectrometry (DIA-MS) approach.

Results: Our siRNA screen revealed that p38β was the major p38 isoform impacting SARS-CoV-2 infection. Proteome and phosphoproteome analysis of SARS-CoV-2-infected, p38-perturbed cells identified 35 putative p38 substrates 30 proteins. A subsequent siRNA screen revealed that depletion of 23 of these proteins significantly increased SARS-CoV-2 infection, suggesting that their phosphorylation counteracts antiviral functions. We identified a cluster of 4 phosphorylation sites on the viral nucleoprotein that were sensitive to p38 inhibition. We generated a recombinant virus mutating these phosphorylated residues and found that phosphomutant virus infectivity was 10-fold reduced compared to a recombinant wild-type virus.

Conclusions: While p38α is by far the most well characterized p38 isoform, this study uncovered a unique proviral function for p38β that is not shared with p38α. By combining genetic and chemical perturbation of p38 pathways, we identified putative host and viral p38 substrates that may drive this phenotype.

PP02.011: Survival Strategy of the Tolerant Coxiella Burnetii Strain to Doxycycline Exposure

Flores-Ramirez G[1], Zuniga Navarrete F[1], Benada O[2], Dresler J[3], Skultety L[1,2]
[1]Biomedical Research Center of the SAS, [2]Institute of Microbiology of the CAS, [3]Military Health Institute, Military Medical Agency

Introduction: Antibiotic resistance is a global threat that is a top healthcare concern. Doxycycline (DOX) is a high cell membrane permeable antibiotic used to treat a broad spectrum of bacteria, including Coxiella burnetii. This pathogen is a causative agent of Q fever, a re-emerging zoonosis found worldwide. Hence, the bacterium has a considerable impact on the farming industry and public health; exploring its adaptation/tolerance strategy to antibiotics is necessary for more effective therapy.

Methods: The tolerant C. burnetii strain Heinzerling was propagated in an axenic medium supplemented with a slightly lower concentration of DOX (4 µg/ml) than its MIC (1). DOX. Bacterial multiplication was assessed by mRNA amplification using qRT-PCR on the 7th day post-inoculation. The Amplex red Hydrogen peroxide Assay (ThermoFisher Scientific) was then performed, and the bacteria were imaged by Nova NanoSEM 450 scanning electron microscope (FEI). To assess the bacterium's response to DOX exposure, proteomic analysis based on the 2-DE technique was performed using LC-MS/MS (2).

Results: Dozens of proteins were detected as significantly altered. The adjustments of the critical proteins were subsequently confirmed by gene expression analysis. Increased hydrogen peroxide concentration in the presence of DOX pointed to deregulated redox balance.

Conclusions: Restoration of protein synthesis, activation of the defense mechanism against oxidative stress, and maintenance of cell envelope integrity, including pH homeostasis, are the key processes ensuring C. burnetii survival under DOX exposure.

Acknowledgments: This study was supported by a grant from the Slovak Research and Development Agency (APVV-19-0519) and the grant agency VEGA (2/0052/19).

1. Zuniga Navarrete, F., et al. 2019 J Proteomics 208; art. no. 103479.
2. Lakhneko, O., et al. 2020 Int J Mol Sci 21(10); 3445.

PP02.012: A Proteomic View of Bacterial and Viral Coinfections in Mice

Gesell Salazar M[1], Michalik S[1], Cuypers F[2], Skorka S[2], Hammerschmidt S[2], Siemens N[2], Völker U[1]
[1]Department of Functional Genomics, University Medicine Greifswald, [2]Department of Molecular Genetics and Infection Biology, University of Greifswald

Introduction:
During seasonal influenza A virus (IAV) infections, further concurrent infection by bacterial commensals such as Streptococcus pneumoniae can occur, leading to severe pneumonia and even the development of sepsis. Apart from the acute Corona pandemic in the last 2 years, lower respiratory tract infections are generally the leading infectious cause of death worldwide and account for approximately 6% of deaths. The aim of our study was to get insights into alterations of the lung tissue proteome of mice upon IAV infection or coinfection with IAV and S. pneumoniae. In detail, we intended to uncover the impact of viral infection or co-infection onto metabolic pathways.

Methods:
For analysis of proteome profiles, lung tissue was disrupted and homogenized. After determination of protein concentration aliquots of samples were reduced, alkylated, tryptic digested and desalted. Tryptic peptide solutions were analyzed on an Ultimate 3000 nano-LC system coupled to a Q ExactiveTM HFx mass spectrometer (Thermo Fischer Scientific) and measured in data independent acquisition mode. Data analyses and statistic tests were performed using Spectronaut version 16 (Biognosys) and R.

Results:
In the lung tissue samples, we identified approximately 44500 peptides assigned to 5640 proteins per sample. In the statistical tests, we detected a large number of proteins that showed statistically significant differences between the different groups of mice compared to the respective control groups. These proteins were included in functional analysis in IPA (QIAGEN).

Conclusion:
Our results of proteomic analysis indicate specific protein signatures for colonization, infection and coinfection with S. pneumoniae and IAV. In general, differently abundant proteins were involved in immune response, acute phase response and inflammatory processes. In the host response to IAV infection, the protein patterns specifically indicated mitochondrial dysfunction. Specific to mice colonized with S. pneumoniae and infected with IAV was an interference with the EIF2 pathway.

PP02.013: Proteomics to Study Candida Albicans-host Interaction: Focus on Stress and Macrophage Responses

Gil C[1,2], Zapico I[2], Borrajo A[1], Malmström J[3], Monteoliva L[1]
[1]Department of Microbiology and Parasitology, Complutense University of Madrid, [2]Proteomics Unit, Complutense University of Madrid, [3]Department of clinical Sciences, Lund University

Introduction

Candida albicans is a commensal fungus that causes systemic infections in immunosuppressed patients. To cope with the changing environment during commensalism or infection, C. albicans must reprogram its proteome. Characterizing the stress- and macrophages-induced changes in the proteome that C. albicans uses to survive should be very useful in the development of new antifungal drugs.

Methods

We studied the C. albicans global proteome after exposure to hydrogen peroxide (H2O2) and acetic acid (AA) and after interaction with human macrophages using a DIA-MS strategy. In addition, a targeted proteomic study of 32 proteins related to apoptosis in yeast was carried out.

Results

C. albicans responded to treatment with H2O2 with an increase in the abundance of many proteins involved in the oxidative stress response, protein folding, and proteasome-dependent catabolism, which led to increased proteasome activity. Treatment with AA resulted in a general decrease in the abundance of proteins involved in amino acid biosynthesis, protein folding, and rRNA processing. A targeted proteomic study of 32 proteins related to apoptosis supported the results obtained by DIA-MS.

Monitoring C. albicans proteome after interaction with THP-1 human macrophages at three time points showed both conserved and specific changes in proteins at each time point. At 3 h, the proteomic changes showed a response to oxidative damage and a slight mitochondrial involvement. After 6 h, an extensive decrease of mitochondrial proteins was observed and correlated with a lower activity of this organelle. After 9 h of interaction, fewer changes in the protein abundance suggested a recovery of C. albicans cells after escaping from macrophage.

Conclusions

These results allowed the discovery of Prn1 as a key protein in the defense against oxidative stress as well the increase in the abundance of Oye32 protein when apoptotic process occurred point them out as possible drug targets.

PP02.014: New Peptidase Inhibitors from Mollusks with Anticryptococcal Properties

Geddes-McAlister J[1], Raouf-Alkadhim1 F[1], Woods M[1], Prosser R[2], Geddes-McAlister J[1]
[1]Molecular and Cellular Biology Department, University of Guelph, [2]School of Environmental Sciences, University of Guelph

Cryptococcus neoformans is an encapsulated human fungal pathogen responsible for 15% of AIDS-related deaths around the world. Although several antifungal drugs are available, a fast generation of resistant strains threatens current treatments. A new strategy to combat fungal infections is to disarm the pathogen using an anti-virulence approach without killing the fungus. In this context, peptidases constitute promising targets, as they are associated with virulence and antifungal drug resistance but are not directly involved in growth; suggesting an important opportunity to combat the pathogen. Notably, mollusks possess a strong innate immune system with numerous compounds, such as peptidase inhibitors (PIs) with antimicrobial properties but, are underexplored in their usage against fungal pathogens. In this study, we explored the intricate relationship between peptidases and virulence mechanisms within C. neoformans using bioinformatics approaches and mass spectrometry, and, assessed the effects of aqueous extracts from six different species of mollusks, including snails, mussels and slugs against cryptococcal virulence factors (e.g., melanin and capsule production, biofilm formation, growth at 37 ˚C, and drug resistance). Several extracts impair the growth of cryptococcal cells and display an additive effect with the antifungal drug, fluconazole. Additionally, several extracts completely inhibit the production C. neoformans biofilms at 30 ˚C with a comparable efficacy to the antifungal, Amphotericin B. Furthermore, clarified fractions of mollusk protein extracts have inhibitory activity over capsule production by cryptococcal cells. Importantly, we also identified reversible strong inhibitory activity against S8 serine peptidases and moderate activity against M4 metallopeptidases. Finally, using mass spectrometry-based proteomics approaches we revealed different peptidase inhibitors within the extracts that may explain the observed anticryptococcal properties. These results highlight the potential of mollusks as promising sources of new PIs with anti-cryptococcal properties that are less prone to develop resistance, improving our ability to combat these deadly pathogens.

PP02.015: Proteomics and Phosphoproteomics to Elucidate the Differences Between M.Bovis BCG Vaccine Strains.

Iradukunda J
[1]Student

Introduction

Tuberculosis (TB) disease remains of global importance, particularly in low-income countries. The ever-increasing incidence of this chronic disease has spiked urgency to develop better therapeutic prophylaxis and measures, in order to alleviate the health burden. M.Bovis BCG, is the only approved anti- tuberculosis vaccine to date. Since discovery, this vaccine has evolved into 13 genetically different daughter strains with different clinical phenotypes. This study employs omics techniques to understand implications of using different M. Bovis BCG vaccines on the host.

Methods

Strains were cultured on 7H10 media and single colonies transferred to 7H9 media and harvested at mid-log phase.THP-1 cells were maintained as monocytes in RPMI media and differentiated into macrophages before 1hr infections at MOI of 5. Proteins were precipitated in ice cold acetone: methanol (8:1) solution and resuspended in Denaturation buffer. Standard mass spectrometry sample preparation techniques were used for protein digestion and desalting. MagReSyn Zr-IMAC used for Phosphopeptide enrichments. For LC-MS/MS analysis, HPLC coupled Q exactive was used. Raw files were then processed by Maxquant software with downstream data analysis using Perseus and R software.

Results

Data quality checks revealed normal distributions and high Pearson correlation scores suggested good reproducibility. Principle component analysis increased our confidence in the biological differences between the different strains with all replicates clustering. Statistical tests of the bacterial proteome revealed significantly differentiated proteins between the M. Bovis BCG strains, offering clues on expected infection phenotypes. Infections revealed significantly differentiated protein clusters GO enrichment highlighting biologically significant pathways, processes etc., offering insights on mechanisms behind different vaccination phenotypes observed after M. Bovis BCG vaccination.

Conclusions

A deep omics analysis of our phosphoproteome, lipidome, ligandome, secretome and MHC peptidome data will shed more light key players (of the innate and adaptive immunity) driving the post-vaccination phenotypes observed.

PP02.016: Time Dependent Characterization of Protein Profiles in Different Tissues Induced by Chronic Infection with Lymphocytic Choriomeningitis Virus

Khan A[1], Steffensen M[2], Abduljabar A[1], Vorum H[4], Sørensen T[3], Nissen M[2], Honoré B[1]
[1]University Of Arhus, Department of Biomedicine, [2]Department of Immunology and Microbiology, University of Copenhagen, [3]Faculty of Health and Medical Sciences, University of Copenhagen, [4]Department of Clinical Medicine, Aalborg University,

We present a comprehensive discovery-based proteomic investigation of mice with chronic infection of lymphocytic choriomeningitis virus (LCMV). Using high resolution liquid chromatography - tandem mass spectrometry (LC-MS/MS) and tandem mass tag (TMT) labelling, we report significant protein changes in different tissues like, retina, RPE-choroid, kidney and spleen in LCMV infected mice. Using bioinformatics, we observed several protein pathways to be perturbed and associated with LCMV mediated inflammation and disease progression.

We have applied a global proteome profiling and quantitative proteomics approach to improve understanding of LCMV mediated immune interactions. We investigated infection with LCMV, which is capable of inducing a chronic infection. We followed the protein changes in a time dependent manner in the different tissues. Validation of the results were done using skyline based targeted proteomics.

The effects of the infection on the cellular proteomes varied substantially between tissues. In the retina at 1 week of the infection, we observed no detectable immune response, which, however, became strong at 8 and 28 weeks. Substantial degeneration of photoreceptors were observed early in the infection at 1 week. In the RPE-choroid a strong immune response was detected from week 1 and throughout to 28 weeks. Degeneration was observed from 8 weeks and some degradation could be detected at 28 weeks. In kidney, a strong immune response was detected throughout the period with initial inhibition of the mitochondrial function. In spleen, no major degeneration was observed in spleen.

Our findings suggest that the response to a systemic chronic infection differs between neuro retina and the RPE/choroid and that the processes induced by systemic infection are not unlike those induced in non-immune privileged organs such as the kidney. Overall, our data suggest that the posterior part of the eye is not an isolated immunologic entity in spite of the existence of immune privilege.

PP02.017: Targeted Proteomics-Driven Computational Modeling of the Mouse Macrophage Toll-like Receptor Signaling Pathway

Manes N[1], Calzola J[1], Kaplan P[1], Armstrong A[1], Fraser I[1], Germain R[1], Meier-Schellersheim M[1], Nita-Lazar A[1]
[1]National Institutes Of Health

Introduction

The Toll-like receptor (TLR) signaling pathway is crucial for the initiation of effective immune responses. Subtle variations in the concentration, timing, and molecular structure of the stimuli (e.g., lipopolysaccharide (LPS)) are known to affect TLR signaling and the resulting pathway dynamics. Tight regulation is essential to avoid acute tissue damage and chronic inflammation. Computational modeling can test mechanistic hypotheses about how regulation is achieved and why it sometimes fails, causing pathologies.

Methods

Mouse (C57BL/6J) bone marrow-derived macrophages were either unstimulated or stimulated with 10 nM LPS for 30 min. A dilution series of stable-isotope labeled internal (phospho)peptide standards was spiked into cell lysates (136 unmodified peptides and 29 phosphopeptides for 54 (phospho)proteins). Targeted mass spectrometry (nanoLC-PRM) was performed using a Q Exactive HF, and the resulting data were analyzed using Skyline. Protein complex structures were predicted using AlphaFold2, and Rosetta was used to idealize and relax the structures. Protein-protein association rates were estimated using Simulation of Diffusional Association and TransComp. Rule-based pathway modeling and simulation is being performed using Simmune.

Results

The protein abundances ranged from 1,332 to 227,000,000 copies per cell. They moderately correlated with transcript abundance values ($r = 0.699$, $p = 1.37e\text{-}17$), and these data were used to make proteome-wide abundance estimates. Abundance increases and decreases in response to LPS were observed for proteins known to be affected by TLR pathway activation. For example, phosphorylated ERK1 increased from 30,000 to 250,000 copies per cell. Hundreds of protein-protein association rates were estimated. The (phospho)protein absolute abundance values and protein-protein association rates are being used as parameters for TLR pathway models.

Conclusions

Experimental and computational techniques are being integrated to generate a strongly data driven model of the TLR pathway. This work was supported by the Intramural Research Program of NIAID, NIH.

PP02.018: Proteomic Analysis of Fish (Labeo rohita) Liver Provides Insights into Interplay of Host and Pathogen during Aeromonas Hydrophila Infection

Nissa M[1], Pinto N[2], Ghosh B[3], Singh U[4], Goswami M[2], Srivastava S[1]
[1]Indian Institute of Technology Bombay, [2]Central Institute of Fisheries Education, Indian Council of Agricultural Research, Versova, [3]Regional Centre for Biotechnology, [4]Department of Biochemistry, Sri Venkateswara College, University of Delhi

Introduction

Aeromonas hydrophila (Ah) is a Gram-negative bacterium and a serious global pathogen causing Motile Aeromonas Septicaemia (MAS) in aquatic system. Investigation of molecular alterations of host tissues, as liver could be a powerful approach to identify mechanistic and diagnostic immune signatures of disease pathogenesis.

Methods:

Proteomic analysis was performed for Control and challenged group (AH) using two strategies; discovery and targeted proteomics. Label-free quantitative analysis was performed to identify the differentially expressed proteins (DEPs). Gene ontology and protein-protein interaction enrichment analysis was performed to obtain an overview of functional annotation of significant proteins and dysregulated metabolic pathways. Further validation of few DEPs was performed for eleven samples (6 Control and 5 AH) using multiple reaction monitoring (MRM) approach.

Results:

A total of 2525 proteins were identified of which 157 were differentially expressed (p-value <0.05, minimum fold change 1.5) during Ah infection. Several metabolic enzymes (as citrate synthase, succinyl ligase), antioxidative proteins (as Glutathione peroxidase, Epoxide hydrolase), cytoskeletal and immune related proteins (Actin-related protein 2, Alpha N-catenin, Cathepsin F, Toll-like receptor 3, C-type lectins) were dysregulated. Pathways like lysosome pathway, apoptosis, metabolism of xenobiotics by cytochrome P450, retinol metabolism, pantothenate metabolism, beta alanine metabolism and drug metabolism were found to be majorly mapped by downregulated proteins. However, innate immune system, signaling of B cell receptor, proteosome pathway, ribosome, carbon metabolism and protein processing in ER were mainly mapped to upregulated proteins.

Conclusions

Our systemic approach revealed the protein dynamics in the host cells to explore the putative biological processes underlying the metabolic reprogramming of the host cells during Ah infection. These findings paved the way for future research into the role of Toll-like receptors (Tlr3), C-type lectins (Clec4e) and metabolic intermediates like Citrate and Succinate in Ah pathogenesis leading towards host directed immunotherapies to tackle the Ah infection in fish.

PP02.019: Multilevel Proteomics Reveals Epigenetic Mechanisms Influencing BCG-mediated Macrophage Activation

Celeste C[1], Iradukunda J[2], Schaefer Z[1], Blackburn J[2], Parker I[1]
[1]University of Florida, J Crayton Pruitt Family Department of Biomedical Engineering, [2]University of Cape Town, Institute of Infectious Disease and Molecular Medicine

Tuberculosis (TB) is a leading cause of death globally, and the bacilli Calmette-Guerin (BCG) vaccine is currently administered at birth in endemic areas to prevent TB transmission. The BCG vaccine has been shown to be particularly immunogenic, and causes trained immunity, where after vaccination innate immune cells mount heightened immune responses to secondary unrelated pathogens. Studies show that monocytes display epigenetic memory of BCG vaccination at the histone level for up to three months, characterized by increased TNF-alpha and IL-6 production upon secondary stimulation with other pathogens. Mass spectrometry- based proteomics and bioinformatic analysis provide insight into epigenetic mechanisms of sustained BCG-mediated immunogenic signaling.
Methods: THP-1 macrophages were infected with BCG for 24 hours, and protein extracted for total proteomic analysis. Histones were isolated to assess global changes in post-translational modifications, and total protein and histone isolates were assayed using tandem mass spectrometry (Q Exactive). Bioinformatic analysis allowed for discovery of differentially regulated cytokines and histone post translational modifications in BCG infected and uninfected macrophages. These data were cross-referenced with phospho-data previously obtained from BCG infected RAW macrophage to assess activation of histone modifiers.
Results: Bioinformatic analysis revealed decreased phosphorylation of four histone acetyltransferase (KAT) peptides, two demethylation (JmjC) peptides, a lysine demethylase peptide, and a lysine methyltransferase peptide, identifying mechanisms for robust upregulation in histone methylation after BCG infection. 105 peptides were differentially regulated ($p<.05$) and pathway analysis identified histone modifications with the ability to promote expression of inflammatory cytokines along with novel targets to explore in future study.
Conclusion: Proteomic analysis allows for comprehensive profiling of BCG mediated immunogenic macrophage activation and reveals a complex interplay of cytokine expression upon initial BCG infection. Our data implies that dynamic histone modification occurs upon BCG infection in macrophages.

PP02.020: Mass Spectrometry Based Template-Assisted De Novo Sequencing of SARS-CoV-2 and Influenza Monoclonal Antibodies

Gadush M[1], Sautto G[2], Chandrasekaran H[1], Bensussan A[1], Ross T[2], Ippolito G[1], Person M[1]
[1]The University of Texas at Austin, [2]University of Georgia

Introduction

Determining the protein sequence of a monoclonal antibody (mAb) has become more feasible due to advances in mass spectrometry and availability of large numbers of nucleic acid sequences of antibodies. When the exact sequence is not present in the database, the MS data can first be matched to a template and then varied at each residue position until the best match is determined. Use of secondary fragmentation to produce w-ions can distinguish between Leu and Ile residues.

Methods

We used enzyme digestion with trypsin, chymotrypsin, elastase and pepsin to produce peptides of varying size and cleavage. These were analyzed via LC-MS/MS with higher-energy collisional dissociation (HCD) and electron-transfer/higher energy collision dissociation (EThcD) fragmentation on an Orbitrap Fusion. The data was searched using Supernovo to generate a complete template-assisted de novo sequence. Glycosylation sites were matched using a set of common human glycoforms.

Results

The method was refined on a mAb of known sequence, a SARS-CoV-1 anti-receptor binding domain (RBD) spike mAb and two SARS-CoV-2 mAbs of known sequence resulting in correct sequences for the variable regions and correct distinction of Ile and Leu residues. We then used the method on a set of twenty-five anti-hemagglutinin (HA) influenza antibodies of unknown sequence and determined high confidence sequences for >99% of the complementarity determining regions (CDRs). The heavy chain and light chain genes were cloned and transfected into cells for recombinant expression followed by affinity purification. The recombinant mAbs displayed binding curves matching the original mAbs with specificity to the HA influenza antigen.

Conclusions

Our findings demonstrate this method results in almost complete antibody sequence coverage with high confidence results for CDR regions on diverse mAb sequences. The results are validated with known sequences and by generating functional mAbs.

PP02.021: Proteomic Tools for COVID-19 Research: Protein Expression and Antibody Production of SARS-CoV-2 3CL Main Protease

Kappelhoff R[1], Pablos I[1], Ramos de Jesus H[1], Butler G[1], Grin P[2], Overall C[1,2]
[1]University of British Columbia, Centre for Blood Research, Department of Oral Biological and Medical Sciences, [2]University of British Columbia, Centre for Blood Research, Department of Biochemistry and Molecular Biology

Introduction: The cysteine protease 3CLpro is the main protease of SARS-CoV-2, which cleaves the coronavirus polyprotein at 11 conserved sites to release 4 structural and 16 non-structural proteins needed for mature virus assembly. As a dimer 3CL catalyzes protein bond cleavages over a catalytic dyad via Histidine-41 and Cysteine-145. To further study this important protease in viral replication we expressed and purified 3CL, the catalytic-inactive-mutant 3CL-C145A, and raised a 3CLpro-specific antibody.

Methods: Plasmids pET21(+)-SARS-CoV-2-3CL and pET21(+)-SARS-CoV-2-3CL-C145A protease (Genbank: NC_045512.2) were custom synthesized. Codon C145A was introduced to create an inactive protease mutant by changing the catalytically important cysteine to alanine. The 6xHis-tagged protein was expressed in an IPTG inducible bacterial system and purified by IMAC. For various applications, several different constructs were synthesized including features like cleavage sites for factor Xa and enterokinase, and a triple FLAG-tag and Myc-tag. Purified inactive protease was used to raise 3CL specific antibodies in rabbits. Antibodies were affinity purified via CNBr4B-Sepharose coupled with 3CL-C145A-His protein from rabbit plasma.

Results: We successfully expressed and purified SARS-CoV-2 3CLpro active and inactive protease. Yield for inactive protease (~60mg/L) was 20x higher than for active protease (~3mg/L). This is a common effect while expressing active enzymes as they already start interacting with the bacteria during expression. We digested peptide libraries prepared by trypsin or GluC digestion of cell lysates with 3CLpro to characterize nonprime (P) and prime (P') side specificities of 3CLpro cleavage using Proteomic-Identification-of-Cleavage-site-Specificity (PICS).

Conclusions: Purified active and inactive 3CL protease and its antibody are essential tools for the characterisation of SARS-CoV-2 infection and pathogenesis including host protein substrate discovery by TAILS. The antibody was used in immunohistochemistry staining of virus-infected tissue samples.

Reference: Pablos, I et al. 2021. Mechanistic insights into COVID-19 by global analysis of the SARS-CoV-2 3CLpro substrate degradome. Cell Reports. 37(4): 109892.

PP02.022: Ultrafast Bacterial Detection in Urinary Tract Infections using High-throughput Proteomics and Artificial Intelligence

Roux-Dalvai F[1], Leclercq M[1], Pelletier S[1], Bekker-Jensen D[2], Gotti C[1], Bache N[2], Guichard A[1], Thibeault M[1], Arrey T[3], Dauly C[3], Bérubé È[4], Boissinot M[4], Bergeron M[4], Droit A[1]
[1]CHU De Québec - Université Laval - Proteomics and Computational Biology laboratory, [2]Evosep Biosystems , [3]Thermo Fisher Scientific, [4]CHU De Québec - Université Laval - Infectiology Research Center

The standard method to identify bacteria responsible for Urinary Tract infections (UTIs) relies on a long bacterial culture (24-48h) prior to MALDI-TOF analysis. During this time, patients receive broad-spectrum antibiotics increasing the bacterial resistances in the whole population. Recently, we demonstrated that LC-MS/MS combined to artificial intelligence allows a fast identification with high sensitivity and specificity. However, non-parallelizable conventional analyses are not compatible with the hundreds of samples to be analyzed by clinical laboratories each day. To overcome this issue, we propose to acquire LC-MS data on ultrafast analyses (5 min) and use automated algorithms to predict bacterial infection without peptide/protein identification.

We analyzed more than 600 urine samples inoculated with the 15 bacterial species representing 85% of all UTIs on an Evosep One LC system interfaced with a Thermo Orbitrap Exploris 480 mass spectrometer with a throughput of 200 samples per day. Raw LC-MS signal was binned in both mass and time dimension and converted into input matrices for automated learning algorithms. Various strategies were tested to escape batch effect, reduced the dimensionality and benchmarked multiple algorithms to discriminate the different groups.

The total number of features after LC-MS binning was approximately 180 million. To avoid fitting on noisy data, we selected 1000 to 10000 using information gain ranking. Batch effect was attenuated using auto-discretization approaches. Then, pre-processed raw data were used to test multiple machine learning models and deep learning methods (Convolutional Neural Networks) to discriminate amongst the bacterial species. We finally ended with a Random Forest model as the best model, reporting a high prediction accuracy of about 90% on a test set.

This new approach is able to identify the 15 bacterial species from 2mL urine samples and provides a species identification without bacterial culture at a throughput of 200 samples par day. (For research only).

PP02.023: Unveiling the OprF Target Sequences Recognized by Specific Antibodies: Step Forward in the Design of a Vaccine against Multidrug-resistant Bacteria

Sánchez Corrales G[1,2], Ortega A[2], Carrascal M[1], Rosell E[2], Abián J[1]
[1]IIBB-CSIC, [2]Reig Jofre Laboratory

One of the most important challenges to global health is the growing problem of antibiotic resistance. Some bacteria have a high capacity to develop resistance and adapt. These include Pseudomonas aeruginosa, Klebsiella pneumoniae and Acinetobacter baumannii, which are considered by the WHO to be the most dangerous. Vaxdyn and Reig Jofré Laboratory are collaborating for using Vaxdyn's technology to deliver vaccine candidates against these pathogens. The drug substances under development display multiple bacterial antigens, and one of antigens for targeting P. aeruginosa is the porin OprF from this bacteria. For the correct characterization of the vaccine candidate, analytical techniques based on immunoassays are being developed to evaluate the potency of the candidate.

The aim of this work was to determine the sequence of OprF recognised by at least two monoclonal antibodies (mAb). For this purpose, the recombinant OprF protein was used, and immunoprecipitation was performed. The protein was digested in situ with trypsin (bound to the antibody). Potential tryptic peptides were determined by MALDI-TOF MS. The eluate was analysed by bottom-up proteomics in LC-MS/MS. This allowed full confirmation, and resolution of the sequence, which would be the peptide containing the epitope recognised by both antibodies. A structural analysis of OprF was also performed with the available resolved structure to locate the theoretical position of the epitope.

This approach allows specific antibodies to be promoted for use in techniques such as immunoassays. The analysis employed confirmed the ability of the mAb to recognise the protein and bind tightly to its epitope. It showed a potentially immunogenic region, which would be exposed in its native form in the pathogen. LC-MS/MS analysis confirmed and resolved the specific sequences. This forms the basis for the development of selective assays and future quantitative analysis of the OprF antigen in a potential vaccine candidate.

PP02.024: Phosphoproteomic Analysis of Murine Alveolar-Like Macrophages Infected with ΔSapM and Wild-Type Mycobacterium bovis BCG

Schell D[1], Ganief N[1], Ganief T[1], Baros-Steyl S[1], Iradukunda J[1], Soares N[3], Blackburn J[1,2]
[1]Division of Chemical and Systems Biology, Department of Integrated Biomedical Sciences, University of Cape Town, [2]Institute of Infectious Diseases and Molecular Medicine, University of Cape Town, [3]Department of Medicinal Chemistry, University of Sharjah

Introduction:
In the early stage of infection, Mycobacterium tuberculosis (Mtb) alters host signalling pathways through the secretion of phosphatases. SapM, a lipid phosphatase, is one of the phosphatases theorised to be essential for Mtb's pathogenesis. It is postulated to be involved in the host-pathogen interaction and signal transduction. When knocked out, Mtb has a significantly reduced ability to infect THP1 macrophages compared to the wild type. Although previous studies have identified some of the phosphatase's possible substrates, these do not necessarily reflect the actual primary site of infection, as it has been demonstrated that alveolar macrophages are the primary site in animal models. Although inhibitors for SapM have recently been identified, a better understanding of the role SapM plays in thwarting the immune response can aid in the generation of better inhibitors.

Methods:
Label-free shotgun LC-MS/MS analysis was conducted on an alveolar-like macrophage cell line infected with wild-type or ΔSapM M. bovis BCG for 1 hour. Standard in-solution digestion was performed and MagReSyn Zr-IMAC was used for phosphopeptide enrichment. The mass spectrometry-based analyses were conducted using MaxQuant, R, and Perseus.

Results:
Preliminary proteomic results showed 59 differentially phosphorylated protein sites. Functional analysis of the infection shows enrichment of proteins related to RNA regulation and the spliceosome pathway.
Most notably, SapM appears to alter the spliceosome pathway in part through the altered phosphorylation state of Srsf1 Ser199, to similar phosphorylation levels of an uninfected macrophage, possibly reducing the frequency of translocation of hyper-phosphorylated Srsf1 to and from the nucleus.

Conclusion:
Preliminary evidence suggests that SapM alters RNA splicing, binding, and spliceosomal complex assembly. Thus, suggesting Mycobacteria may be able to hinder innate splicing events in response to infection. Further analysis of SapM's role in the reduction of phosphorylation of Srsf1 Ser199 and its greater role in the infection would be necessary.

PP02.025: Global Phosphorylation and Proteomic Profiling of Cells Expressing Active Ebola Virus Replication Machinery

Shamorkina T[1], Snijder J[1]
[1]Biomolecular Mass Spectrometry & Proteomics, Bijvoet Center for Biomolecular Research, Department of Chemistry, Faculty of Science, Utrecht University,
Introduction: Ebola virus (EBOV) is associated with re-emerging high-mortality rate outbreaks. Although FDA approved live Ebola Zaire Vaccine (Ervebo) and two monoclonal antibody therapies, no cost-effective treatment is available, highlighting the urgency to develop small molecule antiviral therapies. EBOV is a negative-sense RNA virus and, therefore, relies on its own RNA-dependent RNA polymerase (L protein) to transcribe and replicate the viral RNA genome in the host cell cytoplasm. In these viral replication and transcription processes, viruses highjack host cellular pathways to evade the host immune defenses. Viral highjacking of the post-translational modification (PTM) pathways facilitates fast remodeling of the host environment suitable for the viral undisturbed transcription and replication.
Methods: Here, we apply mass spectrometry-based proteomics and phosphoproteomics to determine the global changes in protein abundances and PTMs in the cells transfected with the model system of EBOV replication/transcription: EBOV minigenome. The EBOV minigenome system consists of EBOV replication/transcription complex (Nucleoprotein NP, co-factor VP35, transcription factor VP30 and L protein) and minigenome itself with original EBOV leader and trailer sequences on the flanks and the original EBOV genome replaced by GFP. To dissect the signaling pathways related either to replication or transcription, we transfect the cells with wild-type L protein, catalytically dead L mutant or leaving out the transcription factor VP30.
Results/Conclusions: We describe the replication- and transcription-dependent changes in host proteome and phosphorylation signaling dynamics. We see enrichment in the disrupted kinase substrates and pathways. Once the kinases involved in EBOV transcription and replication are mapped, the approved inhibitors (e.i. kinase inhibitors) can be screened on its ability to stall EBOV replication/ transcription.

PP02.026: Simultaneous Detection of Respiratory Infectious Diseases using Immunoprecipitation and Liquid Chromatography-tandem Mass Spectrometry

Song Y, Gibson R, Samra S, Hassell K
[1]Thermo Scientific

Introduction: With recent emergences in new infectious diseases and their variants, there is a need to develop faster and more specific analytical tools to detect different respiratory infectious diseases such as SARS-CoV-2 or influenza viruses. Not only are their initial symptoms similar, but they are also both enveloped viruses with several common biological properties, often leading to challenges in disease identification.

Among different viral components, nucleocapsid proteins or nucleoproteins (NP) are highly conserved, contain fewer post-translational modifications, and are mostly specific to each infectious disease virus type. Therefore, targeting NPs could be advantageous for method development, resulting in simpler and more robust methods with minimal subsequent modifications.

This study describes a targeted approach for the simultaneous detection of NPs from different respiratory infectious diseases using immunoprecipitation (IP) and liquid chromatography-tandem mass spectrometry (LC-MS/MS). SARS-CoV-2, influenza virus A and B, respiratory syncytial virus, and human coronavirus (HCoV-229E), were selected to demonstrate that this method can distinguish different disease viruses.

Methods: Samples collected via nasopharyngeal swabs in viral transport media was directly subjected to IP using Thermo Scientific™ Pierce™ MS-Compatible IP Kit (Streptavidin). The IP purified samples were then digested using SMART Digest™ Trypsin Kits and analyzed by a Thermo Scientific™ Vanquish™ MD HPLC system hyphenated to a Thermo Scientific™ TSQ Altis™ MD mass spectrometer. Data processing was performed using TraceFinder™ LDT software 1.0.

Results: Combining IP and LC-MS/MS resulted in a highly sensitive and specific targeted approach, with limits of detection in the sub femtomole range. In addition, it simplified the overall sample preparation process eliminating prior protein precipitation and post sample clean-up. Since the NPs of variants mostly remain unchanged, the method doesn’t need tremendous alterations once established.

Conclusions: This targeted approach can be applied to the detection of other enveloped viruses and may be automated with KingFisher™ purification systems.

PP02.027: Characterizing ClpX and its Therapeutic Potential in Reversing Antifungal Resistance in Cryptococcus Neoformans

Woods M[1], Geddes-McAlister J[1], Bermas A[1], Muselius B[1], Chan N[1]
[1]University Of Guelph

INTRODUCTION

Cryptococcus neoformans is an opportunistic human fungal pathogen known to cause lethal meningitis in immunocompromised individuals (e.g., HIV/AIDS). Treatment options, including the commonly prescribed antifungal fluconazole, are effective; however, prolonged treatment requirements, excessive prescription, and application of azoles in agriculture, has led to the development of antifungal resistance. Such resistance presents a deadly obstacle against effectively treating cryptococcal fungal infections. Our research uses mass spectrometry-based proteomics to identify and characterize mechanisms driving fluconazole resistance in C. neoformans to uncover new strategies to reverse resistance and make current treatment options effective once again.

METHODS

Quantitative proteomics by label-free quantification was used to identify candidate proteins in the fluconazole-resistant strains compared to fluconazole-susceptible strains of C. neoformans. Deletion and complement strains were constructed using biolistic transformation for the lead first candidate, followed by proteomic profiling after the disruption of the protein. Growth and synergistic assays comparing fungal growth after protein interference was completed in 96 well plates measuring OD600. Infection assays were performed to define the impact of protein interference on fungal survival with macrophage (in vitro), as well as host survival and fungal dissemination (in vivo).

RESULTS

Six protein candidates were identified in the quantitative proteomic analysis. ClpX, a hydrolase involved in protein degradation was identified as the lead candidate. Deletion or inhibition of ClpX led to significant remodelling of the proteome and reduced growth of C. neoformans in combination with fluconazole treatment in vitro and in vivo. A synergistic effect was observed in the presence of ClpX inhibitor and fluconazole in the resistant strains.

CONCLUSIONS

Quantitative proteomics has become a valuable tool in the discovery of new antifungal cellular targets. Here, we apply these techniques to reveal a novel function to the fungal protein ClpX and provide a method to reverse azole resistance in C. neoformans.

PP02.028: Label-free ex Vivo Chemical Proteomics Reveals Organ Specific Small Molecule Drug Targets

Batth T[1], Locard M[1], Doncheva N[1], Mendez B[1], Jensen L[1], Olsen J[1]
[1]NNF Center for Protein Research, University Of Copenhagen

Introduction

Proteins are the primary targets of almost all small molecule drugs. However, even the most selectively designed drugs can potentially target number of unknown proteins. Identification of all potential drug targets can enable better drug design and repurposing of existing drugs for different clinical diseases. Current state-of-the-art proteomics methodologies enable screening of 1000's proteins against a small number of small molecules. However, higher throughput is required in order to determine protein targets of large number of drugs.

Methods

Proteins were gently extracted from eight different rat organs and two human cell lines in order to preserve their native state before being subjected to different small molecule drugs. A complete 96-well format workflow was developed for different segments of the experiment, including a modified thermal proteome profiling approach, removal of insoluble proteins, and sample preparation for mass spectrometry analysis. Resulting samples were analyzed using data-independent acquisition analysis on a Thermo Fisher Oribtrap Exploris 480 mass spectrometer. Downstream data analysis workflow was developed in order to confidently identify and visualize drug-protein interactions. Rat targets were further associated to human proteins using ortholog gene mapping.

Results

The 96-well plate workflow lead to the identification of a higher number of drug targets compared to traditional methods that require peptide labelling or centrifugation to remove insoluble proteins. Analysis revealed numerous previously unknown potential drug targets and informed on their tissue specificity. Liver extracts in particular had the highest number of proteins affected by drug treatment followed by muscle and kidney extracts.

Conclusions

The optimized strategy and utilization of rat organ protein extracts enables parallelized and high-throughput screening of drug targets against a large portion of the proteome. Furthermore, these data constitute a valuable resource that can be utilized for repurposing existing drugs for different clinical diseases.

PP02.029: Profiling Reactive and Functional Cysteines of Transcription Factors using Cellular Fractionation and an Electrophilic Probe

Canzani D[1], Fondrie W[1], Allen C[1], Gutierrez A[1], McEllin B[1], Pino L[1], Federation A[1]
[1]Talus Bioscience

Introduction

Gene expression is tightly regulated by interactions between protein transcription factors (TFs) and DNA. Abnormal TF activity drives many human cancers, so TFs are desirable candidates for drug development. However, most TFs are intrinsically disordered and have been considered "undruggable." Covalent drugs that react with cysteine residues offer promise for targeting TFs, but comprehensive mapping of reactive cysteines within TFs has not been achieved to date, and it is unknown if drugging TF cysteines will yield desirable cellular outcomes. We present a strategy to identify reactive and functional TF cysteines from cancer cells that combines a novel electrophilic probe, nuclear protein enrichment, and mass spectrometry.

Methods

Various cancer cell lines were treated with 12.5 uM n-methyl iodoacetamide (NMIAA). The cells were fractionated using chromatin enriching salt separation (ChESS), which uses 4 sequential buffer washes of increasing ionic strength to separate the cellular proteome into cytosolic, nucleoplasm, euchromatin, and heterochromatin fractions. ChESS fractions were digested using trypsin and analyzed using data-dependent acquisition on an Evosep One – ThermoFisher Exploris 480 Orbitrap platform. The results were searched with Comet with the mass of the NMIAA addition as a variable modification on cysteines, then filtered at a 1% FDR with mokapot, and quantified with FlashLFQ.

Results

We observed dozens of NMIAA-modified TFs in multiple cancer cell lines after ChESS fractionation. In addition to characterizing the reactive cysteines of TFs, movement of a TF between ChESS fraction after NMIAA treatment indicated a functional consequence of the cysteine modification. For example, the TF CTCF was ejected from heterochromatin and observed in the nucleoplasm fraction after NMIAA modification, suggesting those cysteines influence CTCF binding to chromatin.

Conclusions

NMIAA treatment with ChESS fractionation is a powerful tool for uncovering reactive and functional cysteines of transcription factors and can be used to drive drug development.

PP02.030: Evaluation of Thermal Proteome Profiling Methodologies to Identify Membrane Protein Targets

Lizano-Fallas V[1], Berlin E[1], Carrasco del Amor A[1], Cristobal S[1]
[1]Linköping University

The thermal proteome profiling (TPP) is a mass spectrometry-based thermal shift assay that could unbiasedly identify the protein targets from small molecules in a studied proteome. The non-ionic surfactants such as Igepal CA-630 or Nonidet P-40 substitute are frequently used for a mild solubilization of membrane proteins. Those detergents were introduced in TPP aiming to include the membrane proteome in the analysis. The aim of this study is to evaluate the thermal dependent dynamics in the membrane proteome based on the theoretically expected changes in size and shape of the non-ionic surfactant micelles. The cloud points of non-ionic detergents are important parameters to consider. Both detergents reached their cloud points within the thermal shift range of temperatures applied in TPP. Here, we analyze the variation in the membrane proteome under the thermal shift assay by mass spectrometry and the modification of the detergent micelles at that temperature range by using dynamic light scattering, and microscopy. Our results showed that the TPP solubilization condition did not facilitate the incorporation of membrane protein into the detergent micelles. We observed that the non-ionic detergent micelles in solution are thermal sensitive, and the thermal shift method surpass their respective cloud points. In the studied conditions is not expected to generate defined layer of detergent-rich micelles due to the high concentration of proteins compared to detergent in micelles, but we could detect changes in micellar size and protein aggregation. Therefore, the thermal-dependent changes in the micelle´s morphology contribute to modification in the membrane proteome. It also induces alteration of the protein solubility that would be independent on ligand binding, the parameter used to identify protein targets in TPP. This study illustrates that the membrane protein solubilization strategy utilized in this methodology should be reviewed in the context of their compatibility with the thermal shift assay.

PP02.032: Proteomics Investigation of Diverse Serological Patterns in COVID-19

Liang X[1,2], Sun R[2], Wang J[3], Zhou K[3], Li J[3], Chen S[3], Lyu M[2], Li S[2], Xue Z[2], Shi Y[2], Xie Y[2], Zhang Q[4], Yi X[4], Pan J[3], Wang D[3], Xu J[3], Zhu H[3], Zhu G[3], Zhu J[3], Zhu Y[2], Zheng Y[3], Shen B[3], Guo T[1,2]
[1]Fudan University, [2]Westlake University, [3]Taizhou Hospital of Zhejiang Province affiliated to Wenzhou Medical University, [4]Westlake Omics Biotechnology Co., Ltd.

Introduction
Serological tests of IgM and IgG titers are crucial to assess COVID-19 patients' status. Seronegative results throughout the disease were frequently observed, whereas the underlying molecular mechanisms remain elusive.
Methods
We systematically investigated the diversity of serum IgM and IgG expressions in 144 COVID-19 patients over two years, via longitudinal clinical manifestation and proteomic profiling.
Results
During ten weeks since COVID-19 onset, 3.5% of the patients remained both IgM and IgG negative. Additionally, 6.3% of the patients exhibited over 20-fold higher titers of IgM than average values starting from the 2nd week. Notably, none of the IgG seronegative patients exhibited severe symptoms whereas over 33.3% of the IgM seropositive patients were severe cases. IgG titers in the convalescent patients declined in a year, which were significantly boosted after vaccination and could persist till two years after disease onset. Longitudinal proteomic profiling of 111 sera samples, together with clinical indices characterization, showed that the continuously negative IgM and IgG expressions during COVID-19 were associated with mild inflammatory reactions and relatively high T cell responses. An inferior immunological basis and insufficient cellular immune responses might lead to compensatory serological responses, causing over-expression of IgM.
Conclusions
The integrative molecular landscape suggests that patients with negative serologies still developed cellular immunity for viral defense, and that high titers of IgM might not be favorable to patient recovery.

PP02.033: Dose-dependent and Proteome-wide Drug Response Profiling by Micro-flow-LC-FAIMS-MS/MS

Eckert S[1,2], Berner N[1,2], Lechner S[1], Brajkovic S[1], Wilhelm S[1], Kuster B[1]
[1]Chair of Proteomics and Bioanalytics, Technical University Of Munich (TUM), [2]German Cancer Consortium (DKTK) and German Cancer Research Center (DKFZ)

Introduction

Mass spectrometry-based proteomics emerges as a powerful read-out for mode of action studies of drugs. We have previously demonstrated that microflow LC-MS/MS can achieve high proteomic coverage in a single shot. Here, we extend the approach to include high field asymmetric waveform ion mobility spectrometry (FAIMS) as a further separation step and employ this technology to profile drug perturbed proteomes.

Methods

For drug profiling, Jurkat cells were treated with five concentrations of the respective drug for 18 hours followed by standard methods to obtain tryptic peptides. Peptides were analyzed using a 60 min gradient at a flow rate of 50 µL/min on a microflow-LC-FAIMS-MS/MS setup applying a single optimized compensation voltage (CV).

Results

Optimizing the coupling and parameters of FAIMS to microflow-LC-MS/MS led to the identification of 7,000 proteins in one hour. We employed this setup to the dose dependent profiling of 117 drugs representing 12 drug classes. Data analysis revealed frequent up and down regulation of protein expression. For instance, Brigatinib, a designated ALK inhibitor, drastically upregulated the dual specificity protein kinases CLK1, CLK3 and CLK4 (up to 12-fold; EC50 of 8, 8491 and 53 nM respectively). This suggests a potent effect of the inhibitor on mRNA splicing in which these kinases play a pivotal role. Similarly, pomalidomide, a Thalidomide derivative and Cereblon E3 ligase modulator, led to the potent degradation of the DNA-binding protein Ikaros (IKZF1; EC50= 11 nM), a transcription factor known to be responsible for the drug's immunomodulatory effects.

Conclusions

The presented method offers a simple yet powerful option to improve the understanding of drug mode of action by measuring the potency of induced protein expression changes in response to treatment.

PP02.034: Novel Blood-Brain Barrier Shuttle Peptides

Hanes J, Majerova P, Olesova D, Kovac A
[1]Institute of Neuroimmunology, Slovak Academy of Sciences

Introduction: Delivery to the brain is a major challenge in central nervous system drug development. The blood-brain barrier prevents access of biotherapeutics to their targets in the CNS and therefore prohibits the effective treatment of neurological disorders.

Methods: To find BBB-shuttle peptides that target the brain, we performed a phage display method against a primary rat BBB cellular model which mimics the characteristics of the BBB.

Results: From the panning experiment of a 12-mer library, the specific peptide sequences were selected and their permeability validated. The permeability of peptides was measured by ultra-performance liquid chromatography-tandem mass spectrometry coupled to a triple-quadrupole mass spectrometer.

Conclusions: Our results showed the importance of in vitro BBB model for the discovery of BBB-shuttle peptides through phage display libraries. The results indicate that the peptides identified by the in vitro phage selection approach could be useful transporters for systemically administrated biopharmaceuticals into the brain with therapeutic benefits.

Acknowledgments: This work was supported by research grants: APVV-21-0254, APVV-21-0321, VEGA 2/0123/21, VEGA 2/0129/21, VEGA 2/0078/22

PP02.035: Proteome Analysis of Rhabdosynochus Viridisi, a Tool for Drug Development against a Parasitic Worm of the Marine Fish Centropomus Viridis

Caña-Bozada V[1], Morales-Serna F[2], Elizalde Contreras J[3], Ruiz-May E[3], Huerta Ocampo J[1,4]
[1]Centro de Investigación de Alimentación y Desarrollo, A.C., [2]Instituto de Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México, [3]Instituto de Ecología, A.C., [4]Consejo Nacional de Ciencia y Tecnología

Introduction: Monopistocotylean monogeneans are parasitic flatworms which can infect farmed fish and lead to diseases and mortality. Particularly, Rhabdosynochus viridisi represents a threat for production of Pacific white snook, Centropomus viridis, which is a new fish species for marine aquaculture (1, 2). Understanding the molecular mechanisms of parasites can contribute to the improvement of antiparasitic compounds; however, the transcriptomic and proteomic information of monogeneans is scarce. The goal of this work was to reveal the proteome of R. viridisi and to identify its peptidases and peptidase inhibitors.
Methods: Proteins of R. viridisi were extracted, trypsin digested and identified by tandem mass spectrometry. Bioinformatic tools were used to annotate the proteins based on the molecular function and biological processes in which they participate. Peptidase and peptidase inhibitors were identified by integrating transcriptomic and proteomic data of R. viridisi, using the MEROPS database as reference.
Results: Identified proteins and their functional annotation are presented. Given their important role during invasion and colonization of parasites, peptidases are being analyzed in more detail. To date, 159 peptidases and 25 peptidase inhibitors had been identified in the transcriptome of R. viridisi.
Conclusions: This work contributes to expanding the omics knowledge in monogenean parasites. The proteome of R. viridisi represents the first reported for a monopistocotylean species. Knowledge of the proteins expressed in monogeneans, mainly peptidases, should be useful for the development of new drugs and control methods against monogeneans.

1. Morales-Serna, F.N., et al. 2020 J Appl Ichthyol 36: 740-744
2. Baldini, G., et al. 2022 Aquacult Rep 23: 101048

PP02.036: Target Protein Identification and Validation and Mode of Action Study of Natural Compound Inducting Autophagy with DARTS-LC/MS/MS

Jang Y[1], Ko M[1], Cho H[1], Kim J[2], Yoo J[2], Kwon H[1]
[1]Yonsei University, [2]Korea Basic Science Institute

Autophagy has been highlighted as a critical regulator of cellular homeostasis, dysregulator of whigh is associated with diverse disease. In addition, Autophagy plays a wide variety of physiological and pathophysiological roles. Particularly, the autophagy of cytoplasmic lipid droplets is known as lipophagy. A link between lipophagy and lipid associated mechanism remains to be addressed. To explore the role of autophagy in lipid regulation, we identified a natural compound (ACA) as a novel anti-NASH agent through autophagy regulation. Notably, ACA suppressed lipid accumulation and attenuated the expression of adipogenesis related factors in 3T3-L1 adipocytes without showing cell toxicity. Moreover, ACA activates autophagic degradation in vitro and exhibitis anti-NASH effect in vivo. To investigate the mode of action for ACA, we applied a label free small molecule target identification method called DARTS with LC-MS/MS analysis. Target protein of ACA (TPA) was identified and validated using DARTS and CETSA. Collectively, these results demonstrated that ACA exhibits anti-NASH activities by inducing autophagy and mTORC1 inhibition resulted in inhibition of lipidosis and improvement of intracellular steatosis via direct binding to TPA.

Acknowledgements. This work was partly supported by grants from the National Research Foundation of Korea, funded by the Korean government (MSIP;2021R1A3B1077371, MSIP; 2015K1A1A2028365,MSIP;2015M3A9C4076321)) and Brain Korea 21 Plus Project, Republic of Korea.

PP02.037: DARTS-LC-MS/MS Analysis Reveals Target Protein of a Natural Compound with Anti-atherosclerotic Activity

Ko M[1], Lee D[1], Lee J[2], Kim J[2], Yoo J[2], Kwon H[1]
[1]Yonsei University, [2]Korea Basic Science Institute

Traditional medicinal plants have been widely used as pharmaceutical agents due to their fascinating biological activities in vivo. Recently, we confirmed that the natural plant Danshen (Salvia miltiorrhiza) is effective in improving atherosclerotic plaque formation in ApoE-/- mice by inducing autophagy. In this study, we revealed that compound CTS, an active principle of Danshen reduces the mitochondria-ER contacts leading to enhance autophagy. To investigate the mode of action of CTS, a combination of drug affinity responsive target stability (DARTS) and LC-MS/MS method was applied to identify the target protein of CTS. Proteomes were analyzed by the averaged quantitative SWATH analysis. As the result, three proteins belonging to the GRP family having a similar domain structure to GRP 75 which is known to play an important role in mitochondria-ER contacts were identified. In addition, target protein of CTS was validated by cellular thermal shift assay (CETSA). Interaction of CTS with the target protein was validated with knockdown of the target gene resulting in increase of the autophagic flux. Collectively, this study provides new insights into the mechanism of an anti-atherosclerotic natural compound in targeting mitochondria-ER contact complex for autophagy induction.

PP02.038: Development of a Mass-spectrometry-compatible Chemical Probe for the Enrichment of Protein Citrullination

Meelker Gonzalez R[1], Laposchan S[1], Lee C[1]
[1]; Young Investigator Group: Mass Spectrometry in Systems Neurosciences, School of Life Sciences, Technical University of Munich

Introduction:Citrullination is a post-translational modification (PTM) on arginine catalyzed by peptidylarginine deiminase (PAD) enzymes. It causes a mass increase of 0.98Da and the loss of a positive charge that alters the protein structure and function. Citrullination has been linked to many autoimmune diseases such as rheumatoid arthritis and multiple sclerosis. However, due to the lack of biochemical enrichment tools, proteomic-wide studies to identify citrullination sites and the altered function of the modified proteins are very scarce. Here, we develop a MS-compatible chemical probe to enrich protein citrullination.

Methods:A pool of 200 synthetic citrullinated peptides was derivatized with glyoxal derivatives followed by a consequent click reaction to a biotin residue with a cleavable linker. After being enriched by streptavidin beads and removing the biotin tag, the peptides were measured in LC-Orbitrap MS/MS. MS Fragger and MaxQuant were used to characterize and identify the derivatized peptides using open search and a custom variable modification. We examined different fragmentation types and acquisition methods and inspected MS2 spectra manually and systematically for potential diagnostic and neutral loss ions. Additionally, the selectivity and specificity of this strategy were assessed by spiking in synthetic citrullinated peptides and in vitro citrullinated cell lysate into cell lysate.

Results:Citrullinated peptides were successfully derivatized with an intensity-based efficiency of more than 80%. The interpretation of the MS2 spectra revealed the consecutive loss of N2 and OH from precursor and fragment ions carrying the derivatization, which can be used to increase site localization. However, these fragment ions show a lower intensity similar to the citrulline effect previously observed. Examining the enrichment in cell lysate shows that the identification accuracy of citrullinated peptides is improved compared to non-derivatized citrullinated peptides without enrichment.

Conclusions:The developed chemical probe for protein citrullination enrichment shows its feasibility and potential in large-scale proteomic studies.

PP02.039: Regulation of Metabolic Pathways for Antifungal Biosynthesis by Amycolatopsis BX17

Palafox Félix M, Cabrera Ruiz R, Huerta Ocampo J
[1]Centro de Investigación en Alimentación y Desarrollo, A.C. , [2]Centro de Investigación en Alimentación y Desarrollo, A.C; CONACyT, [3]Centro de Investigación en Alimentación y Desarrollo, A.C; CONACyT

Introduction: The milpa is an agricultural ecosystem that preserves traditional farming techniques that allow the conservation of beneficial microbial communities in the soil, which establish ecological interactions with plants and provide a decrease in pathogenic microorganisms through the synthesis of metabolites with antifungal activity (1). Within these communities, the Actinobacteria are recognized for their biotechnological importance for synthesizing bioactive compounds (2, 3). In our research group, the microbiology soil of agricultural systems is studied, particularly the traditional agroecosystem milpa, located in the Alto Mezquital region in the state of Hidalgo, where the Amycolatopsis BX17 strain was isolated.

Methods: Culture media with 0 and 20 g/L glucose were selected to grow Amycolatopsis BX17 and analyze the differential accumulation of intracellular proteins. Proteins for each condition were identified and quantified by gel-free proteomic analysis (shot-gun). Using bioinformatics tools, differentially accumulated proteins and metabolic pathways were identified.

Results: The composition of the culture medium is the main factor in the regulation of the biosynthesis of these compounds. The proteomic analysis obtained for Amycolatopsis, cultivated in a glucose medium, suggests that the carbon flux is mainly directed towards cell growth, in which the primary metabolism of the pentose phosphate pathways and glycolysis intervene. Among the proteins identified in the medium without glucose, enzymes corresponding to glutamate metabolism and the Krebs cycle were found, suggesting that Amycolatopsis metabolizes the glutamate present in the medium and uses the pyruvate pathway and the Krebs cycle for the synthesis of antifungal compounds.

Conclusions: The growth of Amycolatopsis BX17 and the synthesis of metabolites with antifungal activity are regulated by the composition of the culture medium.

1. Zizumbo, D., et al. 2012 Econ Bot 66(4); 328–343.
2. Aguirre-Von-Wobeser, E., et al. 2018 PLoS One 13(12); 1–18.
3. Cabrera, R., et al. 2020 Biol Control 147; 10428.

PP02.040: Expeditious Chemoproteomic Target Deconvolution of Nature-made Molecules

Prokofeva P[1], Hoefer S[1], Hornisch M[1], Abele M[1,2], Kuster B[1,2], Médard G[1]
[1]Chair Of Proteomics And Bioanalytics, Technical University Of Munich, [2]Bavarian Biomolecular Mass Spectrometry Center (BayBioMS), Technical University of Munich

Introduction

Deconvolution of cofactor interactomes and identification of the protein targets of bioactive natural compounds contribute to the functional annotation of proteomes and expansion of druggable proteome. We are developing a versatile chemical toolbox for immobilization of microgram quantities of unmodified natural products that allow for their systematic chemoproteomic profiling.

Methods

We have optimized a general protocol for the photoactivated immobilization of microgram quantities of natural molecules to generate their respective tailored affinity matrices, which we used in subsequent affinity-based proteome profiling i.e. dose-response competition pulldown experiments followed by quantitative mass spectrometry readout. We are currently exploiting other nonconventional chemistries to immobilize unmodified natural molecules on beads, including gold-catalyzed alkoxylation, Mitsunobu reaction and enyne metathesis.

Results

We have applied our photo-immobilization assay on 31 natural molecules of very diverse reported activity and biosynthetic origin, of which 25 were successfully immobilized, and 9 found to bind proteins with affinities < 100 µM. We recapitulated known targets of kinase drug staurosporine and antibiotics rifamycin B and geldanamycin in human or bacterial lysates. Additionally, we identified differential interactomes of CoA and acetyl-CoA as well as surprising nanomolar interactions of FAD with dozens of RNA-binding proteins. We have also benchmarked other immobilization procedures using the immunosuppressant drug Tacrolimus and recapitulated its cognate target FKBP12 for all attempted chemistries.

Conclusions

Our experimentally straightforward approach can be easily applied to any natural molecule to quickly probe for protein binding in a wide range of biological systems (lysates). The competition aspect adds an additional level of resolution characterizing the specificity and the strength of the observed interactions. Our chemical toolbox should facilitate target deconvolution of natural molecules, for which preparation of conventional chemical probes is particularly difficult.

PP02.041: LC-MS Methods to Investigate PLP Cofactor Occupancy in an Engineered Leucine Decarboxylase under Development for Potential Treatment of MSUD

Salvato F, Riggins J, Matje D, Yan P, Zhang N, Miao T, Das S, Skvorak K, Mehmood R, Liu J, McCluskie K, Du F, Teadt L, Jenne S, Kruse N, Lao A, Silverman A, Alaoui H, Huisman G
[1]Codexis

Introduction: Maple syrup urine disease (MSUD), an inborn error of branched-chain amino acid metabolism, is characterized by toxic accumulation of leucine in the brain, blood, and urine. Using directed evolution, a bacterial pyridoxal phosphate (PLP)-dependent leucine decarboxylase (LDC) is under development for potential treatment of MSUD. PLP acts as an enzymatic cofactor that is required for LDC catalytic activity. Thus, we developed intact mass LC-MS approaches to evaluate PLP occupancy and mass mapping LC-MS strategies to identify PLP-enzyme adducts localization.

Methods: LDC samples were analyzed by LC-MS in the presence of different PLP concentrations. To measure PLP occupancy in LDC, the PLP-lysine imine was reduced using sodium borohydride to form a chemically-stable amine linkage between PLP and the protein. The reduced protein was analyzed by LC-MS for intact mass measurement, and PLP was detected by a mass shift. PLP occupancy was calculated by taking the proportion of PLP bound-LDC and PLP not bound-LDC intensities. In addition, the reduced protein was enzymatically digested, and PLP-bound peptide(s) were enriched by TiO2 affinity chromatography followed by LC-MS/MS analysis to determine PLP site localization.

Results: Intact mass analysis of reduced LDC samples can accurately determine the fraction of LDC occupied by PLP, and PLP occupancy can be increased by adding soluble PLP to the sample. When a large molar excess of PLP is added,, multiple PLP adducts can form, observed as PLP:LDC occupancy ratios >1. Peptide mapping of reduced samples confirmed PLP binding to the protein active site and showed that PLP can bind to other sites when the free PLP concentration is high.

Conclusions: Our results demonstrate that intact mass analysis of reduced LDC can be used to assess PLP occupancy, while peptide mapping can confirm the presence of PLP in the active site and identify the location of aberrant PLP-enzyme linkages.

PP02.042: A Chemical Probe for Comprehensive Analysis of Protein Succinylation

Umezawa K[1], Tsumoto H[1], Kawakami K[1], Miura Y[1]
[1]Tokyo Metropolitan Institute Of Gerontology

Introduction: Protein succinylation, one of the protein acylation, serves as an important post-translational modification that regulates various physiological functions of intracellular proteins such as gene expression, enzyme activity, and protein-protein interactions. In addition, the level of protein succinylation is considered as a barometer that potentially reflects the intracellular energy metabolism, because succinyl CoA (substrates for protein succinylation) is the important energy metabolite. Thus, analytical methods for comprehensive evaluation of protein succinylation are highly required. Therefore, we aimed to the establishment of a new method for proteomic analyses of succinylated proteins using a chemical tagging technology.

Methods: In this study, we designed and synthesized a new chemical probe for the detection of succinylated proteins. An alkynyl unit was introduced on the succinate for selective conjugation of various azide-containing reporter molecules via Click ligation (Huisgen 1,3-dipolar cycloaddition). Acetoxymethyl esterification of the substrate was also performed for improving cell permeability. The synthesized substrate was incubated with living cells. After cell lysis, azide-containing biotin moieties or fluorescent dyes were conjugated to the succinylated proteins via the Click ligation for the purpose of SDS-PAGE analysis and LC-MS/MS-based proteomic analysis. Also, in-cell Click ligation and subsequent cellular imaging were performed.

Results: The synthesized substrate successfully labeled lysine residues of proteins through intracellular metabolic labeling, and the Click ligation of the lysate with Cy5-azide resulted in the visualization of numerous succinylated proteins on gels. Replacement of Cy5-azide with biotin-azide enabled biotinylation and subsequent enrichment of the succinylated proteins with avidin beads, resulting in the identification of a large number of subcellular succinylated proteins associated with various biological processes. Furthermore, visualization of the subcellular localization of succinylated proteins was succeeded.

Conclusion: We established a new chemical method for comprehensive analyses of protein succinylation, which overcomes the limitations of conventional analytical methods.

PP02.043: BCA-no-more: Seamless, High Throughput Protein Quantification Directly on S-Trap Plates

Wilson J[1]
[1]ProtiFi, Llc

Recent advances in the throughput of proteomics, necessitate concomitant advances in bottom-up sample preparation workflows. Any and all extraneous steps in a sample preparation workflow must be eliminated to increases both throughput and robustness. Post lysis, a protein concentration assay is standard. All assays are neither instant nor impervious to interference. Additionally, if performed in a 96-well plate, they are subject to edge effects. Here, we demonstrate the new concept of direct quantification of cleaned, surface-bound protein on S-Trap 96-well plates using intrinsic protein fluorescence.

S-Trap 96-well plates used as per standard protocols: samples of varied hydrophobicity were bound onto plates and washed recommended; standard BSA curves were also measured. Protein fluorescence in both a wet and dry state was measured with an excitation between 269 and 280 nm and an emission of 325 to 475 nm using a Tecan Sparc plate reader in top-read emission mode. Protein concentrations determined via fluorescence were compared to BCA for limit of detection, reproducibility, and dynamic range.

We determined the optimal z-position (2100 µm) for fluorescence protein concentration quantification. In a dry state, likely due to quenching, fluorescence was inversely correlated to the amount of protein bound up to 300 µg. Protein in digestion buffer fluorescence tracked with protein load at 277 nm excitation and 350 nm emission. The direct-determination method afforded protein quantification in a significantly reduced time compared to BCA assays with dynamic range and sensitivity compatible with standard bottom-up and top-down proteomics workflows. The S-Trap sample preparation workflow successfully removed matrix contaminants prior to protein concentration determination without the need for additional steps. Such on-plate protein concentration determination lends itself directly to deployment in high-throughput clinical settings using automated fluid handlers.

This workflow allows direct determination of protein concentration with intrinsic cleanup removing the need for protein assays.

PP02.044: System-wide Profiling by Proteome Integral Solubility Alteration Assay of Drug Residence Times for Target Characterization

Zubarev R[1], Sabatier P, Beusch C, Meng Z
[1]Karolinska Institutet

Most drugs used in the clinic and drug candidates target multiple proteins, and thus detailed characterization of their efficacy targets is required. While current methods rely on quantitative measurements at thermodynamic equilibrium, kinetic parameters such as the residence time of a drug on its target provide a better proxy for efficacy in vivo. Here, we present Residence Time Proteome Integral Solubility Alteration (ResT-PISA) assay which provides monitoring temporal protein solubility profiles after drug removal ("off-curve") in cell lysate or intact cells, quantifying the lifetime of drug-target interaction. A compressed version of the assay measures the integral under the off-curve enabling the multiplexing of binding affinity and residence time assessments into a single proteomic analysis. We introduce a combined scoring system for three parametric dimensions to improve prioritization of targets. By providing complementary information to other characteristics of drug-target interaction, ResT-PISA approach will be useful in drug development and precision medicine.

PP02.045: Fast and Accurate 4D-LFQ Workflows from Real-Time Results on PaSER

Adams C[1], Park R[1], Brehmer S[1], Krawitzky M[1], Park M[1], Krieger J[1], Srikumar T[1]
[1]Bruker

Introduction

Label free quantification (LFQ) from DDA acquisitions provides essential information on protein and proteome abundance. This quantitative information is critical to understanding protein expression and turnover in complex diseases and drug therapies. Traditional LFQ workflows rely on the complete acquisition of an experimental cohort followed by data transfer, identification, match between runs (MBR) and data output, which, collectively can take many hours or days to complete. Here we demonstrate a LFQ workflow that is based on the real-time results generated on the PaSER platform where as soon as the data acquisition is completed a LFQ workflow is triggered based upon the real-time generated results. Given the elimination of the data transfer and search steps LFQ results are delivered in a fraction of the time as compared to traditional analysis, all while maintaining accuracy and precision.

Methods

Mixed species experiments were performed using tryptic protein digests of H. sapiens, S. cerevisiae and E. coli mixed in two different experiments leading to a ratio of 1:1 (HeLa), 1:2 (S. cerevisiae) and 1:4 (E. coli). NanoLC MS was performed on a timsTOF Pro 2 with and nanoElute LC using various gradient lengths. Data was processed on the PaSER platform using common DDA search parameters and TIMScore.

Results

Using DDA PASEF in the mixed proteome samples we identified greater than 8100 protein groups from 60000 peptides in a 60 min. gradient where the quantitative accuracy was within less than 15% of the expected ratios. Importantly, the MBR functionality of the LFQ workflow boosted the number of quantified proteins by >30%. We also monitored the time savings as compared to commonly used proteomic packages, where 4D-LFQ on PaSER saved more than 80% processing time as compared to leading packages1 while delivering complete and accurate results.

PP02.046: Digitalomics: Digital Transformation across Omics Leading to Precision Medicine and NextGen Healthcare.

Balasubramaniam N[2], Basu S[1]
[1]Oracle, [2]P4ML

Convergence between “Omics applications” and “Digital technologies” is transforming the landscape for biomarker discovery arising from genomics, next generation sequencing (NGS), other Omics studies, and having a positive impact on drug discovery, molecular and companion diagnostics, toxicogenomics, as well as pharmacogenomics. We would like to term this new ecosystem as being “Digitalomics” that leverages the power of high power computing, cloud technologies, autonomous databases, artificial intelligence, machine learning, deep learning, medical Internet of Things (mIoT), data security and protection to derive insights from Omics data and integrating it with Electronic Health Records for NextGen Healthcare. This application has profound impact for the global population ranging from oncology, to infectious, cardiovascular and neurological diseases to name a few.

In this poster, we would like to highlight the challenges and opportunities for Precision Medicine enabled by Digitalomics. Omics analysis generates big data arising both from patient samples, as well as machines, which can be streamlined leveraging cloud computing, managed and integrated to derive meaningful insights of value for both the clinician and the patient. We would like to validate this workflow map and to highlight the power of Digitalomics by presenting evidence from specific case studies. Initiatives that will be highlighted will include the; Global Pathogen Analysis System (GPAS), applications of artificial intelligence and deep learning for classifying breast cancer tumors, development of synthetic vaccines, developing algorithms for validating innate immune fitness, as well as computational tools for predicting cardiotoxicity.

PP02.047: Evaluating Proteomics Knowledge Derived from Trillions of Data Points

Pullman B[1], Wertz J[1], Carver J[1], Bandeira N[1]
[1]University Of California, San Diego

Introduction

The trustworthiness of proteomics knowledge accumulated from tens of thousands of publications strongly depends on the robustness of data analysis protocols used to process billions of spectra in millions of mass spectrometry runs. Stymieing the galloping accumulation of false discoveries thus requires both new automated workflows for controlling community-scale error rates, and community curation resources for critical evaluation of data directly supporting the most surprising reported discoveries.

Methods

New algorithms, resources and processes were developed for aggregation and validation of proteomics discoveries at the repository scale. Methods were implemented in open-source workflows available at MassIVE and altogether consider results of identification, quantification and differential abundance at all levels of peptide, protein and post-translational modifications.

Results

Standardized formats for dataset submissions and open-source workflows for dataset reanalysis enabled the public availability of nearly 1 billion identifications of 21.8 million modified peptides from >365,000 protein identifiers from >75 species across the kingdoms of life. These also support the detection of >165,000 differential protein abundance events in hundreds of MassIVE.quant reanalyses.

Furthermore, new workflows for repository-scale aggregation of search results into reusable resources implement algorithms for assessing the significance of community knowledge derived from tens of terabytes of public data. The resulting new version of the MassIVE-KB human spectral library now contains >6 million precursors from >32,500 protein groups, which combine with new algorithms implementing Human Proteome Project global guidelines to establish the in-vivo detection of >16,500 canonical human proteins.

Finally, new resources enable interactive exploration of protein identifications and differential abundances across a vast collection of publicly available results, as well as for detailed inspection of key identifications using either Universal Spectrum Identifiers or real-time repository-scale modification-tolerant spectrum matching against billions of spectra.

Conclusions

Systematic repository-scale reanalysis yields hundreds of millions of identifications supporting detection and evaluation of fundamental proteomics knowledge.

PP02.048: BrainProt: Expanding Horizons for the Understanding of Human Brain with OMICS

Biswas D[1], Shenoy S[1], Srivastava S[1]
[1]Indian Institute Of Technology Bombay

Introduction: Brain is the most complex organ of the human body. Globally, many studies have supported and unveiled different functional and anatomical paradoxes of the human brain and brain-related diseases. The scientific studies of brain-related diseases have always focused on understanding the molecular level alteration to identify better diagnostic strategies and unveil novel therapeutic modalities. However, understanding and comparing the nature of brain diseases and disorders based on their known markers and biology could aid in connecting the relationship between them. This study attempts to develop an interactive, simple web application by integrating different proteomics and transcriptomics datasets of brain diseases to accelerate biomarker discovery, drug identification, and divulge disease progression mechanisms.

Methods: Global public repositories of Proteomics and Transcriptomics were searched based on appropriate keywords (disease name). All the datasets, which had passed the filters, were matched with the sample metadata or clinical details. The transcriptomics' datasets were analyzed in the arrayQualityMetrics and affylmGUI for Quality check and pre-processing, respectively. Whereas the proteomics' raw files were analyzed in MaxQuant and the pre-processing was done in Perseus. All the processed datasets, and their respective clinical information, are integrated into BrainProt (http://www.brainprot.org/).

Results: Approximately 50 proteomics and transcriptomics datasets were analyzed under the human brain disease map. More than 20,000 transcripts and protein level expressions from ~1200 samples were integrated into BrainProt. In addition to the expression visualization, the portal allows the identification and selection of disease markers curated based on popular keyword mining tools. Lastly, the portal integrates popular drug databases to fetch the best-matched compounds for the query marker of human brain disease.

Conclusion: BrainProt aims to stimulate, accelerate, and support the global research community. It also complements previous efforts toward the global characterization of the human brain and strengthens the initiatives of the Human Brain Proteome Project (HBPP).

PP02.049: Semi-supervised Learning for Sensitive Open Modification Spectral Library Searching Provides Insights in the Role of Modifications during Viral SARS-CoV-2 Infection

Bittremieux W[1], Arab I[2], Adams C[2], Laukens K[2]
[1]UC San Diego, [2]University of Antwerp

Introduction: Semi-supervised machine learning, as introduced by Percolator, can be used to augment the target-decoy strategy to sensitively discriminate between correct and incorrect peptide-spectrum-matches (PSM) and boost the spectrum identification rate. Here we have implemented semi-supervised rescoring of spectrum assignments in the ANN-SoLo spectral library open modification search engine to sensitively identify modified peptides carrying any type of post-translational modification (PTM). Additionally, we have used ANN-SoLo for the reanalysis of public SARS-CoV-2 protein-protein interaction data to obtain hypotheses about the functional role of PTMs during viral infection.

Methods: Semi-supervised rescoring was implemented in ANN-SoLo using the mokapot library. Available machine learning models include a linear support vector machine (SVM), equivalent to Percolator, and a random forest. Features used by the classifier include various spectrum similarity measures, such as the shifted dot product, spectral contrast angle, Pepitome hypergeometric score, spectral entropy, etc., as well as PSM information, such as the precursor charge and precursor m/z deviation. A previously generated affinity purification mass spectrometry (AP-MS) dataset to study the SARS-CoV-2 virus-host interactome by Gordon et al. was reanalyzed with ANN-SoLo.

Results: ANN-SoLo reanalysis of the SARS-CoV-2 AP-MS data boosted the number of identified spectra by 214% compared to standard searching. Notably, the use of a random forest classifier for PSM rescoring provided additional benefits compared to a linear SVM, especially for the identification of spectra corresponding to modified peptides, in which case the non-linear random forest achieved better performance for PSM subgroups with different PTMs. The analysis identified several novel modification sites on SARS-CoV-2 proteins that were investigated in relation to their interactions with human proteins, which revealed important hypotheses about the functional role of modifications, including phosphorylation, ubiquitination, and S-nitrosylation, during viral infection by SARS-CoV-2.

Conclusions: Sensitive identification of modified peptides was achieved using open modification searching and semi-supervised machine learning.

PP02.051: Aligning DIA Proteomics Data in Space: A Large-Scale Citizen Science Project

Callens T[1]
[1]Ghent University

Identification and quantification of large amounts of proteins from a sample has major clinical importance and mass spectrometry (MS) provides a means to perform this in a sensitive way. The majority of proteomic analyses conducted by MS is data dependent acquisition (DDA). Recently, however, data independent acquisition (DIA) became increasingly popular to try and overcome certain problems in DDA by not selecting specific ions, but instead acquiring all fragments simultaneously in a continuous manner, at the price of leading to complex, chimeric data. Hidden in this complex data cluster lies a great amount of information on the proteome of the specific sample. One of the components of correct protein identification and noise reduction is the alignment of data of different mass spectrometry runs. We therefore set up a collaboration with MMOS and NetEase to implement DIA data alignment in the highly popular mobile game EVE Echoes. For this project, data is used in four dimensions: precursor retention time, precursor drift time, fragment mass over charge and fragment intensity. Players of EVE Echoes will align data of two runs of the same sample based on the first three dimensions. We will acquire the exact matches, as well the translation vectors the players made to create these matches. Later, a scoring function based on all dimensions will be used as a means to compare the gamers' alignment to already existing and our newly developed alignment algorithm. Eventually, a machine learning algorithm will be trained on this acquired data to achieve better alignment and filter out noise from these complex datasets.

PP02.052: PeakFilter: A Generative Method to Denoise High-resolution Mass Spectra

Zhang Y[1,2], Xie L[1], Shu K[2], Chang C[1]
[1]State Key Laboratory of Proteomics, Beijing Proteome Research Center, National Center for Protein Sciences (Beijing), Beijing Institute of Lifeomics, [2]School of Computer Science and Technology, Chongqing University of Posts and Telecommunications

Introduction: Liquid chromatography-mass spectrometry (LC-MS) has been widely used in proteomics for years. In MS data, the number of the noise peaks is several times that of the signal peaks, which seriously affects the sensitivity and accuracy of subsequent identification and quantification. Thus, removing the noise in mass spectra is essential. Currently, denoising methods usually only consider one or two dimensions of the basic MS information (mass-to-charge ratio (m/z), intensity, and retention time) using machine learning. However, feature selection relies heavily on the priori knowledge and domain knowledge, which makes spectra denoising insufficiently and difficultly. Here, we propose a generative deep learning method, PeakFilter, to denoise spectra by generating the corresponding theoretical spectra directly. PeakFilter considers the spectra as three-dimensional point clouds, utilizing the whole three-dimensional information of MS data.
Methods: Using the experimental spectra from the raw mass spectrometry data of the Proteometools dataset (10.1038/nmeth.4153) and the theoretical spectra predicted by Prosit (10.1038/s41592-019-0426-7) from the identified peptide sequences, peaks are represented as three-dimensional points. We collected 21,886 spectra and divided the training set, validation set, and test set in 8:1:1. We used (m/z, intensity, retention time) as the input of a PointNet network model (10.1109/CVPR.2017.16). The output is the theoretical spectrum containing the classification result of each peak (noise or signal) and the predicted intensities of all the signal peaks. The model is trained using a generative adversarial network framework.
Results: The performance of PeakFilter was evaluated on a test set: F1-score (0.56), recall (0.82), precision (0.42), and accuracy (0.90 for peak intensity prediction and 0.79 for peak classification).
Conclusions: The generative denoising method (PeakFilter) has shown its potential ability to distinguish the signals with a low signal-to-noise ratio from noise interferences. The performance of PeakFilter still needs to be improved by optimizing the training process.

PP02.053: Development of Proteomics-based Assays for Detection and Prognosis of COVID-19

Srivastava S[1], Suvarana K, Nair D
[1]Indian Institute Of Technology Bombay

Introduction

The history of COVID-19 from December, 2019 to till date is well known. Varying symptoms of the disease has created great challenge in understanding which variable is influencing the diagnosis, severity rate and mortality of the disease. Studies that can quickly predict diagnosis, and prognosis of COVID-19 should be prioritized. We have used plasma and nasopharyngeal swab sample to identify molecules that can be used as a therapeutic target to detect COVID-19 infection. Here we used mass spectrometry based analysis for viral detection and to determine the differentially expressed proteins. From our study we have detected that host proteins like, LDHA, AST, STAT1 and C3 in swab, and AGT, FGG, APOB in plasma samples were differentially expressed in COVID-19 positive samples. Additionally, we have identified some pathways such as neutrophil degranulation, cytokine signaling, and complement activation that are getting altered under COVID-19 infection.

Methods

Plasma and swab samples were collected from COVID-19 infected patients at Kasturba Hospital, Mumbai. Deep proteomic analysis of plasma and swab was performed using Orbitrap Fusion LC-MS/MS. Targeted assays were developed using Triple Quadrupole Altis.

Results

MS analysis could differentiate COVID-19 patients based on the severity. IL-6, LDHA, AST, CRP and ferritin were found to be upregulated in COVID-19 positive swab samples. AGT, SERPINA3, SERPIN1, APOB, LCP1 and CEP were differentially expressed in plasma samples and validated using MRM. Targeted approach could detect viral peptides in COVID-19 positive patient samples. ATR-FTIR based rapid detection method simplified the detection of COVID-19 severity based classification.

Conclusion

MS based analysis of COVID-19 could help us develop MRM-based assay for viral detection and prognostic assay for disease severity.

PP02.054: PeptidoformViz is a Shiny App for Processing, Visualising and Analysing Mass Spectrometry based Intensity Data on Peptidoform Level

Demeulemeester N[1,2], Provez L[1], Corveleyn L[1], Van Puyvelde B[1], Dhaenens M[1], Martens L[1,2], Clement L[1]

[1]Ghent University, [2]VIB

Introduction: Histones are known to carry a plethora of post-translational modifications (PTMs). Changes in these histone PTMs (hPTMs) have been linked to a variety of diseases. Nonetheless, the mapping of hPTMs, with the aid of mass spectrometry based proteomics (MS) has not been extensively described.

Methods and results: Therefore, we have made the first untargeted map of the histone code for 21 T-cell acute lymphoblastic leukaemia cell lines. We have pre-processed the raw data into a fully accessible platform-independent Progenesis QIP project (1). Because data visualisation and pre-processing are important first steps when analysing data, but are often not trivial, we have developed an accompanying shiny app, PeptidoformViz (https://github.com/statOmics/PeptidoformViz), in which users can intuitively visualise the different peptidoforms in the dataset. It facilitates importing, pre-processing and visualization of peptide level intensity data and will be updated to include statistical analyses in future versions. The app currently consists of three panels: data import, pre-processing and visualization. As input, the mentioned histone map or personal data can be easily uploaded and explored. In the different panels, users have the option to normalize the peptidoform intensities towards the average/median peptidoform intensity of a selected protein. Hence, both absolute peptidoform abundances or relative abundances, also referred to as peptidoform usage, can be visualised. Currently, we are expanding the app with formal statistical analyses, i.e. differential abundance (DA) analysis and usage at the peptidoform and ptm level. The statOmics group has previously published msqrob2, an R/Bioconductor package for DA at peptide or protein level (2) (https://www.bioconductor.org/packages/release/bioc/html/msqrob2.html) and we show that it can be used to perform DA at the peptidoform and ptm level.

Conclusions: The PeptidoformViz app currently provides pre-processing and visualization options for peptidoform-level intensity data.

References:

1. L. Provez, bioRxiv, (2022).
2. L. J. E. Goeminne, Mol. Cell. Proteomics. (2016).

PP02.055: MaxQuantAtlas Creates Accurate Large-scale Human Body Protein Concentration Map from Heterogeneous Proteomics Data

Ferretti D[1], Shi Y[2], Sinitcyn P[1], Tiwary S[1], Browne C[2], Rusin S[2], Kuhn E[2], Breitkopf S[2], Walther D[2], Feeney C[2], Cox J[1], Sharma K[2]
[1]Computational Systems Biochemistry, Max Planck Institute Of Biochemistry, [2]Kymera Therapeutics
Introduction. A fundamental understanding of protein abundance of all expressed proteins in human body resolved to various tissues and cell types is key to various applications in biomedical research. With the widespread use of MS-based shotgun proteomics, countless datasets of different human cell types and tissues with deep proteome coverage are constantly being added to repositories providing valuable quantitative information on proteome-wide protein copy numbers. However, it remains largely underused because of technical challenges to compare protein levels across individual studies. Here we introduce MaxQuantAtlas, a software platform for the integration of MaxQuant-processed proteomics datasets over many samples acquired with label-free and label-based quantification strategies and instrument types.
Methods. PSM level data from individual MaxQuant-processed projects are used as input. MaxQuantAtlas generates a unified protein groups meta-table with newly assembled quantification results for all label-free, MS1-labeled, isobaric labeled DDA and DIA samples across all projects. For isobaric labeling samples, we introduce algorithms for ratio decompression and combined MS1-MS2 quantification to obtain cellular protein abundances. MS signals are matched to concentrations using the Proteomic Ruler method. We introduce a novel imputation method that is compatible with combining data from sources with different dynamic ranges. A novel two-dimensional quality score based on estimates of sample dynamic range and correlation of housekeeping proteins allows for detection of problematic samples leading to their automatic exclusion.
Results. Using comparative analyses of expression patterns, we identify differentiated opportunities for selective pairings of E3 ligases with therapeutic targets of interest. The quantitative readouts from MaxQuantAtlas show good correlation with precision method results provided by our LED (ligandability, expression, degradation) strategy as novel protein quality-map control.
Conclusion. MaxQuantAtlas enables assembly of first-in-class human protein concentration atlas over cell lines, primary cells, healthy and diseased tissues which are clustering in biologically meaningful ways, independent of quantification and acquisition technologies.

PP02.056: Prosit: One Model to Predict Them All

Gabriel W[1], Sternlicht V[1], Shouman O[1], Mohamed R[1], Zolg D[2], Lautenbacher L[1], Kuster B[2], Wilhelm M[1]
[1]Computational Mass Spectrometry, [2]Chair of Proteomics and Bioanlytics

Introduction

The prediction of peptides' fragment intensities has gained significant attention over the last few years. However, only a few studies focused on a small set of post translational modifications (PTMs). PTMs play a significant role in protein function, allowing cells to respond to stimuli quickly. Moreover, PTM crosstalk is an essential factor in defining protein function. Here, we present a new Prosit model to predict fragment ion intensities of peptides with multiple and unseen PTMs for CID and HCD fragmentation, all in one model.

Methods

Peptides from ProteomeTools were used for training, covering 60 different peptide classes across a pool of unlabeled, labeled, tryptic, non-tryptic, and ~15 PTMs. We systematically investigated methods of encoding PTMs. Finally, a novel data augmentation strategy enabled Prosit to extrapolate its prediction capabilities to modifications not contained in the training data.

Results

The model with the best accuracy encodes PTMs by listing the atoms gained, lost, and the location of the modification. The new Prosit model achieved very high levels of agreement between predicted and experimentally acquired spectra for seen modifications, reaching a normalized spectral angle (SA) of 0.9 for HCD and 0.86 for CID. Furthermore, we tested the model on 15 modifications not contained in the training data. On average, a prediction performance of 0.87 and 0.84 was achieved for HCD and CID, respectively. We also see high levels of agreement for peptides with multiple PTMs (up to 5) with an SA of 0.85. Because PTMs often result in dominant neutral losses, we extended Prosit to predict the intensities of fragments of relevant neutral losses, achieving an average spectral angle of 0.81.

Conclusions

We have developed a new Prosit model that allows the prediction of tandem mass spectra for any modified peptide, and we show its application on rescoring and confident site localization.

PP02.057: ProteomicsML: An Online Platform for Community-curated Datasets and Tutorials for Machine Learning in Proteomics

Rehfeldt T[1], Gabriels R[2,3], Bouwmeester R[2,3], Gessulat S[4], Neely B[5], Palmblad M[6], Perez-Riverol Y[7], Schmidt T[4], Vizcaíno J[7], Deutsch E[8]
[1]Institute for Mathematics and Computer Science, University of Southern Denmark, [2]VIB-UGent Center for Medical Biotechnology, VIB, [3]Department of Biomolecular Medicine, Ghent University, [4]MSAID Gmbh, [5]National Institute of Standards and Technology, [6]Center for Proteomics and Metabolomics, Leiden University Medical Center, [7]European Molecular Biology Laboratory, European Bioinformatics Institute (EMBL-EBI), [8]Institute for Systems Biology

Introduction

Interest in machine learning (ML) applications in computational proteomics has increased tremendously in recent years. Nevertheless, due to the complex data formats and preprocessing steps required to make proteomics data ready for ML, there is a steep learning curve for anyone trying to enter the field. This hinders both reproducibility of newly published methods and rapid advancements in the field. To alleviate these issues, we present ProteomicsML, an online platform for community-curated datasets and tutorials for ML in proteomics.

Methods

The ProteomicsML platform is centered around proteomicsml.org, a static website that is generated from markdown files and Jupyter notebooks using the Quarto framework. All source files are hosted on GitHub, where everyone in the community is welcome to contribute by opening discussions, issues, or pull requests to update or add new datasets and tutorials. Datasets can be any proteomics input file suitable for machine learning and can be presented in an ML-ready format, or as closer-to-raw data that is then preprocessed in one of the tutorials. The tutorials are presented as Jupyter notebooks that describe one or more steps in a fully functional machine learning workflow, using the provided datasets. Datasets and tutorials are available for various data types, currently including fragmentation intensity, ion mobility, peptide detectability, and retention time. All datasets and tutorials can easily be viewed on proteomicsml.org, and tutorials can be opened for interactive use on Google Colab.

Results and conclusions

ProteomicsML is an open and dynamic online platform that aims to provide newcomers to the field with an easier starting point. All contents are available on github.com/ProteomicsML under CC-BY 4.0 license, and we welcome everyone in the community to join and contribute to the platform.

PP02.058: psm_utils: A High-level Python API, CLI, and Web Interface for Parsing and Handling Peptide-spectrum Matches and Proteomics Search Results

Gabriels R[1,2], Declercq A[1,2], Bouwmeester R[1,2], Degroeve S[1,2], Martens L[1,2]
[1]VIB-UGent Center for Medical Biotechnology, [2]Department of Biomolecular Medicine, Ghent University

Introduction

A plethora of search engine output file formats are in circulation, greatly complicating generic downstream processing of peptide-spectrum matches (PSMs) and PSM files. While standards exist to solve this problem, these are not (yet) universally supported by search engines. Some software libraries are available to read various PSM file formats, but a light-weight package to parse PSM files into a unified data structure has been missing. Here, we present psm_utils, a Python package to read and write various PSM file formats and to handle peptidoforms, PSMs, and PSM lists in unified and user-friendly Python, command line, and web interfaces.

Methods

psm_utils was developed with pragmatism and maintainability in mind: (1) Notations follow the HUPO-PSI ProForma 2.0 and Universal Spectrum Identifier standards; (2) data handling is optimized with Pydantic data classes; (3) existing packages such as Pyteomics and psims are re-used where possible; and (4) the package is split into two sublibraries: psm_utils and psm_utils.io. psm_utils provides a unified, high-level API for search engine data, while psm_utils.io provides the modules for reading and writing various file formats from and into PSM lists, allowing these to be easily added or updated as needed, without changing the core psm_utils API.

Results and conclusions

psm_utils provides a truly generic, developer-friendly Python API and CLI for handling PSMs, and greatly facilitates handling various PSM file formats. Moreover, a user-friendly web interface was built using psm_utils that allows anyone to interconvert PSM files and retrieve basic PSM statistics. psm_utils is freely available under the permissive Apache2 open-source license at https://github.com/compomics/psm_utils, and the community is very welcome to contribute to this project.

PP02.059: Discovery of Novel CSF Biomarkers to Predict Progression in Dementia using Machine Learning

Gogishvili D[1], Abeln S[1], Koppes S[2], Vromen E[2,3], van der Flier W[3], Lemstra E[3], Pijnenburg Y[3], Tijms B[2,3], Visser P[3], Del Campo M[2,4], Teunissen C[2], Vermunt L[2]
[1]Computer Science, Vrije Universiteit Amsterdam, [2]Neurochemistry Laboratory, Department of Clinical Chemistry, Amsterdam Neuroscience, Amsterdam University Medical Centers, Vrije Universiteit, [3]Department of Neurology, Alzheimer Center Amsterdam, [4]Barcelona Beta Research Center

Introduction

A heterogeneous disease course characterizes dementia and there are significant differences in the rate of cognitive decline among patients. It is currently challenging to provide an accurate prognosis for individual patients after the diagnosis. In this study, we aimed to identify CSF proteins predictive of the speed of decline in patients with dementia.

Methods

We used machine learning (ML) techniques to identify a panel of biomarkers that best predict the speed of cognitive decline. The patient sample included 196 cognitively normal individuals and 210 patients with Dementia. We created two progression groups (fast and slow) within the dementia patients based on the longitudinal cognitive measurements. All CSF samples were analyzed with Olink proteomics and used for training ML classifiers. Shapley values and Gini importance measures were used to interpret the model performance and pinpoint promising biomarkers. Lastly, functional analysis was used for identifying enriched pathways.

Results

In this study, we identified several candidate CSF markers that carry predictive value and can potentially disentangle disease progression in dementia patients. LAP TGF beta-1 emerges as an interesting biomarker for the disease progression being downregulated in fast-progressing patients. Proteins showing a negative correlation with fast progression are enriched in cell signaling and immune response pathways, which could indicate either downregulation at the later disease stages or a lack of a protective response in these patients.

Conclusions

Together, these results suggest that our panel of CSF protein leads can potentially offer useful prognostic information: (i) provide patients with more personalized predictions on disease progression, (ii) aid personalized treatment strategies, and (iii) clinical trials.

PP02.060: SIMSI-Transfer: Reduction of Missing Values in Clinical Proteomic and Phosphoproteomic TMT Data using MS2 Spectrum Clustering

Hamood F[1], Bayer F[1], Wilhelm M[1], Kuster B[1], The M[1]
[1]Technical University of Munich

Introduction

Although missing values are rare in single-batch TMT experiments, they rapidly increase when combining multiple TMT experiments. This is especially relevant in large-scale cancer cohort studies, where many experiment batches must be compared. Here, we present SIMSI-Transfer, a tool that uses MS2 spectrum clustering to cluster spectra by similarity and transfer peptide identifications across TMT batches. This increases the information gained for each batch and reduces missing values across experiments.

Methods

Our pipeline applies the MS2 spectrum clustering tool MaRaCluster to process multi-batch TMT datasets. Spectra clustered together are assumed to originate from the same precursor peptides. The generated clusters are merged on spectrum level with database search results from the quantitative proteomics platform MaxQuant, enabling identification transfers between spectra in the same cluster, including across different experiment batches. We applied SIMSI-Transfer to large-scale studies to assess the gain in evidence usable for clinical evaluation. We analyzed different types of sample data, from studies conducted by the CPTAC consortium as well as 203 sarcoma samples from precision oncology programs processed in our labs.

Results

The number of PSMs in three CPTAC cohort studies was increased by 26-45% for global proteome and phosphoproteome samples, while <1% of the clusters were considered false positives. In a masking analysis, a recall of up to 85% of all masked spectra was observed while maintaining an FDR below 1%. Applying SIMSI-Transfer on the sarcoma tissue analysis resulted in each peptide on average being identified in 13-15 patients more compared to the MaxQuant only analysis.

Conclusions

We implemented SIMSI-Transfer, a tool that can be used in conjunction with established quantitative proteomic data analysis pipelines for TMT experiments to significantly reduce missing values across batches. We found that applying SIMSI-Transfer to patient cancer studies increases the cancer proteome coverage, including well-known oncogenes usable for clinical decision-making.

PP02.061: LiBT : an All-in-One Proteomic Analyzer for Three Types of Proteomic Data: LFQ, iBAQ, and TMT

Lee H[1], Ahn J[2,3], Kim H[2,3], Kim K[3], Han D[2,3]
[1]Bioinformatics Collaboration Unit, Department of Biomedical Systems informatics, Yonsei University College of Medicine, [2]Proteomics Core Facility, Biomedical Research Institute, Seoul National University Hospital, [3]Transdisciplinary Department of Medicine and Advanced Technology, Seoul National University Hospital

Introduction :

With the popularization of quantitative proteomics techniques, developing methods to easily and accurately collect and analyze data is of utmost importance. The growing volumes of mass spectroscopic data has led to the development of analytical tools to quantify proteins, consequently resulting in several mass spectroscopy (MS)-based shotgun proteomic applications. Although as powerful as it is, MS-based shotgun proteomic analysis requires adept programming skills and the ability to survey an exhaustive amount of data using R packages.

Methods:

LiBT is a web-based platform built using R-Shiny. The platform customized its function using HTML/CSS, JavaScript, shinydashboard, shinyjs, shinywidgets, and other scripts. With the straightforward web-based interface of LiBT, users can easily navigate a program. Proteome analysis in LiBT uses R libraries, including limma, edgeR, and fgsea to identify differentially expressed protein (DEP), and furthermore, it can produce downstream analysis such as GSA or GSEA. The results from GSA or GSEA can be used to map the KEGG pathway. LiBT-enabled PPI network analysis uses the STRING database.

Results:

To provide a more efficient and accessible solution, we developed “LiBT”, a comprehensive web-based analytics tool that allows easy navigation without substantial need for prior programming knowledge. Compared to the original DEP library, LiBT incorporates more data types, namely LFQ, iBAQ, and TMT. LiBT also does not require adding the experimental design because the GUI allows researchers to choose the design settings themselves. Furthermore, LiBT incorporates additional analyses in an all-in-one setting: GSA, GSEA, and PPI. Our model provides seamless integration of these three additional analyses based on DEA results.

Conclusions:

LiBT provides an easy-to-use platform for analyzing labeled as well as label-free proteomic data. With its straightforward design and easily accessible open platform, LiBT allows easy utilization of bioinformatics tools without the need for extensive background knowledge in bioinformatics

PP02.062: Unsupervised Identification of Protein Assemblies in a High-Resolution Yeast Mitochondria Complexome Dataset

Schulte U[1], Haupt A[1], Müller C[1], Bildl W[1], Pfanner N[2], Fakler B[1], Becker T[3]
[1]Institute of Physiology II, Faculty of Medicine, University of Freiburg, [2]Institute of Biochemistry and Molecular Biology, ZBMZ, Faculty of Medicine, University of Freiburg, [3]Institute of Biochemistry and Molecular Biology, Faculty of Medicine, University of Bonn

Introduction

Complexome profiling is an emerging powerful approach for identifying protein assemblies and their subunit composition in biological samples through combination of biochemical fractionation of complexes with quantitative mass spectrometric analysis. In practice, its performance is strongly limited by the resolution of the fractionation step. Recently, we obtained the first comprehensive high-resolution complexome dataset of yeast mitochondria ("MitCOM") by combining blue native gel electrophoresis with cryo-slicing sampling and mass spectrometric analysis (csBN-MS). Protein profiles were obtained for >90% of the known mitochondrial proteome, revealing an unexpected complexity (>5200 resolved peaks). This dataset served to develop an advanced processing pipeline for unsupervised identification of protein complexes and subunits (analogous to ComplexFinder [Nolte and Langer, 2021]).

Methods

Protein profiles were decomposed into multiple Gaussian-shaped components. Identified peak centroids were used to guide fitting of a multi-Gaussian composite model, separating even largely overlapping components. After filtering, each component and its corresponding protein profile section was compared to matching sections in all other profiles using a custom similarity metric. The resulting distance matrix served as input for hierarchical clustering (DBSCAN) and visualization (t-SNE).

Results

Application of our evaluation pipeline to MitCOM provided a highly dense map resolving more than a thousand high-confidence protein assemblies. Well-characterized protein complexes from literature were used to assess selectivity and sensitivity of the approach. In addition, we identified and AP-MS-verified a number of novel complexes and complex subunits even from overlapping peaks.

Conclusions

- MitCOM is a valuable, comprehensive resource for analyzing the molecular organization of mitochondrial machineries and pathways.
- The effective resolution and sampling of the fractionation step as well as the precision of the protein quantification are key parameters of complexome profiling.
- The presented unsupervised data processing pipeline provided deep insight into the molecular organization of the yeast mitochondrial proteome with unmet precision and reliability.

PP02.063: Pipelines And Systems for Threshold Avoiding Quantification of LC-MS/MS data (PASTAQ)

Sànchez Brotons A[1], Eriksson J[6], Kwiatkowski M[7], Wolters J[4], Kema I[2], Barcaru A[1], Kuipers F[4], Bakker S[5], Bischoff R[1], Suits F[3], Horvatovich P[1]
[1]Department of Analytical Biochemistry, University of Groningen, [2]Department of Laboratory Medicine, University Medical Center Groningen, [3]IBM Research - Australia, [4]Department of Pediatrics, University Medical Center Groningen, [5]Department of Internal Medicine, University Medical Center Groningen, [6]Department of Biomedical Engineering, Lund University, [7]Functional Proteo-Metabolomics, Department of Biochemistry, University of Innsbruck

Introduction

Lower intensity signals in proteomics LC-MS/MS data may contain relevant biological information and should not be discarded. Furthermore, the majority of existing tools rely on MS/MS identification for quantitative pre-processing, which is due to the stochasticity of the precursor ion selection in data-dependent acquisition (DDA) methods lead to the quantification of a fraction of the measured biological compounds. This work presents a single-stage (MS1) LC-MS(/MS) pre-processing pipeline, which extracts all compound relevant signals irrespective of identification annotation or peak abundances.

2. Approach

The Pipelines And Systems for Threshold Avoiding Quantification (PASTAQ)[1] offers a toolset for accurate quantification and pre-processing of DDA or MS1 only LC-MS(/MS) datasets. The pre-processing algorithms in PASTAQ operate on MS1 data, with support for annotations and identifications linkage in the standard mzIdentML format such as obtained from MS-Fragger[2] or SearchGUI/PeptideShaker[3].

PASTAQ is implemented in C++ and offers Python bindings for the creation of tailored pipelines or integration with an existing ecosystem of mass spectrometry tools. The code is available at https://github.com/PASTAQ-MS/PASTAQ. This pipeline is simple to parametrize, requiring only to adjust the expected width of chromatographic peaks, type of mass spectrometry instrument used as well and its resolution at the reference m/z.

3. Results and discussion

We showed the performance of PASTAQ with three different datasets: a proteome mixture at different ratios, a HeLa matrix spiked in with an artificial concatemer and a publicly available human serum dataset.

PASTAQ identification annotation currently is limited to proteomics application, however the MS1 capability of PASTAQ allow to process untargeted metabolomics, lipidomics, or mixed omics samples and can be used to pre-process stable-isotope labeled proteomics and metabolomics LC-MS(/MS) data. Current development includes pseudo spectra extraction from DIA LC-MS/MS data and visualization of raw data, smoothed resampled LC-MS/MS data and location of detected peak using OpenGL API.

Code: https://github.com/PASTAQ-MS/PASTAQ

PP02.064: SILAC Proteomics Reveals that SARS-CoV-2 Spike Glycoprotein Regulates the Proteome of Kidney Epithelial Cells

Onat B[1], Farkona S[3,4], Clotet-Freixas S[3,4], Boshart A[3,4], Soosaipillai A[5,6], Diamandis E[5,6,7], Yılmaz E[1,2], Konvalinka A[3,7,8,9,10]
[1]Ankara University Biotechnology Institute, [2]Ankara University, [3]Toronto General Hospital Research Institute, University Health Network, [4]Renal Transplant Program, Soham and Shaila Ajmera Family Transplant Centre, University Health Network, [5]Division of Clinical Biochemistry, Department of Laboratory Medicine & Pathobiology, University of Toronto, [6]Department of Laboratory Medicine and Pathobiology, University of Toronto, [7]Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, [8]Dept. of Medicine, Division of Nephrology, University Health Network, [9]Institute of Medical Science, University of Toronto, [10]Multi-Organ Transplant Program, University Health Network, [11]Laboratory Medicine and Pathobiology, University of Toronto

Introduction: COVID-19 causes acute kidney injury. Evidence suggests that SARS-CoV-2 virus directly infects kidney epithelial cells. SARS-CoV-2 enters cells mainly through spike protein-mediated binding to the ACE2 receptor and subsequent internalization. We postulated that spike protein alone can trigger endoplasmic reticulum stress, a typical response of the cells to most viruses, and alter the proteome of human kidney epithelial cells.

Methods: For quantitative proteomic analysis using SILAC, HK-2 kidney tubular epithelial cells were cultured in the presence of heavy arginine (13C6) and lysine (13C615N2) for 7 doubling times, as reported before(1). Light-labeled HK-2 cells were treated with 10nM of recombinant SARS-CoV-2 spike protein or vehicle for 6, 12, 18hrs. Equal total protein amounts of heavy-labeled and light-labeled cell lysates were mixed and analyzed together. After protein denaturation, reduction, alkylation and digestion with trypsin, peptides were analyzed using LC-MS/MS on Q-Exactive HFX instrument. RAW files were analyzed using MaxQuant. Heavy-to-light protein ratios between the different treatments and time-points were analyzed using Perseus. Pathway and interaction analyses were performed using pathDIP and IID.

Results: Spike protein treatment, at 10nM dose, resulted in increased HK-2 cytotoxicity after 8 hrs. From 2927 quantified proteins (FDR<0.01), 74 were significantly altered by spike protein treatment (two-way ANOVA, $p<0.05$). Similarly, 34 proteins were significantly altered by both treatment and time (two-way ANOVA interaction, $p<0.05$). PathDIP analysis revealed enrichment in spliceosome ($q=0.006$), RNA metabolism ($q=0.007$), translation initiation ($q=0.007$), and apoptosis ($q=0.037$) among proteins significantly altered by spike treatment. Interestingly, the pathway “protein processing in endoplasmic reticulum” ($q=0.043$) was also enriched among these proteins. Beta-catenin and Heterogeneous nuclear ribonucleoprotein-F were the hub proteins in the two protein-protein interaction networks.

Conclusions: SARS-CoV-2 spike protein regulates the proteome of kidney cells in vitro. Further validation would support SARS-CoV-2 spike protein’s potential regulatory roles in kidney cells.

References: (1)J. Biol.Chem. 288, 24834–24847(2013).

PP02.065: Deep Multiomics Gene Expression Prediction Convolutional Model

Ilgisonis E[1], Pichugin I[1], Valdman B[1], Lisitsa A[1]
[1]Institute Of Biomedical Chemistry

Deep neural network has become increasingly popular tool for the biomedical research during the recent years. The most often deep learning applications in proteomics include classification, biomarker identification, retention time prediction, MS/MS spectrum prediction, de novo peptide sequencing, PTM prediction, major histocompatibility complex-peptide binding prediction, and protein structure prediction.

With the aim to predict quantitative proteomes we integrated different data domains and adopted visual transformers for experimental data extrapolation in silico.

Original experimental data used for the model training phase included the results of transcriptomic profiling (RNA-seq and Oxford Nanopore) and deep proteomic profiling using two-dimensional fractionation of 6 cell lines. In addition to our original data, we assembled data from GO, neXtProt, Protein Atlas etc. Vectorization of data for model input involves using of main genecentric bases of knowledge and a module to search between IDs systems of each database. The model has an ability to imitate expression of mutated genome region by directly modifying aminoacids or nucleotides sequences.

The architecture is a fully convolutional light-weighted residual deep network with 10 channels height-width variable input with embedding analysis with attention mechanisms and ability of transfer learning.

Using our model, we have found the correlation between predicted and measured protein concentrations. We propose the deep learning network as a promising tool to predict quantitation for missing proteins.

This work was supported by the Russian Science Foundation (RSF Grant 20-15-00410; http://www.rscf.ru/)

PP02.066: ScanBious: Survey for Obesity Genes Using PubMed Abstracts and DisGeNET

Ilgisonis E[1], Tarbeeva S[1], Kozlova A[1], Lisitsa A[1], Ponomarenko E[1]
[1]Institute Of Biomedical Chemistry

Obesity is a disease that is the result of general metabolic disorders or lipid metabolism disorders. In our study DisGeNET data were compared with PubMed/MEDLINE automated processing data to analyze the current level of knowledge about the molecular mechanisms of obesity and to identify key concepts. The DisGeNET resource was used as a platform for collecting information on genes associated with obesity, the UniProt resource as a source of gene names, and PubMed as a source of articles. Downloading publications from PubMed, their abstracts, MeSH terms, as well as analyzing the frequency of occurrence of MeSH terms and visualizing the relationships between them were performed using the ScanBious web tool (a free system for highlighting key concepts identified from PubMed articles https://scanbious.ru/).

We analyzed over 100,000 obesity-related publication abstracts using the ScanBious web tool. Comparing them with UniProt's Gene Name Dictionary, we came up with a set of 622 genes that could potentially be associated with obesity. Data on the relationship of these genes to each other was then obtained using ScanBious, which is directly linked to the PubMed database.

Information exhaustion was carried out: out of hundreds of thousands of publications and hundreds of genes, the extremely broad problem of obesity was reduced to just a few clusters, which made it possible to narrow the vast field of knowledge to a set of concepts that reflect current development trends and key molecular factors of obesity (LEP, TNF, and so on).
Combining the DisGeNET database with ScanBious's processing of PubMed-based articles has reduced the vast field to a limited number of relationships, highlighting several underestimated factors of inflammation, glucose metabolism regulation, and heredity as integral aspects of obesity.
The study was supported by RFBR grant No. 19-29-01138.

PP02.067: Transfer Learning to Tune Prosit to Predict Intensities for Cleavable Cross-linked Peptides

Kalhor M[1], Wilhelm M[1]
[1]Computational Mass Spectrometry

Introduction: Chemical cross-linking mass spectrometry (XLMS) is an effective tool for analyzing protein structure and protein-protein interactions. However, due to the large search space, identifying cross-linked peptides remains a challenging task. Prior research has shown that incorporating fragment intensity information into the matching process can circumvent this problem. Here, we extend Prosit to predict the fragment ion intensities for cleavable cross-linked peptides using minimal data.

Methods: The main challenge is the limited number of training data. For this reason, we systematically evaluated methods to fine-tune a pre-trained model of Prosit to allow the prediction for unknown modifications using the concept of transfer learning. To provide training and test datasets, XL datasets were analyzed using plink2. In the end, the accuracy of the prediction model was improved by considering pre-trained unmodified amino acids for modified amino acids.

Results: We designed an experiment to determine the minimal set of peptides necessary to fine-tune a pre-trained Prosit model for unmodified amino acids to predict TMT-labeled peptides. The results suggest that Prosit can be fine-tuned with ~100,000 TMT-labeled peptide spectra, where modified amino acids reuse the learned embedding of the pre-trained model from unmodified amino acids. The achieved results demonstrate that the prediction performance of this model is as good as training a model from scratch using >1M spectra. We applied this concept to cleavable cross-linked peptides, by using ~130,000 high-quality spectra of cleavable cross-linked peptides. The final model achieved an average spectral similarity (normalized spectral contrast angle) between predicted and experimental spectra of >0.75 (R>0.9).

Conclusions: In this work, we reused a pre-trained model of Prosit as a starting point and transfer learned it to predict fragment ion intensities for cleavable cross-linked peptides. This concept is applicable to any cross-linker.

PP02.068: Extending the Functional Proteomic Map by Integration of Gene Expression and Biological Annotation

Karlsson M[1], Bueno Alvez M[1], Shi M[1], Fagerberg L[1], Uhlen M[1]
[1]KTH Royal Institute of Technology - Science For Life Laboratory

Introduction: A grand objective of basic life science is to produce a complete annotation of the human proteins. The integration of gene expression data on transcriptomic and proteomic level has the potential to extend the annotated map encompassing the spatial and functional roles of proteins. Here, we report on a genome-wide effort to annotate all protein-coding genes based on single cell transcriptomics data representing major tissues and organs in the human body, integrated with bulk transcriptomics and antibody-based tissue profiling.

Method: We introduce a new genome-wide classification tool based on clustering protein-coding genes of similar expression profiles across 444 single cell type clusters and 54 tissue types, visualized using dimensional reduction (UMAP).

Results: Many genes with similar expression profiles across cell types share functional identities. As such, stratifying the transcriptional landscape into local neighbourhoods provides a framework to generate hypotheses of protein function of uncharacterized proteins. With this approach, we extend known functional and spatial annotation to uncharacterized proteins in a manner of “guilt-by-association”, generating testable hypotheses. Integration of the transcriptomic expression profiles with antibody-based tissue profiling enabled validation on protein level.

Conclusion: This analysis has allowed us to annotate all human protein-coding genes with regards to function and spatial distribution across individual cell types in all major tissues and organs in the human body. A new version 21 of the open access Human Protein Atlas (www.proteinatlas.org) has been launched to enable researchers to explore the new genome-wide annotation on an individual gene level.

PP02.069: Deep Learning Peptide Properties for Targeted Assay and Spectral Library Optimization

Lautenbacher L[1], Schmidt T[2], Blankenhagen L[1], Gabriel W[1], Kuster B[2], Wilhelm M[1]
[1]Computational Mass Spectrometry , [2]Chair of Proteomics and Bioanalytics

Introduction

Deep learning has been shown to be useful for MS based proteomics by accurately predicting peptide properties like fragment intensity, retention time and ion mobility. While this covers important aspects of the LC-MS/MS workflow, other properties such as the expected precursor charge and proteotypicity, describing the probability of identifying a peptide, are underutilized in computational workflows due to the lack of accurate good predictors. Here we present a series of neural networks that aim to fill these gaps with their ability to accurately predict precursor charge, proteotypicity as well as ion mobility.

Methods

All introduced models use an encoder-decoder architecture based on Prosit. The proteotypicity model was trained as a siamese neural network with the goal to rank peptides by their proteotypicity, using data from ProteomicsDB. The ion mobility model was trained on collision cross section (CCS) data (Bruker timsTOF) and drift time data (Waters SYNAPT G2-S).

Results

The presented models show a high level of accuracy. The predominant precursor charge state can be predicted with an accuracy of 91%. For CCS, 95% of the predictions are contained within 7.8% of the available separation capabilities, achieving similar performance as other state-of-the-art methods while being applicable to instruments from multiple vendors. The proteotypicity model reaches a Spearman correlation of 0.82 between experimental and predicted proteotypicity showing that the model successfully learns to rank peptides of a protein by their proteotypicity. In addition to the predictors, we highlight some applications of these for tasks such as optimizing the content (and size) of spectral libraries for peptide identification, prioritizing peptides for targeted assays and rescoring database search engine results.

Conclusions

We present multiple new models for the prediction of peptide properties, replacing heuristics by accurate predictors for various steps of the bottom-up MS-based proteomics workflow.

PP02.070: PAEA: Pathway Association Enrichment Analysis for Unrevealed Experimental Metabolites

Anwar A[1], Ahmed E[1], Tanios A[1], Osama A[1], Ezzeldin S[1], Mahjoub S[1], Magdeldin S[1]
[1]Children's Cancer Hospital Egypt 57357

In the last decade, metabolomics has been evolved dramatically to better elaborate metabolome dynamics in different biological samples and disease statuses. To achieve this target, metabolomics strategies are categorized into two main approaches; targeted and untargeted. The latter approach is employed as an unbiased analytical method to identify all metabolite compositions, offering an opportunity for target small molecule discovery. However, more complex data are attained. Bioinformatics tools have been developed for metabolites pathway enrichment analysis in which a group of detected metabolites is tested statistically for over-representation in a specific pathway. However, enrichment pathway analysis per se will suffer from a bias in most cases caused by missed metabolites detection in untargeted Metabo-profiling. Consequently, the list of detected metabolites used may fail to enrich some pathways that may be critically relevant to a study. In the current study, we introduced a novel method utilizing the association between undetected and detected metabolites to enrich more possible pathways, named Pathways Association Enrichment Analysis (PAEA). Testing different case study scenarios with different multi-omics approaches (proteomics and metabolomics) validated that PAEA could enrich biological significant pathways that could not be seen using the traditional enrichment analysis method. Finally, we developed a free online shiny application in the R environment https://proteomicslab57357.shinyapps.io/PAEA/ for ease of use. Full documentation on how to use the PAEA tool application is available as a PDF file https://github.com/AliYoussef96/PAEA/blob/main/Documentation.pdf and as a tutorial video https://drive.google.com/file/d/1OKhkrU4O7niL-UA25bD4dy4t1VU9UiBq/view. The code is also available on the GitHub repository for re-usability at https://github.com/AliYoussef96/PAEA.

PP02.071: UniprotR: Retrieving and Visualizing Protein Sequence and Functional Information from Universal Protein Resource (UniProt Knowledgebase)

Magdeldin S[1], Mostafa Anwar A, Ali Ahmed E, Osama A, Mahjoub S, soudy m
[1]Children Cancer Hospital 57357

Introduction: UniprotR is a software package designed to easily retrieve, cluster and visualize protein data from UniProt knowledgebase (UniProtKB) using R language. The package is implemented mainly to process, parse and illustrate proteomics data in a handy and time-saving approach allowing researchers to summarize all required protein information available at UniProtKB in a readable data frame, Excel CSV file, and/or graphical output. Methods: R language is used to construct an R package available in CRAN. Results: UniprotR generates a set of graphics including gene ontology, chromosomal location, protein scoring and status, protein networking, sequence phylogenetic tree, and physicochemical properties. In addition, the package supports clustering of proteins based on primary gene name or chromosomal location, facilitating additional downstream analysis. Conclusion: In this work, we implemented a robust package for retrieving and visualizing information from multiple sources such UniProtKB, SWISS-MODEL, and STRING. UniprotR Contains functions that enable retrieving and cluster data in a handy way and visualize data in publishable graphs to facilitate researcher's work and fulfill their needs. UniprotR will aid in saving time for downstream data analysis instead of manual time consuming data analysis.

Soudy M, Anwar AM, Ahmed EA, Osama A, Ezzeldin S, Mahgoub S, Magdeldin S. UniprotR: Retrieving and visualizing protein sequence and functional information from Universal Protein Resource (UniProt knowledgebase). J Proteomics. 2020 Feb 20;213:103613. doi: 10.1016/j.jprot.2019.103613. Epub 2019 Dec 14. PMID: 31843688.

PP02.072: Integrative Multiomics Analysis to Infer COVID-19 Biological Insights.

Yahia M[1], Khalaf H[2], Anwar A[1], Fekry A[1], Ahmed E[1,3], Mahgoub S[1], Ezz El-Din S[1], Tanios A[1], Alfishawy M[4], Said A[5], Mohamed M[6], Sayed A[7,8], Magdeldin S[1,9]

[1]Proteomics and Metabolomics research program, Basic research department, Children's Cancer Hospital 57357 (CCHE-57357), Cairo, Egypt, [2]Intensive care unit, As-Salam International Hospital, [3]Department of Pharmacology, Faculty of Veterinary Medicine, Suez Canal University, [4]Infectious Diseases Consultants and Academic Researchers of Egypt (IDCARE), [5]Department of Pulmonary Medicine, Faculty of Medicine, Minia University, Minia, [6]Department of Anesthesia and Intensive care, kasralainy, Cairo University, [7]Genomics Program, Department of Basic Research, Children's Cancer Hospital 57357, [8]Department of Biochemistry, Faculty of Science, Ain Shams University, [9]Department of Physiology, Faculty of Veterinary Medicine, Suez Canal University

Introduction: On December 31, 2019, a cluster of “Viral pneumonia” cases from an unknown source was reported in Wuhan, China, with a nearly consistent plethora of symptoms similar to severe acute respiratory syndrome (SARS). First, it was named the new coronavirus 2019 (nCOV-2019) until it was recently renamed a (SARS-CoV 2) by the international committee on taxonomy of viruses due to its high homology to the older (SARS-COV). The mystery of the SARS-CoV 2 has been primordially deciphered without a clear understanding of the biological changes on the molecular level of biomolecules such as proteins, metabolites, and lipids.

Methods: To infer the most possible biological interactions in severe patients infected with the SARS-CoV-2 virus, a cohort of 57 control and severe COVID-19 patients was subjected to plasma proteomics and metabolomics profiling. Both proteome and metabolome were analyzed with different depths and approaches to unleash more details about the actual pathogen complexity, including single and different multi-omics methodologies.

Results: Our findings revealed the outperformance of the integration between the knowledge-based and statistical-based multi-omics approaches over other omics methods in unveiling the molecular landscape of COVID-19 patients. The superiority of this approach relies on combining both experimental statistical-based correlated molecules and the pre-defined knowledge-based cascade correlation.

Conclusion: The versatile usage of knowledge-statistical multi-omics integration method could provide a reliable mechanistic overview for a better understanding of the molecular mechanisms behind any biological system and provide multi-dimensional therapeutic solutions, targeting more than one pathogenic factor at the same time.

PP02.073: SWATHAtlas: A Multi-organism Spectral Assay Library Resource for DIA-based Proteomics

Midha C[1], Bader S[1], Kusebauch U[1], Midha M[1], Campbell D[1], Mendoza L[1], Sun Z[1], Deutsch E[1], Moritz R[1]
[1]Institute For Systems Biology

Introduction: Advancements in data-independent acquisition (DIA) methods have immensely increased targeted quantitative analyses to over 10,000 proteins per analysis using mass spectral libraries. These spectral assay libraries need to be comprehensive, accurate and above all, trusted. Over many years, we have established and continually refined a trusted, rich multi-organism resource, SWATHAtlas (www.swathatlas.org), to facilitate the exchange, customization, assessment, and repairing of high-quality, experimentally-derived published spectral libraries. No other public resource allows quality assessment of the contents of their libraries. SWATHAtlas's main functions include: (i) Download– Allow users to download OpenSWATH/TraML/PeakView/Speclib/Spectronaut format libraries; (ii) Customize- Refine existing libraries based on listed parameters as per experimental needs; (iii) Quality assessment- New libraries can be fully quality assessed using our DIALib-QC tool (1); (iv) Upload Library- Facilitate and encourage to upload error-free new libraries from different organisms and MS instruments by the scientific community.
Methods: SWATHAtlas libraries of OpenSWATH, PeakView, and Spectronaut formats were assessed by DIALib-QC which reports 64 library attributes categorized as Complexity, Characteristics, Modification, Completeness, and Correctness. DIALib-QC can be run via the SWATHAtlas webpage. For large-size libraries, DIALib-QC can be downloaded (https://github.com/CharuKMidha/DIALib-QC) and used for in-house library assessment. SWATHAtlas is built in PHP, javascript with backend scripts for library assessment, and customized libraries are written in Perl.
Results: We host over 50 error-free reference spectral libraries for 12 organisms along with their assessment reports. The SWATHAtlas workflow allows users to assess, repair, and customize spectral assay libraries.
Conclusions: SWATHAtlas is an open-source repository of high-quality DIA peptide fragment assay libraries that are designed to aid in SWATH\DIA data processing across multiple species. It allows storing and sharing of comprehensive multi-organism, high-quality, experimental spectral assays that will capitalize on the invested time and efforts for broadly applicable DIA analysis.

Midha MK et al., Nat Commun. 2020, 11(1):5251, doi: 10.1038/s41467-020-18901-y

PP02.074: LC-MS Data Analysis Pipelines: Scaling Beyond the Desktop Environment

Mohtashemi I[1], Kitano H[1], Nicholes A[1], Just S[1], Wang J[1], Platt T[1], Batzoglou S
[1]Seer Inc

Introduction

Liquid chromatography coupled with mass spectrometry (LC-MS) has grown into a ubiquitous detection platform due to its speed, sensitivity, and applications. While instrumentation hardware continues to improve, the concurrent increase in translation from data to insight remains a bottleneck. Previously, we have demonstrated a cloud-based serverless task-based infrastructure where closed-source legacy algorithms are deployed as containerized applications leveraging AWS elastic container service. These algorithms are orchestrated with AWS services such as lambda functions and step functions. In this work, we focus on scaling label-free LC-MS data analysis workflows to enable large cohort studies using open-source algorithms leveraging distributed computing models in our AWS infrastructure

Methods

We have curated an AWS proteomics data analysis workflow with choices, error handling, and exception fallbacks including automated file transfer to the cloud and conversion to standard mzML, parquet and HDF5 filetypes, automates single file analysis for every injection upon raw data file arrival. User-specified group run analyses with pre-defined recipes and settings (possible with 1000s of files) and Spark-accelerated modular workflows built on top of open-source software.

Results

We demonstrate on-demand processing of thousands of DDA and DIA LCMS runs with the label-free quantitation workflow and benchmark several commonly used algorithms such MaxQuant, MSFragger, DIA-NN and Alphapept. Algorithm components are deployed in the AWS infrastructure. Modularization of pipelines, efficient data access at scale and distributed implementations of key algorithms components show promise for enabling statistical power in massive cohort studies where computational bottlenecks persist.

Conclusion

A next-generation platform capable of analyzing large cohort proteomics studies supporting fleets of vendor neutral LC-MS instruments. Supporting hundreds of terabytes of incoming LCMS data annually enabling large cohort group runs using Spark-accelerated workflows in the cloud computing framework supporting thousands of FDR-controlled group runs analysis.

PP02.075: Simple and Robust Workflow for the Processing of Ion Mobility-mass Spectrometry Raw Data from Complex Biological Samples

Mouton Barbosa E[1], Gonzalez De Peredo A, Bouyssié D, Burlet-Schiltz O
[1]Institut de Pharmacologie et de Biologie Structurale (IPBS), Université de Toulouse, CNRS, UPS, Toulouse, France

New generation of liquid chromatography – tandem mass spectrometry (LC-MS/MS) instruments associated to the use of ion mobility provide high complexity information that can extend our knowledge of the proteome of complex biological samples and even recover information from a single cell.
Although, such massive data is ultimately not complicated to obtain, the real bottleneck for the interpretation of this information is the processing of the raw files.
Here we present a user-friendly and reliable bioinformatics workflow to analyze Raw data from different manufacturers based on Data Dependent Acquisition (DDA) or Data Independent Acquisition (DIA), with or without the use of ion mobility.
This workflow includes MSAngel, an interface producing peaklists and launching search engine (Mascot, Xtandem, MSGF+, MSAmanda) it also includes Proline, a tool for peptide/protein validation through a target/decoy or a Percolator approach, and quantification, in both DDA label-free or isotopic labeling (TMT (Thermo)). Finally, Proline enables the visualization of quantified peptides and proteins from DDA experiments or from DIA experiments through the use of DIA-NN results.
We demonstrated the efficiency and the ease-of-use of our workflow through the processing of a Thermo raw file dataset acquired on an Orbitrap Exploris 480 with a FAIMS device.
The whole workflow is open source.

PP02.076: Serum Proteomics Study of COVID-19 Patients Over 90 Years

Quiñones-Vega M[1,2], Sosa-Acosta P[1,2], S. Guedes J[1,2], Pinto De Almeida N[1,2], Nascimento D[2], Silva M[3], Vidigal M[3], Naslavsky M[3], Passos-Bueno M[4], Zatz M[3], Nogueira F[1,2], Domont G[1]
[1]Proteomics Unit, Institute of Chemistry, Universidade Federal do Rio de Janeiro, [2]Laboratory of Proteomics (LabProt), LADETEC, Institute of Chemistry, Universidade Federal do Rio de Janeiro, [3]Human Genome Studies Center, Universidade de São Paulo, [4]Department of Biology, Universidade de São Paulo

Introduction: The mortality rate in SARS-CoV-2 infection is drastically increased with age. To study intrinsic/individual factors that may confer protection or susceptibility to infection in patients over 90 years we analysed the plasma proteome of elderly COVID-19 patients that came to death compared with the control group and recovered patients.
Methods: Groups of 3 samples each were investigated: (1) infected patients who had a fatal outcome, (2) recovered patients (collected between 20-30 days post-infection), and (3) non-infected control subjects. Samples were digested using S-trap protocol and quantified by label-free nLC-MS/MS. Also, 62 differentially expressed proteins were selected to corroborate the results by SRM technique.
Results: Analysis of altered proteins during infection compared to the control group shows up-regulated processes related to acute immune response and complement activation. Down-regulated proteins are present in the phosphatidylcholine metabolic process. Proteins that remain altered in the recovery group contrasted with controls are associated with vasoconstriction regulation. Targeted proteomics of 62 differentially expressed proteins, corroborate that A2M, APMAP, and ADIPO remain down-regulated 30 days of pos-infection.
Conclusions: Proteins that are differentially up-expressed during SARS-CoV-2 infection are primarily associated with immunity and complement activation. Proteins related to lipid transport decrease in plasma abundance even after 30 days of infection, indicating possible sequelae by the virus in elderly patients.

PP02.077: The 2022 Metrics of the HUPO Human Proteome Project

Omenn G[1], Lane L, Overall C, Pineau C, Packer N, Cristea I, Lindskog C, Weintraub S, Roehrl M, Nice E, Liu S, Bandeira N, Chen Y, Guo T, Aebersold R, Moritz R, Deutsch E
[1]University Of Michigan

The 2022 metrics of the HUPO Human Proteome Project (HPP) show that protein expression has now been credibly detected (neXtProt PE1 level) for 18 407 (93.2%) of the 19 750 predicted proteins coded in the human genome, a net gain of 50 since 2021 from datasets generated around the world and reanalyzed by the HPP. Conversely, the number of neXtProt PE2, PE3, and PE4 missing proteins has been reduced by 78 from 1421 to 1343. This represents continuing experimental progress on the proteome parts list across all the chromosomes, as well as significant reclassifications. Meanwhile, the application of proteomics in a vast array of biological and clinical studies continues to yield important findings and growing integration with other omics platforms. We present highlights from the Biology and Disease-driven HPP teams and the Resource Pillars.

PP02.078: Multiomics Data Integration using Shared Embeddings

Picciani M[1], Wilhelm M[1]
[1]Computational Mass Spectrometry

Introduction

How cells respond to drug treatment can be the result of a complex interplay between different biological processes. Thus, integrating data from multiple omics was suggested to enable accurate drug response prediction. However, true integration of multiple omics remains a challenging task and existing methods often investigate them separately. Here, we present a method for integration of arbitrary omics using a machine learning model that projects multiple inputs into a shared embedding and, thus, learns how to integrate multiomics.

Methods

Proteomics expression from 375 cell lines from CCLE and corresponding transcriptomics expression were projected into a shared embedding using two independent neural networks. Same cell lines map to the same location in the shared embedding while others are far apart. To estimate robustness, 5 replicates of proteomics data with 5% random artificial missing values are used. Cross validation was used to avoid overfitting of the results.

Results

Prior to training, the median distance between both correctly and incorrectly matching samples (cell lines) of proteomics and transcriptomics was 1.38 in euclidean space. After training, the median distance between correct cell line pairs dropped below 0.46 while the median distance between incorrect pairs increased to 1.41. Furthermore, for 80% of the 53 cell lines in the holdout the closest samples in the embedding were matching samples.

Conclusions

Our results suggest that the model learned to project proteomics and transcriptomics data into a shared embedding while retaining information necessary to separate the cell lines and thus biology. The method is extensible to other omics and shared embeddings of more than two inputs at the same time. The learned embeddings are a promising approach for multiomics data integration and open up a number of relevant applications, such as drug response prediction.

PP02.079: HaDeX: Easy-access Complex HDX-MS Data Analysis

Puchała W[1], Grzesiak K[2], Burdukiewicz M[3], Rafacz D[1], Kistowski M[1], Dadlez M[1]
[1]Institute of Biochemistry and Biophysics Polish Academy of Sciences, [2]University of Wrocław, [3]Autonomous University of Barcelona

Introduction: Hydrogen-deuterium mass spectrometry (HDX-MS) is an analytical tool for monitoring the dynamics and interactions of proteins. Unlike crystallography-based methods, HDX-MS allows a unique insight into the dynamics of the protein structure. Such data is complex and requires a dedicated solution. Following the needs of the HDX-MS community closely, we significantly improved our software HaDeX (1) and are happy to present a new version of the application.

Methods: HaDeX is a versatile software for processing, analyzing, and visualizing output data from existing tools used in HDX-MS experiments. The application provides a complete analytic workflow, with precise uncertainty calculations (2) and reporting features recommended in the community guidelines (3). The variety of visualization methods (including novel ones) ensures in-depth data exploration. HaDeX is freely available as a unique combination of web-server (https://hadex2.mslab-ibb.pl/), standalone application, and an open-source R-package.

Results: The application is significantly extended compared to its prior version. HaDeX contains all forms of visualizations employed by the HDX-MS community, e.g., chiclet, butterfly, manhattan, and volcano plots - for state and differential uptake. The profound quality control allows tracing the journey of the measurement data carefully. The analysis is adjusted for multi-state experiments, including novel deuterium uptake curve classification. All of the figures are of publication quality, featuring ISO-based uncertainty of the measurement.

Conclusions: As HDX-MS is getting more recognition, there is a growing need for standardized analysis and data processing following the community recommendations. Our answer to this issue is HaDeX, an open-source software ready to tackle all challenges associated with analyzing the HDX-MS data.

1. W. Puchała et al., Bioinformatics, doi:10.1093/bioinformatics/btaa587.
2. D. D. Weis, J. Am. Soc. Mass Spectrom. (2021), doi:10.1021/jasms.0c00475.
3. G. R. Masson et al., Nature Methods. 16, 595–602 (2019).

PP02.080: A User-friendly and Interactive Platform for the Quantitative Investigation of Proteomics Data

Schwämmle V[1], Bouyssié D[2], Jensen O[1], Rogowska-Wrzesinska A[1]
[1]University Of Southern Denmark, [2]University of Toulouse

Introduction:
Quantitative analysis and biological interpretation of proteomics data can be a demanding process. Different biological questions and respective experimental designs require the application of appropriate statistical methods as well as a careful assessment of their output. The field is lacking a user-friendly and highly interactive toolkit for the supervised analysis of quantitative peptide and protein expression profiles including modern methods for statistical testing, quantitative QC, clustering and the investigation of the quantitative behavior of protein complexes.

Methods:
We developed a user-friendly software interface for the analysis of quantitative profiles on peptide or protein level given as text or Excel file. This includes consistency checks of uploaded data tables, filtering for harmful features like unsuitable samples, harmonization of statistical power, and flexible integration of post-translationally modified peptides. The interface is implemented as an interactive application in R and Javascript and is available as web service on computproteomics.bmb.sdu.dk.

Results:
The resulting tool provides directly controlled, deep proteomics analysis by pre-processing a given table with minimal user effort for further extensive biological interpretation and visualization. More specifically, we provide integration with statistical testing including a test for missingness without the need for imputation (PolySTest), variance-sensitive clustering for improved recognition of co-regulated protein groups (VSClust) and extensive analysis of protein complex behavior (ComplexBrowser). The software is entirely based on the web browser, does not require any local installation, and has been designed to be easily plugged with other proteomics web applications. Extensive and flexible parametrization and visualization of the results allows full control of the data output and thus reveals the relevant biological content.

Conclusions:
We provide a highly user-friendly solution for in-depth investigation of the biological processes in multi-dimensional proteomics data sets, by determining the highly regulated proteins and giving deep insights into the behavior of co-regulated protein groups and complexes.

PP02.081: Highly Sensitive Proteomics Analysis of ZenoTOF DDA and DIA Data

Shan B[1], Xin L[1], Bian H[1], Shan B[1]
[1]Bioinformatics Solutions Inc.

Introduction: SCIEX recently introduced its innovative ZenoTOF 7600 system with the aim of capturing the daunting complexity of proteome. By integrating Zeno trap pulsing, electron activated dissociation (EAD) fragmentation, and Zeno SWATH, it enabled high-throughput and high-coverage proteomics experiments. Extensive research efforts are now devoted to algorithm development for the massive MS data that it generates.

Methods: Several analysis workflows dedicated to ZenoTOF DDA and SWATH DIA data have been implemented in our PEAKS proteomics platform. Based on the unique ion fragmentation patterns observed in the ZenoTof data, we refined algorithms in PEAKS to improve the peptide and protein identification greatly. We optimized the parameter setting to detect MS1 features, to estimate precursor charges, as well as the tolerances used in associating MS2 scans with MS1 features.

Results: We tested the performance of our analysis workflows in PEAKS on three ZenoTOF datasets that we obtained from SCIEX by courtesy. The first dataset comprises the fractionated DDA data collected from HeLa and K562 samples. The second and third are the Zeno SWATH DIA data collected from K562 sample at various diluting levels and from HeLa sample at various SPD throughputs, respectively. For DDA data, our DB search can identify 20% more peptides at 1% FDR and about 10,000 proteins (10,585 proteins from HeLa and 9,984 from K562). For two Zeno SWATH DIA datasets, we performed library search against spectral libraries that we built from ZenoTOF DDA data of the respective samples. More than 6,000 proteins can be identified from the HeLa sample at 30 SPD and also from the K562 sample of 200 ng load.

Conclusions: Our ZenoTOF data-specific analysis workflows in PEAKS can achieve high sensitivity and high accuracy in peptide and protein identification, which would help the ZenoTOF instruments to deliver superior results in proteomics analysis.

PP02.082: Automated Workflow for Evaluation, Correction and Visualization of Batch Effect in DIA Proteomics Data.

Sundararaman N[1], Bhat A, Venkatraman V, Binek A, Pandey R, Washington K, McArdle A, Manalo D, Rivas A, Phebus C, Raedschelders K, Ayres M, Haghani A, Becker L, Zhgamadze M, Vegesna M, Van Eyk J

[1]Cedars Sinai Medical Center

Introduction: Quantification of proteins in DIA-MS analysis can be affected by variations in technical factors such as sample preparation and data acquisition conditions leading to batch effects, which adds to noise in the dataset. This may in turn affect the effectiveness of any biological conclusions derived from the data. Here we present a workflow for analysis and correction of batch effect through an automated, versatile, and easy to use web-based tool with the goal of eliminating technical variation in cerebrospinal fluid (CSF) samples from Parkinson's Disease Patients.

Methods: Raw intensity data for peptide fragments is extracted from DIA files using the open source openSWATH workflow against the Human Twin population plasma peptide assay library. Normalized transition-level data is processed using the automated batch correction workflow available as an open-source R/Shiny based user-friendly web interface to check for presence of batch effects. Data is first filtered to remove transitions that are largely missing in at least one batch. This is followed by batch correction using ComBat with the R package "proBatch". Missing values are then imputed using random forest imputation with the R package "ranger", after which batch corrected data is visualized using Principal Component Analysis (PCA) and Principal Variance Component Analysis (PVCA) to evaluate the effectiveness of the correction method.

Results: In the CSF dataset, 2695 samples were digested with Trypsin across 16 digestion batches. The pre-corrected PVCA revealed that the digestion batch showed the highest variability in the dataset, with the biological variability being lower. Correction on the digestion batch removed this variability, allowing differences in data stemming from biological factors to be the dominant effect.

Conclusions: Increase in high-throughput mass spectrometry approaches increases the potential for batch effects. Batch correction is therefore extremely important to curb any concerns about the validity of any biological conclusions.

PP02.083: Mass++ ver.4 -MS Data Viewer Meets Online Databases-

Tanaka S[1,2], Murase M[1], Kato M[1,3], Yamamoto H[1,4], Tabata T[1,5], Kusano M[1,6], Kawano S[1,7], Goto S[8], Ishihama Y[5], Yoshizawa A[1,5,7]
[1]Mass++ Users Group, [2]Trans-IT, [3]Data Knowledge Organization Unit, RIKEN Information R&D and Strategy Headquarters, RIKEN, [4]Human Metabolome Technologies, Inc., [5]Grad. School of Pharma. Sci., Kyoto Univ., [6]School of Medicine, Showa Univ., [7]Toyama Intl. Univ., [8]DBCLS, DS, ROIS

Introduction

As studies using mass spectrometry increase, many types of analytical software have been published. However, in any study, it is necessary to examine the mass spectrum for accurate results. We have thus developed Mass++ ver. 4 as a user-friendly spectrum viewer; since the release of ver. 4 alpha in September 2019, 1823 copies have been downloaded through July 1, 2022. This presentation will focus on the features extended in the latest version.

Methods

We have enhanced the fundamental viewer functions of this software, and also implemented several practical functions – mainly the external software execution function which has the ability to set parameters for an external program, input data and have it executed, and then automatically receive the results.

Results

In the newest version, the following viewers are currently available for: TIC, XIC, MS1 spectrum, MS/MS spectrum, 2D/3D-heatmap, and mirror plots (two spectra are displayed simultaneously).
A zoom function was also added for these displays. Additionally, as these views previously could only be displayed on the screen, now they can be saved as images.
Using the external software execution function, Mass++ now allows to perform peak-picking by ProteoWizard and database search by Comet, read the results, and annotate data by marking the corresponding mass peaks on a heat map by specifying the identification results from the table.
Similarly, a function to compare and display MS/MS spectra with the specified identification results together with the corresponding identified peptide spectra downloaded from PeptideAtlas in a mirror plot has also been implemented.

Conclusions

The newly implemented features are expected to enhance the annotation environment for mass spectra by the ability of Mass++ to display spectra in high speed and detail. Mass++ has been developed under a non-profitable operation without any financial support, and released as a BSD 3-clause software at https://www.mspp.ninja/.

PP02.084: Clusters of Co-regulated Proteins in Brain Cortex Associated with Fronto-temporal Lobar Degeneration

Van Gils J[1], Bridel C[2,3], Miedema S[1], Hoozemans J[2], Pijnenburg Y[2], Smit A[1], Rozemuller A[2], Abeln S[1], Teunissen C[2]
[1]Vrije Universiteit Amsterdam, [2]Amsterdam UMC, [3]Geneva University Hospital
Introduction: Frontotemporal dementia (FTD) is an early-onset neurodegenerative disease characterized pathologically by neurodegeneration predominating in the frontal and temporal lobes (frontotemporal lobar degeneration, FTLD). Two major non-overlapping pathological subtypes of FTLD have been described: FTLD-tau, and FTLD-TDP. We aimed to identify proteomic changes in the frontal lobe cortex associated with FTLD-tau and/or FTLD-TDP, and uncover their dysregulated biological processes. Methods: Protein expression levels in FTLD-tau, FTLD-TDP and control groups from post-mortem brain samples were determined using LC-MS/MS. Current methods (e.g. GSEA) often require functional annotations to identify functional protein groups, which biases them towards pre-existing knowledge. We developed a workflow that combined existing clustering (weighted co-expression network analysis) and network-based tools (Hierarchical HotNet) to identify functional groups of coexpressed proteins independent of functional annotation of the proteins, and developed a novel method to validate these modules. Results: We identified 17 modules of coexpressed modules, with both molecular functions known in brain disease and novel insights. We used two datasets with protein expression levels in FTLD-tau and control samples (one set from the medial frontal cortex and one from the temporal cortex of the brain) to validate the identified modules. Eight modules could be validated in the frontal validation set, and ten in the temporal validation set. Conclusions: Taking these samples of FTD subtypes, we show that our workflow can extract meaningful biological insight from protein expression data, and highlight pathways and proteins that could be interesting for further research to understand and eventually treat FTD.

PP02.085: A Pipeline for the Time Efficient Analysis of Thousands of LC/MS Runs in High Performance Computing Environment

van Zalm P[1]
[1]Boston Childrens Hospital

Introduction

Combining robust proteomics instrumentation with high throughput enabling LC systems (e.g., timsTOF Pro and the Evosep One system, respectively) enabled mapping the proteomes of 1000s of samples. Fragpipe is one of the few computational protein identification and quantification frameworks that allows for time-efficient analysis of such large datasets. However, it requires large amounts of computational power and data storage space that leave even state-of-the-art workstations underpowered when it comes to the analysis of proteomics datasets with 1000s of LC/MS runs.

Methods

To address this issue, we developed and optimized a Fragpipe-based analysis pipeline for a high-performance computing environment and analyzed 5000+ plasma samples (8.6 TB) that were longitudinally collected from hospitalized COVID-19 patients under the auspice of the Immunophenotyping Assessment in a COVID-19 Cohort (IMPACC) study.

Results & Conclusion

Our pipeline reduced the total runtime by ~90% from 116 (theoretical) days to just 9 days in the parallelized high-performance computing environment. All code is open-source and can be deployed in most high-performance computing environments, enabling the analysis of large-scale high-throughput proteomics studies.

PP02.086: Mass Dynamics 2.0: A Modular Web-based Platform for Accelerated Insight Generation and Decision Making for Proteomics.

Triantafyllidis A[1], Bloom J[1], Condina M[1], Quaglieri A, Burton Ngov P[1], Infusini G[1], Webb A[1,2,3]
[1]Mass Dynamics, [2]Department of Medical Biology, University of Melbourne, [3]The Walter and Eliza Hall Institute

As the field of Proteomics matures and becomes more accessible to a broader scientific community, it is essential to ensure reproducibility, quality and integrity of data analysis and its interpretation. Currently, for researchers with limited experience in either proteomics, bioinformatics or statistics, there is a lack of accessible tools to facilitate their downstream data analysis. Additionally, beyond straightforward quality control, assessment of experimental health and statistical analysis, insight generation and decision making from complex proteomics datasets are often left to biologists that are provided with little to no support.

Here, we introduce MD 2.0, an extensible modular web-based platform that simplifies and templates complex proteomics analysis, facilitating rapid insight generation and decision making from complex proteomics datasets. The extensible environment is built using progressive and scalable architecture (with continuous integration and continuous deployment practices) that allows rapid module development of future statistical, visualization, processing and knowledge insight generation modules.

Currently available “drag and drop” modules assess quality control (PCA, RLE, CV, Missingness, UpsetR and correlation plots), visualize differential expression (volcano, violin, dot, log/log and heatmap plots) and compute enrichment and over-representation analyses (via STRING-DB and Reactome). Unlike other available tools, MD 2.0 utilizes a cloud-based architecture to enable researchers to store, share and manage their data analysis. Further, web browser access to unlimited collaborators enables seamless discussions and remote collaboration, which supports rapid project insights and efficient decision making. MD 2.0 also supports community-based template generation, with workflows available for quality control, simple LFQ experiments, multi-batch experiments, bio-marker analysis, single cell analysis, time-course and dose response analysis.

Overall, the MD 2.0 platform is designed to optimize collaboration between proteomics experts and biologists. This flexible and seamless data analysis environment accelerates research teams’ abilities to extract interpretable information and maximize what their proteomics experiments can deliver.

PP02.087: Detection of Mutant Peptides of SARS-CoV-2 Variants by LC/MS in DDA Approach using Inbuilt Database

Rajoria S[1], Halder A, Tarnekar I, Pal P, Bansal P, Srivastava S
[1]Indian Institute Of Technology, Bombay

Introduction

Equipped with a dramatically high mutation rate, SARS-CoV-2 has trampled across the globe infecting individuals of all ages and ethnicities. As the variants of concern loom large, definitive detection of SARS-CoV-2 strains becomes a matter of utmost importance. Also, unveiling the structural and molecular basis of SARS-CoV-2 entry, pathogenesis, and immune targeting has been a key priority. Mass spectrometry (MS) has been extensively used for such studies. Current databases lack the mutational information of SARS-CoV-2 variants that can be used for MS-based studies. Thus, we have constructed a Mutant Peptide Database (MPD) for the MS-based identification of mutated peptides of SARS-CoV-2.

Methodology

Genomic sequences for the Indian-originated variants of SARS-CoV-2 were downloaded from GISAID followed by insilico translation using Emboss Transeq. Acquired protein sequences were used to build the database. Stringency-check was performed with the alignment studies done with Jalview using the Wuhan strain as a reference. Peptides identified using MPD were further validated using targeted proteomics. Applicability of the database was also demonstrated with the already reported mutated peptides.

Result

In total, 21549 SARS CoV-2 sequences belonging to 11 proteins were obtained from 36 regions of India and were used to construct the MPD. The functionality of MPD was tested on swabs and plasma samples. Variant peptides for Nucleoprotein and Replicase were identified from both types of specimens that were successfully validated using a multiple reaction monitoring-based targeted approach.

Conclusion

MPD will support broad-spectrum MS-based studies like detection, pathogenesis, and therapeutics regarding SARS-CoV-2 variants. We believe that it could be of high value to the researchers as no such database is currently available.

PP02.088: Proteomic Characterization of Indian COVID-19: First Wave Versus Deadly Delta Wave

Srivastava S[1], Rajoria S, Nair D
[1]Indian Institute Of Technology Bombay

Introduction:

India, along with the whole world has witnessed devastating multiple waves of COVID-19. The deadliest second wave in India, driven by the delta variant (B.1.617.2) has resulted in nearly 30 million cases and >363,079 fatalities. The emergence of coinfections and novel symptoms like multiple organ failure, and breathlessness resulted in high disease severity and complicated the understanding of the molecular mechanisms behind the disease pathology. Exploring the factors underlying disease severity at the molecular level can help in patient stratification and identify the therapeutic targets. This will further help in better management and preparedness for the upcoming waves of COVID-19.

Methods

In the current study, a mass-spectrometry-based proteomic analysis was performed using nasopharyngeal swabs collected from COVID-19 patients from the peak of the second wave. A cohort of 59 patients that tested COVID-19 positive for RT-PCR was considered. Patients were further classified into severe and non-severe groups based on the clinical parameters. Analyzed results were compared with our first wave study (Renuka et al., 2021).

Results

We have identified 23 significant proteins, differentially regulated in severe patients as a host response to infection from the second wave patients. In addition to the previously identified innate mechanisms of neutrophil and platelet degranulation, this study revealed significant alterations of the anti-microbial peptide pathway in severe conditions, illustrating its role in the severity of the infectious strain of COVID-19 during the second wave. Furthermore, myeloperoxidase, cathepsin G, and profilin-1 were identified as potential therapeutic targets of the FDA-approved drugs dabrafenib, ZINC4097343, and ritonavir.

Conclusions

This study has enlightened the role of the anti-microbial peptide pathway associated with the second wave in India and proposed its importance in potential therapeutics for COVID-19.

PP02.089: Strategies to Reduce O-glycopeptide Search Times

Wilson G[1], Bern M[1]
[1]Protein Metrics Inc.

Introduction

The most common informatics approach to MS-based proteomics generates modified peptides from a protein database and then scores these candidate “peptidoforms” against tandem mass spectra. Combinatorial expansion of possible peptidoforms, however, can result in long processing times, especially for the case of O-linked glycosylation. Even a modest search of 8 proteins against 9 glycan compositions, allowing a maximum or 2 glycan modifications per peptide, results in hundreds of millions of glycopeptide candidates. There is substantial commonality in the candidates, as many differ only by the placement of modifications at serine, threonine, or N-glycan motifs (NxS/T). We hypothesized that candidates that share the same peptide sequence and total modification mass (“isobaric peptidoforms”) will give similar database search scores. Here we report on a search speed-up that omits the scoring of subsequent isobaric peptidoforms against a set of spectra if the first form scored does not reach a threshold score.

Methods

All computational methods were implemented and tested within the Byonic search engine (Protein Metrics Inc., Cupertino CA). Raw data from Riley et al., J Proteome Res. 2020;19(8):3286-3301 served as the focus of this study.

Results

A novel search strategy, controlled by user input, was implemented into the Byonic search engine to filter isobaric peptidoform candidates from being scored. Using a modest Byonic score filter of 50, meaning that the first isobaric peptidoform had to score at least 50 for subsequent candidates to be scored, we observed a >2-fold reduction in search time while maintaining equivalent sensitivity of glycopeptide identifications.

Conclusions

The method that we have provided here, to reduce the processing time of glycopeptide searches, provides one solution for a common problem that glycoscientists encounter. Further optimizations to glycoproteomic search engines will undoubtedly accelerate researchers in their pursuit to discover novel biology within the human proteome.

PP02.090: ProtyQuant: An Open Software for Comparative Shotgun-Proteomics, using an Integration of Peptide Probabilities for Protein Inference and Quantification

Winkler R[1]
[1]Cinvestav UGA-Langebio

Introduction:
Comparing Shotgun-Proteomics datasets requires several bioinformatic operations: After matching spectra to peptide sequences, the protein inference needs to be analyzed. Quantification from label-free data is possible with spectral counting. However, conventional methods lose the information on peptide probability in this step.
Methods:
Instead of simply counting protein peptides as absent or present (O,1), ProtyQuant integrates peptide probabilities for estimating the 'Protein Presence.' The software employs three algorithms implemented by the PIPQ program of He et al. (2016): Multiple counting, equal division, and linear programming. The workflow starts with validated HUPO pepXML files, e.g., from the Trans-Proteomic Pipeline (TPP). The final output comprises flat text tables for individual samples and comparative studies.
The code was written in C++ and Python. Linux, Windows, and docker versions were built. A GUI permits interactive use and console scripts batch data processing.
Results:
ProtyQuant was tested with reference data from 48 spiked human proteins in yeast lysate. The number of identified proteins with a false-positive rate cut-off of 1% was comparable and, in some cases, even higher than with the ProteinProphet. The multiple counting algorithm was the most sensitive method. Furthermore, the average calculated fold-change was determined with 2.072 (theoretical value: 2.000), thus demonstrating the suitability of ProtyQuant for protein quantification. The code is licensed as open source (GLP3) and available from https://bitbucket.org/lababi/protyquant/.
Conclusions:
The 'Protein Presence,' obtained by combining peptide probabilities, is an integrative concept for estimating the protein probability and quantity. ProtyQuant enables the user-friendly post-analysis of HUPO pepXML files and can be attached to existing community workflows.
References:
He, Zengyou, Ting Huang, Xiaoqing Liu, Peijun Zhu, Ben Teng, and Shengchun Deng (2016) "Protein Inference: A Protein Quantification Perspective." Comp. Biol. Chem. 63:21, https://doi.org/10.1016/j.compbiolchem.2016.02.006.
Winkler, Robert (2021) "ProtyQuant: Comparing Label-Free Shotgun Proteomics Datasets Using Accumulated Peptide Probabilities." J Prot. 230:103985, https://doi.org/10.1016/j.jprot.2020.103985.

PP02.091: A Unifying, Spectrum-centric Approach for the Analysis of Peptide Tandem Mass Spectra

Zolg D[1], Seefried F[1], Schmidt T[1], Gessulat S[1], Graber M[1], Rathke-Kuhnert M[1], Ben Fredj S[1], Samaras P[1], Kuster B[2], Wilhelm M[2], Frejno M[1]
[1]MSAID GmbH, [2]Technical University of Munich

Introduction:
Mass spectrometry-based proteomics data is acquired using either data dependent (DDA), data independent (DIA) or targeted acquisition (PRM). Typically, the former is analyzed using spectrum-centric algorithms assuming that it generates non-chimeric spectra, while the latter two are analyzed in a peptide-centric fashion. Here, we introduce a spectrum-centric approach that deconvolutes spectra irrespective of isolation window size and demonstrate that it generalizes to any chimeric MS2 spectrum, unifying the analysis of DDA, DIA and PRM data.
Methods:
Our library-free and spectrum-centric algorithm compares predicted and experimental MS2 spectra using various intensity-based scores. All promising peptides in each MS2 isolation window are considered simultaneously and compete for experimental fragment ion intensity in one concerted step. Our algorithm aims at explaining as much experimental intensity with as few candidate peptides as possible and distributes the intensity of shared fragment ions to peptide spectrum matches given their estimated proportional contribution to the experimental MS2 spectrum, resulting in the deconvolution of chimeric spectra. FDR-control is performed using Percolator.
Results:
First, we demonstrate the benefits of our algorithm on 1h DDA measurements of HeLa cell lysates with varying isolation width, resulting in an identification rate of up to 80% and over 120k PSMs. Next, we compare DDA and DIA measurements of the same samples and benchmark various DIA tools against each other. We observe a large overlap in peptide identifications and a high correlation of quantitative values. Finally, we demonstrate the accuracy of our automated quantification method on a PRM dataset, achieving similar performance as manual peak integration using Skyline.
Conclusions:
Conceptually, a DDA MS2 spectrum is indistinguishable from a DIA or PRM spectrum acquired with the same isolation window. Here, we developed a spectrum-centric approach that unifies the analysis of DDA, DIA and PRM data without the need for separate search algorithms.

PP02.092: Substrate Sequence Specificity Determination of the SARS-CoV-2 3CL Protease by Proteomic Identification of Cleavage Substrates

Ramos De Jesus H[1], Solis N[2], Machado Y[1], Pablos I[1], Bell P[1], Grin P[1], Kappelhoff R[1], Butler G[1], Overall C[1]

[1]University of British Columbia, [2]AbCellera Biologics

In a short period, SARS-CoV-2 has transmitted worldwide, killing millions and impairing the quality of life of those who survive it. A resurgence of cases around the world has proven that more definitive treatments are paramount to overcome the crisis. The virus comprises four structural proteins and two viral proteases: 3CLpro and PLpro. These proteases are vital to viral protein replication and are validated therapeutic targets to block viral life cycle progression and to treat COVID-19. Discussions regarding non-prime (P) side of 3CLpro substrates has significantly shaped queries on inhibitors development and have dominated research worldwide, while the contribution of the prime-side (P') has been overlooked. Here we describe the full-substrate specificity for the SARS-CoV-2 3CLpro using our previously described method Proteomic Identification of Cleavage Substrates (PICS). In addition to cleavage site specificity validation and kinetic analysis using synthetic peptides, we performed molecular modeling to unveil structural determinants of 3CLpro-substrate interaction. We observed that the substrate P', despite being neglected, plays a key role on the enzyme-substrate recognition and its consequent specificity. The stabilization of the peptide P' is driven by 3CLpro domain I, while the P interactions occur via 3CLpro domain II. On the prime side, P1' is the most relevant position as S1' can only accommodate small residues (Gly, Ala, Ser) due to the steric hindrance imposed by Thr25, Leu27 and His41 side-chains. In addition to bulky residues, P1' is prohibitive for charged -residues. Furthermore, 3CLpro has a preference for histidine at P3' that forms a stabilizing 2.0Å hydrogen-bond with Thr25-Oγ. Three threonine in tandem, Thr24-26, on domain I are the major P'-stabilizing residues. In summary, the findings provided here shed light toward 3CLpro-structural determinants and can support further rational design of SARS-CoV-2-3CLpro inhibitors. Moreover, we describe two highly sensitive and efficient probes to monitor 3CLpro activity.

PP02.093: SARS-CoV-2 Whole Proteome Peptide Microarray: Insights into the Immunogenic Response to Infection Severity and Mutant Viral Strains

Ray A[1], Acharjee A[2,4], Salkar A[2], Bihani S[2], Tuckley C[1], Duttagupta S[3], Srivastava S[2]
[1]Centre for Research in Nanotechnology & Science, Indian Institute of Technology Bombay, [2]Department of Biosciences and Bioengineering, Indian Institute of Technology Bombay, India, [3]Department of Electrical Engineering, Indian Institute of Technology Bombay, India, [4]Department of Zoology, University of Allahabad, India

Introduction:
Peptide microarrays can help build high-resolution immune response maps. This can be used for COVID-19 severity stratification and identify the impact of mutations on immune response1,2.

Methods:
We used PEPperCHIP® SARS-CoV-2 Proteome microarray containing 4932 peptides representing the viral proteome and mutant peptides from B1.1.7, 501.V2, and P.1 lineage. All peptides were 15aa-long with 13-aa-long overlap; printed in duplicates. We studied IgA and IgG responses in 14 patients. The intensities of the individual spots were background corrected using the "limma" package of R. Then, the average intensities from the duplicate spots were log10 transformed. Peptides with a Z-score > 3, in at least 1 patient were considered immunogenic. Mann–Whitney U test was applied to identify immunogenic peptides with disease discriminatory potential3.

Results:
We, present the epitope map of IgA and IgG against the SARS-CoV-2 proteome in a small Indian cohort. Here, we identified 204 (137) and 676 (412) peptides (epitopes) for IgA and IgG, respectively. In addition, we identified 6 and 443 peptides with discriminatory potential (p-value <0.05) based on the IgA and IgG responses across disease severity.
As a novel finding, we report 5 (IgA) and 10 (IgG) peptides with mutations from B1.1.7, 501.V2, and P.1 eliciting an immunogenic response. Especially, we identified increased immunogenic response against N501Y mutation in Spike, whereas decreased immunogenic response against K417T and D138Y.

Conclusion:
We identified IgA and IgG epitopes having disease severity discriminatory potential. Moreover, for the first time, we could identify altered immune responses against mutant viral epitopes.

References :
1. Acharjee, A. et al. Expert Rev. Proteomics 0, 1–16 (2022).
2. Acharjee, A., Barpanda, A., Ren, J. & Yu, X. Multi-Pronged Omics Technol. Understand COVID-19 (2022).
3. Wang, H. et al. ACS Cent. Sci. 6, 2238–2249 (2020).

PP02.094: Dual Laser Strategy allows the Profiling of Virus and Host Surface Proteins on SARS-CoV-2 Viral Particles

Sahi M[1], Andersson S[1], Mattson C[1], Dale M[1], Hofström C[2], Persson-Lotsholm H[2], Fredolini C[1]
[1]Science for Life Laboratory (SciLifeLab) Affinity Proteomics-Stockholm, Div. of Affinity Proteomics, Dept of Protein Science, School of Engineering Sciences in Chemistry, Biotechnology and Health (CBH), KTH Royal Institute of Technology, [2]Science for Life Laboratory (SciLifeLab) Divison of Drug discovery and development, Dept of Protein Science, School of Engineering Sciences in Chemistry, Biotechnology and Health (CBH), KTH Royal Institute of Technology

Introduction

Previous studies on enveloped viruses, such as HIV, have demonstrated that host cell proteins can be incorporated into newly formed virions during replication affecting host-virus interaction and disease pathogenesis. Using mass spectrometry (MS) and proximity extension assays (PEA) the proteome of viral particles from the enveloped virus SARS-CoV-2 replicated in human cells was profiled, identifying 87 host proteins. The aim of this project was to develop a dual detection bead-based assay to validate the presence of the host proteins identified on the surface of SARS-CoV-2

Methods

UV-inactivated cell supernatant from uninfected VeroE6 cells and cells infected with SARS-CoV-2 was collected. Serial dilutions of the supernatant were prepared in PVXCas-based buffer. Luminex magnetic beads were coupled to recombinant human ACE2. Viral particles captured on the ACE2 beads were detected using the Luminex dual laser system Intelliflex. Two detection channels were used for detecting PE and BV421 labeled Spike1 (S1) detection antibodies. Assay development and optimization was performed using antibodies and antibody fragments targeting different epitopes of the viral Spike protein.

Results

Dose dependent curves of SARS-CoV-2 supernatant were generated (replicate CVs <20%). As a proof of concept two antibodies targeting independent epitopes were applied simultaneously for detecting S1. Good assay performance in both channels confirmed that two virus surface target proteins are detectable in parallel.

Conclusions

The assay set up exploits Luminex Intelliflex technology to obtain a triple detection of viral particles where binding of S1 by two indipendent affinity interactions, ACE2 and an antibody, provides strong specificity and confirms the capture of intact virus particles. A third detector channel is used to verify the presence of host proteins on the surface of the same particle. The assay will be implemented to verify the presence of the 87 SARS-COV-2 host proteins suggested by the previous virus proteomic profile.

PP02.095: Proteomic Analysis of COVID-19 Disease Progression: A Pilot Study

Shareef S[1], Matthews E[1], Daly M[1], Bannard-Smith J[2], Gethings L[3], Fowler S[1], Felton T[1,4], Simpson A[1,4], Mills E[1]
[1]Division of Infection, Immunity & Respiratory Medicine, University of Manchester, [2]Manchester Royal Infirmary, Manchester University NHS Foundation Trust, [3]Waters Corporation, [4]Wythenshawe Hospital, Manchester University NHS Foundation Trust

Introduction: Early in the pandemic, SARS-CoV-2 infection resulted in a range of clinical presentation from asymptomatic to severe disease and death. Defining the mechanistic basis for this is essential to understand how future mutations may affect clinical presentation, and prepare for any future pandemic caused by a novel pathogen. A multi'omics approach is being undertaken to profile changes in the circulating proteome and metabolome. Initially, proteomic analysis has been conducted for selected patients to pilot sample handling and analysis protocols.
Methods: Demographic and clinical data along with plasma and serum samples were collected opportunistically from March 2020–January 2021 from 429 patients with SARS-CoV-2 infection recruited into the ManARTS Biobank (REC Approval 15/NW/0409). Serum taken at admission from selected patients stratified for disease severity were subjected to reduction, alkylation and trypsin digestion after depletion. Mass spectrometry data were acquired using a data independent approach utilising a SYNAPT XS QTOF. Proteins were identified based on a 1% FDR using Progenesis QI for proteomics. Modifications were set as cysteine carbamidomethylation (fixed), and methionine oxidation, asparagine/glutamine deamidation, and serine/threonine/tyrosine phosphorylation (variable).
Results: Principal components analysis allowed separation of patients with mild symptoms from those with severe COVID-19. Semi-quantitative, label-free analysis of protein identifications revealed increased immune dysregulation in severe disease, evidenced by changes in acute-phase and complement system proteins. Gene ontology analysis showed increased immune system activation, coagulation, and angiogenesis.
Conclusion: The pilot analysis showed significant changes in the serum proteome, related to their disease trajectory, had already occurred in patients at admission. There is thus a good prospect to identify prognostic markers for severe COVID-19 in the wider cohort. Proteomic analysis of the entire ManARTS COVID-19 Cohort is underway and will confirm identification of prognostic markers and provide invaluable information on the immune response of a naïve population to an emerging respiratory pathogen.

PP02.096: Exploring Cross-reactivity of SARS-CoV-2 Antibodies to the Human Proteome

Skoglund L[1], Jernbom Falk A[1], Andersson E[1], Hellström C[1], Olofsson J[1], Sjöberg R[1], Havervall S[2], Thålin C[2], Tegel H[3], Hober S[3], Pin E[1], Anggraeni Andersson M[1], Månberg A[1]
[1]Department of Protein Science, KTH Royal Institute Of Technology, Scilifelab, [2]Division of Internal Medicine, Department of Clinical Sciences, Karolinska Institutet, Danderyd Hospital, [3]Division of Protein Technology, Department of Protein Science, KTH Royal Institute of Technology

Introduction: COVID-19 infection can cause a range of symptoms and a dysregulated immune response has emerged as a key contributor in both acute and long-term disease. Sequence comparison of viral and human proteins indicate similarities that potentially could lead to molecular mimicry and autoimmune reactions. We have established a high-performance and high-throughput serological assay for SARS-CoV-2 antibody detection (1), and further developed this method to allow bead-assisted enrichment of antibodies from convalescents for subsequent characterisation. The aim of this project is to analyse antibodies directed to specific viral proteins for cross-reactivity to human proteins using high-density antigen arrays.

Methods: Three convalescent plasma samples with previously confirmed high titres of antibodies towards SARS-CoV-2 proteins were used in this study. The antibody enrichment was accomplished by using a bead-based assay for capturing antibodies, followed by elution through sequential pH and temperature treatments. The obtained eluates were evaluated using a multiplex bead array as well as analysis on our in-house developed high-density antigen arrays containing 42000 protein fragments and 1500 full-length proteins, together representing 94% of the human protein-encoding genes.

Results: Antibodies could successfully be enriched from human plasma samples using protein-coupled microbeads. The collected eluates showed high signals towards the proteins from which the antibodies were enriched and low signals towards other proteins. We are currently investigating cross-reactivity of COVID-19 antibodies to human proteins using our high-density antigen arrays.

Conclusions: Antibodies have been successfully enriched from plasma samples as proven by subsequent analysis in a multiplex bead-based assay. Identifying potential cross-reactivity could help explain the observed heterogeneity in both disease course and outcome of COVID-19 patients.

References: (1) Hober ... Nilsson, Clin Transl Immunology. 2021

PP02.097: Altered Viral Protein Expression as a Mediator of Enhanced Innate Immune Evasion Among SARS-CoV-2 Variants

Ummadi M[1,2,3,4], Zuliani-Alvarez L[1,2,3,4], Bouhaddou M[1,2,3,4], Thorne L[5], Reuschel A[5], Polacco B[1,2,3,4], Pelin A[1,2,3,4], Batra J[1,2,3,4], Rojc A[1,2,3,4], Jang G[1,2,3,4], Stevenson E[1,2,3,4], Xu J[1,2,3,4], Zhou Y[1,2,3,4], Harjai B[1,2,3,4], Fossati A[1,2,3,4], Memon D[6], Fabius J[1,2,3,4], Obernier K[1,2,3,4], Kaake R[1,2,3,4], Beltrao P[1,6], Swaney D[1,2,3,4], Jolly C[5], Towers G[5], Krogan N[1,2,3,4]
[1]QBI Coronavirus Research Group (QCRG), University of California San Francisco, [2]Quantitative Biosciences Institute (QBI), University of California San Francisco, [3]J. David Gladstone Institutes, [4]Department of Cellular and Molecular Pharmacology, University of California San Francisco, [5]Division of Infection and Immunity, University College London, [6]European Molecular Biology Laboratory (EMBL), European Bioinformatics Institute, Wellcome Genome Campus

Introduction:
More than 6 million people worldwide have died of COVID-19, the largest disease outbreak in recent history. While many studies have described how mutations in Spike affect escape from adaptive immunity, little is known about how mutations outside of Spike can alter the immunological response to SARS-CoV-2.

Methods:
Calu-3 human lung epithelial cells and primary differentiated human airway epithelial (HAE) cells were infected with five SARS-CoV-2 variants of concern (e.g. Alpha, Beta, Gamma, Delta, and Omicron) and collected at 10h and 24h post infection. Each sample was analyzed using unbiased abundance proteomics, phosphoproteomics, and RNA sequencing to assess viral and host gene expression and modulation of host immune signaling pathways.

Results:
We found increased RNA and protein production of Orf9b after Alpha, Beta, Gamma, and Delta infections and Orf6 following Alpha and Beta infections. Prior studies show Orf6 interacts with the nuclear pore complex and inhibits RNA export of ISGs during infection. We hypothesize that upregulation of Orf6 can strengthen this interaction and further antagonize this process. Additionally, we show that Orf9b can antagonize innate immune activation through its interaction with TOM70, an activator of the MAVS-TBK1 pathway. We found a phosphomimetic S53E version of Orf9b could block its interaction with TOM70 and subsequently disable its ability to antagonize innate immune activation. Surprisingly, we found the kinase downstream of MAVS, TBK1, to be the kinase responsible for phosphorylating Orf9b-S53, revealing a novel feedback loop from a host antiviral kinase.

Conclusions:
Using unbiased proteomic approaches to compare SARS-CoV-2 variants enabled us to characterize how upregulation of specific viral proteins modulates signaling pathways and ultimately leads to enhanced innate immune suppression, likely contributing to observed higher transmissibility of the virus.

References:
Thorne, L.G., et al. 2022 Nature 602; 487-495
Gordon, D.E., et al. 2020 Nature 583; 459-468

PP02.098: Insight into the Novel Role of Lysosome-Associated Membrane Protein-2a During SARS-CoV-2 Infection by RNA-Protein Interaction Analysis using Proteomics

Verma R[1], Saha S[2], Kumar S[1], Mani S[1], Maiti T[2], Surjit M[1]
[1]Translational Health Science And Technology Institute, [2]Regional centre for Biotechnology

Introduction:
Severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2), responsible for the ongoing COVID-19 pandemic, is a positive-strand RNA virus belonging to the coronaviridae family. Similar to other positive-strand RNA viruses, this virus is capped at the 5'-end and has poly(A) tailed at the 3'-end. The 5' and 3' end of the virus genome consist of untranslated region (UTR) regions. The function of UTRs is mediated through interaction with host/virus-encoded proteins or self-interaction between the RNA regulatory elements present within the 5' and 3' UTRs.
Methods:
We used an in-vivo approach, RNA-protein interaction detection (RaPID) assay coupled to liquid chromatography with tandem mass spectrometry for identification of the host interaction partners of SARS-CoV-2 5'- and 3'- UTRs and generated an RNA-protein interaction network (RPI). We combined previously known protein-protein interaction data proposed to be involved in virus replication with the RNA-protein interaction data, and created the RNA-protein-protein interaction (RPPI) network.
Results:
Generated network likely to be involved in controlling SARS-CoV-2 replication. The bioinformatics analysis of the RPPI network divulged the enrichment of factors involved in translation initiation and RNA metabolism. Lysosome-associated membrane protein-2a (Lamp2a) is one of the host proteins that interact with the 5'UTR. It is the receptor for chaperone-mediated autophagy. Lamp2a was found to be upregulated after SARS-CoV-2 infection in cells and the absence of Lamp2a isoform enhanced the viral RNA level, whereas its overexpression significantly reduced the viral RNA level. Co-localization study of Lamp2a and viral RNA in the infected cells showed an increased autophagic flux, although there was no change in the formation of autophagolysosomes.
Conclusion:
In summary, this study provides the useful resource of SARS-CoV-2 5' and 3' UTR binding proteins and demonstrates the importance of Lamp2a protein during SARS-CoV-2 infection.

PP02.099: Proteomic Perturbation in Patients with COVID-19: A Meta-analysis Database

Zhang F[1,2,3,4], Luna A[5], Tan T[1,2,3,4], Chen Y[6], Guo T[1,2,3,4,6]
[1]Westlake Laboratory of Life Sciences and Biomedicine, Key Laboratory of Structural Biology of Zhejiang Province, School of Life Sciences, Westlake University, [2]Institute of Basic Medical Sciences, Westlake Institute for Advanced Study, [3]Research Center for Industries of the Future, Westlake University, 600 Dunyu Road, [4]Center for Infectious Disease Research, Westlake University, 18 Shilongshan Road, [5]Department of Data Sciences, Dana-Farber Cancer Institute, [6]Westlake Omics (Hangzhou) Biotechnology Co., Ltd.

Introduction
The ongoing pandemic COVID-19 caused by SARS-CoV-2 has yet limited treatment options partially due to the lack of understanding of the molecular dysregulation of the infected patients. We aimed to examine the protein modulators underlying COVID-19 patients to unveil potential therapeutic targets and diagnostic biomarkers.
Methods
We performed a meta-analysis using proteomic expression data from COVID-19 patients published before May 2022. The data comprehensiveness was guaranteed by both manually curation of all proteomic data deposited on the ProteomeExchange and PubMed search. The datasets were further organized into tables of patient information, sample information and protein expression. Protein expression by disease subgroups across projects were compared. We also visualized differentially changed pathways and proteins. Moreover, circulating proteins that differentiated severe cases were identified as the predictive biomarkers. Finally, the proteins with opposite expression by specimen types were characterized.
Results
This study was compiled from 41 original studies from 32 hospital sites with 3077 patient cases from 19 types of clinical specimens. 53 protein expression matrices were collected, reporting a total of 5434 samples with 14,403 unique quantified proteins. Lipopolysaccharide-binding protein (LBP) was identified in the greatest number of projects and significantly expressed in blood samples of severe patients. A panel of co-expressed differential proteins were found to separate the severe patients from non-severe patients. Five test sets reached a mean AUC of 0.87 and ACC of 0.80. Blood samples were enriched with complement and coagulation cascade proteins compared with urea and tissue samples.
Conclusions
An online database https://www.guomics.com/covidPro/ was reported with integrated analysis toolkits that enables more hypotheses testing using the same datasets to compare their own results. This database offers a unique data resource for the management of COVID-19 to the druggable proteins and pathways.

PP02.100: KChIP3 Impacts Cognitive Ability in the 5XFAD Model by Inhibiting Proteins Involved in Long-term Potentiation (LTP)

Arcos-Encarnación B[1], Hernández M[2], Goméz-Caudillo L[2], Encarnación-Guevara S[2], Pedraza-Alva G[1], Pérez-Martínez L[1]
[1]Universidad Nacional Autónoma De México, Instituto de Biotecnología, [2]Universidad Nacional Autónoma de México, Centro de Ciencias Genómicas

Introduction: KChIP3 is an important neuronal protein due to its multiple functions. It is known by other names, such as Calsenilin for its calcium-binding motifs and DREAM exalting its transcriptional regulator function. KChIP3 interacts with presenilins 1 and 2. The presenilins are part of the γ-secretase enzymatic complex. This complex participates in the generation of the peptides β-amyloid (βA), a hallmark of Alzheimer´s disease (AD). KChIP3 overexpression has been reported in post-mortem brains of patients with AD as well as in AD murine models. Therefore, here we propose that KChIP3 participate in the development of AD on one hand, by stimulating βA deposition, and on the other, through its transcriptional activity maintaining an inflammatory state that negatively impacts cognitive functions. Methods: To test this hypothesis, we have used the KChIP3 knockout mouse (KChIP3-/-) and a murine model of AD (5XFAD). We crossed these two genotypes to generate a transgenic model in which KChIP3 is silenced in the AD context (5XFAD/KChIP3-/-). To characterize the molecular mechanism mediated by KChIP3 in the development of AD, we performed a quantitative proteomic analysis by iTRAQ from two regions of the brain; hippocampus and cortex. Results: The protein analysis was performed by principal components (PC) meanwhile the enrichment of pathways and biological process by DAVID GO and PANTHER. These analyses showed that the absence of KChIP3 in the context of the AD model rescues pathways like long-term potentiation (LTP), cholinergic synapse and neurotrophin signaling.
Conclusions: As expected, 5XFAD down-regulated proteins are involved in functions such as learning, synaptic transmission, memory, neural development and neuron migration. These findings support a mechanism by which KChIP3, impairs LTP necessary for process like learning and memory.

PP02.101: Deep Proteomics Coverage of Human Serum Samples by Trapped Ion Mobility Mass Spectrometry

Assis D[1], Santos J[2], Moraes J[2], Campos P[2], Gordon E[1], Silva M[2], Oliveira M[2], Willetts M[1], Borges M[2]
[1]Bruker Scientific LLC, [2]Fundaçao Ezequiel Dias - FUNED

Introduction

Advanced mass spectrometry such as dia-PASEF have demonstrated a continual improvement in proteomics research. These benefits also extend to plasma proteomics accelerating biomarker discovery, drug development, and thus benefit diagnostics and treatments. In this work, we show how dia-PASEF enables a deep evaluation of yellow fever pathophysiology through proteomic studies using serum samples, with potential to detect novel biomarkers and improve diagnostic of this disease.

Methods

Pooled serum samples (non-depletion and acetonitrile depletion) from a control group, a group recovered from Yellow Fever infection and from patients who died were analyzed on a timsTOF Pro 2 instrument combined to a nanoElute (Bruker Daltonics) operating with an Aurora nano column (25 cm x 75 µm ID, C18 - IonOpticks, Australia) on DDA or dia-PASEF modes with long and short gradients. Data were processed using MSFragger or DIA-NN 1.8.

Results

From depleted serum prepared with a simple protocol identification of 1,300 and 2,000 protein groups in DDA and dia-PASEF modes, respectively. Additionally, the number of identified proteins changed significantly when 3 different groups were compared: control, death and recovered patients. Protocols using depletion were the best approach in this study and even using a shorter gradient, dia-PASEF identified about 50% more proteins with an overlapping of 95% compared to DDA. The number of peptides were higher for non-depleted serum, but the number of proteins were lower in this sample preparation due to the presence of highly abundant proteins. In addition to a high number of identifications, using label free quantification several proteins that were up or down regulated across the 3 patient groups could be measured. Here, we show regulated proteins related to the clinical condition of patients where bleeding episodes and liver damage are common symptoms in severe disease manifestation.

PP02.102: Proteomics for the Non-invasive Diagnosis and Molecular Pathway Analysis of Mycosis Fungoides

Qureshi H[1,2,3], Fernandez-Penas P[1,2,3], Wells J[1,3], Azimi A[1,2,3]
[1]Faculty of Medicine and Health, The University Of Sydney, [2]Centre for Cancer Research, The Westmead Institute for Medical Research, [3]Department of Dermatology, Westmead Hospital

Introduction: Mycosis Fungoides (MF) is clinically and histopathologically difficult to differentiate from common skin dermatoses. Its diagnosis also requires a skin biopsy, which may lead to complications such as pain, bleeding, and scarring. Therefore, the aim of this study was to combine tape stripping with data-independent acquisition mass spectrometry (DIA-MS) proteomics to identify novel biomarkers and disrupted biofunctions that may aid in the diagnosis and better understanding of MF pathogenesis.

Method: Lesional and normal human stratum corneum samples were obtained noninvasively by the application of adhesive tape strips on the skin of 28 MF patients
followed by DIA-MS proteomic analysis and bioinformatic analyses using Ingenuity Pathway Analysis (IPA) bioinformatics platform.

Result: In total, 1303 proteins were identified across the samples. The top-250 most variant proteins efficiently separated the samples based on their clinical diagnosis. Also, 290 proteins were significantly changed in the MF cohort compared to normal skin. Of these, the top differentially abundant proteins (GSDMC, PDIA4 and ERP29) were identified as novel biomarkers. IPA analysis predicted the significant inhibition of cell death, and significant activation of immune biofunctions including T-lymphocytes, antigen-presenting cells and recruitment of mononuclear leukocytes in MF lesions. In addition, MF lesions were associated with novel upstream regulators relating to oncogenic processes and immune dysfunction.

Conclusion: The application of tape-stripped sample collection together with DIA-MS technique could transform the diagnosis of MF by reducing the need for traditional invasive biopsy. Additionally, disrupted biofunctions and upstream regulators identified may serve as useful biomarkers to predict MF progression.

PP02.103: Integrated Omics Approaches to Understand Pituitary Adenomas Disease Biology

Banerjee A[1]
[1]Indian Institute of Technology Bombay, [2]Indian Institute of Technology Bombay

Introduction

PAs (Pituitary Adenomas) such as Cushing's and Acromegaly are considered rare conditions with comorbidities leading to high mortality, whereas NFPA (Non-functional PAs) are diagnosed incidentally.

In our study, we built the first Pituitary Proteome map from healthy individuals. Studied Auto-Ab signatures from Cushing's, Acromegaly and NFPAs followed by their metabolomics fingerprinting using vibrational spectroscopy such as Confocal Raman, ATR-FTIR and Orbitrap Mass Spectrometry. Further studied disease pathogenicity of Cushing's, Acromegaly and NFPAs were studied using Orbitrap Mass Spectrometry.

Methods

I. Normal Pituitary Proteome matched Anterior and Posterior lobes.
II. Autoantibody Signatures profiling using HuProt™ (Human Proteome Microarray).
III. Metabolomics Profiling using Confocal Raman, ATR-FTIR and HRMS
IV. Proteomic Profiling of PAs

Results

The study identified and validated hormones GH and TSHβ exclusively to the anterior lobe, while OXT and AVP to the posterior lobe, proteins POU1F1, POMC, PCOLCE2 and NPTX2 were identified as pituitary enriched proteins and was validated for their lobe specificity.

Autoantibody profile of PAs led to the identification of proteins such as AKNAD1, NINJ1and PTP4A1 in Acromegaly. Protein ABR, ST6GALNAC6, ANXA8 in Cushing's patient's and RTN4 exhibited very high antigenic response in NFPA patients.

Metabolic fingerprinting and LC-MS/MS-based analysis identified Sphingosine derivatives, lipids and Choline derivatives differentially regulated in each tumour cohort.

Proteomics analysis of Acromegaly, Cushing's and NFPA led to the identification of protein-hubs related to calcium-binding and regulation and collagen functioning only in Acromegaly and Cushing's, whereas as Adenyl-cyclase signalling, Apo-lipoprotein and Cullin based protein-hub in NFPA.

Conclusion

The study provided us with the first Pituitary Proteome Map, Identified AutoAb signatures for early disease progression and further metabolomic profiling which lead to the identification of various biomolecules dysregulated in each PAs. Proteomics analysis of PAs also led to the identification of protein hubs in each cohort which played a crucial role in disease pathobiology.

PP02.104: Unbiased Profiling of the Senescent Monocyte Proteome and Surfaceome for Human Aging and Disease Biomarker Discovery

Tsitsipatis D[1], Banarjee R[1], Ram A[1], Dey A[1], Cui L[1], Rossi M[1], Gorospe M[1], Basisty N[1]
[1]National Institute On Aging, NIH

Introduction: Senescent cell accumulation drives aging and many pathological processes. Selective elimination of senescent cells is a promising therapeutic approach to treat multiple diseases of aging in humans. However, development of drugs to eliminate senescent cells in humans requires biomarkers and therapeutic targets for senescent cells. Here, we performed comprehensive and quantitative proteomic profiling of secreted, surface, and intracellular proteomes of senescent monocytes with data-independent acquisition (DIA) analysis to identify biomarker candidates of monocyte senescence burden in human peripheral blood.

Methods: We induced senescence in cultured monocytes with ionizing radiation and confirmed senescent phenotypes using a panel of canonical senescence and viability markers. The cell surface proteome was enriched using an optimized cell-surface glycopeptide capture approach. Surfaceome, secretome, and intracellular proteome samples were analyzed LC-MS/MS analysis. Acquisition of all samples was performed on the Q-Exactive HF Orbitrap mass spectrometer. Protein identification and quantification was performed in Spectronaut.

Results: We developed stable and viable models of senescence in monocyte cell lines that were phenotypically validated by expression of senescence markers (p21, p16, DPP4, and IL6, etc). To model the full range of physiological oxygen levels experienced by circulating monocytes in vivo, we established these models under both hypoxic and hyperoxic conditions and analyzed their secretomes and intracellular proteomes. We report the first comprehensive and unbiased assessment of the senescent monocyte intracellular proteome and secretomes, quantifying levels of over 4,000 intracellular proteins (24,444 peptides) and over 2,800 secreted proteins (16,667 peptides). To identify candidate biomarkers peripheral biomarkers of senescence burden in humans, we compared proteomic profiles of senescent monocytes with published protein biomarkers of aging in human plasma. Analysis of the monocyte surfaceome reveals novel cell surface targets for further validation.

Conclusions: These results lay the foundation for therapeutic targets or biomarkers to develop senescence-targeted therapies for chronic inflammation and age-related decline.

PP02.105: Exposures to Polycylic Aromatic Hydrocarbons (PAHs): A LC-MSE Label-Free Proteomic Analysis to Determine Protein Biomarkers of Effects in Rat Plasma

Bourgoin S[1,2], Grenard A[1], El Hajjar M[1], Michelland S[1,2], Cunin V[1,2], Marques M[1,2], Habert D[3,4], Vallée B[3,4], Sparfel-Berlivet L[5], Courty J[3,4], Persoons R[1,2], Maître A[1,2], Seve M[1,2], Demeilliers C[1,2], and consortium miR-Ex-HAP EST-19-010[1,2,5]

[1]Univ. Grenoble-Alpes, CNRS, Grenoble INP, TIMC, EPSP, [2]Univ. Grenoble-Alpes, CNRS, Grenoble INP, CHU Grenoble Alpes, TIMC, EPSP, [3]AP-HP, Hopital Henri Mondor, Département d'Hématologie-Immunologie, [4]Univ Paris Est Creteil, INSERM, IMRB, [5]Univ Rennes, Inserm, EHESP, Irset (Institut de recherche en santé, environnement et travail), UMR_S 1085

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous pollutants of hundreds of molecules that result from incomplete combustion of organic matter (grilled/smoked food, tobacco smoke) or from distillation of coal/petroleum. They are always found as complex mixtures whose composition and toxicity are highly variable depending on emitting sources. PAH exposure may induce several diseases including cancers (lung, skin, bladder). Therefore, several PAHs are classified as priorities at European level such as pyrene and benzo[a]pyrene (B[a]P), and only B[a]P is classified as a proven carcinogen for humans. Several exposure biomarkers are used for the biomonitoring of exposed populations and health risk assessment, but their interpretation is limited by the lack of toxicological reference values. Moreover, the relationship between exposure biomarkers and the occurrence of toxic effects is poorly documented. Therefore, there is a major interest in identifying effect biomarkers to better understand molecular mechanisms involved in the pathologies induced by PAHs exposure.

Herein, label-free quantitative proteomics was used to investigate protein modulations in plasma upon PAH exposure of rats with the following groups (no exposure, B[a]P, pyrene, industrial coal mixture at different concentrations, n=10 per group). The proteome profiles were obtained after a low abundant protein enrichment, a trypsin/lys-C digestion and a mass spectrometry analysis by using a NanoAcquity-C18/SYNAPT-G2Si mass spectrometer system operating in a high-definition LC-MSE mode.

This study allowed us to decipher the proteome responses to PAH/B[a]P exposure and to determine several effect biomarker candidates of B[a]P exposure. One protein was specifically regulated upon a B[a]P dose effect (not for pyrene) after an exposure to B[a]P alone or in industrial coal mixtures. A GEPIA analysis showed that this protein is related to an unfavorable prognosis in lung, renal, pancreatic and mesothelioma carcinoma. This protein should be further investigated for PAHs human exposure monitoring and cancer health risk assessment.

PP02.106: Global Proteome Analysis of Peripheral Tissues to Understand AD Progression

Bowser B[1], Arul A[1], Robinson R[1]
[1]Department of Chemistry, Vanderbilt University

Introduction: Alzheimer's Disease (AD) is a neurodegenerative disease that's modeled with double transgenic mice with mutations in amyloid precursor protein (APP) and presenilin 1 (PS1). APP/PS1 mice display cognitive decline and plaque accumulation, which furthered our understanding of molecular mechanisms that contribute to AD. Here, we apply high throughput proteomics to study system-wide effects of AD with disease progression in brain and peripheral tissues.

Methods: Brain, heart, liver, and kidney tissues from wild type (N=6) and APP/PS1 (N=6) transgenic mice were harvested at ages 3-, 7- and 12-months. All tissues (N=164) were prepared by an automated combined precursor isobaric labeling and isobaric tagging (cPILOT) chemical labeling workflow with multiplexing capacity up to 36 samples. Each of the five 36-plex batches of samples were fractionated into 24 fractions with high pH reversed phase separation. Each fraction was analyzed using nanoflow liquid chromatography-tandem mass spectrometry (LC-MS/MS) and MS3 on an Orbitrap Fusion Lumos mass spectrometer. Data analysis was performed with Proteome Discoverer software (v. 2.5).

Results: APP/PS1 mice at 3, 7, and 12-months of age have no, little, and advanced amyloid beta plaque deposition and increased cognitive challenges. Data analysis resulted in ~6000 quantified proteins, whereby 90% of these proteins were identified across tissue types. Proteins associated with AD pathology such as apolipoprotein 1 and clusterin changed similarly to previous reports and Gene Ontology analysis confirmed inflammation, amyloid secretase, and presenilin pathways change with disease progression and tissue type. Here, we present an in-depth analysis of significant protein changes across tissue types and with disease progression. Technical considerations for quality control, automated sample preparation, and data normalization are also presented.

Conclusions: Insight into system wide changes in an AD mouse model can be achieved with expanded multiplexing capacity of cPILOT and help to further understanding of AD.

PP02.107: Optimising DIA Library Production

Bucio Noble D[1], Koh J, Humphries E, Smith D, Williams S, Sykes E, Loudon C, Xavier D, Lucas N, Robinson P, Haines P
[1]Children's Medical Research Institute

Introduction: Data Independent Acquisition (DIA) strategies are an integral part of proteomic studies involving large cohorts. DIA-MS data is traditionally analysed using spectral reference libraries (SRLs) created from separate Data-Dependent Acquisition (DDA) experiments. However, it is also possible to generate SRLs using DIA. Our aim was to compare the performance of SRLs derived from either DDA or DIA.
Methods: 1,261 fresh frozen cancer samples encompassing 73 cancer types from 27 tissue types were grouped to produce 39 pools. Each pool was fractionated using high-pH RP-HPLC and the 15 fractions were analysed across various Triple TOF 6600 instruments in technical duplicate (one run per MS). Four approaches were evaluated using DIA-NN for data analysis: DIA-SRLs and DDA-SRLs acquired from DIA or DDA mode respectively, SRL-free made using unfractionated sample DIA files, and combined-SRL using both DIA-SRLs and SRL-free.
Results: In a squamous cell lung carcinoma pool, the DIA-SRL, DDA-SRL, SRL-free, and combined-SRL, approaches identified 9,451, 5,632, 6,956, and 8,614, proteins, respectively with a protein overlap of 36%. Whilst the DIA-SRL had the highest number of identifications, the SRL-free method had the lowest number of missing values at 38% compared to 51%. Analysis of the proteins uniquely identified in each show that the SRL-free library produced the most intense values and the highest confidence score peptides.
Conclusions: Conventional DDA-SRLs performed poorly relative to the other methods. Fractionated SRLs have a cost of a high proportion of missing values and low intensity peptides. The SRL-free approach offers a faster alternative for large scale clinical proteomic analysis where sample availability may be limited.

PP02.108: Proteomic and Phosphoproteomic Study of the Molecular Mechanisms Unlerlying Progressive Familiar Intrahepatic Cholestasis Type 3

Corrales F[1], Guerrero L[1], Carmona-Rodríguez L[1], Stark L[2], Vicent D[2]
[1]CNB-CSIC, [2]IdiPAZ

Introduction

Progressive familiar intrahepatic cholestasis type 3 (PFIC3) is a severe rare liver disease which affects between 1/50,000 to 1/100,000 children (1). In physiological conditions, bile is produced by the liver and stored in the gallbladder, then it flows to the small intestine to play its role in fat digestion. To prevent tissue damage, bile acids are kept into fatty acids micelles. In cholestatis this bile flow is disrupted. Mutations in phosphatidyl choline transporter ABCB4 lead to intrahepatic accumulation of free bile acids that results in liver damage since early ages (2). Currently, the only treatment of this disease is liver transplantation.

Materials and methods

We performed a quantitative proteomic and phosphoproteomic analysis of 8 samples from control and PFIC3 patients. We have extracted and digested with trypsin proteins from liver tissue and then we have performed an isobaric labelled based quantification using TMT11plex. We separated the peptides in 10 fractions that where analyzed independently by LC-MS in an Orbitrap Exploris 240. Moreover, we performed a phosphopeptide purification with TiO2 beads to carry out a phosphoproteomic analysis. For data analysis we used Proteome Discoverer software for protein identification and quantification, and Ingenuity Pathway Analysis for functional analysis.

Results and conclusions

We identified 56,345 peptides corresponding to 6,246 proteins. 324 proteins showed differential expression between control and PFIC3 conditions (t-test: adj p value<0,05). Regarding phosphoproteomic analysis, we identified 1,964 proteins, 5,731 peptides and 5,090 phosphopeptides. 215 peptides were differentially phosphorylated in PFIC. IPA analysis revealed that canonical pathways including lipid metabolism, inflammation, cell survival and cytoskeleton organization were affected in PFIC3.

References

1. Shagrani, M., et al (2017). Genetic profiling of children with advanced cholestatic liver disease. Clinical genetics, 92(1), 52-61.
2. Gonzales E, et al. Liver diseases related to MDR3 (ABCB4) gene deficiency (2009). Frontiers in Bioscience .;14(11):4242-4256.

PP02.109: High Throughput Analysis of Parkinson's Disease PBMC with DIA-FAIMS

Flinkman D[1,2], Hong Y[1], Deshpande P[1], Gnjatovic J[1], Fagerholm V[1], Kaasinen V[3], James P[1,2], Coffey E[1]
[1]Turku Bioscience, University Of Turku And Åbo Akademi, [2]Lund University, [3]University of Turku

Introduction:
Parkinson's disease (PD) is the second most common neurological disorder with the latest estimate of 9.4 million individuals affected (2020). The disease is characterized by debilitating motor symptoms ranging from dyskinesia to bradykinesia, as well as non-motor symptoms such as constipation and sleep disturbance, which can precede the motor symptoms by a decade. PD is difficult to diagnose, there is no cure or disease-causing mechanism known, which is why it is critical to study patient material for insight for both diagnosis and treatment. In this study we seek to find biomarkers and biology behind the PD using peripheral blood mononuclear cells (PBMC) from two independent PD patient cohorts containing samples from sporadic and genetic PD patients, motor disease patients without PD diagnosis (nonPD), prodromal stage and healthy individuals +/- PD mutation.

Methods:
PBMC samples were obtained from South-Western Finland catchment area (27 PD, 31 nonPD and 32 healthy) and from Parkinson's Progression Marker Initiative consortium from 387 individuals with three time points per individual (71 with PD, 39 prodromal, 88 with genetic PD, 173 genetic unaffected, and 26 healthy) with total of 1162 samples. High throughput digestion with S-trap was used to generate tryptic peptides. Each digestion batch contained two digestion controls from a standard sample. Resulting tryptic peptides were analysed with Evosep 30 samples per day method on Oribitrap Lumos interfaced with FAIMS-pro with a single compensation voltage, in data independent acquisition mode with a daily run control.

Results:
Preliminary data-analysis with Spectronaut 16 shows over 6.5k protein groups identified per sample, with digestion controls showing ~0.99 average Pearson correlation at protein level.

Conclusions:
Robust and reproducible data was obtained without visible batch effects in the standard samples. Deep dive into the obtained DIA data is underway and will reveal biomarkers indicative of both PD and PD progression.

PP02.111: Protein Phosphorylation is Robustly Dysregulated in The Primary Auditory Cortex of Schizophrenia.

Gilardi J[1], Dumrongprechachan V[1], Garver M[1], Salisbury R[1], Newman J[1], Ding Y[1], Grubisha M[1], DeGiosio R[1], Xu K[1], Sweet R[1], MacDonald M[1]
[1]University of Pittsburgh Department of Psychiatry

Introduction: Pathologic alterations to synaptic protein networks are believed to underlie synapse loss and disease symptoms in schizophrenia. Phosphorylation plays an important role in synaptic protein trafficking and activity. Here, we utilize phosphoproteomic mass spectrometry to analyze human postmortem brain tissue from schizophrenia and matched control subjects, identifying synaptic phosphoprotein and phosphoprotein network alterations linked to spine loss in schizophrenia.

Methods: Homogenates were prepared from right hemisphere auditory cortex grey matter from 43 schizophrenia and 41 matched control subjects. Samples were digested with trypsin, barcoded with TMT, subject to phosphopeptide enrichment by Fe3+ cartridges on an AssayMAP Bravo, and analyzed on an Orbitrap Eclipse Mass Spectrometer.

Results: We observed 11,720 phosphopeptides, 7,240 of which were quantified with > 50% presents call. Of these phosphorylation sites, 1807 were significantly dysregulated in schizophrenia ($q < 0.05$) accounting for multiple hypothesis testing. Proteins with dysregulated phosphosites were significantly enriched for terms relating to both postsynaptic ($p = 3.95E-48$) and presynaptic function ($p = 4.93E-32$). We are currently investigating associations between altered protein phosphorylation and dendritic spine loss in this cohort. One phosphorylation site on the PALM1 (S116) was significantly associated with spine loss in tissue, and expression of the phosphomimic (S116D) induced spine loss in primary neuronal culture.

Conclusion: This study employs phosphoproteomic mass spectrometry to investigate the dynamic post translational modification, phosphorylation, and its role in the synaptic alternations observed in schizophrenia. Individuals with schizophrenia exhibit altered phosphoproteome expression. We are currently attempting to map kinase activation states in this cohort.

PP02.112: All-in-one: Comprehensive, Sensitive, and Reproducible Digitization of Clinical Biospecimen using Informed hybrid DIA/PRM-MS

Goetze S[1,2,3], Canterbury J[4], Fort K[5], van Drogen A[1,2,3], Robbiani D[6], Ghandi T[6], Makarov A[5], Moehring T[5], Wollscheid B[1,2], Xuan Y[5]
[1]Institute of Translational Medicine (ITM), Dep. of Health Sciences and Technology (D-HEST), ETH Zurich, [2]Swiss Institute of Bioinformatics (SIB), [3]Institute of Translational, [4]Thermo Fisher Scientific, [5]Thermo Fisher Scientific (Bremen) GmbH

Introduction

MS-based proteotyping has been widely employed for biomarker discovery, yet the clinical/translational proteotyping community requires strategies that not only enable the discovery of novel biomarker candidates but that also boost the probability of establishing protein-based biomarker assays, enhance analytical and clinical validation speed, and resolve the issue of data missingness. Here we present an intelligent data acquisition Hybrid-DIA strategy enabling the comprehensive digitization of a clinical specimen on the proteotype level while at the same time enhancing measurement sensitivity for a specific set of markers of clinical interest.

Methods

Hybrid-DIA uses the API of the Exploris or the Orbitrap Fusion Lumos. It combines standard DIA with isotope-triggered, multiplexed (msx) PRM optimized for sampling of the light, endogenous peptide. We established and optimized our Hybrid-DIA workflow on proteotypic peptide mixes for tumor-associated antigens as well as on isotopically-labeled AQUA-grade melanoma markers. We finally benchmarked our optimized Hybrid-DIA method on a melanoma patient cohort.

Results

Hybrid-DIA was applied to a melanoma patient cohort to capture clinically actionable information of limited biological specimens while at the same time digitizing the underlying proteotype for exploratory studies. In msxPRM-mode we monitored c.a. 50 classic diagnostic melanoma markers (level-1 information) and proteins of potential clinical relevance (level-2 information). We benchmarked the analytical validity of our optimized Hybrid-DIA method both in terms of DIA performance as well as limit of detection and quantification of msxPRM. We found that Hybrid-DIA allows for the robust detection and quantification of endogenous marker proteins with virtually no impact on DIA proteome profiling (level-3 information).

Conclusions

Hybrid-DIA minimizes sample consumption and measurement time by merging different acquisition schemes while at the same time targeted PRM measurements circumvent the issue of data missingness thereby preventing imputation. Also, Hybrid-DIA allows to absolutely quantify peptides in a specimen thereby providing enhanced diagnostic validity.

PP02.113: In-depth Identification and Accurate Quantification of Mitochondrial and Lysosomal Crosstalk Proteins

Cho B[1], Goo Y[1,2]
[1]Mass Spectrometry Technology Access Center at the McDonnell Genome Institute, Washington University School of Medicine, [2]Department of Biochemistry and Molecular Biophysics, Department of Genetics, Washington University School of Medicine

Introduction

Mitochondria and lysosomes coordinate a pivotal role in cellular metabolism by physical and functional interaction. The alterations in their crosstalk are known to be involved in muscular and neurodegenerative disorders such as Parkinson's, Alzheimer's, Huntington's, and amyotrophic lateral sclerosis. We established a simple method for organelle-specific in-depth proteome profiling without the use of enrichment, followed by targeted quantification of crosstalk proteins for mitochondria and lysosomes by dia-PASEF and prm-PASEF.

Methods

Three different human cell lines, HeLa, K562, and HEK293, were analyzed by dia-PASEF and prm-PASEF methods using timsTOF Pro2 with nanoElute system. The dia-PASEF data was queried against Bruker's built-in spectral library. The experimental collision cross section (CCS) and the normalized retention time (RT) values of identified peptides were applied to the prm-PASEF analysis. The prm-PASEF data was processed using the Skyline software.

Results

By employing the dia-PASEF analysis using whole cell lysate without the organelle-specific enrichment, over 1200 and 500 mitochondria- and lysosome-specific proteins, respectively, were identified from three human cell lines. The spectral library was generated for all identified peptides based on the CCS and the normalized RT values. The prm-PASEF analysis was developed to target 103 mitochondrial and 84 lysosomal proteins based on the three molecular functions, amino acid metabolism (109 protein targets), autophagy (80), and calcium homeostasis (27), known for their functional crosstalk between organelles. To evaluate the analytical performance, we analyzed 15 independent prm-PASEF datasets generated by three biological/five technical replicates and confirmed that the majority of the target peptides showed less than 15% of the coefficient of variation across all the data.

Conclusions

Our unbiased approach demonstrated that the use of dia-PASEF and prm-PASEF provides in-depth organelle-specific profiling and accurate quantification for tracking key organelle proteins involved in disease-specific crosstalk.

PP02.114: Portraying Primeval Immune Defense in Protein Folding Diseases: the Complement System in Amyloid

Gottwald J[1], Lux A[1], Treitz C[2], Mühle H[1], Gericke E[1], Behrens H[1], Daniel C[3], Amann K[3], Tholey A[2], Röcken C[1]
[1]Department of Pathology, Christian-Albrechts-University Kiel, [2]Systematic Proteome Research & Bioanalytics, Institute of Experimental Medicine, Christian-Albrechts-University Kiel, [3]Department of Nephropathology, Institute of Pathology, Friedrich-Alexander University Erlangen-Nürnberg

Introduction: Amyloidoses are a group of protein folding diseases with 36 known fibril-forming proteins. These fibrils agglomerate into insoluble amyloid deposits at different tissue sites, causing various clinical patterns and outcomes. These amyloid deposits are accompanied by amyloid-associated compounds called signature proteins, e.g., apolipoprotein E (apoE). Their role in amyloid formation and progression is mostly unknown. Recently, we and others reported on amyloid constituents associated with the complement system, an innate immune defense mechanism. Here, we present our findings on the co-occurrence of complement-associated components with several amyloid types. As the complement system is known to react to a broad range of pathological structures, mapping these compounds in amyloid deposits contributes to a better understanding of amyloidosis and could point to new leverage points for future therapies.

Methods: Amyloid is diagnosed by Congo red staining of formalin-fixed and paraffin-embedded tissue sections. Immunohistochemistry (IHC) depicts co-localization of complement components and signature proteins to these deposits. Both amyloid and the surrounding tissue are further investigated by liquid chromatography and tandem mass spectrometry (LC-MS/MS).

Results: Complement components C3 and C9 co-localized with amyloid deposits as depicted by IHC. Interestingly, C9 was found as regularly as the amyloid signature protein apoE in eight amyloidosis- and various tissue-types (1). Furthermore, LC-MS/MS analysis unraveled 22 complement-associated compounds correlating to transthyretin abundance in transthyretin amyloidosis of carpal tunnel tissue, including activators and final pathway components (2). Additionally, IHC unraveled potential evidence on complement activators enriched in amyloid lambda light chain in kidney tissue.

Conclusions: The repeated detection and enrichment of complement-associated compounds in amyloid hints towards an important role in the amyloid pathogenesis. Future investigations need to close the knowledge gap between the complement system and amyloid formation and/or progression.

References:
(1) Lux, A., et al. 2021 Amyloid 28(3)
(2) Treitz, C., et al. 2021 Amyloid 29(2)

PP02.115: Proteomics Signature in the Redox Dysregulation of Amyotrophic Lateral Sclerosis (ALS)

Greco V[1,2], Spalloni A[3], Scarano B[1], Roncada P[4], Longone P[3], Urbani A[1,2]
[1]Department of Basic Biotechnological Sciences, Intensivological and Perioperative Clinics, Catholic University Of The Sacred Heart, [2]Fondazione Policlinico Universitario Agostino Gemelli IRCCS, [3]Department of Experimental Neuroscience, Molecular Neurobiology Unit, Fondazione Santa Lucia, IRCCS, [4]Department of Health Sciences, University of Catanzaro Magna Græcia

Introduction: Amyotrophic lateral sclerosis (ALS) is a fatal multifactorial disease characterised by the death of motor neurons. Despite considerable research efforts, the exact mechanism underlying the pathogenesis of ALS is still not fully understood. Redox imbalance and mitochondrial dysfunction have long been associated with ALS, and recent evidence suggests that such dysregulation may act as a real driver of motor neuron degeneration. Central redox regulatory mechanisms, including the thioredoxin and glutathione systems, are disrupted in ALS. Furthermore, in recent years, our group has proposed an adaptation of hydrogen sulphide (H_2S)-driven metabolism as an emerging player in disease progression. The mechanisms underlying this alteration are elusive. Therefore, this study aims at an in-depth characterisation of the ALS proteome in order to highlight the molecular features, especially by looking at the remodelling of the redox profile to shed light on this still hidden H_2S-ALS crosstalk.
Methods: An in depth redox proteomics approach has been performed looking at protein thiol oxidation and persulfides in spinal cord tissues (total extracts and mitochondrial enriched fractions) prepared from ALS SOD1G93A and SODWT mice. Label free proteomic analysis has been performed using HRDDA approach on Orbitrap Fusion Lumos Tribrid Mass Spectrometer (Thermo Scientific). In addition, mapping of persulfidation sites have been developed. Peptide identification and quantification have been performed by Peaks and MaxQuant softwares respectively; bioinformatics analysis was performed by Ingenuity Pathway analysis (Qiagen-IPA).
Results: Significant protein hallmarks and a metabolomic reshaping have been highlighted in ALS SOD1G93A compared to SODWT mice. Moreover, redox investigation shed light on the proteome imbalance towards the oxidation of protein thiol and persulfides in SOD1-fALS. Conclusions: This investigation provides a comprehensive characterization of the redox scenario of ALS revealing molecular features hitherto unexplored.

PP02.116: From Patient Skin to the Brain: Understanding Neurodegeneration with Fibroblast-iPSC-neuron Proteomics

Frankenfield A[1], Li H[1], Hasan S[2], Gropman A[3], Chiaramello A[1], Ward M[2], Hao L[1]
[1]George Washington University, [2]National Institute of Neurological Disorder and Stroke, [3]Children's National Medical Center

Introduction: Patient skin fibroblasts can be reprogrammed into iPSCs and further differentiated into neurons, enabling the study of live human neurons that were previously inaccessible. Despite the identification of many genetic variants that cause brain diseases, the molecular mechanisms remain unclear. Here, I'll describe major efforts in our lab to develop proteomic strategies and decipher lysosomal and mitochondrial dynamics in human fibroblasts and fibroblast-iPSC-neuron models of neurodegeneration.

Methods: Dermal fibroblasts were obtained from both healthy subjects and diseased patients harboring genetic mutations that can cause inherited neurodegeneration. CRISPR-Cas9 technology was used to introduce genetic mutations in healthy subjects or correct genetic mutations in the diseased patients, creating multiple pairs of comparison groups. Fibroblasts can be directly used for proteomics and metabolomics and can also be reprogrammed into iPSCs and derived to neurons via the advanced i3Neuron technology. Proximity labeling proteomics was conducted to capture organelle microenvironment. (1,2) Dynamic SILAC proteomics was conducted to understand proteostasis in combination with other cell biology assays.

Results: DDA and DIA proteomics of patient fibroblasts carrying mitochondrial DNA mutation revealed OXPHOS complex deficiency and deficit in arginine biosynthesis, congruent with patient's chronic energy deficit. (3) Increased arginine administration may be suggested to improve patient care. Proximity labeling and dynamic SILAC proteomics in neurons carrying lysosomal gene mutation revealed deficit in lysosomal degradative function and altered lysosomal pH which could be targeted to design new therapies. (unpublished)

Conclusions: Using patient fibroblasts and fibroblasts-iPSC-neuron models of neurodegenerative diseases, we identified key therapeutic targets involved in mitochondrial and lysosomal dysfunction caused by inherited genetic mutations.

References

1.Frankenfield, M et al, 2020, Anal. Chem. (23), 15437-15444.
2. Frankenfield, M et al, 2022, J. Proteome. Res. (23), 15437-15444.
3. Li, H et al 2022, Mol. Omics, 18, 196-205.

PP02.117: Intermittent Fasting Induces Sexually Dimorphic Hepatic Interferon Alpha Signaling

Harney D[1], Cielesh M[1], Viengkhou B[1], Hofer M[1], Larance M[1]
[1]University Of Sydney

Introduction

Intermittent fasting (IF) is a beneficial dietary treatment for obesity which improves metabolic health independent of weight loss. Recently, we completed the first proteomic analysis of IF in male and female mice, which uncovered a sexually dimorphic response to IF in the liver. This response included a divergence in fatty acid metabolism between the sexes and an EODF induced potent interferon-□□(IFNα) signalling in female mice.

Methods

Male and female C57B/6J mice were subject to a 2-week every-other-day fasting (EODF) model with chow and compared against an ad libitum control. Metabolic health was assessed throughout the model, tissues were snap frozen on LN2 and submitted to a standard trypsin digest and stage-tip cleanup. Samples were analysed using conventional DIA proteomics on a QExactive HFX-fusion type instrument. Data was analysed using the DIA-NN search engine and subject to statistical analysis by two-way ANOVA.

Results

These analyses consistently identified >4,500 proteins across the cohort. Animals of both sexes had increased fatty acid catabolism, but this change was much greater in females whereas males had greater increases in fatty acid synthesis. Interestingly, females had increased interferon-alpha signalling after IF and increased abundance of downstream IFNα targets, whereas males had minimal change. Then we applied IF to castrated mice, which showed testosterone signalling represses IFNα pathway induction in the liver after IF. Next, mice that had one of the IFNα receptors knocked out (IFNAR1) before IF, where these animals produced no IFNα signalling response demonstrating that receptor engagement is necessary.

Conclusions

We hypothesis that IFNα signalling is tied to lipid biosynthetic flux and as this pathway is downregulated during the fasting period it engages IFNα through the STING pathway. This work highlights an interesting intervention that improves metabolic health in the absence of weight loss and is a novel opportunity for metabolic disease treatment.

PP02.118: Urinary Proteomic Characterization and its Relationship with the Metabolome of Women with Gestational Diabetes Mellitus through the Multiomics Approach

Herrera Van Oostdam A[1], Salgado Bustamante M[2], Oropeza Valdez J[3], Monárrez Espino J[4], Martínez Martínez E[5], Hernández M[6], Encarnación-Guevara S[6], Lopez Y[3]
[1]Centro de Investigación en Ciencias de la Salud y Biomedicina, Universidad Autónoma de San Luis Potosí, [2]Department of Biochemistry, Universidad Autónoma de San Luis Potosí, [3]CONACyT, Metabolomics and Proteomics Laboratory, Universidad Autónoma de Zacatecas, [4]Christus Muguerza Hospital Chihuahua-University of Monterrey, [5]Laboratory of Cell Communication and Extracellular Vesicles, Instituto Nacional de Medicina Genómica, [6]Centro de Ciencias Genómicas, Universidad Nacional Autónoma de México

Gestational Diabetes Mellitus (GDM) is defined as glucose intolerance that begins during pregnancy. It has been proposed that, in an adverse intrauterine environment, such as that generated by glucose intolerance, it promotes changes at the transcriptomic, proteomic and metabolomic levels. In a study carried out by our work team, the presence of urinary exosomes of placental origin was determined in women with GDM. In turn, the transcriptomic analysis of these vesicles was carried out based on a group of microRNAs, which showed a differential expression from the second trimester of pregnancy, being more evident in the third trimester. On the other hand, a metabolomic study was carried out in pregnant women with GDM during the third trimester of pregnancy, where an alteration of 14 metabolites was found, which were related to metabolic pathways such as steroid hormone biosynthesis and tryptophan biosynthesis. Therefore, a comprehensive study is carried out using multi-omic strategies to determine the urinary protein profile of women with GDM and to evaluate its association with the expression of the urinary metabolomic phenotype in patients with this pathology. Pregnant women were included in the study and a urine sample was requested at 12 and 24 weeks of gestation. We worked from composite samples (pools) homogenized from the age and body mass index of the participants. In a first phase of the study, the concentration of the urine samples was carried out by means of ultracentrifugation and subsequently the proteomic analysis was carried out through peptide labeling using stable isobaric isotopes (iTraq). Finally, a bioinformatic analysis will be carried out to correlate the possible interaction of the microRNAs profile (determined in the initial phase), the urinary proteomic content profile and the urinary metabolomic profile of women with GDM in early stages of pregnancy.

PP02.119: Utility of Quantitative Proteomics in Solving Rare Disease Diagnosis

Hock D[1], Semcesen L[1], Caruana N[1,2], Compton A[1], Amarasekera S[1], Stait T[1], Muellner-Wong L[1], Simons C[1], Christodoulou J[1], Thorburn D[1], Stroud D[1]
[1]University of Melbourne, [2]Victoria University

Introduction: Diagnostic yields from exome (ES) or genome sequencing (GS) in suspected rare diseases are typically ~50% of the cases. Large numbers of candidate genes with variable genotype-phenotype correlations complicate variant curation and diagnosis, leading to some patients and families waiting for decades for a definitive diagnosis. Delayed diagnosis not only places a burden in public health systems but also prevents any possible patient intervention. Undiagnosed patients can have zero to tens of candidate variants that may warrant functional follow-up to determine genetic diagnosis.
Methods: Label Free Quantitation (LFQ) and TMT-based proteomics were used to analyse primary fibroblasts from a retrospective cohort (N=10) of patients suspected of rare mitochondrial disease where ES/GS were inconclusive. We also comprehensively analysed (N=26) primary fibroblasts from diagnosed mitochondrial disease patients to validate and investigate the broad utility and limitations of the technique in the clinical setting.
Results: We achieved 80% diagnostic rate in the retrospective cohort with most variant types being intronic, missense and copy number variants. We also showed that proteomics was more sensitive and specific in detecting protein defects compared to classical enzymology test, currently the only accredited test for confirmation of mitochondrial disease in Australia. Including the retrospective cohort, our quantitative proteomic approaches have helped achieve a genetic diagnosis for over 30 rare disease patients, and contributed to the identification of 5 new disease genes that can allow faster diagnosis in the future.
Conclusions: Mass spectrometry-based quantitative proteomics has the potential to identify the functional impact of genetic variants by quantifying thousands of proteins in a single test, being a suitable approach for clinical accreditation and use in functional investigation of ES/GS inconclusive cases.

PP02.120: An Omics Approach to Identify Biomarkers Describing Duchenne Muscular Dystrophy Pathology

Jiménez Requena A[1], Johansson C[1], Al-khalili Szigyarto C[1]
[1]KTH University

Introduction

There is an unmet clinical need for diagnosis and disease progression biomarkers to monitor dystrophinopathies such as Duchenne Muscular Dystrophy (DMD). This study aims to identify novel potential biomarkers that characterise disease-related pathological changes. Exploring databases for information generated by omics methods such as RNA -sequencing, and immunohistochemistry can be used to more effectively select biomarker candidates for discovery studies. DMD is a severe disorder caused by mutations in the dystrophin gene. The affected individuals experience loss of muscle function, with a fatal ending. Current studies within DMD identified proteins as biomarkers primarily involved in muscle function or specifically expressed in muscle. That neglects the effect of dystrophin absence on other tissues than muscle and lacks the means to follow up pathological changes in other tissues and organs. With this approach, we aim to identify biomarker candidates to study disease-associated events such as increasing fibrosis and inflammation processes or, proteins involved in smooth muscle and/or brain function.

Methods

Whole transcriptome and proteome studies on the anatomic level can help identify and prioritise new biomarker candidates for DMD. Thus, we present a strategy to select biomarkers using biological information from the Human Protein Atlas, Gene Ontology, EURETHOS, etc. along with literature to monitor pathological changes and explore disease events in DMD.

Results

The strategy resulted in a scoring system for DMD biomarker candidates hypothesised to be associated with DMD. Among them, the top ten are explored with bead-based antibody arrays to determine their association with the disease.

Conclusions

This strategy of identifying novel biomarker candidates can facilitate our understanding of the DMD pathology. Further validation steps would allow these biomarkers to be used as tools to follow the diagnosis, prognosis and treatment of dystrophinopathies.

PP02.121: Proteomic Analysis of Urothelium in Patients with Overactive Bladder Disease

Kim G[1,4,5], Shin J[2], Na Y[2], Chang Y[3]
[1]Korea Basic Science Institute, [2]School of Medicine Chungnam National University, [3]School of Medicine Konyang University, [4]University of Science and Technology, [5]Graduate School of Analytical Science and Technology (GRAST) Chungnam National University

Overactive bladder (OAB) syndrome is a condition that has four symptoms: urgency, urinary frequency, nocturia, and urge incontinence and negatively affects a patient's life. Recently, it is considered that the urinary bladder urothelium is closely linked to pathogenesis of OAB. However, the mechanisms of pathogenesis of OAB at the molecular level remain poorly understood, mainly as a result of lack of modern molecular analysis. The goal of this study is to identify a potential target protein that could act as a predictive factor for effective diagnosis and aid in the development of therapeutic strategies for the treatment of OAB syndrome. We performed the first proteomic analysis on the mucosal layer (urothelium) of the bladders of sham control and OAB patients. As a result of proteome analysis, 199 proteins in the non-OAB group and 144 proteins in the OAB group were identified in more than two-thirds of the samples in each group. Signaling pathway analysis revealed that the differentially expressed proteins were mainly involved in the inflammatory response and apoptosis. Our findings suggest a new target for accurate diagnosis of OAB that can provide essential information for the development of drug treatment strategies as well as establish criteria for screening patients in the clinical environment.

PP02.122: FABP3 and IGFBP7 as Potential Biomarkers for Early Onset of Diabetic Cardiomyopathy

Junior A[1], Kim A[1,2], Han J[1,2]
[1]Inje University College Of Medicine, Department of Physiology, Cardiovascular Metabolic Disease Center, [2]Inje University College of Medicine, Department of Health Sciences and Technology
Introduction: Diabetic cardiomyopathy is a disorder of myocardial function that mainly affects diabetics and is initially reversible. However if left untreated, it quickly progresses to heart failure and systolic dysfunction, both terminal conditions. Thus, a distinct diagnostic marker for the early onset of diabetic cardiomyopathy is a dire need. The primary goal of this study was to confirm whether FABP3, IGFBP7, and MYL7, individually or in combination, could serve as biomarkers for screening the early onset of the disease.
Methods: Male C57BL/6J and db/db (diabetic) mice were studied for 13 weeks without intervention. Echocardiography was performed 3 days before sacrifice at 7, 9, 11, and 13 weeks. ELISA was used to determine plasma levels of FABP3, IGFBP7, and NT-proBNP; Western blot was used to determine the protein, and RT-PCR was used to quantify the mRNA levels of FABP3, IGFBP7, and MYL7.
Results: The echocardiography results remained normal throughout the study, but protein levels of FABP3, IGFBP7, and α-SMA gradually increased in the db/db group, following the same pattern for body weight and fasting blood glucose. MYL7 levels, on the other hand, were discovered to be lower and declining over time. In the db/db group, plasma levels of FABP3 and IGFBP7 increased over time, while the level of NT-proBNP between groups showed insignificant changes.
Conclusion: MYL7 is known to modulate cardiac development and contractility, and FABP3 and IGFBP7 protein levels are directly related to the severity of glucose dysregulation, suggesting great relevance and potential as biomarkers. While 2D and 3D echocardiograms can detect real-time structural and functional changes in DCM, they cannot detect the initial molecular changes that occur before structural changes such as fibrosis and cardiac hypertrophy appear. Our findings support the notion that FABP3 and IGFBP7 may hold great potential as early indicators of diabetic cardiomyopathy.

PP02.123: Global Phosphoproteomic Profiling of Signaling Pathway Aberrations in Human and Mouse Cardiomyopathies with Different Etiologies

Kuzmanov U[1,2], Reitz C[1,2], Wang E[2,3], Tavassoli M[1,2], Kim D[1,2], Hadipour-Lakmehsari S[1,2], Shah S[4,5], Teng A[1,2], Billia F[6,7], Emili A[8], Radisic M[2,3], Oudit G[4,5], Gramolini A[1,2]

[1]University Of Toronto, Department of Physiology, [2]Ted Rogers Centre for Heart Research, [3]University of Toronto, Institute of Biomaterials and Biomedical Engineering, [4]University of Alberta, Department of Medicine, [5]Mazankowski Alberta Heart Institute, [6]Toronto General Research Institute, [7]University Health Network, Division of Cardiology, [8]Boston University School of Medicine, Departments of Biochemistry and Biology and the Centre for Network Systems Biology

INTRODUCTION: More than 18 million deaths worldwide are attributed to cardiovascular diseases (CVDs) annually, and CVDs remain one of the leading causes of mortality in the developed world. CVD patient prognosis and outcomes rely heavily on patient etiology. However, due to the underlying complexity the majority of underlying molecular and signaling mechanisms have not been fully elucidated. Phosphorylation is a major point of protein regulation with dynamic and profound effects on function and activity of signaling networks. Currently, there is a limited breadth of comprehensive proteomic and phosphoproteomic studies examining CVD patients with differing etiologies.

METHODS: We have conducted studies to identify the signaling changes associated with ischemic cardiomyopathy (ICM), hypertrophic cardio myopathy (HCM), dilated cardiomyopathy (DCM) in patient tissue explants utilizing isobaric tag (TMT) based (phospho)proteomics. Additionally, further (phospho)proteomic profiling and validation experiments were performed on Biowire organ-on-a-chip and the mouse pressure-overload model. A 2-dimensional LC-MS methodology was applied utilizing hydrophilic interaction chromatography (HILIC) and TiO2-based phosphopeptide enrichment.

RESULTS: Overall, the combined proteomic and phosphoproteomic analysis identified and quantified more than 5,000 cardiac proteins with greater than 13,000 corresponding phosphorylation sites. We subjected the totality of this data to functional enrichment analysis to identify aberrant signaling pathways and other annotated biological processes from a compendium of publicly available databases resulting in the identification of more than a 1000 etiology-specific or common pathway annotations associated with ICM, HCM and DCM. These analyses resulted in the further investigation of the role of GSK3 in cardiac fibrosis and the identification of a dilated cardiomyopathy-specific hyper-phosphorylation cluster in the cardiomyocyte intercalated disc protein, αT-catenin (CTNNA3) in Biowires and/or mouse.

CONCLUSIONS: These studies provided a global overview into protein, phosphorylation, and pathway level changes occurring in CVD patient-derived cardiac tissue explants identifying unique attributes and molecular signatures with clinically actionable biological inferences.

PP02.124: Proteome Profiling of Peripheral Blood Mononuclear Cells in Dog with Mammary Gland Tumor

Lee J[1], Kyoung Y[1], Lee D[1], Kim J[1], Cho J[1]
[1]Seoul National University

Introduction: Peripheral blood mononuclear cell (PBMC) is a subpopulation of blood cells, including various immune cells. Due to less-invasive sampling, many researchers used them and found novel marker of disease. Intriguingly, lots of studies showed that the PBMCs in the cancer patients, which have met tumor tissue, were somehow different from those in the normal individuals. However, PBMC proteome has rarely been studied in dogs diagnosed with cancer.

Methods: We collected the blood samples from 30 dogs; 6 Normal dogs, 12 dogs with benign tumor, and 12 dogs with malignant tumor. Then, PBMCs were isolated from each sample using Ficoll and processed for proteomic analysis. These samples were labeled with TMTpro, and 36-fractionated through Nanomate. Finally, LC-MS3 analysis of the proteins from the PBMCs were done by Orbitrap Eclipse Tribrid mass spectrometer.

Results: We could found that the PBMCs in dogs bearing benign tumor were quite more different from normal than those in cancer patients. In particular, the proteins related to autophagy were down-regulated in benign group. Otherwise, the proteins related to IL-8 regulation were down-regulated in cancer group.

Conclusions: We profiled the proteomic dynamics of PBMCs related to canine mammary gland tumor. This profiling showed how the immune cells are affected by their neighboring tumors. Further studies are needed to understand their downstreams.

PP02.125: Investigation of Proteomic Changes in Biological Process Related to Neuroinflammation using Data-independent Acquisition Methods in Mouse CNS Cell Lines.

Lee G[1,2], Kim H[1,2], Park M[1,2], Jeon E[1,2], Kim S[2,3], Cheon S[1,3], Han D[1,2]
[1]Proteomics Core Facility, Biomedical Research Institute, Seoul National University Hospital, [2]Transdisciplinary Department of Medicine and Advanced Technology, Seoul National University Hospital, [3]School of Biological Sciences and Technology, Chonnam National University

Introduction : Inflammatory response is essential for eliminating invading pathogens as well as the encouragement of angiogenesis and wound healing inside the brain. However, persistent CNS inflammation has been linked to a number of neurodegenerative illnesses. Because Lipopolysaccharides are well known to induce neuro-inflammation associated with neurodegeneration, we focused proteomic alteration in inflammatory response to LPS-stimulation in three CNS cell lines including BV2(microglia), HT22(neuron), and C8-D1A(astrocyte).
Methods : The LPS was treated to the BV2, C8-D1A, and HT22 cell lines at five different concentrations depending on the cell density. To detect nitric oxide as an inflammatory mediator, nitrite assay kit was used. Expression level change of NOS2 induced by LPS stimulation were verified by western blot. For proteomic analysis, proteins from whole cell lyates and secretome were digested with Strap method. Peptide desalted with a C18 stage tip, and analyzed by Q-Exactive LC-MS/MS. Data-independent acquisition method was applied to quantification of proteome in three cell lines. MaxQuant and Spectronaut were used to process MS raw data files. Using Perseus software, all statistical analyses of MS data were carried out.
Results : For spectral library, we identified 10,000 proteins in mouse CNS cell line proteome using DDA method. DIA quantification showed that several hundred proteins were significantly modulated by LPS stimulation in three CNS cells. Of the differentially expressed proteins in stimulated cell line, we classified pathways that were related to immune-inflammatory responses, cellular metabolism, and extracellular matrix remodeling. Comparison analysis suggest that distinct biological process that associated with neurodegenerative disease are significantly enriched among three cell lines.
Conclusion : In conclusion, our proteomic study highlights how proteomes specific to different types of mouse CNS cell lines are activated, which may help us better understand the various pathological mechanisms underlying neurodegenerative disease associated with neuroinflammation.

PP02.126: Proteomics Investigation of Extracellular Matrix Remodeling Associated with Atherosclerotic Plaque Destabilization

Lorentzen L[1], Yeung K[2], Eldrup N[2], Eiberg J[2], Sillesen H[2], Davies M[1]
[1]Københavns Universitet, [2]Rigshospitalet

Aim: Cardiovascular disease (CVD) is the leading cause of death worldwide. Atherosclerosis, characterized by cholesterol and lipid accumulation in the artery wall, is the major underlying cause of CVD. While often asymptomatic for decades, destabilization and rupture of an atherosclerotic plaque can arise suddenly and cause an acute myocardial infarction or stroke. While remodeling of the extracellular matrix (ECM), is believed to play a major role in plaque destabilization, the mechanisms involved are not fully understood.

Methods: We compared 21 atherosclerotic plaques (7 hard plaques, 7 soft plaques, and 7 mixed/intermediate plaques) from patients with recently symptomatic carotid artery disease. Plaques were solubilized in 80% trifluoroacetic acid to ensure efficient extraction of both intra- and extracellular proteins. After neutralization with Tris, proteins were captured on magnetic microparticles for enzymatic digestion. Peptides were then analyzed by LC-MS/MS on a Bruker TIMS-TOF Pro with either DDA-PASEF or DIA-PASEF acquisition.

Results: By utilizing an efficient single-step extraction method in combination with state-of-the-art mass spectrometry, we were able to identify and quantify >4500 proteins from human carotid artery plaques, including 211 ECM proteins. Thus, we obtained unprecedented coverage of both the intra- and extracellular proteome across 21 samples with high reproducibility. We identified 630 proteins with differential abundances between stable and unstable lesions. We observe an enrichment of proteins involved in inflammation and ECM remodeling with upregulation of a range of proteolytic enzymes with concomitant loss of ECM proteins in soft lesions. This was also overserved, though to a lesser extent, in intermediate plaques.

Conclusions: The data presented offers a unique insight into the inflammatory mechanisms and ECM remodeling in plaque destabilization. The data provide a framework for the identification of novel biomarkers that can help distinguish stable and unstable plaques and thereby guide strategies for clinical intervention.

PP02.127: Cx43 Modulates the Protein Profile of Chondrocytes' Exosomes Promoting Cellular Senescence and Contributing to the Progression of Osteoarthritis

Varela-Eirin M[1,2], Carpintero-Fernández P[1], Guitián-Caamaño A[1], Varela-Vázquez A[1], García-Yuste A[1], López-Díaz I[1], Sánchez-Temprano A[1], Bravo-López S[3], Yañez-Cabanas J[4], Fonseca E[1], Largo R[5], Mobasheri A[6,7,8,9,10], Caeiro J[4], Mayán M[1]
[1]Instituto de Investigación Biomédica de A Coruña (INIBIC), Servizo Galego de Saúde (SERGAS). Universidade da Coruña, [2]European Research Institute for the Biology of Ageing (ERIBA), University Medical Center Groningen (UMCG), University of Groningen (RUG), [3]Instituto de Investigación Sanitaria de Santiago de Compostela (IDIS), Complexo Hospitalario Universitario de Santiago de Compostela (CHUS), Universidade de Santiago de Compostela (USC), [4]Complexo Hospitalario Universitario de Santiago de Compostela (CHUS), Universidade de Santiago de Compostela (USC), [5]Instituto de Investigación Sanitaria de la Fundación Jiménez Díaz (IIS-FJD), Universidad Autónoma de Madrid (UAM), [6]Faculty of Medicine, University of Oulu, [7]State Research Institute Centre for Innovative Medicine, [8]University Medical Center Utrecht, [9]First Affiliated Hospital of Sun Yat-sen University, [10]World Health Organization Collaborating Centre for Public Health Aspects of Musculoskeletal Health and Aging

Introduction: The accumulation of senescent cells is a key characteristic of aging, leading to the progression of age-related pathologies such as osteoarthritis (OA). Previous data from our laboratory has demonstrated that high levels of connexin43 (Cx43) are associated with a senescent phenotype in chondrocytes from OA cartilage. OA has been reclassified as a musculoskeletal disease characterized by the breakdown of the articular cartilage affecting the whole joint, subchondral bone, synovium, ligaments, tendons and muscles. However, the mechanisms that contribute to the spread of pathogenic factors throughout the joint tissues are still unknown.

Methods: We have isolated small extracellular vesicles (sEVs/exosomes) from chondrocytes with different Cx43 levels and analyzed their protein cargo by LC-MS/MS using a 6600 triple TOF, as well as their biological function in recipient cells (chondrocytes, bone and synovial cells).

Results: We show for the first time that sEVs released by OA chondrocytes contain high Cx43 protein levels and are able to induce a senescent phenotype in targeted primary chondrocytes, synovial and bone cells contributing to the formation of an inflammatory and degenerative joint environment by the secretion of senescence-associated secretory associated phenotype (SASP) molecules. Interestingly, LC-MS/MS analysis of OA chondrocytes-derived Cx43-sEVs revealed an altered protein profile, with an enrichment in proteins involved in stress responses (e.g. HSP90B1), DNA damage (e.g. PARP1, XRCC5), p53-signalling (e.g. TP53, IGFPB3, PAI-1) and catabolic processes (e.g. MMP-1, MMP-2), among others. Our results indicate a dual role for exosomal Cx43 activating cellular senescence and inflammation mediated by NF-kB, as well as dedifferentiation through the overexpression of ERK1/2, contributing to the loss of the fully differentiated phenotype in different cell types from the joint.

Conclusions: These results highlight the importance for futures studies to consider sEVs positive for Cx43 as new biomarkers of disease progression and new targets to treat OA.

PP02.128: Development of a Human Brain Proteome Frontotemporal Dementia Resource -Using Protein Profiles of Hereditary FTD to Explore Sporadic Disease Cases

Miedema S[1], Mol M[2], de Boer S[3], Dijkstra A[3,4], Koopmans F[1], Hondius D[1], van Nierop P[1], Menden K[5], de Veij Mestdagh C[1,3,6], van Rooij J[2,7], Ganz A[1,3], Paliukhovich I[1], Bakali C[1], Melhem S[2], Li K[1], Holstege H[3,8], Rizzu P[5], van Kesteren R[1], van de Berg W[9], van Swieten J[2,10], Heutink P[5], Pijnenburg Y[3], Smit A[1]
[1]Department of Molecular and Cellular Neurobiology, Vrije Universiteit Amsterdam, [2]Department of Neurology, Erasmus Medical Center, [3]Alzheimer Center Amsterdam, Amsterdam UMC location VUmc, [4]Department of Pathology, Amsterdam UMC location VUmc, [5]German Center for Neurodegenerative Diseases (DZNE) - Tübingen, [6]Department of Clinical Pharmacy and Pharmacology, University Medical Center Groningen, [7]Department of Internal Medicine, Erasmus Medical Center, [8]Department of Clinical Genetics, Amsterdam UMC location VUmc, [9]Department of Neuroscience, Amsterdam UMC location VUmc, [10]Department of Clinical Genetics, Erasmus Medical Center

INTRODUCTION: Frontotemporal dementia (FTD) is a major cause of a lethal early-onset dementia. FTD is hereditary in 30% of cases, mainly induced by mutations in the C9orf72 (FTD-C9), progranulin (FTD-GRN), and microtubule associated protein tau (FTD-MAPT) genes. The remaining 70% (sporadic FTD) can be conceived as a complex trait disorder. Our research aims to determine key cell types and disease mechanisms for hereditary FTD and to construct a disease framework to enable profiling of sporadic cases. This work forms the basis of a protein resource for frontotemporal dementia.
METHODS: Laser-dissected frontal and temporal cortex tissues from FTD-C9 (n=16), FTD-GRN (n=9), FTD-MAPT (n=13), and non-demented controls (n=11) were analysed by LC-MS/MS using an Ultimate3000 system coupled to the TripleTOF5600 running in DIA mode. FTD-MAPT data was also compared to an Alzheimer's Disease (AD) proteomic signature (n=10).
RESULTS: Differential abundance analysis ($q<0.05$) revealed brain area-specific protein signatures, with minor regulation in the frontal cortex for FTD-C9 (n=41), and major regulation in the frontal cortex for FTD-GRN (n=579) and temporal cortex for FTD-MAPT (n=488). Gene ontology analysis indicated the presence of genetic subtype-specific disease mechanisms. Using scRNAseq resources we deduced the involvement of major brain cell types. In FTD-GRN, we observed a role for immune processes related to endothelial cells and for mitochondrial dysregulation in neurons. In FTD-MAPT, we observed involvement of dysregulated RNA processing, oligodendrocyte dysfunction, and axonal impairments. Comparison of FTD-MAPT with AD indicated that alterations in RNA processing and oligodendrocyte function are specific to FTD-MAPT.
CONCLUSION: Our results demonstrate cell type-specific biological processes distinctive for genetic FTD subtypes. Proteomic profiling of multiple brain regions of sporadic cases is currently ongoing. With our efforts we aim to build a resource that can be used to explore potential mechanisms of disease and to pave the way for development of disease subtype-specific treatment.

PP02.129: Deep Plasma Protein Profiling in Alzheimer's Disease (AD) with a Novel Unbiased and Scalable Proteogenomics Approach.

Motamedchaboki K, Zamanighomi M[1], Guturu H[1], Wang J[1], Huang T[1], Benz R[1], de Geus M[2], Das S[2], Kivisakk P[2], Batzoglou S[1], Arnold S[2], Siddiqui A[1]
[1]Seer, [2]Massachusetts General Hospital

Introduction

Biofluids are a rich source of protein biomarkers for early detection of diseases, but the large dynamic range of protein concentrations in some biofluids like plasma necessitates complex workflow and trade-offs between throughput, coverage, and precision. Here, we use multiple physicochemically distinct nanoparticles to provide broad, unbiased coverage of the plasma proteome at scale.

Methods

Plasma samples from 100 AD and 100 non-AD healthy age- and sex- matched controls (HC), were interrogated using the Proteograph™ Product Suite (Seer Inc.) and liquid-chromatography mass-spectrometry analysis.

Results

Across the samples, data-independent acquisition yielded 39,699 peptides and 5,060 plasma proteins. Data-dependent acquisition (DDA) yielded 36,496 peptides and 4,706 proteins.

A pQTL analysis was conducted on a subset of the individuals (n=139) genotyped on the Global Screening Array platform. We have applied a simple linear model with a χ^2 statistic with 1-degree of freedom to establish significance of association between every SNP and Protein:Nanoparticle intensity. We further performed permutation tests for FDR < 0.01 to achieve high confidence in our associations. We were able to identify 73 cis-pQTLs associated with 20 proteins and 41 SNPs, and approximately 1,527 trans-pQTLs from DDA.

Finally, power analysis demonstrates that a minimum sample size of 84 is sufficient to detect a fold change of 1.5x between AD and HC with 90% power at an FDR of 5%. Projecting the power analysis to a larger cohort size demonstrates that a sample size of 600 will detect a fold change of 1.2x with over 90% power at an FDR of 5%.

Conclusions

The plasma proteomics analyses in this cohort has identified a combination of known and potential new candidate plasma protein markers, demonstrating the Proteograph platform's ability to perform unbiased, deep, and rapid interrogation of the plasma proteome, enabling large-scale studies to detect novel insights with clinically relevant potential.

PP02.130: Deciphering CSF Protein Signatures in FTD

Mravinacová S[1], Gómez de San José N[2], Bergström S[1], Olofsson J[1], Steinacker P[3], Anderl-Straub S[2], Danek A[5], Diehl-Schmid J[6], Fassbender K[7], Fliessbach K[8], Holger J[9], Kornhuber J[10], Landwehrmeyer B[2], Lauer M[11], Ludolph A[2,4], Oeckl P[2,4], Prudlo J[8], Schneider A[8], Schroeter M[12], Wiltfang J[13], Otto M[3], Nilsson P[1], Månberg A[1], German FTLD consortium
[1]Department of Protein Science, SciLifeLab, KTH Royal Institute of Technology, [2]Department of Neurology, Ulm University Hospital, [3]Department of Neurology, Martin Luther University of Halle-Wittenberg, [4]German Center for Neurodegenerative Diseases (DZNE e.V.), [5]Department of Neurology, Ludwig-Maximilians-University Munich, [6]Department of Psychiatry, Technical University of Munich, [7]Department of Neurology, Saarland University, [8]Department of Neurodegenerative Diseases and Geriatric Psychiatry, University of Bonn and DZNE Bonn, [9]Department of Psychiatry, University Hospital Hamburg, [10]Department of Psychiatry, Friedrich-Alexander University Erlangen-Nuremberg, [11]Department of Psychiatry and Psychotherapy, University of Würzburg, [12]Clinic for Cognitive Neurology, University Clinic Leipzig, and Max Planck Institute for Human Cognitive and Brain Sciences, [13]Department of Psychiatry and Psychotherapy, University Medical Center Goettingen, and DZNE

Introduction: Frontotemporal dementia (FTD) encompass a spectrum of dementias characterized by neurodegeneration in frontal and/or temporal lobe. Due to the diversity of clinicopathological symptoms and similarity with other neurodegenerative disorders, FTD is difficult to diagnose. Detailed understanding of the processes and proteins involved is critical in order to identify new biomarkers for accurate diagnosis, diseases progression and use in clinical trials.

Methods: We used a targeted multiplex antibody-based suspension bead array technology to analyze the relative levels of > 350 proteins in CSF from approximately 600 individuals with different variants of FTD (mostly sporadic), other neurodegenerative disorders and healthy individuals. The measured proteins were thoroughly selected from the Human Protein Atlas (www.proteinatlas.org) based on their RNA expression level in brain tissues, brain tissue specificity and reliability of the available antibodies. Additionally, selected targets from literature and previous in-house neuroproteomic efforts were included.

Results: Correlation clustering analysis will be performed to identify protein clusters associated with FTD pathology. The protein clusters will be further analyzed in relation to clinical presentation of patients.

Conclusions: We aim to provide insights into proteins involved in FTD pathology and unravel the association between CSF brain proteins and clinical measurements. The ultimate goal is to provide a comprehensive overview of these protein signatures also in the context of other diseases, expanding our current understanding of neurodegenerative disorders.

PP02.131: Deciphering the Proteomic Signature of Xeroderma Pigmentosum C Disease at Basal State and After UV Irradiation in Human Keratinocytes

Nasrallah A[1], Bourgoin S[2], Urs A[2], Sulpice E[1], Couté Y[3], Belmudes L[3], Gidrol X[1], Sève M[2], Rachidi W[1]
[1]Univ. Grenoble Alpes, INSERM, CEA, BIOMICS U1292, [2]Univ. Grenoble Alpes, CNRS, TIMC U5525, EPSP-PROMETHEE Proteomic Platform, [3]Univ. Grenoble Alpes, INSERM, CEA, UMR BioSante U1292, CNRS, CEA, FR2048

The sun's DNA-damaging ultraviolet (UV) radiation remains the major extrinsic risk factor for skin cancer development. Nevertheless, mammalian cells exploit the presence of the nucleotide excision repair (NER) pathway as a protective shield to eliminate the accumulation of photoproducts generated by UVB radiation. Xeroderma Pigmentosum C (XPC) is a key multifunctional protein implicated in the NER pathway that acts as a sensor for helical distortions found in DNA lesions. Loss of function mutations in the XPC gene would render the patient’s skin cells to be highly photosensitive with photoproducts accumulation and 10,000 folds more prone to skin cancers compared to normal population. Unfortunately, until date, the absence of a reproducible XPC cellular disease model seems to be a barrier for further research advancements. We therefore generated via CRISPR-Cas9 genome editing tool, the first reproducible XPC cellular disease model recapitulating the two major key phenotype characteristics, which are UVB photosensitivity as well as the delay or absence of DNA damage repair. WT cells and XPC knockout cellular model were then used to map the specific proteomic signature of XPC cells at basal state and its modifications post UVB irradiation. For this, total proteomes of WT and XPC cells subjected or not to UVB irradiation (power, time) were prepared and analysed by mass spectrometry-based quantitative proteomics using isobaric labelling. Peptides and proteins were identified and quantified using MaxQuant software. The statistical analysis of the quantitative datasets was performed using ProStaR. Surprisingly, a unique XPC disease proteomic signature of around 400 proteins was significantly, specifically, and differentially altered in UVB irradiated XPC KO versus WT cells. This novel signature will pave the way to understand the behaviour of XPC cells photosensitivity and repair capacity, and unravel key biomarkers for skin cancer phenotype for novel therapeutics.

PP02.132: Toxicoproteomics Reveals an Effect of Clozapine on Autophagy in Human Liver Spheroids

Nury C[1], Celine M[1], Eb-Levadoux Y[1], Bovard D[1], Porchet M[1], Maranzano F[1], Loncarevic I[1], Yepiskoposyan H[1], Marescotti D[1], Hoeng J[1], Ivanov N[1], Renggli K[1], Titz B[1]
[1]Philip Morris International

Introduction

Clozapine is a potent atypical antipsychotic drug used for severe schizophrenia. However, it has a narrow therapeutic window, and many patients experience severe side effects, including liver enzyme abnormalities, underscoring the need for a safer first-line therapy. Clozapine's molecular effects have been explored using diverse test systems, but in-depth proteomics investigations on clozapine's effects are rare. We performed a Data-Independent Acquisition (DIA)-based quantitative proteomics investigation of clozapine-treated human liver spheroid cultures.

Methods

HepaRG spheroids were treated with clozapine, and proteins were extracted using the PreOmics iST kit as described in the manufacturer's protocol. DIA analysis was performed using an EASYnanoLC 1200 connected to a Q-Exactive HF. Raw data were analyzed using directDIA on Spectronaut software. Autophagy markers were validated using Parallel Reaction Monitoring (PRM) and western blot.

Results

We quantified a total of 4,479 proteins across all the groups. Clozapine treatment induced 36 differentially expressed proteins (FDR < 0.05). Gene-set enrichment analysis indicated perturbation of several gene sets, including those involved in interferon-gamma signaling (e.g., IFNGR1) and prominently autophagy-related processes (e.g., upregulation of SQSTM1, MAP1LC3B/LC3B2, GABARAPL2, and NCOA4). Moreover, four autophagy-related markers demonstrated a statistically significant dose response to clozapine treatment compared to the control set response. We further confirmed the SQSTM1 response using PRM and western blot. Finally, we could conclude that it was unlikely that a strong pro-inflammatory response was caused by clozapine and found that the measured effects in our assays were likely related to clozapine itself rather than its metabolites.

Conclusions

This work exemplifies how proteomics can help elucidate physiological and toxicological mechanisms. Our results were consistent with prior literature and suggest that clozapine's therapeutic effects as well as its side effects broadly contribute to autophagy.

PP02.133: Plasma Proteomics to Study Hypertension as an Alzheimer's Disease Risk Factor in African American Adults

Oliver N[1], Robinson R
[1]Vanderbilt University

Introduction:
Many factors contribute to higher incidence of hypertension and Alzheimer's disease (AD) in African American/Black adults, but it is unclear how molecular changes in hypertension increase risk for AD. In the Proteomics of Hypertension and Alzheimer's Disease in African Americans (PrHADAA) study, we seek to understand this risk by first measuring plasma proteins to characterize hypertension in adults from the Southern Community Cohort Study (SCCS). We applied a robust automated sample preparation workflow for plasma proteomics to samples from SCCS and here focus on its application including assessment of quality control (QC) metrics towards mass spectrometry (MS) analysis.

Methods:
Plasma samples (N=808) from normotensive and hypertensive African American/Black adults were analyzed using a previously established automated plasma proteomics workflow. Samples were grouped into batches and subjected to immunodepletion, BCA assay, tryptic digestion, TMTpro labeling, and fractionation. Digestion and labeling steps were performed on a robotic liquid handler accompanied with QC checks using liquid chromatography (LC)-MS/MS analysis on a Q-Exactive HF.

Results:
Immunodepleted plasma samples from the SCCS required ~1728 HPLC injections and resulted an average protein concentration of 1.371 µg/µL. Given the variability in protein concentration, sample volumes and robotic liquid handler processing steps were designed to handle consistent amounts of protein in each sample batch. QC checks with LC-MS/MS led to 294 proteins identified with 3.30% coefficient of variation (CV) across batches. TMTpro labeling efficiencies and LC fractionation retention times had <5 %CVs. This presentation will provide an overview of study design, QC specifications, and analytical performance of automated workflow as applied to samples from SCCS.

Conclusions:
An automated plasma proteomics workflow with robust QC metrics has enabled the preparedness of plasma samples to understand how hypertension increases AD risk in African-American/Black adults.

PP02.134: CSF Protein Profiles in Relation to Disease Progression Rates in Patients with Amyotrophic Lateral Sclerosis

Olofsson J[1], Bergström S[1], Kläppe U[2], Ingre C[2], Anggraeni Andersson M[1], Månberg A[1]
[1]Department of Protein Science, KTH Royal Institute of Technology, Scilifelab, [2]Department of Clinical Neuroscience, Karolinska Institutet

Introduction: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by degeneration of motor neurons which leads to muscle weakness and paralysis. No efficient treatments exist, and most patients die within 2-5 years after disease onset. However, the heterogeneity of the disease is reflected in different progression rates with a small subset of the patients surviving for over 10 years. Currently, specific markers for ALS and prediction of progression rate are missing. We aim to identify proteins in CSF from ALS patients that might increase understanding of the disease and aid in discriminating between fast and slow progressing patients.
Methods: An antibody-based suspension bead array was used to analyze levels of 154 proteins in CSF from 423 individuals from a well characterized Swedish cohort including ALS patients, ALS mimics, healthy controls, and other neurological controls. Longitudinal ALSFRS-R scores as well as up to four samples from different time points were available for the ALS patients.
Results: So far, a subset of the samples have been analyzed and preliminary results show several proteins with significant differences in levels between ALS patients and controls. Among these, chitotriosidase (CHIT1) and osteopontin (SPP1) were found at elevated levels in patients compared to controls (CHIT1; p=2e-6, SPP1; p=1e-4). Furthermore, neurofilament medium (NEFM) showed significant differences in protein levels between fast and slow disease progressors when stratifying patients based on ALSFRS-R (p=0.004).
Conclusions: Several proteins, including CHIT1, SPP1 and NEFM, were observed as interesting in a subset of the samples. We are currently analyzing the full cohort to verify our findings and explore additional proteins and patterns able to predict disease progression rate. We will also compare protein profiles with other neurological diseases including FTD and MS. Our results could lead to a more personalized treatment and improved quality of life for the patients.

PP02.135: Histological and Proteomic Analysis for the Evaluation of Perfusion Culture System of Spheroids

Ono T[1], Noguchi R[1], Sin Y[1], Osaki J[1], Akiyama T[1], Adachi Y[1], Nakano A[1], Yanagihara K[3], Amari K[4], Sugihara Y[5], Yoshimatsu Y[4], Kodera Y[6], Kondo T[1]

[1]Division of Rare Cancer Research, National Cancer Research Institute , [2]Department of School of Biomedical Science, Nagasaki University, [3]Biospecimen Laboratories Inc., [4]Division of Patient-Derived Cancer Model, Tochigi Cancer Center Research, [5]Division of Cancer Proteogenomics, Tochigi Cancer Center Research , [6]Center for Disease Proteomics, Kitasato University School of Science

Introduction

Constant nutrient supply by blood vessels is important for tumor maintenance and growth. In the static culture, the medium is changed every several days, which is different from the natural environment. Perfusion culture provides a constant supply of fresh medium to the tumor tissue and eliminates waste products. The comprehensive analysis allows us to evaluate the usefulness of perfusion culture from multiple perspectives at the molecular level. However, there are few cases in which the difference between static and perfusion culture was compared in a comprehensive analysis.

Methods

In this study, we investigated the characteristics of perfusion culture. The spheroids were used instead of tumor tissue. The patient-derived undifferentiated polymorphous sarcoma cell line NCC-UPS4-C1 was used for the fabrication of the spheroids. Histological observations and proteomic analysis were performed to examine the properties of perfusion-cultured spheroids. For histological observation, spheroids were paraffin-embedded, sectioned, and stained with hematoxylin-eosin. Phase transfer surfactant was added to the spheroids, and proteins were extracted using an ultrasonic disrupter. The extracted proteins were enzymatically digested and examined by LC-MS/MS.

Results

Histological observations showed that perfusion culture prevented necrosis inside the spheroids. LC-MS/MS confirmed the expression of 2400 proteins. Among the 1000 proteins for which comparative analysis was possible with high accuracy, we identified proteins whose expression was upregulated by perfusion culture. Prevention of internal necrosis by perfusion culture is important to distinguish between cell death by the culture stress and the anti-cancer drugs. One of the proteins up-regulated by perfusion culture is the E3 ubiquitin-protein ligase HUWE1, which ubiquitinates the anti-apoptotic factor Mcl-1, and its expression may influence drug sensitivity testing.

Conclusions

The perfusion culture is a promising method for in vitro drug sensitivity tests using spheroids. To further demonstrate the practical utility of perfusion culture, we plan to analyze the perfusion-cultured tumor tissues.

PP02.136: Proteome Analysis of FFPE and Fresh-frozen Meningeal Tumor Tissue

Østergaard O[1], Maier A[2,3], Mirian C[1,2], Scheie D[3], Mathiesen T[2,4,5], Olsen J[1]
[1]NNF Center For Protein Research, Copenhagen University, [2]Department of Neurosurgery, Rigshospitalet, [3]Department of Pathology, Rigshospitalet, [4]Department of Clinical Medicine, University of Copenhagen, [5]Department of Clinical Neuroscience, Karolinska Institutet,

Introduction

Meningiomas are the most common primary intracranial tumor in adults. Differences in the proteome from formalin-fixed paraffin-embedded (FFPE) meningioma samples and frozen meningioma tissue are unknown. FFPE material is more readily available for large, population-based, retrospective studies. Thus, investigation of differences in tissue preservation type and age of meningioma sample is pertinent for proteomics research into the meningioma proteome.

Methods

Meningioma tissue showing different grades of malignancy was collected during surgery and either frozen directly or preserved by FFPE embedding and biobanked. Paired samples of frozen and FFPE tissue (n=6) were selected and after deparaffinization, both types of samples were subjected to protein extraction with boiling 4% SDS. Extracted protein was quantified, and equal amounts digested using eLysC and Trypsin. Desalted peptides were loaded on EvoTips and analyzed by 30SPD on EvoSep One HPLC connected to an Orbitrap Exploris 480 MS in data-independent acquisition (DIA) mode. Resulting raw files were processed using directDIA in Spectronaut v16, and bioinformatics of resulting protein matrices performed in Perseus and Rstudio.

Results

Protein yields were higher from frozen tissue relative to FFPE tissue, but MS analysis using equal peptide loading amounts resulted in quantification of comparable number of identified proteins (5100-5800 proteins) from FFPE and frozen tissue. Hierarchical clustering showed two main clusters representing the preservation methods and a subcluster representing a patient who contributed with more than one sample. 26% of the proteins showed significant differences between FFPE and frozen tissue. FFPE samples showed higher variation between young samples (3.5 yrs) and old samples (18 yrs) in comparison to frozen samples. Abundance of certain proteins seems to correlate with malignancy.

Conclusion

Differential proteome analysis of meningiomas is feasible when pre-analytical variables are controlled for.

PP02.137: Snapshots of Chronic Heart Failure. Can Proteomics Discriminate between Left-ventricular and Bi-ventricular Heart Failure?

Behounek M[1], Lipcseyova D[1], Melenovsky V[2], Benes J[2], Petrak J[1]
[1]BIOCEV, First Faculty of Medicine, Charles Universit, [2]Institute for Clinical and Experimental Medicine (IKEM)

Introduction

In chronic heart failure (HF), heart muscle is unable to pump enough blood to meet the body's demand for blood and oxygen. Left ventricular heart failure (LVHF) is more common, right-ventricular dysfunction develops often because of the LVHF presence, resulting in bi-ventricular heart failure (biVHF). Patients with biVHF have worse prognosis than LVHF patients, and respond differently to standard therapies. Characterization of molecular differences between biVHF and LVHF could assist diagnosis and therapy of biVHF. The aim of our project was to identify proteins differentially expressed in the hearts of patients with biVHF compared to patients with LVHF.

Methods

Using LFQ LC-MS/MS we analyzed myocardial samples of patients with LVHF and biVHF undergoing heart transplantations, and from control non-failing heart donors . To reduce the content of highly abundant myofibrilar proteins, we used a "mild" detergent Triton X-100 for sample solubilisation. To remove the detergent SP3 sample processing was used.

Results

The analysis resulted in identification of roughly 3000 proteins in both heart ventricles. Numerous differentially expressed proteins were identified in both types of heart failure relative to control myocardium. However, the differences between hearts of LVHF and biVHF patients were rather subtle. Only 9 and 23 proteins were differentially expressed in the left and right ventricle, respectively. Differential expression was confirmed by western blotting (periostin, fibulin 5, myosin 10, UCHL-1, myoferlin…). Selected proteins with extracellular localization were quantified in HF patient blood plasma using ELISA to determine their diagnostic potential.

Conclusions

Among the proteins differentially expressed in the heart of patients with biVHF relative to patients with LVHF, secreted protein fibulin 5 was confirmed as significantly upregulated in biVHF. More importantly, fibulin 5 concentration in blood plasma of patients with biVHF was significantly increased compared to healthy volunteers, preliminarily suggesting its potential as a circulating marker of biVHF.

PP02.138: Mass Spectrometry-based Proteomics on Parkinson's Disease Post-mortem Human Brain.

Pita Illobre D[1], Koopmans F[1], Hoozemans J[2], Smit A[1], Li K[1], Rozemuller A[2]
[1]Department of Molecular and Cellular Neurobiology, Center for Neurogenomics and Cognitive Research, Amsterdam Neuroscience, Vrije Universiteit Amsterdam, [2]Department of Pathology, Amsterdam Neuroscience, Amsterdam University Medical Centres, Location VUmc

Introduction: Parkinson's disease (PD) is the most common neurodegenerative movement disorder. It is characterized clinically by parkinsonism and pathologically by the loss of neurons in the substantia nigra pars compacta and the presence of Lewy bodies in the cytoplasm of neurons, which are positive for alpha-synuclein. PD is a multifactorial disease and even when some of the main molecular mechanisms involved in its development are known, a definite etiology or unifying sequence of molecular events cannot be established. To unravel the proteome changes, we performed quantitative mass spectrometry-based proteomics. In addition, we selected the top protein candidates for immunohistochemistry analysis of the tissue.

Methods: Human fresh frozen post-mortem putamen from PD and control patients (n= 9 and 10 respectively) were analyzed using Evosep LC system coupled to TimsTof Pro2 mass spectrometer. A gradient of 30 samples per day with dia-PASEF program was used. Database search was performed on DIA-NN and sample quality control and statistical analysis were performed on MS-DAP R script (q-value < 0,05).

Results: Approximately 5.000 proteins and 33.000 peptides were identified and quantified per sample. Of these proteins, 77 were found to be downregulated and 109 upregulated in PD when compared to control, showing an important downregulation in synaptic processes as result of the downregulation of the dopaminergic pathway, proteins like tyrosine hydroxylate (FC=-1,93) and Aldehyde Dehydrogenase 1 Family Member A1 (FC=-2,06) were identified; as well as an upregulation in mitochondrial and metabolic processes. Proteomics findings were confirmed with immunohistochemistry analysis.

Conclusions: The upregulation of metabolic and mitochondrial processes in our data has brought an interesting difference between PD and other neurodegenerative disorders, where most of these processes are downregulated. Further study in this research line may lead to the discovery of PD specific targets.

PP02.139: Increased Levels of Cytoplasmatic TDP-43 can Influence Cell Fate Beyond Stress Granules Formation.

Rosa Fernandes L[1,2], Cheng F[1], Chapman T[1], Davidson J[1], Rayner S[1], Morsch M[1], Chung R[1], Lee A[1]
[1]Macquarie University Centre for Motor Neuron Disease Research, Macquarie Medical School, [2]University of Sao Paulo

Introduction: TAR DNA-binding protein 43 (TDP-43) is a DNA/RNA binding protein involved in the biogenesis and processing of non-coding and protein-coding RNA. TDP-43 proteinopathies are a common feature of related neurodegenerative disorders, including amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD). The pathological hallmarks comprise nucleus to cytoplasmic mislocalization, where protein deposits can act as inclusion bodies in patients' brain, spinal cord, and muscle tissue. TDP-43 has defined roles in the cytoplasm, including stress granule (SG) regulation, mRNA stability and trafficking, and autophagy. Effects of increased cytoplasmic TDP-43 levels and respective cellular functions remain largely unknown as most studies focus on the loss of TDP-43 function with increased SG formation. Methods: We used a label-free MS-based proteomics approach combined with a bioinformatics pipeline to evaluate the influence of TDP-43 overexpression in neuronal cells before SG formation. Results: Overexpression of TDP-43 alone modulates the expression of 344 proteins ($q<0.05$) in N2A cells, being 165 and 179 up and downregulated, respectively. Aberrant protein expression expanded through all major cell compartments, including nuclear and plasma membranes. Disrupted biological processes included negative regulation of nucleosome binding and mRNA catabolic process, protein acetyltransferase complex, and positive regulation of JUN kinase activity. Moreover, the known TDP-53 regulated metabolic genes Cox3, Cox6a, Cox11, Cox20, Rraga, and Lamtor4 were also modulated. Conclusions: Increased levels of cytoplasmatic TDP-43 can influence cell fate beyond the formation of SG aggregates. Functional studies are needed to determine the role of the observed modulations on the course of TDP-43 proteinopathies and their possible impact on ALS and FTLD patient outcomes.

PP02.140: The Synaptic Proteome of Autism Spectrum Disorder Across Postnatal Development in Human Visual Cortex

Ruiz S[1], Xu K[2], Salisbury R[2], MacDonald M[1,2]
[1]University Of Pittsburgh, [2]University of Pittsburgh Medical Center

Introduction:
Despite diversity in symptoms and severity in autism spectrum disorders (ASD), spine alterations are observed in many genetic (ASD) models and subject tissue. Genetic studies of ASD have identified rare variants and common loci that implicate the synaptic protein networks crucial for spine formation and stabilization. Here, in a preliminary study, we assayed synaptic protein levels in primary visual cortex tissue from postnatal ASD and matched neurotypical subjects.

Methods:
Postmortem primary visual cortex tissue from 31 pairs of ASD and neurotypical subjects (ages 4-33) matched for sex, age and postmortem interval were obtained from the University of Maryland branch of the NIH NeuroBioBank. Homogenate and synaptosome enriched samples were digested with trypsin, TMT-labeled, fractionated, and analyzed on an Orbitrap Eclipse with SPS and real time search. Peptide and protein identification and quantification were performed in Proteome Discoverer 2.5.

Results:
7,577 proteins were identified in synaptosome enrichments across all subjects. Synaptosome levels of 1,462 proteins were significantly associated with age ($q > 0.05$); with 860 decreasing during postnatal development and 539 increasing. Interestingly, the 860 proteins that decreased with age were significantly enriched for the GO term synapse in SYNGO ($q = 5.82e\text{-}15$, relative to the 7,577 proteins quantified) while the proteins that increased with age were not. Similarly, the proteins that decreased with age included 40 ASD risk genes while increasing proteins included only 4 ASD risk genes. We are currently assaying homogenate protein expression and phosphorylation in this cohort.

Conclusions:
Synaptic protein levels are robustly altered across postnatal development in the human primary visual cortex. The decreased synaptosome levels of canonical synaptic proteins and ASD risk genes likely reflects the rearrangement and loss of synaptic proteins that accompanies synaptic pruning in postnatal development and the importance of ASD genes in prenatal synaptic development.

PP02.141: Deciphering New Roles of Glucosamine-6-phosphate Isomerase 2 (GNPDA2) in Neurodegeneration through Proteomics

Lachén-Montes M[1], Iñigo-Marco I[1], Cartas-Cejudo P[1], Ballesteros C[1], Fernández-Irigoyen J[1], Santamaría E[1]
[1]Clinical Neuroproteomics Unit, Navarrabiomed, HUN, UPNA, IdiSNA

Introduction: Glucosamine-6-phosphate isomerase 2 (GNPDA2) participates in the glucose metabolism, converting D-glucosamine-6-phosphate into D-fructose-6-phosphate and ammonium. Traditionally linked to obesity, our group revealed a significant increment in olfactory GNPDA2 protein levels in Alzheimer´s and Parkinson´s diseases. However, the role of GNPDA2 in neurodegeneration (ND) is largely unknown.

Methods: To evaluate the effects of GNPDA2 in different neurological contexts, we have used: i) a zebrafish transgenic Line that overexpress the human mutant of Tau (P301L) specifically in neurons and ii) human nasal epithelial cells (hNECs) treated with neuropathological insults. Real time cell analysis and proteomics were used to characterize GNPDA-dependent effects as well as its protein interactome. GNPDA2 levels were measured in the sera from 214 individuals with four different NDs (n=40/disease).

Results: GNPDA2 shuttles to the nucleus in overexpressing conditions in hNECs. Its protein interactome is mainly constituted by protein mediators of nucleus organization, gluconeogenesis and IFN signaling. GNPDA2 overexpression with amyloid-beta oligomer stimulation induced higher toxicity than amyloid-beta oligomers treatment alone in hNECs. GNPDA2 inhibition promoted a significant increment in hNEC proliferation rate, attenuating the amyloid-beta oligomer-induced toxic effects.

Mass-spectrometry allowed us to characterized the proteomic imbalance induced by h.Tau (P301L) in zebrafish embryos. Human GNPDA2 did not modify neither the hyperphosphorylated Tau levels nor axonal motoneuron extension and neuronal death in h.Tau (P301L) zebrafish embryos. However, GNPDA2 overproduction interfered with intracellular vesicle trafficking, modulating the expression of protein such as SEC31B, Atlastin GTPase 2 and taxilin beta. Serum GNPDA2 levels were significantly increased in AD, PSP, DLB and ALS patients respect to control population.

Conclusions: Our preliminary results suggest that GNPDA2 interferes with amyloid-beta toxicity. The increment in GNPDA2 serum levels is a common consequence during the neurodegenerative process across very distinct neurological disorders, demonstrating that GNPDA2 may be considered a marker of neurodegeneration.

PP02.142: Differential Sexually Dimorphic Mechanisms in the Sirtuin (SIRT) Signaling across the Olfactory-entorhinal-amygdaloid Axis in Alzheimer´s and Parkinson´s Diseases

Cartas-Cejudo P[1], Lachén-Montes M[1], Iñigo-Marco I[1], Ballesteros C[1], Cortes A[1], Mendizuri N[1], Ausín K[1], Ferrer I[2], Fernández-Irigoyen J[1], Santamaría E[1]
[1]Clinical Neuroproteomics Unit, Navarrabiomed, HUN, UPNA, IdiSNA, [2]Department of Pathology and Experimental Therapeutics, UB, IDIBELL, CIBERNED-ISCIII
Introduction: Smell impairment is one of the earliest features in Alzheimer's (AD) and Parkinson's diseases (PD). However, the underlying molecular mechanisms associated to the olfactory dysfunction are poorly understood. To further characterize commonalities and differences during the olfactory neurodegeneration in both neurological syndromes, sex- and neuropathological stage-dependent proteomics analysis were performed at the level of the olfactory tract (OT). In addition, secondary olfactory structures were also evaluated.
Methods: We applied SWATH-MS in 57 postmortem OTs derived from non-demented (n=6F/11M), AD (n=4F/13M) and PD (n=7F/16M) subjects. Complementary molecular analyses were performed in entorhinal cortex and amygdala areas (n=10/structure/group; 50%F/M). Human nasal epithelial cells (hNECs) treated with neuropathological insults (beta-amyloid, tau and alpha-synuclein) were used for in vitro experiments.
Results: 11% of the quantified proteome varied between groups. 35 and 20 proteins were commonly deregulated across both sexes in AD and PD, respectively. Sex-dependent differences in terms of olfactory proteostasis, pathway modulation and neuropathological staging were identified. Specifically, olfactory-entorhinal-amygdaloid sirtuin (SIRT) signaling was divergent between sexes across both pathologies, leading to changes at acetylome level. The expression of specific SIRT members was directly modulated by neuropathological substrates in hNECs.
Conclusions: OT protein dyshomeostasis was more severe in AD than in PD. Moreover, protein expression changes were more abundant in females than males independent of the neurological syndrome. Mechanistically, the tangled SIRT profile observed across the olfactory-entorhinal-amygdaloid axis in AD and PD points out differential NAD (+)-dependent deacetylase mechanisms, directly governed, in part, by neuropathological substrates.

PP02.143: Early, Sex-dependent and Progressive Proteomic Imbalance in the Amygdala during Alzheimer´s Disease Evolution

Dominguez J[1], Cartas-Cejudo P[1], Lachén-Montes M[1], Mendizuri N[1], Ausín K[1], Díaz R[1], Ferrer I[2], Fernández-Irigoyen J[1], Santamaría E[1]
[1]Clinical Neuroproteomics Unit, Proteomics Platform, Navarrabiomed, HUN, UPNA, IdiSNA, Pamplona, Spain, [2]Department of Pathology and Experimental Therapeutics, UB, IDIBELL, CIBERNED-ISCIII, Barcelona, Spain

Introduction: The amygdala is involved in expression of emotions, memory processing and managing stimulatory input. Amygdala atrophy is evidenced in early stages of Alzheimer's Disease (AD). Late AD is associated with large amount of A-beta and neurofibrillary tangles composed by hyperphosphorylated Tau aggregates (NFTs) in this area. However, the molecular mechanisms disrupted in early neuropathological stages are unknown.
Methods: We applied label-free quantitative proteomics using an Orbitrap Exploris 480 mass-spectrometer in 24 postmortem amygdala specimens derived from non-demented (n=3F/5M), AD-Braak stage I-II (with no discernible or a few isolated NFTs) (n=4F/4M) and AD-Braak stage III-IV (with low-moderate numbers of NFTs). Data analysis was performed using MaxQuant software. AlzData repository as well as Metascape were considered for data interpretation.
Results: A progressive proteomic impairment was observed across Braak stages. 66 and 153 differential proteins (DEPs) were exclusively detected in Braak I-II and Braak III-IV stages respectively (FC 30%; p-val<0.05). Although multiple metabolic routes were commonly deregulated across staging, early dyshomeostatic proteome specifically impacted on amyloid binding, autophagy and ER-Golgi intermediate compartment. Considering not only the neuropathological stage but also the sex dimension, the amygdaloid proteome was more severely affected in women than in men. 49 and 105 DEPs were identified in men across Braak staging, whereas 81 and 139 DEPs were observed in women. 6 and 17 proteins were commonly deregulated across Braak staging in men and women respectively. Part of the differential datasets
showed a gene expression correlation with AD pathology in Abeta and/or Tau line AD mouse models.
Conclusions: Early AD-dependent protein dyshomeostasis was evidenced at the amygdaloid level. This proteomic imbalance was more severe in women than in men independent of the neuropathological stage. Some DEPs are differential features in AD mouse models before AD pathology, suggesting a relevant role during the initial steps of neurodegenerative process.

PP02.144: Metabolic Dyshomeostasis Induced by SARS-CoV-2 Structural Proteins Reveals Immunological Insights into Viral Olfactory Interactions

Lachén-Montes M[1], Mendizuri N[1], Ausín K, Echaide M[2], Blanco E[2], Chocarro L[2], de Toro M[3], Escors D[2], Fernández-Irigoyen J[1], Kochan G[2], Santamaría E[1]
[1]Clinical Neuroproteomics Unit, Proteomics Platform, Navarrabiomed, HUN, UPNA, IdiSNA, [2]Oncoimmunology Unit, Navarrabiomed, HUN, UPNA, IdiSNA, [3]Genomics and Bioinformatics Platform, Centro de Investigación Biomédica de La Rioja (CIBIR)
Introduction: One of the most common symptoms in COVID-19 is a sudden loss of smell. SARS-CoV-2 has been detected in the olfactory bulb (OB) from animal models and sporadically in COVID-19 patients. However, the specific role over the SARS-CoV-2 proteome at olfactory level is not known.
Methods: We characterized the in-depth molecular imbalance induced by the expression of GFP-tagged SARS-CoV-2 structural proteins (M, N, E, S) on mouse OB cells by a multi-omics approach (RNA-seq and SWATH-MS).
Results: Transcriptomic and proteomic trajectories uncovered a widespread metabolic remodeling commonly converging in extracellular matrix organization, lipid metabolism and signaling by receptor tyrosine kinases. The molecular singularities and specific interactome expression modules were also characterized for each viral structural factor. The intracellular molecular imbalance induced by each SARS-CoV-2 structural protein was accompanied by differential activation dynamics in survival and immunological routes in parallel with a differentiated secretion profile of chemokines in OB cells. Machine learning through a proteotranscriptomic data integration uncovered TGF-beta signaling as a confluent activation node by the SARS-CoV-2 structural proteome.
Conclusions: Taken together, these data provide important avenues for understanding the multifunctional immunomodulatory properties of SARS-CoV-2 M, N, S and E proteins beyond their intrinsic role in virion formation, deciphering mechanistic clues to the olfactory inflammation observed in COVID-19 patients.

PP02.145: Intracellular Protein Glycation Influences the Cellular Machinery for Impaired Protein Clearance

Senavirathna L[1], Chen R[2], Pan S[1]
[1]Brown Foundation Institute of Molecular Medicine, McGovern Medical School / University of Texas Health Science Center at Houston, [2]Section of Gastroenterology and Hepatology, Department of Medicine, Baylor College of Medicine

Introduction:
Autophagy and the ubiquitin-proteasome system [UPS] are important cellular mechanisms to clear the impaired proteins and recycle the cellular components. Protein glycation with reducing sugars forms stable advanced glycation end products [AGEs], which can accumulate and aggregate intracellularly to disturb cellular homeostasis, inducing stress on UPS and autophagy. Ultimately, the proteome impairment can cascade various cellular signaling, leading to the pathogenesis of many diseases, including cancer.

Method:
Normal and malignant pancreatic ductal epithelial cells were treated with glyceraldehyde and cell lysates were harvested for mass spectroscopy-based proteomic analysis. Western blot was performed to validate the expression of E3 ligases, and determine the activities of autophagy and ubiquitination. Functional assays were carried out to evaluate UPS activity and protease resistance. Bioinformatic analyses were performed to reveal the functional changes and predict vulnerable protein sites that are prone to protein AGEs formation and aggregation.

Results:
Glycation is more favorable when the neighboring amino acids are acidic and hydrophobic. Dose-dependent AGEs accumulation was observed more prominently in lysine [K] residues compared to arginine [R] residues. Protein aggregation and resistance to protease were observed due to protein AGEs formation. The expression of E3 ligases was decreased during protein AGEs accumulation, and the overall ubiquitination was reduced, likely due to the competition of glycation on the K sites, all of which lead to the decrease of UPS activity. In contrast, autophagy activity was found increased following AGEs formation.

Conclusion:
Protein AGEs formation and accumulation lead to proteome impairment, which negatively impacts cellular homeostasis, overwhelming and altering UPS and autophagic activities. Understanding the functional impacts of intracellular AGEs accumulation on UPS and autophagy could pave the way for future development of pharmaceutical agents targeting AGEs-related diseases, including pancreatic cancer.

PP02.146: Proteomic Analysis to Identify Candidate Biomarkers Associated with Skin Co-exposure to Ultraviolet Radiations and Benzo[a]pyrene

Fayyad-Kazan M[1], Kobaisi F[1], Nasrallah A[1], Dorr M[2], Matarrese P[2], Fitoussi R[2], Bourgoin S[3], Seve M[3], Rachidi W[1]
[1]Université Grenoble Alpes, CEA, Inserm, BIG-BGE U1038, [2]Laboratoires Clarins, Centre de Recherche, [3]Université Grenoble Alpes, CNRS, TIMC U5525, EPSP

Human skin, and mainly the outer epidermis, is continuously exposed to environmental stressors, mainly air pollutants. Air pollutants can significantly impair, via oxidative damage, the normal functioning of human skin's proteins, lipids and/or nucleic acids, thus triggering different skin disorders including aging, psoriasis, inflammatory reactions and skin cancer. Among air pollutants that can harm the skin are ultraviolet radiations (UVR) and Polycyclic aromatic hydrocarbons (PAHs ; such as Benzo[a]pyrene). Exposure to UVA has been associated with skin aging whereas UVB alone could account for sunburn. UVA, along with UVB, could trigger photoimmunosuppression and development of different cutaneous cancers (photocarcinogenesis). Exposure to BaP could trigger different pathologies including hepatotoxicity, neurotoxicity, immunotoxicity and placental toxicity. Intriguingly, skin tumor incidence increases in response to BaP + UVA treatment, compared to BaP- or UVA-treatment alone. Till date, a detailed analysis of the biological processes that are altered following coexposure of human skin to BaP + UVR has not been performed. In this study, we used a mass-spectrometry-based proteomic analysis to identify differentially expressed proteins in human epidermal skin cells exposed to either no pollutant, UVR alone, or UVR + BaP.
With the help of pathway analysis, the deregulated proteins were grouped into different pathways found to be modified post pollutant exposure. Intriguingly, autophagy appeared to be deregulated upon such exposure. Western blot and immunohistochemistry analysis were then performed to validate the effect of pollutant exposure on autophagy. Altogether, the identified components could serve as potential biomarkers to the effects of UVR + BaP on the physiology of the skin.

PP02.147: Effect of Curcumin in the Liver of Mice Fed a High-fructose Diet: A Proteomic Study

Silva Gaona O[1], León García M[1], Hernández M[2], Vargas Ortiz K[1], Ramírez Emiliano J[1], Garay Sevilla M[1], Encarnación-Guevara S[2], Perez Vazquez V[1]
[1]Universidad de Guanajuato, [2]Centro de Ciencias Genómicas, UNAM

Introduction: High fructose intake has been related to the development of metabolic diseases due to changes in protein expression, altering metabolic and signaling pathways. Curcumin is an antioxidant with a regulatory effect on genes, metabolic pathways, and glycated proteins. The aim is to identify changes in protein expression and the effect of curcumin on glycated protein by high fructose and curcumin intake in mice livers.
Methods: Four groups (n = 4/group) of male mice (C57BL6J) of six-weeks-old were formed. One group received a standard diet; another received curcumin at 0.75 % w/w in the feed; one more received 30 % w/v fructose in drinking water; and one group received 30 % w/v fructose in drinking water and 0.75 % w/w curcumin in food; for 15 weeks. Accumulation of glycated proteins was evaluated by immunoblot using anti-CML and anti-AGE antibodies. Proteomic analysis was performed by LC-MS/MS using the label-free technique with the MaxQuant program for identification and Perseus for expression change analysis. Differentially expressed proteins (fold change ≥ 1.5 and $p < 0.5$) were analyzed by gene ontology and KEGG.
Results: Fructose-fed mice accumulated glycated proteins in seven bands and curcumin prevented the accumulation of glycated proteins in a 70 kDa band generated from fructose. 1047 proteins were identified, of which 113 changed their expression in mice fed fructose compared to the control group; and curcumin modified the expression of 64 proteins in mice fed fructose and curcumin compared to mice fed fructose alone. Curcumin prevented the change in expression of 13 proteins: NDUFB8, NDUFB3, ATP5L, PSMA5, HIST1H1D, THRSP, DGAT1, ECI1, and ACOT13.
Conclusion: Curcumin prevented the accumulation of 70 kDa glycated proteins produced from fructose and the change in expression of 13 hepatic proteins of fructose-fed mice involved in oxidative phosphorylation, cellular stress response, and lipid metabolism.

PP02.148: Proteomic Analysis of Bone Marrow-derived Macrophages (BMDM) Polarized to Proinflammatory and Anti-inflammatory Phenotype: The Role of Mitochondria-targeted Hydrogen Sulfide

Stachowicz A[1], Czepiel K[1], Suski M[1], Olszanecki R[1]
[1]Chair of Pharmacology, Jagiellonian University Medical College

Introduction

Macrophage polarization plays a pivotal role in the development of low-grade chronic inflammation and macrophage phenotype switching from proinflammatory M1 to anti-inflammatory M2 may represent promising strategy for the treatment of chronic inflammatory diseases. The M1 macrophages rely on glycolysis, pentose phosphate pathway and fatty acid synthesis, while M2 macrophages are characterized by increased oxidative phosphorylation, Krebs cycle and enhanced fatty acid oxidation. Recently, it has been demonstrated that hydrogen sulfide (H2S) exhibits modulatory properties in supporting cellular bioenergetics. H2S derived from mitochondrial enzyme: 3-mercaptopyruvate sulfurtransferase (3-MST) was shown to complement the Krebs cycle as an alternative source of electrons for oxidative phosphorylation and ATP production. Thus, the aim of our study was to investigate the influence of mitochondria-targeted H2S produced by 3-MST on the reprogramming of mitochondrial metabolism in macrophages and elucidate mechanism involved using proteomic methods.

Methods

Bone marrow cells were isolated from the femurs and tibiae of 6-8 week-old male C57BL/6J mice and differentiated to BMDMs for 7 days. Then, BMDMs were silenced with 3-MST siRNA and polarized to M1 and M2 phenotype for 24 h with LPS and INF-γ or IL-4, respectively. For proteomic analysis lysed cells were digested using the FASP method with trypsin and Lys-C and analyzed on Orbitrap Exploris™ 480 mass spectrometer in data-independent acquisition mode.

Results

Our proteomic data showed that BMDM polarized to proinflammatory M1 and anti-inflammatory M2 phenotypes differs in the expression of proteins related to metabolism, e.g. oxidative phosphorylation, glycolysis, Krebs cycle and fatty acid oxidation. Importantly, silencing of 3-MST, an enzyme responsible for H2S production modulated polarization of macrophages to M1 and M2 phenotypes.

Conclusions

Taken together our results indicate that proinflammatory and anti-inflammatory macrophages exhibit distinct proteomic profile, which is modulated by mitochondria-targeted H2S. The exact functional consequences of the revealed alterations require further investigation.

PP02.149: Performance Comparison of TMT and Label-free DIA Methods for the Analysis of Heart Tissue Samples

Suárez Artiles L[1,2,3], Popp O[1], Radke M[2,3], Gotthardt M[2,3,4], Mertins P[1,2]
[1]Proteomics Platform, Max-Delbrück-Center for Molecular Medicine and Berlin Institute of Health, [2]German Center for Cardiovascular Research (DZHK), partner site Berlin, [3]Department of Neuromuscular and Cardiovascular Cell Biology, Max Delbrück Center for Molecular Medicine in the Helmholtz Association, [4]Department of Cardiology, Charité Universitätsmedizin Berlin

Introduction
The heterogeneity of clinical muscle samples and the presence of highly abundant proteins make comprehensive proteomics analysis of the heart challenging. Isobaric labeling techniques like tandem mass tags (TMT) allow in-depth coverage in proteome and post-translational modifications (PTM) studies. However, the recent advances in technology and analytic software tools make the data-independent acquisition (DIA) methods very attractive, providing higher data completeness for large sample cohorts with less effort in sample preparation. The improvement of DIA methods and the increasing interest in mathematical modeling are shifting the attention currently toward label-free techniques.
Methods
To better understand the effect of applying these different methods to heart tissue, we compared a standard data-dependent acquisition (DDA) TMT workflow with two DIA methods, using the orbitrap Exploris 480 and the timsTOF Pro2. These differences were tested using heart tissue from two mouse models of diastolic disfunction.
Results
We present a comparative study of the performance of TMT and label-free DIA methods in cardiac tissue at a global proteome and PTM level.
Conclusions
Continuous developments in mass spectrometry-based proteomics allow us to overcome complex challenges in the field. However, it is crucial to fully understand what the newest techniques provide when applied to different biological contexts. With this work, we gain a comprehensive insight into the advantages and disadvantages of using DDA and DIA methods to study cardiac diseases.

PP02.150: Secretome Analysis of Human Astrocytes: Brain Renin–angiotensin System in Astrocyte–microglia Crosstalk

Suk K[1]
[1]Kyungpook National University School Of Medicine
Introduction: Astrocytes are major supportive glia and immune modulators in the brain; they are highly secretory in nature and interact with other cell types via their secreted proteomes. Understanding the astrocyte secretome may provide insights into the regulatory mechanisms of neuroinflammation through astrocyte-mediated communication.
Methods: To understand how astrocytes communicate during neuroinflammation, we profiled the secretome of human astrocytes following stimulation with proinflammatory factors.
Results: A total of 149 proteins were significantly upregulated in stimulated astrocytes, and a bioinformatics analysis of the astrocyte secretome revealed that the brain renin–angiotensin system RAS is an important mechanism of astrocyte communication. We observed that the levels of soluble aminopeptidase N (sANPEP), a RAS component that converts angiotensin (Ang) III to Ang IV in a neuroinflammatory milieu, significantly increased in the astrocyte secretome. To elucidate the role of sANPEP and Ang IV in neuroinflammation, we first evaluated the expression of Ang IV receptors (ATRs) in human glial cells because Ang IV mediates biological effects through its receptors. The expression of AT1R was considerably upregulated in activated human microglial cells but not in human astrocytes. Moreover, interleukin-1β release from human microglial cells was synergistically increased by cotreatment with sANPEP and its substrate, Ang III, suggesting the proinflammatory action of Ang IV generated by sANPEP. In a mouse neuroinflammation model, brain microglial activation and proinflammatory cytokine expression levels were increased by intracerebroventricular injection of sANPEP and attenuated by an enzymatic inhibitor and neutralizing antibody against sANPEP.
Conclusions: Our results indicate that astrocytic sANPEP-induced increase in Ang IV exacerbates neuroinflammation by interacting with microglial proinflammatory receptor AT1R, highlighting an important role of indirect crosstalk between astrocytes and microglia through the brain RAS in neuroinflammation. Our findings also suggest the diagnostic and therapeutic potential of sANPEP in neuroinflammatory diseases.

PP02.151: Brain Proteomic Atlas of Alcohol Use Disorder

Teng P[1], Barakat W[1], Tran S[1], Tran Z[1], Bateman N[1], Conrads K[1], Zhou M[2], Hood B[1], Leggio L[3], Maxwell G[2], Lee M[3], Conrads T[2]
[1]Gynecologic Cancer Center of Excellence, [2]Women's Health Integrated Research Center, Women's Service Line, Inova Health System, [3]Section on Clinical Psychoneuroendocrinology and Neuropsychopharmacology, National Institute on Alcohol Abuse and Alcoholism and National Institute on Drug Abuse, National Institutes of Health

Introduction: Alcohol Use Disorder (AUD) represents a significant social and medical burden with lifetime prevalence in the United States of nearly 30% (1). Here we conducted a comprehensive proteomics analysis of six anatomic brain regions from antecedents with the goal of characterizing neuroadaptations in AUD.
Methods: Formalin-fixed, paraffin-embedded (FFPE) tissues from the amygdala, hippocampus, hypothalamus, nucleus accumbens, prefrontal cortex and ventral tegmental area from AUD (n = 11) and control (n = 16) individuals were processed using barocycler-assisted digestion (2320EXT, Pressure BioSciences), labeled and multiplexed with tandem mass tags (TMTpro, ThermoFisher Scientific) and analyzed by nanoflow liquid chromatography (EASY-nLC 1200, ThermoFisher Scientific) coupled online with tandem mass spectrometry (Q-Exactive HF-X, ThermoFisher Scientific). Ingenuity Pathway Analysis was used for pathway analysis of significantly altered proteins (fold change > 1.5 and $p < 0.01$).
Results: Proteomic analysis of brain tissues quantified > 6,000 proteins and identified numerous uniquely altered proteins in AUD antecedents within each of the six brain regions assessed. The region with the greatest number of significantly altered proteins in AUD antecedents was the amygdala, followed by the hypothalamus. Pathway analysis indicated that the α-adrenergic pathway and dopaminergic signaling were the most significantly enriched in AUD antecedents in the amygdala and hypothalamus, respectively. In addition, the oxytocin pathway was predicted to be activated in the amygdala in AUD. Our study also identified proteins significantly elevated in AUD that represent possible therapeutic targets, such as CSNK1D (PF-670462) and GABAB receptor (Baclofen).
Conclusion: We report a comprehensive proteomic study examining the effects of AUD in six brain regions known to play a role in the pathogenesis of addiction. Our results provided insights to understand the molecular pathways associated with AUD and identified potential novel drug targets.

1. Grant, B.F., et al. 2015 JAMA Psychiatry 72(8); 757-66

PP02.152: Interaction Proteomics of Alzheimer's Disease-associated Proteins from Post-mortem Human Brains

Thanou E[1], Hondius D[1,2], Koopmans F[1], Smit A[1], Li K[1]
[1]Center For Neurogenomics And Cognitive Research, Department Of Molecular And Cellular Neurobiology, Vrije Universiteit, [2]Department of Pathology, Neuroscience Campus Amsterdam, VU University Medical Center, Amsterdam, The Netherlands

Introduction: Alzheimer's disease (AD) is the most common neurodegenerative disorder. Previous quantitative proteomics have demonstrated many differential expressed brain proteins in AD patients, in particular microtubule-associated protein tau (MAPT), glial fibrillary acidic protein (GFAP), and (aberrant processing of) Amyloid Beta Precursor Protein (APP). In the present study, we characterized the protein complexes of these AD-associated proteins from extracts of AD and non-demented healthy control brains.

Methods: Hippocampi from AD and non-demented healthy control post-mortem brains were extracted in 0.5% n-Dodecyl β-D-maltoside buffer. Extracts were used for a) immunoprecipitation to isolate protein complexes of interest, run on SDS-PAGE gel, and b) fractionation of native proteins on BN-PAGE gel. Proteins were trypsin digested in gel, and analyzed by evosep-Timstof pro2 with the 30 samples per day program.

Results: Proteomics analysis of BN-PAGE fractions revealed the masses of native protein complexes. MAPT showed several distinct complexes of different masses that were present in both AD and control cases. Interestingly, AD cases showed an additional high mass (>1000 kDa) MAPT complex representing probably the oligomer form. The alternative immunoprecipitation experiments revealed known and novel interactors of the AD-associated bait proteins.

Conclusions:
We revealed the composition and sizes of the brain protein complexes and demonstrated some subtle differences between the AD and non-demented healthy control cases. Our data highlight more precise focus areas that could be useful for further studying the disease.

PP02.153: Profiling the Cellular Signalling State by Targeted Phoshoproteomics

Van Bentum M[1,2], Zauber H[1], Klinger B[2], Haji M[1], Mertins P[1], Blüthgen N[2], Selbach M[1,2]
[1]Max Delbrück-centre Berlin, [2]Charité University Medicine

Introduction

Normal cell functioning is regulated by a complex signalling network that responds to various extra- and intracellular signals like growth factors, cytokines and nutrients. Aberrations in cellular signalling often lead to disease, for example cancer. Understanding the functioning of the cellular signalling network, as well as changes in the network between healthy and diseased cells is a key challenge in biology research.

Methods

We have developed a targeted phospho-proteomic assay, that aims to provide a high-throughput, quantitative, reproducible profile of the cellular signalling state. The assay enables sensitive measurement of the phospho-sites that give a comprehensive overview of the cellular signalling state. The panel includes 443 phospho-peptides mapping to 249 proteins in all major signalling pathways. The phospho-peptides are measured in a targeted fashion, using internal standard triggered PRM (Surequant).

The data is analysed with a newly developed in-house tool: Vali. This tool enables semi automatic analysis of PRM data and is optimised to work directly with Picky generated spectral libraries. We confirmed sensitivity and accuracy of quantification of the PRM-Vali pipeline using a SILAC dilution series. We observed linearity in quantitation down to 63-127 fold ratios using the described setup in a pure automated fashion.

Results

Performance of the assay was determined by measuring perturbations of HCT-116 colon cancer cells with growth factors and cytokines. We were able to validate previously identified signatures of these perturbations. Furthermore, the assay is applied to a colon cancer cell line panel to uncover previously unknown synergistic feedback mechanisms, in response to MEK inhibition.

PP02.154: Quantitative Proteomics for Net Amount of Urinary Tract Tissue-derived Urine Proteins by Subtraction of Plasma proteins from Urine proteome

Yamamoto K[1], Uchimoto T[1], Elguoshy A[1], Yanagita K[1], Shimada S[1], Yamamoto T[1,2]
[1]Niigata University, [2]Shinrakuen Hospital

Introduction: Urine has been used to discover biomarkers for urinary tract tissue injuries by proteomics. Recent advances in quantitative proteomics have enabled to quantify ~2,000 proteins in urine. Since urinary excretion of plasma proteins was enhanced when the kidney glomerulus was injured in kidney diseases such as diabetic kidney injury, urinary proportion of plasma proteins often affected more or less net amount of urinary tract tissue derived proteins. We developed a method to subtract plasma protein contents from urine proteome for quantitative comparison of urine proteins derived from urinary tract tissues between healthy volunteers (HV) and diabetic patients with or without kidney injuries (DM).

Methods: Urine samples were collected from 20 HV and 20 DM. The peptide samples of 200 ng each were analyzed by LC_MS (Bruker Daltonics, NanoElute-timsTOF Pro) using PASEF mode both in data-dependent acquisition (DDA) and data-independent acquisition (DIA) manners. Quantitative data was obtained by Spectronaut (Biognosys AG) analysis. After subtraction of plasma protein contents from urine proteome, proportions of urine proteins derived from the urinary tract organs were compared between HV and DM.

Results: About 2,000 proteins were identified by the DDA analysis and quantitated by DIA analysis. A small proportion of plasma proteins, such as serum albumin were quantitated even in urine from HV, however, the population increased in DM patient urine. By the subtraction of the plasma proteins, several proteins were found as up or down-regulated in the DM urine, suggesting that the subtraction method was useful for discovery of biomarkers of kidney injuries.

Conclusion: In this study, we compared the proportion of urinary tract-derived proteins after subtraction of plasma protein contents from urine proteome to know more precise proportion of urinary tract tissue-derived proteins. We could pick-up several urine proteins as the biomarker candidates.

PP02.155: Search for Biomarkers for Early Detection of Renal Impairment in Diabetic Patients

Yanagita K[1], Yamamoto K[1], Elguoshy A[1], Uchimoto T[1], Yamamoto T[1,2]
[1]Biofluid Biomarker Center, Niigata University, [2]Clinical laboratory, Shinrakuen Hospital

Diabetes mellitus (DM), a lifestyle-related disease, causes systemic organ and tissue damage as it progresses. In this study, we introduce our established quantitative proteomics platform for discovery of urinary biomarkers to detect early renal damages in DM patients. DM patients often develop kidney injuries, which are detected by trace albuminuria (microalbuminuria) at the beginning. Since the kidney injuries assiduously progress to kidney failure, which is treated with kidney replacement treatment is necessary, more early detection of the onset of renal injuries are important to prevent the progression.

To discover urinary biomarkers for various diseases, we have collected about 110,000 urine samples over the past four years from patients with various diseases and from people who had no critical diseases and visited at hospitals for physical examinations (healthy volunteers, HV). These urine samples stored in -20C freezers and clinical data were anonymized to create our urine sample database. From these samples, we selected urine samples from DM patients, who developed microalbuminuria during 4-year tracing, and from HV to analyze their urine proteomes by quantitative proteomics using LC-MS (SCIEX, 6600+) and SWATH.

We found that several proteins were increased or decreased in urine samples from the DM patients, who developed microalbuminuria, comparing to those in HV urine. These proteins were selected as candidate biomarkers for early kidney injuries in DM patients.

We have established a quantitative proteomics platform for urine biomarker discovery using LC-MS and SWATH, and identified urinary proteins that correlated with development of microalbuminuria in DM patients.

PP02.156: Cerebrospinal Fluid Proteome Map Reveals Molecular Signatures of Reversible Cerebral Vasoconstriction Syndrome (RCVS)

Yang J[1,2,3], Chen S[4,5,6,7,8], Wang Y[4,5,8], Chang C[9], Fuh J[4,5,8], Chow L[8,10], Han C[11], Chen Y[1,2,12], Wang S[4,5,8]
[1]Institute of Chemistry, Academia Sinica, [2]Sustainable Chemical Science and Technology, Taiwan International Graduate Program, Academia Sinica and National Yang Ming Chiao Tung University, [3]Department of Applied Chemistry, National Yang Ming Chiao Tung University, [4]Department of Neurology, Neurological Institute, Taipei Veterans General Hospital, [5]Brain Research Center, National Yang Ming Chiao Tung University, [6]Division of Translational Research, Department of Medical Research, Taipei Veterans General Hospital, [7]Institute of Clinical Medicine, College of Medicine, National Yang Ming Chiao Tung University, [8]College of Medicine, National Yang Ming Chiao Tung University, [9]Department of Chemistry, National Central University, [10]Department of Anesthesiology, Taipei Veterans General Hospital, [11]Master Program in Clinical Genomics and Proteomics, College of Pharmacy, Taipei Medical University, [12]Department of Chemistry, National Taiwan University

Introduction: reversible cerebral vasoconstriction syndrome (RCVS) is a complex neurovascular disorder characterized by hyperacute severe headaches and reversible vasoconstrictions. Despite its significance, the pathophysiological molecular mechanism remains underexplored and the unmet need for molecular diagnosis is lacking. To provide insight into the pathogenesis of RCVS, an RCVS-CSF proteome library resource was established to apply data-independent acquisition mass spectrometry (DIA-MS) for quantitative proteomic profiling of the cerebrospinal fluid (CSF) in patients with RCVS and control individuals. A panel of biomarker candidates was identified and a machine learning model suggests a protein signature discriminating RCVS patients from controls.
Methods: The CSF was collected from 17 patients with RCVS compared to 20 age- and sex-matched controls and analyzed using DIA-MS. Functional protein networks were constructed by protein-protein interaction and functional enrichment analysis from the RCVS proteome. A supervised learning statistical approach was performed for disease classification.
Results: A CSF proteome library resource of 1,054 proteins was established for RCVS. Quantitative profiling of RCVS patients and controls revealed abundant upregulated proteins enriched in the brain and blood-brain barrier (BBB). This RCVS-CSF proteomic profile and alteration in patients reveal potential implications involving the interplay between the complement system and extracellular matrix in the RCVS pathogenesis. Moreover, a machine learning model based on the panel of biomarker candidates suggests a protein signature that allows differentiating RCVS patients and controls with high accuracy (AUC: 0.985).
Conclusions: This study reveals the first RCVS proteome and a potential pathogenesis mechanism of alteration in BBB permeability and nominates potential biomarker candidates that are mechanistically plausible for RCVS, which may serve as potential diagnostic and therapeutic targets in the future.

PP02.157: Mass-spectrometric Analysis of APOB Polymorphism rs1042031(G/T) and its Influence on Serum Proteome of CAD Patients: Some Genetic Derived Proteomics Consequences

Zafar M[1,2,3], Mirza M[1], Awan F[3], Malik I[2], Larsen M[4], Choudhary M[1]
[1]ICCBS, PCMD. University Of Karachi, [2]University of Sargodha, [3]Health Biotechnology Division, National Institute for Biotechnology and Genetic Engineering (NIBGE), [4]Department of Biochemistry and Molecular Biology, University of Southern Denmark, Odense, Denmark

Introduction: Cardiovascular disease is considering as complex and multifactorial diseases involving several biological and environmental risk factors. The currently available platforms for early diagnosis and for deep pathophysiology understanding, are still insufficient to provide holistic information. In the current study, APOB (rs1042031) genotype-guided proteomic analysis was performed in a cohort of Pakistani population.
Methods: A total of 700 study subjects, including Coronary Artery Disease (CAD) patients (n = 480) and healthy individuals (n = 220) were included as control group. Genotyping was carried out by using tetra primer-amplification refractory mutation system-based polymerase chain reaction (T-ARMS-PCR) and validated through sequencing, whereas orbitrap LC/MS was used for label free quantification of serum samples.
Results: Genotypic frequency of GG genotype was found to be 70%, while 27% was for GT genotype and 30% was for TT genotypes in CAD patients. In the control group, 52% healthy subjects were found to have GG genotype, 43% had GT genotype, and 5% were with TT genotypes. Significant (p = 0.004) difference was observed between genotypic frequencies in the patients and the control group. The dominant allele GG was found to be strongly associated with the CAD (OR: 2.4 (1.71 - 3.34), as compared to the control group in both dominant and allelic genetic models (p = 0.001). Using label free proteomics, altered expression of 40 significant proteins was observed. Enrichment analysis of these protein showed up-regulated pathways, including chylomicron remodeling and assembly, complement cascade activation, plasma lipoprotein assembly, apolipoprotein-A receptor binding and metabolism of fat soluble vitamins in G allele carrier of rs1042031 (G>T) as compared with the mutant type T allele carriers.
Conclusion: This study provides a deeper insight into CAD pathobiology with reference to proteogenomics of Apo-lipoprotein B, and proves the genotypic-phenotypic relationship of rs1042031 (G>T) of APOB with CVD.

PP02.158: Parallel Global Proteome and Succinylome of Human Brain Reveal Altered Succinylation of Key Proteins Linked to Alzheimer's Disease Pathology

Zhang S[1], Yang Y[2], Tapias V[2], Xu H[2], Bhawal R[1], Anderson E[1], Gibson G[2]
[1]Cornell University, [2]Burke Neurological Institute

Introduction:

Although amyloid-beta (Aβ) deposition and tau neurofibrillary tangles in brain are hypothesized as a central force driving Alzheimer's disease (AD) pathogenesis, the mechanism underlying pathology is still unclear. Lysine succinylation is a common modification regulating metabolic processes. However, no lysine succinylation study was reported in human brains yet. Here we analyzed proteome and succinylome of human brain in parallel to explore succinylation roles in AD pathology.

Methods:

Proteins were extracted from Broca's area of human brains of 10 controls and 10 AD patients in two cohorts. TMT10-plex labeling was used for global proteomics and affinity enrichment of succinylated peptides was performed for succinylome. All samples were analyzed by nanoLC-MS/MS and data processed by Proteome Discoverer. Significantly changed succinylated sites/peptides in AD were further explored.

Results:

In global proteomics analysis, out of 4,678 proteins identified and quantified in both cohorts, 868 proteins show statistical differences in ADs. Meanwhile, 1,908 succinylated peptides from 314 unique proteins were confidently identified and quantified in both cohorts.

Subcellular localization analysis showed that about 90% of the succinylated proteins with >2 succinylation sites were mitochondrial proteins including eight enzymes of the TCA cycle and their multiple subunits. One striking finding was that 29 succinylated peptides from 20 proteins were significantly changed in AD. Among them, the hallmark proteins APP (K612) and tau (K311) at their critical sites were succinylated in 9 out of 10 AD patients but no succinylation was found in controls. Succinylation at K612 of APP was demonstrated to be pathologically important in promoting Aβ42 production by inhibiting cleavage by α-secretase and contributing to Aβ42 oligomerization. Succinylation at K311 of tau promoting pathological tau aggregation to tangles and impairing microtubule assembly was also validated by tau aggregation assay and electron microscopy.

Conclusions:

Our results suggest that metabolism-linked succinylation appears associated with AD pathogenesis.

PP02.159: Functional Characterization of Dermokine in Epidermal Differentiation

Canbay V[1], Tian W[1], Goletz S[1], auf dem Keller U[1]
[1]Technical University Of Denmark

Introduction. Keratinocyte differentiation and epidermal renewal are decisive for skin homeostasis and maintenance of the skin's barrier capacity, which is disturbed upon injury and in inflammatory skin diseases. Dermokine is increased in abundance under these conditions, but its functional contribution to epidermal stratification has been poorly understood, due to alternative splicing events resulting in the major isoforms α, β and γ. We conducted proteoform ablation to monitor the putative function of dermokine isoforms in human keratinocyte differentiation.

Methods. We have generated two proteoform-specific and one proteoform-independent dermokine-knockout keratinocyte phenotype using CRISPR/Cas9 technology, α/β, β/γ or α/β/γ. To characterize the secreted dermokine we utilize skin organotypic cultures allowing us to characterize the modified effects on keratinocyte differentiation and activation of downstream signaling via phosphoproteomics. Here we performed tissue immunohistochemistry and targeted proteomics analysis, monitoring epidermal proteases.

Results. We demonstrate that ablation of dermokine proteoforms disrupts the normal stratification of the human epidermis in 3D skin models. Applying directDIA and DIA-NN, we identify 5983 proteins and provide evidence for the distortion of the epidermal protease network, which is crucial for the proper formation of a fully stratified epidermis. Immunohistochemistry and Surequant confirmed differential abundance levels of epidermal proteases and their inhibitors in dermokine-ablated keratinocytes.

Conclusions. The function of dermokine is associated with epidermal differentiation, but the full capacity and mechanistic role of dermokine in the epidermis remains elusive. Here, we present indications for the necessity of dermokine for the proper stratification of keratinocytes by generation of proteoform-specific dermokine knockout phenotypes and their disturbed epidermal development in human organotypic skin cultures.

PP02.160: Developing a Method to Analyze Proteins and Metabolites in Human Saliva

Chauhan M[1], Simmons D[1]
[1]Ontario Tech University

Introduction: The quality of data from biological fluid samples critically relies on the sample preparation method. Choosing the appropriate method, which is dependent upon the intrinsic properties of a molecule of interest, allows for the best acquisition of data. Given the complex nature of saliva, various methods have been employed in attempts to create a standardized method.
Methods: In this method development, we compared commonly used protein and metabolite extraction methods including the modified Folch method, known as the Bligh and Dyer method, with centrifugal separation and ultrafiltration with the goal of defining an optimum saliva sample preparation strategy. Centrifugation was also factored in to assess its impact on the different sample preparation methods. All samples were analyzed using liquid chromatography tandem high resolution mass spectrometry (LC-QTOF) using a C18 column and a HILIC column for proteins and aqueous metabolites, respectively.
Results: Looking at the overall human salivary proteome results, a total of 1026 proteins were found and 222 of them were found to be significantly greater in abundance when both centrifugation and ultrafiltration were used ($p < 0.05$). Looking at the overall metabolomic results, a total of 32 metabolites were detected using all methods, however only 21 metabolites had sufficient abundance ($p < 0.05$) to be included in comparison analyses.
Conclusions: Preliminary data suggests that centrifugation alone results in the loss of data. The most effective method for proteins was ultrafiltration in combination with centrifugation. Metabolites were more variable in all methods, and it was less clear which sample preparation method performed the best overall due to a lack of statistical power.

PP02.161: Expression of Uncharacterized Chromosome 19 Proteins in Breast and Ovarian Cancer: Human Proteome Project (HUPO)

Gonzalez Tolentino D[1], Flores Palma E[1], Torres Rojas F[1], Rodriguez Ruiz H[1], Bello Rios C[1], Alarcon Romero L[2], Catalan Castorena O[2], Saavedra Herrera M[4], Navarro Tito N[5], Cayetano Salazar L[5], Illadez Aguiar B[1], Leyva Vazquez M[1], Jimenez Lopez M[4], Garibay Cerdenarez O[1], Encarnación-Guevara S[5]
[1]Lab. de Biomedicina Molecular, Facultad de Ciencias Químico-Biológicas, Universidad Autónoma de Guerrero, [2]Lab. de Citopatología e Histoquímica, Facultad de Ciencias Químico-Biológicas, Universidad Autónoma de Guerrero, [3]Lab. de Biología Molecular del Cáncer, Facultad de Ciencias Químico-Biológicas, Universidad Autónoma de Guerrero, [4]Instituto Estatal de Cancerología "Dr. Arturo Beltrán Ortega", [5]Centro de Ciencias Genómicas, Universidad Nacional Autónoma de México

Abstract

Introduction: Cancer is one of the leading causes of death globally (Sung et al., 2021). One of the challenges currently in diagnosing and treating the disease is due to a few proteins that have not yet been characterized (uPE1), of which their biological role in the cell, location, and level of expression is known as "dark proteins," are unknown. To characterize all the proteins encoded by the genes in each human chromosome, in 2001, the Human Proteome Project (HPP) was initiated as a resource to elucidate the biological and molecular function of each protein (Legrain et al., 2011). In Mexico, the chromosome 19 consortium is responsible for investigating the characterization of the unknown proteins C19ORF47, CCDC61, CCDC97, LENG8, and TMEM160 of this chromosome (Gil et al., 2017). This work aims to evaluate the expression at the level of mRNA and protein of C19ORF47, CCDC61, CCDC97, LENG8, and TMEM160 in breast and ovarian cancer. Methodology: The expression of C19ORF47, CCDC61, CCDC97, LENG8 and TMEM160 in biopsies of patients with breast and ovarian cancer by qPCR and immunohistochemistry were analyzed. Results: an overexpression of C19ORF47, LENG8, and TMEM160 was observed in breast and ovarian cancer, while CCDC61 and CCDC97 have no changes in the level of expression in breast and ovarian cancer. Conclusion: the positive expression of C19ORF47, LENG8 and TMEM160 in breast and ovarian cancer, suggests these types of cancer as exemplary models of studies to continue the functional analysis of these proteins.

Reference

1.- Gil, J., et al. (2017). The Journal of Biological Chemistry, 292(44), 18129-18144.
2.-Legrain, P., et al. (2011). The Human Proteome Project: Current State and Future Direction. Molecular & Cellular Proteomics: MCP, 10(7).

PP02.162: The Chromosome 19 Consortium Moves Forward in Search of the Missing Proteins.

Hernández M[1], Morales-Tarré O[1], Sánchez-Castellanos N[1], Martinez-batallar A[1], Encarnación-Guevara S[1]

[1]Centro de Ciencias Genómicas. UNAM

Introduction: Chromosome 19 encodes for 1434 genes. The proteins by chromosome have been grouped into categories in terms of protein evidence, all genes with evidence at the protein level integrated by 1327 proteins (PE1 group). Missing proteins (MPs) are grouped into four categories according to their confirmation degree. PE2 represents those genes that were confirmed at transcript level. PE3 corresponds to genes inferred from homology. PE4 belongs to the group predicted, and PE5 is classified as uncertain. Currently, the number of MPs in chromosome 19 is grouped as follows: 67 in PE2, 10 in PE3, 1 in PE4, and 30 in PE5 (neXtProt-2022-06). The missing proteins are undetectable because the protein expression differs significantly between tissue and cell types. In addition, C-HPP aims to map specific protein variations such as post-translational modifications (PTMs), alternative splicing, and protease-processed variants. Outstanding characteristics belong to this chromosome. More than half of MPs are Zinc finger proteins, approximately 10% are olfactory receptors, and 33% involved membrane proteins, complicating the identification process.
Methods: To obtain deep protein identification, we used alternative enzymes additional to trypsin to digest using total and sub-proteome from cancer biopsies and lung cancer cell lines.
Results: As a strategy to expand our coverage in protein identification, proteins have been identified by acquiring data in the Data Independent Acquisition mode and Data Dependent Acquisition. We will show preliminary results.
Conclusions: This set of experiments has identified MPs. However, the further you go, the more difficult it is to identify PMs. Therefore, analyzing sub-proteome combined with DIA allows for broadening the search horizon.

PP02.163: Chromosome-Centric Human Proteome Study of Chromosome 11 Team using neXtprot and SAAVpedia

Park Y[1], Park M[2], Yoo J[1], Kim J[1], Hwang H[1]
[1]Research Center for Convergence Analysis, Korea Basic Science Institute, [2]Department of Bio-Chemical analysis, Korea Basic Science Institute

Introduction

The Chromosome-Centric Human Proteome Project (C-HPP) aims to define the full set of proteins encoded in each chromosome through development of a standardized approach for analyzing the massive proteomic data sets currently being generated from various teams. Our team have developed a few algorithms for accurate identification of missing proteins, alternative splicing variants, single amino acid variants, and characterization of function unannotated proteins. We have found missing proteins, novel and known ASVs, and SAAVs using LC-MS/MS data from human brain and olfactory epithelial tissue, where we validated their existence using synthetic peptides.

Methods

Herein, we used the latest released neXtProt database (2022-02) which incorporates PeptideAtlas human (2022-01 build) and MassIVE, where they contains 2 and 7 new sample types (e.g. normal muscle, cancer from other female reproductive organ), respectively.

Results

According to the neXtProt database, the number of missing proteins in chromosome 11 shows a decreasing pattern. The development of genomic and transcriptomic sequencing techniques make the number of protein variants in chromosome 11 tremendously increase. For the 70 uPE1 in chromosome 11, we have studied the function annotaion of CCDC90B (NX_Q9GZT6), SMAP (NX_O00193), and C11orf52 (NX_Q96A22).

Conclusions

As a part of C-HPP, we have developed a bioinformatic method for accurate identification, and discovery of missing proteins, ASVs, SAAVs as well as function annotation of uPE1 in chromosome 11. However, 217 missing proteins and 70 uPE1 are still remained in the darkside of proteome. We will further explore them with our collaborators. So it is important to use a database that is updated annually.

PP02.164: Analysis of Popular Proteins in the Knee Joint and their Role in Osteoarthritis within the RAD-HPP Initiative

Paz González R[1], Lourido L[1], Calamia V[1], Fernández Puente P[2], Quaranta P[1], Blanco F[1,2], Ruiz-romero C[1,3]

[1]Unidad de Proteómica. Grupo de Investigación de Reumatología (GIR), Instituto de Investigación Biomédica de A Coruña (INIBIC), Complexo Hospitalario Universitario de A Coruña (CHUAC), Sergas. C/As Xubias de Arriba 84, 15006, A Coruña, Spain, [2]Universidade da Coruña (UDC), Grupo de Investigación de Reumatología y Salud (GIR-S), Departamento de Fisioterapia, Medicina y Ciencias Biomédicas, Centro de investigaciones Avanzadas (CICA), 15008, A Coruña, Spain., [3]Centro de Investigación Biomédica en Red de Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN), Av. Monforte de Lemos, 3-5. Pabellón 11, 28029, Madrid, Spain

Introduction: One of the goals of the Rheumatic and Autoimmune Diseases (RAD) initiative of the Human Proteome Project (RAD-HPP) is the proteomic characterization of the human joint tissues using literature-mining strategies to extract meaningful information from the scientific literature and focus the research efforts on high-priority proteins related to rheumatic disorders. The knee is the most common location of osteoarthritis (OA), the most prevalent and highly disabling rheumatic disease. The aim of this work has been to provide an organized picture of the proteins most closely related to the different knee joint tissues following a text-mining strategy.

Method: A large-scale bibliometric analysis applying the PubPular software was performed on references curated in PubMed to provide prioritized lists of proteins associated with the knee joint and to explore their relationship with knee OA.

Results: The proteins associated with the six different knee components (articular cartilage, subchondral bone, synovial membrane, synovial fluid, meniscus, and cruciate ligament) were retrieved and ranked by their relevance in the tissue. Their role in OA was also described. The majority of the top proteins reported for each knee component are related to extracellular matrix and proteases. However, only eight proteins were identified in all the knee components within the top 100: MMPs -1, -3 and -13, ADAMTS -4 and -5, ACAN, PRG4 and COMP. Gene ontology analyses showed the biological functions in which these popular proteins are involved.

Conclusions: This systematic and prioritized description of the proteins most frequently cited in the literature related to the knee joint provides a landscape of the pathogenic molecular processes that occur within the different knee tissues in OA. It also facilitates the development of targeted proteomic studies to characterize biomarkers that can be used in therapeutics development, treatment monitoring and a basis for future precision medicine strategies.

PP02.165: Proteins in Municipal Wastewater Hold Valuable Information about Community Health

Simmons D[1], Lara-Jacobo L, Islam G, Tannouri N, Desaulniers J, Kirkwood A
[1]Ontario Tech University

Introduction: The COVID-19 pandemic challenged the world to invent new ways of doing research and science. During this time, wastewater epidemiology emerged as a convenient and informative tool to measure SARS-CoV-2 infection at the community level. Wastewater contains biological and chemical information including pathogens, human health biomarkers, drugs, and pesticides. Working in partnership with our regional public health unit and the Ontario Government, our research team worked to concentrate and purify proteins and RNA from wastewater, and in doing so, discovered novel ways of observing the health of our community.

Methods: Using centrifugation and ultrafiltration devices, we concentrated proteins from large volumes of wastewater, and then analyzed them using a shotgun proteomics approach with formic acid and heat for protein digestion, and an Agilent 1260 Infinity HPLC with a 6545 Q-TOF as the detector. We searched the peptide sequences obtained from the spectral data against various databases, including SARS-CoV-2 proteins, flu and flu-like viruses, bacterial pathogens like H. pylori, and human protein databases that include biomarkers for urogenital and gastric cancers using Spectrum Mill software (previously Agilent and now the Broad Institute).

Results: Novel biomarkers of SARS-CoV-2 were detected during the second wave of the COVID-19 pandemic which correlated weakly but significantly with clinical cases, and now we are detecting biomarkers of cancer and proteins from other pathogens.

Conclusions: The untapped potential of wastewater for understanding population-level health and for monitoring future pandemics is enormous. A shift from the stringency of clinical settings is required to make useful inferences with this type of data, with an eye on long-term trajectories and broad-level screening. Knowledge translation focusses on trends and thresholds for actionable decision making.

PP02.166: Chemical Acetylation of Proteins: A Strategy for the Identification of Missing Proteins from Chromosome 19

Vargas-Lagunas C[1], Gil Valdés J[2], Hernández M[1], Morales-Tarre O[1], Encarnación-Guevara S[1]
[1]Centro de Ciencias Genomicas. UNAM, [2]Division of Oncology, Section for Clinical Chemistry, Department of Translational Medicine, Lund University.

Introduction: The HPP Human Proteome Project (HPP) is a collaborative effort of teams worldwide to make significant progress in understanding the human proteome. One of the main objectives is to obtain definitive evidence of at least one protein product from each protein-coding gene in the human genome and identify and characterize their functions. Chromosome 19 encodes for 1434 genes, most of which have corroborated their existence at protein level. The neXtProt-2022-06 listed 108 missing proteins on Chromosome 19.

Methods: We performed an in-depth analysis of cervical cancer cell lines using different fractionation/separation protocols along with other protein extraction to identify the most significant number of missing proteins belonging to chromosome 19. All lysine residues of proteins were chemically acetylated with heavy isotopes. In consequence, the lysine residues of all proteins are fully acetylated. Due to trypsin cannot hydrolyze the peptide bond when acetyl-lysine is present, the generated peptides will be delimited by arginine residues, decreasing the de number of peptides in the sample.

Results: Mapping proteins identified against the neXtProt database revealed 6 MPs (PE2–PE4) belonging to Chromosome 19. To validate these missing proteins, 22 unique peptides were selected based on visual inspection of PSMs and synthesized. The peptides were mixed and analyzed by LC-MS/MS (Q Exactive Plus, Thermo Fisher Scientific) to acquire higher energy collisional dissociation (HCD) fragmentation spectra for comparison with the initial spectra in the closest possible conditions.

Conclusions: Based on the results obtained, we consider that chemically acetylating all proteins and reducing the number of peptides generated after tryptic digestion allowed us to identify more protein identities in the MS, and consequently, we suggest having identified 6 missing proteins belonging to chromosome 19.

PP02.167: Plant Protein Extraction Methods for Protease Inactivation and their use in LC-MS/MS-based Proteomics

Bojórquez-Velázquez E[1]
[1]Instituto de Ecología, A.C.

Introduction: In proteomic studies, having a good protein extraction method is the first requirement to cover, since it must allow obtaining the largest possible number of these molecules while preserving their integrity. Despite the use of chaotropic, reducing, and denaturing agents that facilitate protein solubilization, breaking of disulphide bonds, and the loss of tertiary and quaternary structure, some enzymes can retain their activity, including proteases. For this reason, in this work we evaluate different protein extraction systems using pineapple pulp as a model, a sample with a high content of proteolytic activity.

Methods: Five different solutions were used to extract proteins from pineapple pulp, which include combinations of urea, thiourea, 2-mercaptoethanol, protease inhibitors, SDS, sucrose, NaCl and Phenol, at room temperature or incubation at 90°C. Protein extracts were analysed by SDS-PAGE, zymography, and LC-MS/MS.

Results: Three of the five methods showed electrophoretic protein profiles with an adequate quality for proteomic studies, in which no protease activity was observed by zymography. LC-MS/MS analysis allowed the identification of more than 3000 proteins by each method individually.

Conclusions: The use of direct phenol resulted in the simplest and fastest option for protein extraction, since it does not require the preparation of other solutions or prolonged incubation steps, in addition, it was successful for the inactivation of the proteases present in the sample without the need to use inhibitors.

PP02.168: Exploring the Role of Proteins in Brushite Biomineralization Using an Extract of Wheat Bran as a Scaffold: A Proteomic Approach

Carreño-Márquez I[1], Carreño-Márquez I[1], Huerta Ocampo J[2], Encarnación-Guevara S[3], Hernández M[3], Azamar-Barrios J[4], Mendoza-Wilson A[1], Ramos-Clamont Montfort G[5]
[1]Centro de Investigación en Alimentación y Desarrollo, A.C., Coordinación de Tecnología de Alimentos de Origen Vegetal, [2]CONACYT-Centro de Investigación en Alimentación y Desarrollo, A.C., [3]Centro de Ciencias Genómicas, UNAM, [4]Centro de Investigación y de Estudios Avanzados del IPN, Unidad Mérida, Departamento de Física Aplicada, [5]Centro de Investigación en Alimentación y Desarrollo, A.C., Coordinación de Ciencia de los Alimentos

Introduction: Calcium phosphate dihydrate (brushite) has important biomedical usages. The spherical morphology is of outstanding interest because of its dental and orthopedical applications, but also is the most difficult to obtain (1). Earlier, by using an aqueous extract of wheat bran as a scaffold for biomineralization, we reported a simple method to precipitate multilayer ordered brushite crystals, arranged radially to form microspheres (2). In the present work, through a proteomic approach, it was explored whether the proteins of the extract are part of the structure of brushite spheres to gain insights on the biomineralization mechanism.

Methods: Brushite, precipitated as previously reported (2), was thoroughly washed with methanol, and demineralized by digestion with 0.5 N HCl. Demineralized brushite was precipitated in ammonium acetate and washed with acetone. Proteins were suspended in RH buffer (8 M urea, 2% CHAPS) and subjected to SDS-PAGE. Soluble proteins were reduced, alkylated, and digested with MS-grade trypsin. After digestion, detergent was removed with ethyl acetate and peptides desalted with Sep-Pak C18 (Waters). Samples were analyzed by nano-LC-MS/MS using an Ultimate 3000, RSLCnano UHPLC system coupled to a Q-Exactive Plus. MS/MS spectra datasets were searched against the Triticum aestivum subset of the RefSeq protein database (142,442 sequences, July 2022) using the MaxQuant software.

Results: Electrophoretic pattern shows protein bands in the molecular mass range of 10-100 kDa. Gene ontology allowed the classification of identified proteins into different categories of biological processes in which they are involved.

Conclusions: The presence of proteins within the brushite spheres suggests that they play a role in the biomineralization mechanism. The development of mechanistic models will allow to delve into the role of proteins in biomineralization processes in general.

1. Bohner, M, et al. 2013 Biomatter 3; e25103
2. Zavala-Corrales, J. L, et al. 2020 Food and Bioprod Process 121; 238-249

PP02.169: Mass Spectrometry-based Analysis of Food-grade Protein Extracts of Nannochloropsis Oculata

Hamzelou S[1], Belobrajdic D[1], Broadbent J[2], Colgrave M[2]
[1]CSIRO, [2]CSIRO

Introduction: Microalgae have immense potential as an important source of sustainably produced, high-quality proteins in the human diet. A marine microalgae, Nannochloropsis has been commercially produced, primarily for its lipid content, but it is also rich in protein and essential amino acids. Although this is promising for food applications, protein extraction needs to be employed as Nannochloropsis has a thick cell wall, and little is known about the potential allergenicity of the extracted protein. Thus, we aimed to compare a range of different food-grade protein extraction methods on the proteome profile. We also sought to explore the presence of potential food allergenic peptides in line with understanding the safety of novel foods.
Methods: Food-grade protein extracts of Nannochloropsis oculata were obtained using different cell disruption methods. In parallel, the commonly used proteomic research extraction method using TCA-acetone and the same cell disruption techniques were used as controls that assumed comprehensive extraction. The resulting tryptic peptides from both food-grade and research-grade extractions were measured by data-independent acquisition using a SCIEX 6600 LC-QqTOF and DIA-NN data processing.
Results: We highlight the differing effects of extraction methods on proteome composition (qualitative and quantitative) of the N. oculata proteome. Stark differences in protein abundance were observed between food-grade and research-grade extraction techniques. Disruption techniques showed greater effects on proteome composition in food-grade than the research-grade extraction methods.
Conclusions: The choice of cell disruption approaches used in food-grade protein extraction impacts the composition of the algal proteome and can inform the best practice for ingredient preparation dependent on the desired outcome.

PP02.170: Phospho-proteomic Analysis and Phylogenetic Perspective on Conserved Plant Immune Signalling

Lu J[1], Zampronio C[1], Bottrill A[1], Ntoukakis V[1], Jones A[1]
[1]School Of Life Science, University Of Warwick

Due to the climate change and agrochemicals, many important food crops have been confronted with serious challenges from various pests and diseases. To efficiently respond to the complex environmental stresses, plants have evolved an immune system, involving biochemical molecules and signal pathways, to recognize and defend against pathogens. Phosphorylation is one of post-translational modifications which plays such a crucial role in signal transduction. Due to the great advances in experimental technique and data analysis, high-throughput Mass Spectrometry-based phosphoproteomics has been widely applied to quantitative profiling, post translational modifications, signaling pathways, and protein–protein interactions. In the green revolution of agriculture, many important food crops are worthy to be researched on their resistance to biotic stresses, such as oil-rape, cabbage, tomato, maize, wheat. Many approaches, like genomic, proteomic data or their combinations have been applied to investigate plant tolerance features and try to identify superior genotypes. Based on MS-based phsopho-proteomics and proteogenomics, this project provides a more comprehensive understanding of immune signalling and their relationship with biotic stress. We also explored the conserved proteins and evolutionary origin of the immunity pathways by phylogeny science which allows for analytical integration across different species in the green lineage. This project has given better annotation to these less-known plant species and make their biological processes more complete, which will contribute to plant breeding in Precision Agriculture.

PP02.171: Improving the Mango Peel Proteome Coverage by High- and Low-resolution MS/MS Acquisition and Multiple Search Engines

Ruiz-May E[1], Bautista-Valle M[1], Elizalde Contreras J[1], Bojórquez-Velázquez E[1], Zamora Briseño J[1]
[1]Instituto de Ecologia, A.C.

Studying any organism's proteome requires optimizing workflows at different levels, including extraction, processing methods, mass spectra acquisition, and bioinformatics. The complexity increases exponentially when studying non-model organisms with no standardized protocols and dealing with recalcitrant tissues such as peels, seeds, or plant resins. In this study, we inspected mango peels proteome using a hybrid mass spectrometer Orbitrap Fusion™ Tribrid™ series by two types of mass spectrometry (MS) acquisition methods independently, including an Orbitrap analysis for precursor ions (MS1) followed by Ion-Trap (OT-IT) for fragment ions (MS/MS) whereas in the second scheme both MS1 and MS/MS analysis where done by the Orbitrap (OT-OT). The obtained datasets of each MS workflow were submitted to Andromeda, Amanda, Byonic, MASCOT, and SEQUEST search engines. Our results show that the OT-IT acquisition outperforms the OT-OT in terms of number. We identified more than 2000 unique proteins IDs with the OT-IT than OT-OT while more than 1000 proteins IDs with OT-OT than OT-IT. Therefore, both types showed to be complementary, allowing an increasing proteome coverage of mango peels identifying more than 5000 proteins in mango peels. All search engines contributed to improving the sequence coverage of several proteins, but Byonic was the best search engine for protein identification.

PP02.172: Root Proteomics of Pistachio Rootstock, UCB-1 Revealed Specific Adaptive Mechanisms for Salt Tolerance

Katam R, Akbari M, Singh R, Vishnudasan D, Survajhala P
[1]Florida A&M University, [2]Pistat Research Center, Nazari Business Group , [3]Florida State University, [4]Amrita Vishwa Vidhyapeetham, [5]Amrita Vishwa Vidhyapeetham

Pistachio (Pistacia vera L.) is an economically important tree nut that commonly thrives in semi-arid and arid environments. P. vera is a highly adaptable to various abiotic stresses, and it can tolerate drought and salinity stresses, which makes it suitable for reforestation of arid and salinized zones. However, the mechanisms underlying the salinity tolerance of this plant are not well understood. The present study was aimed at physiological and molecular investigations to unravel the metabolic pathways associated with the salt tolerance mechanisms in UCB-1cultivar. Five one-year-old pistachio rootstocks were treated with four saline water regimes for 100 days. The rootstocks adopted Na+ exclusion strategy to resist the salinity stress. Total proteins were isolated from the roots and treated with different NaCl concentrations. The proteins were characterized using high throughput LC-MS/MS spectrometry searched against the Citrus database. Over 1600 protein IDs were detected, among which the comparative analysis revealed 245 more abundant and 190 low abundant proteins to three stress levels. The proteins associated with amino acid metabolism, cell wall organization, protein homeostasis, response to stress, signal transduction, TCA cycle, and vesicular trafficking were constantly overexpressed at all stress levels. At low and moderate stress levels, the chromatin and cytoskeleton organization lipid metabolism proteins were overexpressed, while at higher salt concentrations, they were unaffected. Transcription and translation processes were affected by all stress levels, as the proteins showed down-regulation in response to all stress levels. Transcription proteins were downregulated at low and moderate stress while overexpressed at high salt stress treatment. Protein interaction network with all the orthologous proteins mapped to Arabidopsis thaliana and the clusters associated with these proteins revealed the cytosolic, carbohydrate, and amino acid metabolism are associated with salinity stress tolerance. The proteome data were validated with corresponding changes in transcripts.

PP03.001: Deciphering the Signaling Pathways of the C-type Lectin Receptor Dectin-1 in Myeloid Cells using Quantitative Phosphoproteomics

Abderrahim R[1], Raymond B[1], Sanchez T[1], Lecabeque V[1], Rombouts Y[1], Gonzalez De Peredo A[1], Schiltz O[1], Neyrolles O[1]
[1]Institut de Pharmacologie et de Biologie Structurale, Université de Toulouse, CNRS

Intoduction:

Dectin-1 is a Pathogen Recognition Receptor belonging to the hemi-ITAM-containing C-type lectin Receptors (CLR) family. It is expressed by myeloid cells and is known to be involved in many diseases such as fungal infections, inflammation, autoimmune diseases and cancer.

Through the recognition of β-glucans present on pathogens, the phosphorylation of tyrosine residues of Dectin-1 leads to the activation of SYK-dependent and independent signaling pathways triggering the secretion of pro-inflammatory cytokines and chemokines as well as the production of reactive oxygen species and nitric oxide.

Even though Dectin-1 is one of the most studied CLRs, its signaling mechanisms remain unclear. In this project we used unbiased global proteomic approaches to explore Dectin-1 signaling and identify some potential medical targets.

Methods:

We generated ER-Hoxb8 immortalized murine cell lines from either WT or Dectin-1 KO progenitors, that were in-vitro differentiated into either macrophages or dendritic cells(DC). Cells were stimulated with depleted Zzymosan, a canonical ligand for Dectin-1, during different time length. Phosphorylated peptides obtained from TiO2 and phospho-Tyrosine antibody_enrichment were analyzed on an Orbitrap-Exploris instrument equipped with a FAIMS interface. Secretome analysis was performed after 24h of stimulation to identify inflammatory mediators..

Results:

We identified more than 300 phosphosites significantly regulated upon Dectin-1 stimulation, showing different kinds of kinetic profiles . These included sites on known proteins of the Dectin-1 signaling pathway, as well as novel targets. Secretomics also provided a detailed characterization of the DC or macrophage response, with more than 500 proteins significantly over-represented in the secretome of activated cells, including many inflammatory mediators.

Conclusion:

We generated proteomic datasets offering a global and dynamic overview of Dectin-1 signaling and phenotype in DCs and macrophages. This study allowed monitoring Dectin-1-dependent phosphorylation of novel targets that could be interesting for medical purposes.

PP03.002: Proteomic Analysis of the Molecular Biological Changes Induced by Fatty Acid-Binding Protein-5 inhibitor in Hepatocellular Carcinoma

Adachi Y[1,2], Noguchi R[1], Yookil S[1], Ono T[1], Akiyama T[1], Osaki J[1], Nakano A[1], Yanagihara K[1,3], Amari K[4], Sugihara Y[5], Yoshimatsu Y[4], Yokoo H[2], Kondo T[1,5]
[1]Division of Rare Cancer Research, National Cancer Center Research Institute, [2]Division of Hepato-Biliary-Pancreatic Surgery and Transplant Surgery, Department of Surgery, Asahikawa Medical University, [3]Biospecimen Laboratories Co., Ltd., [4]Department of Patient-Derived Cancer Model, Tochigi Cancer Center, [5]Department of Cancer Proteogenomics, Tochigi Cancer Center
Introduction: Therapeutic outcomes for hepatocellular carcinoma are improving with the advent of molecular-targeted agents and the maturation of surgical techniques. However, the current effectiveness of drug therapy for hepatocellular carcinoma is limited. Therefore, novel therapies are required for the treatment of unresectable hepatocellular carcinoma. Fatty acid-binding proteins (FABPs) are a type of lipid chaperone family that transports fatty acids and other hydrophobic ligands within cells. FABPs have 12 isoforms with similar structures and are named after the organ or tissue in which they are found. Epidermal fatty acid-binding protein (Epidermal FABP/FABP5) is abundant in skin and eyes and is also highly expressed in hepatocellular carcinoma. Recent studies have also revealed that the FABP5 is a poor prognostic factor, leading to malignant transformation via epithelial-mesenchymal transition. This study aims to investigate whether the FABP5 inhibitor can be a novel therapeutic agent for suppressing the progression of hepatocellular carcinoma.
Methods: The response to SBFI-26, a FABP5 inhibitor, was assessed in four hepatocellular carcinoma cell lines (Li-7, HLE, HepG2, and Hep3B) by cell proliferation assay, cell migration and invasion assay, fatty acid uptake assay, and glucose uptake assay. In addition, changes in protein expression by the FABP5 inhibitor were investigated using mass spectrometry.
Results: The assay's results showed that the inhibitory effects on hepatocellular carcinoma progression and the proteomic analysis revealed altered protein expressions by the FABP5 inhibitor. Further analysis is underway to identify these changes' specific effects on hepatocellular carcinoma progression and their molecular biological mechanisms.
Conclusions: The FABP5 inhibitor may be a promising new therapeutic candidate in hepatocellular carcinoma. Given the differential response of hepatocellular carcinoma cell lines to the FABP5 inhibitor, predictive diagnostic techniques for efficacy need to be developed.

PP03.003: Identification of Novel Target Signal Networks of Cancer Stem Cells by the Functional Integrated Proteomics

Araki N[1], Panawan O[1,2], Chang C[1], Yokota T[1], Nishisyama-Ikeda Y[1], Niibori-Nambu A[1], Silsirivanit A[2]
[1]Kumamoto University, [2]Khon Kaen University

Cancer stem cells (CSCs) have been demonstrated as the most responsible target for chemotherapy resistance, metastasis, and recurrence in malignant tumors. However, the study of CSC is limited because of the lack of precise molecular information on CSCs. In this study, we successfully established cancer-stem-like cells from extremely incurable cancer such as glioblastoma tissues/cells that have the potential to differentiate into glioblastoma in animal models. CSCs commonly showed a high expression of stem cell markers, resistance to anti-cancer drugs, and low proliferation compared to their parental cells. To understand the molecular pathway controlling CSCs, we analyzed the molecular dynamics during their differentiation of 0, 24, 48, 72 hr after the FCS-induced differentiation. Six glioma stem-like cell clones (GSCs) with 4 replicates of each time course were subjected to both global and phospho-proteomics by EASY-nLC-OrbiTrap Fusion Tribrid with DDA/DIA-LFQ mode. We identified a total of 9,854 proteins in global proteomics. The hierarchical clustering analysis revealed the two common up and down regulatory clusters in GSC during differentiation. We found 24 proteins are commonly upregulated in GSCs and decreased during their differentiation in a time-dependent manner. Interestingly, 20 of the 24 proteins were found to be important key enzymes related to the lipid metabolic pathway. Moreover, in the parallel study of phospho-proteomics by HAMMOC, we identified 31,873 phospho-sites in total which is corresponding to 5,429 proteins across 6 clones of GSCs. Interestingly, the AMPK signaling pathway, which is a major pathway controlling the phosphorylation of key proteins related to cholesterol and fatty acid biosynthesis, was found to be up-regulated in GSC. These findings suggest that lipid metabolism are essential for the stemness maintenance of GSCs, and manipulation of these metabolisms can be an attractive therapeutic target for the eradication of GSCs.

PP03.004: Proteomic Profiling of Cutaneous Melanoma Identifies Novel Molecular Signatures of Tumour Metastasis

Azimi A[1,2,3], Patrick E[2,4], Kim J[5], Fernandez-Penas P[1,2,3]
[1]Faculty of Medicine and Health, The University Of Sydney, [2]Centre for Cancer Research, The Westmead Institute for Medical Research, [3]Derpartment of Dermatology, Wesmtmead Hospital, [4]School of Mathematics and Statistics, Faculty of Science, The University of Sydney, [5]Department of Tissue Pathology and Diagnostic Oncology, Institute of Clinical Pathology and Medical Research, Westmead Hospital

Introduction: Despite recent developments in targeted therapy and immunotherapy for the management of metastatic melanomas, the rate of patients' response to these therapies and their overall survival remains low. Therefore, the current study aims to better understand the proteome-wide changes associated with melanoma metastasis that will assist with the identification of novel and more effective therapies.

Methods: The latest development in data-independent acquisition mass spectrometry-based proteomics together with extensive bioinformatics analysis were used to investigate the molecular changes in 60 formalin-fixed and paraffin-embedded samples of primary as well as lymph node (LN) and distant organ metastatic melanomas. Proteomic measurements were confirmed in silico by assessing their melanoma tissue expression using publicly available immunohistochemistry images from the Human Protein Atlas database, and genomic datasets from Gene Expression Omnibus (GSE46517).

Results: A total of 4,631 protein groups were identified across the samples studied, of which 72 and 453 were significantly changed between the LN and distant organ metastatic melanomas compared to the primary lesions (adj. p-value <0.05). An increase in proteins such as SLC9A3R1, CD20, and GRB2, and a decrease in CST6, SERPINB5, and ARG1 were associated with regional LN metastasis and its increased invasion and decreased apoptosis. In contrast, increased metastatic activities with reduced apoptosis in distant organ metastatic melanomas were related to higher levels of CEACAM1, MC1R, AKT1, and MMP3-9, and decreased levels of CDKN2A, SDC1, SDC4, and HTRA1 proteins. Furthermore, the application of support vector machine (SVM) analysis classified the lesions with up to 92% accuracy based on their metastatic status.

Conclusion: Overall, the findings from this study provide up-to-date proteome-level information about the progression of melanomas to regional LN and distant organs, leading to the identification of novel therapeutic targets that could improve patients' response and survival.

PP03.005: Deciphering ECM Proteome Remodeling in Human Lung Chronic Inflammation-Associated Carcinoma using an Optimized Data-Independent Acquisition Workflow

Bons J[1], Pan D[2], Shah S[1], Bai R[2], Chen-Tanyolac C[2], Basisty N[1], O'Broin A[1], Rose J[1], Sangwan V[3], Camilleri-Broët S[4], Gascard P[2], Ferri L[3], Tlsty T[2], Schilling B[1]
[1]Buck Institute for Research on Aging, [2]Department of Pathology, University of California, [3]Division of Thoracic and Upper Gastrointestinal Surgery, Montreal General Hospital, McGill University Health Centre, [4]Department of Pathology, McGill University

Introduction

Chronic inflammation is associated with 20-25% cancers worldwide. Early events during both chronic inflammation and cancer lead to significant remodeling of the extracellular matrix (ECM), which greatly affects its composition and functional properties. However, the mechanisms by which the early ECM changes can promote tumor progression are not well understood. Here, we present a refined proteomics pipeline, combining ECM enrichment from fresh human tissue specimens, comprehensive data-independent acquisition (DIA) and stringent data processing, that we applied in the context of lung squamous cell carcinoma (LSCC).

Methods

To investigate ECM remodeling, a cohort of fresh tumor and patient-matched normal lung specimens were collected by surgery from 10 patients. After enrichment for ECM components, proteins were solubilized using a refined procedure and in-gel digested. Proteolytic peptides were further deglycosylated with PNGase F and desalted. Samples were acquired in DIA mode on a TripleTOF 6600 system, and data were processed using Spectronaut (Biognosys).

Results

When applied to LSCC samples, our pipeline provided robust quantification of 1,802 protein groups (2+ unique peptides), including 162 protein groups reported in MatrisomeDB. The application of very stringent filtering revealed that 529 protein groups were confidently significantly altered in Tumor vs Matched Normal. More particularly, we observed a coordinated loss of basement membrane proteins, including laminins, nidogen-1, and perlecan, and small leucine-rich proteins, such as biglycan, decorin, and mimecan. Upregulated proteins included desmoplakin and junction plakoglobin, two desmosomal components, periostin, and Hsp47 (SERPINH1), which interacts with collagens in the ECM. Immunofluorescence verification confirmed Hsp47 dramatic increase in cancer-associated fibroblasts in LSCC and also esophageal adenocarcinoma.

Conclusions

Altogether, our work presents an efficient and robust proteomic workflow to get deeper insights into ECM remodeling in chronic inflammation-associated cancers and to propose an ECM signature with key components that represent biomarker candidates and potential therapeutic targets for ECM repair.

PP03.006: Discovery of Biomarkers in Urine of Patients with Bladder Cancer and Chronic Kidney Disease via the Investigation of Deep Proteomics

Bruderer R[1], Hoch D[2], Raess L[1], Tognetti M[1], Feng Y[1], Reiter L[1]
[1]Biognosys AG, [2]Thermo Fisher Scientific

Signatures of altered proteomes in body fluids are promising in the screening and diagnosis of diseases especially those related to bladder and kidney. Investigation of urinary biomarkers are particularly attractive due to costs, and the noninvasive nature but poses challenges due to biological and preanalytical variance. We address this unmet need by applying a novel mass spectrometry-based discovery workflow which leads to unprecedented depth.

Urine samples of patients with bladder cancer (BC, n= 27), chronic kidney disease (CKD; n= 26) and matched healthy (n= 27) were obtained from biobanks. The urine samples were analyzed on a Thermo Scientific Orbitrap Exploris 480 equipped with a FAIMS. Data were analyzed using Spectronaut.

With our discovery proteomics workflow, we analyzed 81 urine specimens from patients. This resulted in 11,123 proteins identified in the study. This study reports the deepest healthy urine proteome map to date with 9645 proteins. PCA analysis revealed partial clustering of the conditions. Exploratory analysis revealed 55 variants of post translational modifications including citrullination, hydroxylation and methylation.

We compared healthy to BC and found 3058 proteins to be significantly differential abundant. We could recapitulate previously described markers like interferon-alpha receptor 2 and urokinase plasminogen activator surfacereceptor. Next, we compared proteins between CKD and healthy and found 3010 proteins to be differential abundant. As expected, a general elevation of protein in urine due to CKD was detected and elevated levels of C-reactive protein, Bisphosphoglyceratemutase and high-affinity copper uptake protein 1.

When comparing BC to CKD, 2158 proteins are significantly differential abundant. They are enriched in programmed cell death, and keratinocyte and epidermal cell differentiation. The generation of predictive models is ongoing.

Harnessing the power of the latest advancement in mass spectrometry-based technique, we generated a comprehensive and quantitative map of proteomes from urine in different disease states which provided biological insights.

PP03.008: Proteomic Analysis of Brain Tissue from Mice with Distant Tumors.

Contreras-Martinez S[1], Morales Tarré O[1], Martinez-batallar A[1], Encarnación-Guevara S[1], Hernández M[1], Quesada H[2]
[1]CCG/UNAM, [2]Lab. Inmunologia y Proteomica, HIM Federico G.

Introduction: Just as cancer cells can alter their surrounding tissues to create a permissive and supportive environment, there is also evidence that the interactions of tumors with their hosts go beyond the microenvironment, since these are capable of actively perturbing distant organs and tissues. It has been shown that tumors are helped by other organs for successful metastasis and that they have the ability to alter distant organs to induce changes that confer advantages for their development, However the mechanism by which the tumor acts on distant tissues is not fully described. The effects of cancer on the nervous system can be direct, such as primary or metastatic tumors, or indirect, such as neurological paraneoplastic symptoms caused by distant tumors. In biological systems, proteins play a predominant role as biologically active molecules for most cellular functions such as growth, proliferation, apoptosis, senescence, and metabolism, among others. The challenge and main objective of proteomics in the study of cancer is the characterization of oncoproteins for diagnostic and treatment approaches.

Materials and methods:Total protein extracts from individual mouse brain samples corresponding to 4 different cancer tumors SiHa (HPV16 cervical cancer), B16 (melanoma), MDA-MB-231 (breast cancer), COLO 205 (colon cancer) were compared with a non-cancerous control group using liquid chromatography-mass spectrometry (LC-MS/MS), isobaric tags for relative and absolute quantitation (iTRAQ), principal component analysis (PCA) and gene set enrichment analysis (GSEA).

Results: We found differences in protein expression in the brain as a consequence of the presence of distant tumors that allow differentiating between the effects of different classes of tumors.

Conclusions : The results show that the presence of a distant tumor induces changes in the brain proteome that facilitate cancer progression and could serve as markers to predict the formation of metastasis.

PP03.009: Proteomic Analysis of Superficial and Muscle Invasive Bladder Cancer Highlights Distinct Subgroups

Dinh T[1,2,3], Cosenza-Contreras M[1,2,3], Huynh A[1], Pinter N[1], Kurowski K[1], Glavynskyi I[1], Braun L[1], Espadas G[4,5], Brombacher E[2,6,7,8], Hause F[1], Werner T[1,8], Kreutz C[6,7], Werner M[1,9], Sabidó E[4,5], Bronsert P[1,9], Grabbert M[10], Schell C[1], Schilling O[1,9]
[1]University of Freiburg, Institute for Surgical Pathology, Faculty of Medicine, [2]University of Freiburg, Faculty of Biology, [3]University of Freiburg, MeInBio Graduate School, [4]Center for Genomic Regulation, Proteomics Unit, [5]Universitat Pompeu Fabra, [6]Faculty of Medicine and Medical Center - University of Freiburg, Institute of Medical Biometry and Statistics, [7]Centre for Integrative Biological Signalling Studies, [8]University of Freiburg, Spemann Graduate School of Biology and Medicine, [9]German Cancer Consortium and German Cancer Research Center, [10]Medical Center, University of Freiburg, Department of Urology

Introduction:
Urothelial bladder cancer ranks among the top ten malignancies worldwide, with approximately 20% of cases presenting as a muscle-invasive entity with worse prognosis. We investigated the proteome biology of superficial and muscle invasive bladder cancer (sBC/MIBC) with a cohort consisting of 17 and 51 cases, respectively.

Methods:
Protein was extracted from formalin-fixed, paraffin-embedded (FFPE) samples after macrodissection and analyzed by data-independent acquisition (DIA), yielding an average proteome coverage of >6000 quantified proteins per sample.

Results:
Comparison of MIBC vs. sBC highlighted an enriched proteome signature of extracellular matrix (ECM) and immune response components in MIBC together with depletion of lipid metabolism and cell-cell adhesion.
Semi-tryptic data analysis suggests elevated levels of proteolytically truncated proteins in MIBC, indicative of increased endogenous proteolytic processing. Moreover, we can observe an enrichment of matrix-metalloproteinases and cathepsins in MIBC.
Unsupervised clustering of the MIBC proteomes produced three distinct clusters with signatures of metabolism, immune-functionality, and ECM. Our clusters resemble transcriptomic MIBC clusters (Kamoun et al., 2020), namely luminal-papillary, basal-squamous, and stroma-rich.
The metabolic subgroup is enriched with proteins associated with immune exclusion and non-muscle invasive properties, e.g. PPARgamma, GATA3. In line with this observation, the metabolic cluster exhibits an immune excluded phenotype and clusters close to sBC in principal component analysis, indicative of proteomic similarities. Cox regression analysis suggests a tendency for prolonged progression free survival (PFS) for the metabolic cluster and a shortened PFS for the ECM cluster as well as proteins of endoplasmic reticulum and Golgi complex.
Currently, we are investigating genome-proteome correlations, function of cell surface proteases, and co-regulated proteins of tumor and stroma using patient-derived bladder cancer xenografts.

Conclusion:
Our study provides a deep insight into the proteome biology of early and advanced bladder cancer. In addition, we could identify clusters that resemble clinically relevant mRNA expression subtypes of MIBC.

PP03.010: Histone Deacetylase Inhibitor Resistance in High-grade Serous Ovarian Cancer Cells

Duda J[1], Thomas S[1]
[1]University Of Minnesota

Introduction

High-grade serous ovarian cancer (HGSOC) is the most lethal gynecologic malignancy in women. Its low survival rate is attributed to late detection, relapse, and drug resistance. The lack of effective second line therapeutics remains a significant challenge. There is an opportunity to incorporate histone deacetylase inhibitors (HDACi) into HGSOC treatment to sensitize cells to poly-ADP ribose polymerase inhibitors (PARPi) or chemotherapy. Therefore, we set out to elucidate proteomic alterations following HDACi treatment in HGSOC cells to determine the mechanism and efficacy of HDACi in the context of BRCA-1/2 mutation status.

Methods

Vorinostat, Panobinostat, and Romidepsin (HDACi) IC50 values in Caov3 (BRCA wt), COV362 (BRCA-1-/-) and PEO1 (BRCA-2-/-) cells were established using a Sulforhodamine B cytotoxicity assay. HGSOC cells were treated with HDACi and analyzed for alterations in HDAC activity levels. LC-MS/MS was used to analyze alterations in global protein regulation followed by an analysis of the acetyl-lysine landscape in enriched samples. Samples were analyzed on an Orbitrap Fusion mass spectrometer (ThermoFisher Scientific). Peptide and protein identifications were performed using Proteome Discoverer 2.5.0.400 with SEQUEST. Relative quantitation analysis was carried out using SILAC metabolic labeling and label-free quantitation.

Results

HDAC6, a target of Vorinostat, was significantly upregulated in untreated BRCA-1/2 wildtype cells when compared to BRCA-1/2 deficient HGSOC cells. HDAC6 deacetylates non-histone proteins such as tubulin and heat shock proteins, and its upregulation is associated with poor prognosis and chemoresistance. The IC50 of Vorinostat in each of the cell lines was > 10-fold higher than treatment with Panobinostat or Romidepsin. This corresponded to an increase in HDAC activity following Vorinostat treatment. Acetylated proteins involved in glycolysis were significantly upregulated following Vorinostat treatment.

Conclusions

This work will elucidate individual HDACi functions to determine the benefit of incorporating HDACi into HGSOC treatment protocols to sensitize cells to PARPi treatment and chemotherapy.

PP03.012: Mass Spectrometry Imaging Spatially Identifies Complex-type N-glycans as Putative Cartilage Degradation Markers in Human Knee Osteoarthritis Tissue

Lee Y[1,2,3], Briggs M[2], Young C[2], Condina M[2], Kuliwaba J[3], Anderson P[1], Hoffmann P[2]
[1]Clinical and Health Sciences, Health and Biomedical Innovation, University of South Australia, [2]Clinical and Health Sciences, University of South Australia, [3]Discipline of Orthopedics and Trauma, Adelaide Medical School, The University of Adelaide

The majority of human proteins are being modified by covalent attachment of complex oligosaccharides, called N-glycans. They are involved in virtually all physiological and pathological processes. However, the involvement of N-glycans in knee osteoarthritis (KOA) progression at the tissue level, especially within articular cartilage, is still poorly understood. Thus, the aim of this study was to spatially map and identify KOA-specific N-glycans from formalin-fixed paraffin-embedded (FFPE) osteochondral tissue of the tibial plateau relative to cadaveric control (CTL) tissues. Human FFPE osteochondral tissue from end-stage KOA patients (n=3) and CTL individuals (n=3), aged >55 years old, were analyzed by matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI) and liquid chromatography-tandem mass spectrometry (LC-MS/MS). Overall, it was revealed that 22 N-glycans were found in the cartilage region of KOA and CTL tissue. Of those, 15 N-glycans were more prominent in KOA cartilage than CTL cartilage. We then compared sub-regions of KOA and CTL tissues based on the Osteoarthritis Research Society International (OARSI) histopathological grade (1 to 6), where 1 is an intact cartilage surface and 6 is cartilage surface deformation. Interestingly, three specific complex-type N-glycans, (Hex)4(HexNAc)3, (Hex)4(HexNAc)4, and (Hex)5(HexNAc)4, were found to be localized to the superficial fibrillated zone of degraded cartilage (KOA OARSI 2.5-4), compared to adjacent cartilage with less degradation (KOA OARSI 1-2) or relatively healthy cartilage (CTL OARSI 1-2). Our results demonstrate that N-glycans specific to degraded cartilage in KOA patients have been identified at the tissue level for the first time. This discovery may lead to the development of novel biomarker assays or therapeutic targets to slow down or prevent the progression of KOA. Finally, our next step is to investigate the presence of these specific N-glycans in synovial fluid collected from KOA patients in order to develop a new non-invasive method to assess the progression of KOA.

PP03.013: Uncovering the Proteolytic Landscape of Human Aortic Aneurysms using Integrated Forward and Reverse Degradomics

Martin D[1], Bhutada S[1], Cikach F[1], Germano da Silva E[1], Willard B[1], Ramkhelawon B[1], Ramamurthi A[1], Blackstone E[1], Roselli E[1], Apte S[1]
[1]Cleveland Clinic

Introduction: Dysregulated proteolysis is causally implicated in thoracic (TAA) and abdominal (AAA) aortic aneurysm pathogenesis, but the complete proteolytic landscapes (degradomes) of aneurysmal and normal aorta and the contributions of individual proteases therein are yet undefined. Here, a proteome-wide approach was used to delineate TAA and AAA degradomes and pathways and define contributions of two secreted proteases, mast cell chymase (CMA1) and matrix metalloprotease-9 (MMP9) to aortic remodeling.
Methods: Terminal Amine Isotopic Labeling of Substrates (TAILS), comprising labeling/blockage of free protein N-termini with reductive dimethylation or iTRAQ, enrichment of peptides with labeled/blocked N-termini, off-line fractionation, high-resolution LC-MS/MS, and positional peptide annotation was applied to Marfan syndrome TAAs (n=5), AAAs (n=16) and corresponding non-diseased aorta (n=4 each) to define their substrate and protease degradomes. 8-plex iTRAQ-TAILS was used for quantitative four-way comparison of degradomes. CMA1 and MMP9 substrates and cleavage sites were sought by digestion of aortic proteins and quantitative TAILS. Biglycan proteolysis by both proteases was specifically investigated using Amino-Terminal Oriented Mass Spectrometry of Substrates (ATOMS).
Results: The analysis identified 16,923 proteolytically derived peptides (substrate degradome) and 78 proteases (protease degradome). Quantitative degradome comparisons identified differentially modulated pathways and networks in TAA and AAA. CMA1 and MMP9 substrate degradomes comprised over 300 cleavage sites and many of these, including biglycan cleavages, were identified in the disease degradomes, specifying their contribution to aortic remodeling.
Conclusions: A systematic, unbiased, proteome-wide forward degradomics analysis of the aortic wall from TAA, AAA and undiseased tissue provided a comprehensive view of their hitherto occult proteolytic landscapes, demonstrating widespread cell and matrix remodeling. The specific contributions of CMA1 and MMP9 were mapped on the aortic degradomes using reverse degradomics, illustrating a strategy for defining the activities of all proteases involved in aortic disease. The findings provide insights into aortic aneurysm pathways and potential biomarkers.

PP03.014: Comparison of CX-4945 and SGC-CK2-1 as Inhibitors of CSNK2 using Quantitative Phosphoproteomics

Menyhart D[1], Gyenis L[1], Jurcic K[1], Roffey S[1], Litchfield D[1]
[1]University Of Western Ontario

Introduction:
Specificity is a limiting factor when using small-molecule inhibitors for assignment of protein kinase-substrate relationships. We previously devised a strategy with an inhibitor-resistant kinase mutant to validate the on-target effects of a small-molecule kinase inhibitor. When combined with mass spectrometry-based phosphoproteomics, this enabled a systematic strategy for confident identification and validation of CSNK2 substrates. In this study, we compared CX-4945, a clinical stage CSNK2 inhibitor, and SGC-CK2-1, a chemical probe selectively targeting CSNK2.

Methods:
We engineered U2OS cells expressing exogenous wild type CSNK2A1 (WT) or a triple mutant (TM, V66A/H160D/I174A) with substitutions at residues important for inhibitor binding. In contrast to CSNK2A1-WT, CSNK2A1-TM retains activity in the presence of inhibitor enabling confident identification and validation (i.e. rescue of phosphorylation in the presence of the inhibitor) of CSNK2 substrates. SILAC (Stable Isotope Labelling of Amino Acids in Cell Culture) labelled cells were treated with 30 µM CX-4945 or 5 µM SGC-CK2-1 for 4 and 24 hours and analyzed by mass spectrometry. Phosphoproteomic and proteomic analyses were performed using MaxQuant with additional downstream analyses conducted in R.

Results:
CX-4945 treatment resulted in inhibition of phosphorylation of >300 phosphosites after 4 hours and >750 phosphosites after 24 hours with expression of CSNK2A1-TM rescuing phosphorylation of a minority of these phosphosites (approximately 15% at 4 hours and 5% at 24 hours). SGC-CK2-1 resulted in inhibition of phosphorylation for a similar number of phosphosites (>250 after 4 hours and close to 400 after 24 hours) with a significantly higher proportion of phosphosites rescued by expression of CSNK2A1-TM (>50% at both 4 and 24 hours). With both CX-4945 and SGC-CK2-1, the majority of rescued sites adhere to the consensus for CSNK2.

Conclusions:
Overall, our results indicate that SGC-CK2-1 exhibits dramatically more selectivity towards CSNK2 than CX-4945.

PP03.015: Targeted Degradation and Phosphoproteomics to Uncover Plk2 Kinase-substrate Relationships

Mercado J[1], Howarth C[1], Gerber S[1]
[1]Dartmouth

Plk2 is a member of the polo-like kinase family of serine/threonine protein kinases, shown to have a role in normal cell division, centriole duplication, DNA damage signaling, and neuronal activity. However, little is known about the biochemical mechanisms that underlie these processes.

The auxin-inducible degron (AID) system can control the abundance of AID-tagged proteins by addition of the plant hormone auxin to cells, including those contained within protein complexes, and is active in all phases of the cell cycle. TurboID allows for the biotinylation of its immediate environment. Here, we use the auxin-inducible degron (AID) technology combined with TurboID (TID) to rapidly and specifically degrade endogenously degron-tagged Plk2 and enrich proximal proteins to identify candidate substrates. We then employ multiplex quantitative phosphoproteomics and proteomics to study Plk2 signaling in hydroxyurea-arrested cells. Quantitative phosphoproteomics will allow us to determine phosphorylation events in the presence or absence of Plk2 in order to find candidate substrates.

We used CRISPR-Cas9 technology and homology-directed repair to generate homozygous, endogenously degron (AID)-tagged Plk2 HeLa cell lines that exhibited rapid Plk2 degradation with a half-life of 30 minutes upon addition of auxin. Phosphoproteomics experiments were conducted comparing the phosphoproteome in the presence and absence of Plk2; however, few phosphorylation sites demonstrated significant decreases upon loss of AID-Plk2, possibly due to the low abundance of Plk2. This led us to modify our strategy and create a double tagged cell line containing the AID and TID, allowing us to enrich for only those phosphoproteins proximal to Plk2 through streptavidin pulldowns of biotinylated interactors. By tagging all three alleles, no untagged wild-type Plk2 remained in the cell. Following the creation of these cell lines, we conducted phosphoproteomics/proteomics studies and discovered novel substrates of Plk2 relevant to its roles in physiology with targets related to centrioles, dna damage, and neuronal biology.

PP03.016: Multiplexed Surveillance of Post-translationally Modified Proteins

Moen J[1], McKetney J[1], Swaney D[1], Zuliani-Alvarez L[1], Krogan N[1]
[1]UCSF

Introduction: Post-translational modifications (PTMs), in which a single amino acid is chemically modified, are the cell's preferred method for the dynamic regulation of protein function. Although several hundred PTMs have been identified there is a lack of understanding surrounding PTM-PTM interactions. Technical challenges related to established sample preparation workflows have limited overall depth and scope. We have since overcome many of these limitations by combining an SP3-based digestion strategy with on-bead TMT-labeling to identify PTM crosstalk following simultaneous enrichment of phosphorylated, ubiquitinylated, and ADP-ribosylated proteins.

Methods: Proteomic workflow optimization enhanced sensitivity while expanding the experimental range by systematically accommodating diverse PTM chemistries, e.g., tuning reaction pH and temperature to improve ADP-ribosylation stability during solid-phase protein digestion. Combinatorial application of our optimized workflow with Cell Signaling Technology's PTMScan platform and sample multiplexing through on-bead TMT-labeling enabled unprecedented analytical depth for multiple PTMs.

Results: We found the efficacy of SP3-based digestion strategies could be fine-tuned through systematic control of reaction temperature, alteration of cysteine reduction/capping schemes and proteolytic digests. On-bead labeling strategies improved overall sample depth, particularly with downstream peptide fractionation and MS analysis using EThcD fragmentation. Altogether, our approach resulted in the putative assignment of PTMs conditionally dependent on the modification status of distinct secondary PTM sites, highlighting the understudied biological complexities of PTM-PTM interactions.

Conclusion: Multi-PTM enrichment coupled with TMT-labeling enables in-depth proteomic surveillance and facilitates the construction of high-value PTM-PTM interaction networks.

PP03.017: Characterization of the Multi-acetylated Sites of Human Histone 2A Variants by Capillary Electrophoresis and Electrospray Ionization Mass Spectrometry (CESI-MS)

Yang Z[1], Demianova Z[1], Faserl K[2], Sarg B[2], Mollah S[1], Lindner H[2]
[1]SCIEX, [2]Medizinische Universität Innsbruck

Introduction

Histones are heavily modified by dynamic post-translational modifications (PTMs), which can affect chromatin structures and regulate DNA transcription and replication. Separation of isomeric histone peptides can be challenging using liquid chromatography (LC) due to their similar hydrophobicity. Capillary electrophoresis (CE) separates analytes by hydrodynamic radius and can separate positional isomers. We employed CESI-MS in this work to separate histone H2A peptides containing multiple acetylation sites with proven high sensitivity. Combined with data-independent acquisition (DIA), we achieved reliable identification and quantification of these peptides.

Methods

Nuclei from human lymphoblastic T-cells were extracted with 0.2M sulfuric acid and precipitated in 20% trichloroacetic acid. Nuclear proteins were then re-dissolved in 0.1% β-mercaptoethanol and fractionated by RP-HPLC (C4). Fractionated proteins were digested overnight at 37°C using 1:20 endoproteinase Arg-C. Peptides were analyzed using a CESI 8000 Plus system coupled with the TripleTOF 6600+ system. Both data-dependent acquisition (DDA) and SWATH acquisition (DIA) were implemented for MS analysis.

Results

SWATH acquisition provided histone protein identifications with good sequence coverage. H2A type 1-D and H2A type 2-A were identified as the major components of the H2A1 and H2A2 fractions, respectively. CESI-MS successfully differentiated isomeric acetylated peptides, which can be a challenge using LC-MS. Two isomeric peptides, GKQGGKAR with monoacetylation at either the K2 or K6 site, coeluted in LC separation but were separated clearly by CESI-MS approach. The site-specific fragments were identified from two MS/MS spectra, confirming the separation and identification of the two isomeric peptides. Moreover, CESI-MS showed baseline-resolution separation of peptides with different levels of acetylation (for example, 1-ac, 2-ac and 3-ac) and obtained good quality MS/MS spectra confirming their identities. Relative abundance of the peptides with varying levels of acetylation was also determined.

Conclusion

High-resolution separation capability of CESI-MS helps in separation and identification of Isobaric peptides with different acetylation modifications.

PP03.018: Analysis of Post-translational Modifications using Fast electron Activated Dissociation

Morrice N[1], Antonoplis A[2], Hunter C[2], Mollah S[2]
[1]SCIEX, [2]SCIEX

Introduction

Electron activated dissociation (EAD) is a fragmentation mode that involves capture of electrons by molecular ions to form radicals that dissociate into fragment ions. EAD often preserves labile post-translational modifications (PTMs) and provides peptide sequence information that is complementary to collision induced dissociation (CID). This work focuses on localization of peptide phosphorylation and glycation sites using EAD in a bottom-up liquid chromatography-mass spectrometry (LC-MS) workflow.

Methods

A total of 100 µg of digested HeLa cell lysate was fractionated using reverse-phase HPLC. The resulting 44 fractions were separated using a 20-min microflow LC gradient with a 6 µL/min flow rate. For glycated peptide analysis, human serum albumin was used with similar separation conditions. Data dependent acquisition (DDA) was performed using the ZenoTOF 7600 system with Zeno MS/MS and either CID or EAD fragmentation. Mascot was used for database searching and results were imported into Scaffold for analysis.

Results

From the CID analysis of HeLa cell lysate, 93,866 peptides were identified at 1% FDR using Mascot as the database search engine. With EAD fragmentation, 52,905 peptides were identified at 1% FDR. The orthogonal fragmentation mode provided complementary sequence information and identification of peptides not found using CID, increasing the number of identified peptides by 11%.

Next, data were mined for PTMs with a specific focus on identifications that are challenging to achieve with CID data. EAD spectra from phosphopeptides identified the sequence and site of phosphorylation using complete c’ and z• (z+1) ion series, including localization of phosphorylation sites on peptides with multiple serines. Additionally, analysis of glycated peptides using EAD enabled site-specific localization of a hexose modification.

Conclusions

Zeno EAD DDA analysis using microflow chromatography enabled confident identification and automatic site assignment of peptide phosphorylation and glycation sites.

PP03.019: Regulation of Vesicular Proteome Dynamics by Post-translational Modifications in Lysine

Morales Tarré O[1], Popa-Navarro X[2], Hernández M[1], Encarnación-Guevara S[1]
[1]Center For Genomic Sciences, [2]National Cancer Institute, Mexico

Introduction: Post-translational modifications in lysine residues play a fundamental role in the regulation of the activity of various proteins and the control of cellular processes. in particular, acetylation and ubiquitination have been described as the most abundant and there is extensive literature relating their regulation, in particular, to the development of cancer. The vesicular proteome of exosomes and ectosomes, has gained interest in recent years since these vesicles have been linked to functions as coagulation, intercellular signaling, T-cell activation and transferring antigens to the surface of professional antigen presenting cells. In particular, there are reports of changes in the composition of exosome cargo during the development of different types of cancer. Vesicles generated by malignant cells isolated from cancer patients have a higher content of proteins related to extracellular matrix remodeling, angiogenesis, organotropism, chemoatraction and epithelial-mesenchymal transition mediators. However, the mechanisms used by tumors to modulate exosomal cargo of proteins in a way that benefits cancer progression are not completely described. Here, we ascertain how global levels of acetylation and ubiquitination modify the vesicular proteome in non-small cell lung cancer models.

Methods: The cells were treated with suitable inhibitors to modify the levels of acetylation and ubiquitylation. Vesicle fractions were isolated by well-established methods of differential ultracentrifugation. Proteins were analyzed by means of Liquid chromatography coupled to mass spectrometry using both DDA and DIA methods.

Results: We found several differentially expressed proteins in cells, ectosomes and exosomes, as a result of all treatments. These were involved in redox homeostasis, carbon metabolism and cytoskeleton organization.

Conclusions: Altered protein expression profiles in extracellular vesicles content may indicate the status of the PTMs inside their parent cells and could be used to predict the outcome of targeted treatments.

PP03.020: ConNekting the Dots: Using Targeted Degradation and Enrichment Strategies to Identify Substrates of Mitotic Nek Kinases Lacking Chemical Inhibitors

Morris E[1]
[1]Dartmouth College
[1]Emily Morris, [1]Charles Lincoln Howarth, [1]Rufus Hards, [2]Mark Adamo, and [1],[2]Scott A. Gerber

[1]Department of Biochemistry at Dartmouth College
[2]Geisel School of Medicine at Dartmouth College

Introduction: The NIMA-related (Nek) kinases, Nek6, Nek7, and Nek9 are essential regulators of mitotic phospho-signaling and are frequently dysregulated in cancer. However, due to a lack of specific chemical inhibitors, there is no comprehensive list of substrates for these enzymes, making them difficult to elucidate their specific roles in cell cycle control. To establish a proteome-wide set of mitotic substrates, we applied the auxin-inducible degron (AID) system for endogenous protein degradation in combination with proximal labeling strategies and phosphoproteomic mass spectrometry to the Nek6/7/9 signaling module. In addition, we used chemical phosphoproteomics in conjunction with AID to isolate Nek-specific substrates from those of their upstream regulator, Plk1.

Methods: Endogenously tagged cell lines homozygous for each AID-Nek were generated using CRISPR/Cas9 from a pool of HeLa cells. Individual clones, isolated by FACS, which completely degraded the kinase of interest upon addition of auxin were then used to compare degraded vs. non-degraded phosphoproteomes in mitotically arrested cells (under various conditions) using tandem mass tagging (TMT) phosphoproteomic mass spectrometry.

Results: Quantitative analysis revealed a specific pool of phosphopeptides that decreased in abundance upon addition of auxin for each Nek. Comparisons of chemically inhibited Plk1 and AID-Nek degradation were also performed to assess potential substrate overlaps in this signaling pathway.

Conclusions: To date, this is the first study to elicit an in cellula set of candidate substrates for the Nek6/7/9 signaling module. Follow up work to assess the biological relevance of these substrates will provide novel insights in mitotic phosphoregulation. We have also established an efficient workflow that can be used to identify substrates of other inhibitor-free kinases in the future.

PP03.021: Targeted Proteomics for Discovery of Novel Early-stage Biomarker for Breast Cancer Diagnosis

Eiriksson F[1,2], Holm K[1], Ulfarsson M[1], Jonsdottir K[1], Bodvarsdottir S[1], Thorsteinsdottir M[1]
[1]Univeristy Of Iceland, [2]ArcticMass

Introduction: Breast cancer (BC) is one of the most prevalent cancers in women across the world and the second leading cause of cancer deaths. It is a heterogeneous disease with different patient outcomes based on clinical factors such as age, grade, and molecular status. There is a clinical need for improved diagnostic tool for early detection of BC due to mammography having significant error rate and is insufficient for BC detection in younger women. To improve BC prognosis, we conducted a targeted proteomics study of well-defined Icelandic BC study cohort to search for novel biomarkers in human plasma for early BC diagnosis.
Methods: 418 biobank-based plasma samples were analyzed using PeptiQuantTM protein human plasma MRM kits with UPLC-MRM-MS/MS. The kit contained a panel of one synthetic light peptide and matching heavy peptide for each of the 131 proteins quantified. Plasma samples were proteolytically cleaved with trypsin and sample cleanup done by solid-phase-extraction utilizing an Tecan robot. Data analysis was conducted using Skyline and SIMCA Pro-17.
Results: The assay was successfully implemented for the quantification of 131 proteins in human plasma samples. Preliminary data from 25 BC patients and 25 healthy controls indicate that 97 proteins were successfully quantified in all the plasma samples with acceptable precision and accuracy. Statistical analysis including unsupervised (PCA) and supervised (OPLS-DA) analysis indicate a potential difference between cases and controls. Data analysis for the whole cohort is being conducted, and in addition biomarker profiles will be analyzed in relation to patient BC subtypes, clinicopathological variables and prognosis and our focus will be on the BC high risk BRCA2 germline mutation carriers with possibility of more accurate diagnosis at BC onset.
Conclusion: Targeted proteomics using UPLC-MRM-MS/MS facilitated discovery of novel early-stage BC biomarker in human plasma for early detection of BC.

PP03.022: Exploring Cellular Signaling Networks in ProteomicsDB: Interactive Visualization of Differentially Regulated Post-Translational Modifications in Biochemical Pathways

Müller J[1], The M[1], Bayer F[1], Küster B[1]
[1]Chair Of Proteomics And Bioanalytics, Technical University Of Munich

Introduction

Cells react to perturbations by adjusting their signaling mechanisms, commonly by means of post-translational modifications such as phosphorylation. Studying these adjustments in the context of pathways greatly improves our understanding of cellular signaling and mode of action of drugs. Here, we present a web-based graphical interface to explore proteomics datasets from a pathway-centric viewpoint.

Methods

We collected information from publicly available databases of biochemical pathways (KEGG, Reactome, WikiPathways) and PTM databases (e.g. PhosphoSitePlus) and processed the pathways into a uniform format. Using the JavaScript frameworks Vue.js and D3.js, our software overlays information about regulated peptides onto pathway diagrams. Our application makes use of ProteomicsDB, a comprehensive resource on mass-spectrometry based proteomics data, to integrate information on site, peptide, gene, protein, and pathway level into a single application. We will release the tool as an open-source Vue component available within ProteomicsDB.

Results

Users of our software can choose between either visualizing data contained in ProteomicsDB or uploading their own experiments. Furthermore, they may customize pathway diagrams by moving, adding and removing genes or interactions. One possible application of our tool is to assess whether a protein is directly targeted by a drug or whether the the target of the compound actually is an upstream kinase that regulates the activity of the protein of interest via a phosphorylation signaling cascade. Using our tool, we confirmed known effects of Afatinib (an EGFR inhibitor) in the MAPK pathway and learned about previously unknown interactions between kinases and phosphorylation sites in this pathway.

Conclusions

We designed a software tool to analyze how a cell propagates information in the form of post-translational modifications. Due to its ability to process both inhouse and custom user data, we envision that it will become a useful asset for proteomics research.

PP03.023: A Proteome-wide Analysis of Protein Methylation Function in Pluripotency

Sayago C[1], Sanchez J[1], Garcia F[1], Zarzuela E[1], Ximenez P[1], Lafarga V[1], Prieto P[1], Ortega S[1], Fernandez-Capetillo O[1], Munoz J[2,3]
[1]CNIO, [2]Ikerbasque, Basque Foundation for Science, [3]IIS Biocruces Bizkaia

Introduction
Embryonic stem cells (ESCs) rely on an exquisite control of their epigenome to maintain pluripotency. This is partially attained by a set of methyl-transferases and de-methylases that configure histone marks. However, it is now clear that these enzymes regulate multiple substrate proteins beyond histones, which have important roles in gene transcription, protein synthesis, and signal transduction. Not surprisingly, protein methylation is emerging as an important modification for pluripotency control. However, our knowledge of the proteins and functions that are regulated by this modification in ESCs is very limited.
Methods
Here, we characterize the arginine and lysine methyl-proteomes comprehensively, reporting the identification of hundreds of novel sites and methylated proteins, including key factors for pluripotency. Most importantly, we present a strategy to deconvolute the relationships between methyl-transferases and their downstream methylated substrates based on the Proteome Integral Solubility Alteration (PISA) assay.
Results
We demonstrate that different molecular and structural events driven by methylation can be revealed by alterations in the thermal stability of the cognate proteins. We exploit this strategy to identify substrates, functions, and processes for two methyl-transferases, Prmt5 and Ezh2, that have important functions in pluripotency.
Conclusions
Our data represent a rich resource to study the role of protein methylation in pluripotency and show that ESCs are particularly sensitive to perturbation in the homeostasis of protein methylation networks. The thermal stability analyses reported here provide novel insights into the functions and processes controlled by protein methylation and represent a great addition to the proteomic toolbox for studying this modification.

PP03.024: Deephos: Predicted Spectral Database Search for TMT-labeled Phosphopeptides and its False Discovery Rate Estimation

Na S[1], Paek E[1]
[1]Hanyang University

Introduction

Tandem mass tag (TMT)-based tandem mass spectrometry (MS/MS) has become the method of choice for the quantification of post-translational modifications in complex mixtures. Many cancer proteogenomic studies have highlighted the importance of large-scale phosphopeptide quantification coupled with TMT labeling. Herein, we propose a predicted Spectral DataBase (pSDB) search strategy called Deephos that can improve both sensitivity and specificity in identifying MS/MS spectra of TMT-labeled phosphopeptides.

Methods

Deephos predicts fragment ions of peptides using a hybrid model that combines BiLSTM network and fully connected network. The model takes a phosphopeptide as input, and outputs relative intensities of possible fragment ions. Each cleavage site in an input phosphopeptide is converted to a feature vector of size 96. Relative intensities of eight fragment ion types per cleavage site are predicted, including singly- and doubly-charged b/y fragment ions as well as their neutral loss ions, which are prominent marker ions from phosphopeptides.

Results

With deep learning-based fragment ion prediction, we compiled a pSDB of TMT-labeled phosphopeptides generated from 8000 human phosphoproteins annotated in UniProt. Deep learning could successfully recognize the fragmentation patterns altered by both TMT labeling and phosphorylation. In addition, we discuss the decoy spectra for false discovery rate (FDR) estimation in the pSDB search. We show that FDR could be inaccurately estimated by the existing decoy spectra generation methods and propose an innovative method to generate decoy spectra for more accurate FDR estimation. The utilities of Deephos were demonstrated in multi-stage analyses (coupled with database searches) of glioblastoma, acute myeloid leukemia and breast cancer phosphoproteomes.

Conclusions

Deephos provides a predicted spectral database, its specialized decoy model, and software program to perform the search easily. Deephos improved both sensitivity and specificity of TMT-labeled phosphopeptide identification.

PP03.025: µPhos: A Highly Sensitive Platform for Functional (phospho-)proteomics

Oliinyk D[1], Fleischmann M[2], Tschernjawski C[1], Schneidmadel F[1], Scholl S[2], Hochhaus A[2], Meier F[1]
[1]Functional Proteomics, Jena University Hospital, [2]Hematology and Internal Oncology, Jena University Hospital

Introduction:
Protein phosphorylation is a key regulator of cellular processes and numerous strategies have been developed to analyse this post-translational modification via mass spectrometry (MS)-based proteomics. State-of-the-art protocols typically start from hundreds of µg to mg of protein input material to cover > 10,000 phosphosites (1). However, especially in clinical settings, sample amounts can be limited to just a few micrograms of protein extracted from thousand cells or miniscule tissue biopsies.
Methods:
(Phospho-)proteomics data were acquired with a throughput of 20 samples per day on a hybrid trapped ion mobility quadrupole time-of-flight MS (Bruker timsTOF HT) coupled to a nanoElute liquid chromatography system. Samples were analysed with dia-PASEF using a variable isolation window scheme adapted to the two-dimensional precursor ion density. Data were processed with Spectronaut v16 in library-free mode (directDIA).
Results:
We developed µPhos, an end-to-end sample preparation platform for sample-limited phosphoproteomics. Avoiding sample transfer steps and minimizing liquid volumes to <200 µL allows us to process samples directly in 96-well plates. We optimized enrichment and washing conditions, resulting in an >85% selectivity for modified vs. unmodified peptides. In combination with rapid and sensitive dia-PASEF acquisition, we identified ~3000 phosphopeptides mapping to ~1,000 proteins starting from 10,000 HeLa cells, which corresponds to only about 2,5 µg protein mass. Next, we applied µPhos to generate quantitative drug response profiles of acute myeloid leukemia cell lines to anti-cancer drugs in 96-well format. Even with this low input material, the analysis highlighted relevant perturbations of cellular pathways upon drug treatment with high quantitative accuracy.
Conclusions:
The demonstrated capability to work with small cell counts makes µPhos attractive for functional studies that aims to disentangle the dynamic regulation of the phosphoproteome in various conditions, cellular states or time-resolved.

References:
1. Oliinyk D., et al. Biorxiv.

PP03.026: Role of Olink Explore 3072 in integrative multi-omics analysis in infectious diseases

Rucevic M[2], Ambikan A[1], Pawar V[2], Neogi U[1]
[1]The Systems Virology Lab, Division of Clinical Microbiology, Department of Laboratory Medicine, Karolinska Institute, [2]Olink Proteomics AB

The clinical outcome and disease severity in infectious diseases are heterogeneous, and the progression or fatality of the disease cannot be explained by a single factor such as age, ethnicity, or co-morbidities. Using network-based system biology methodology, we aim to stratify patient groups based solely on omics data signatures and to identify the mechanism associated with disease severity in the acute phase of infection and dysregulated immune function and its impact on aging in chronic infectious diseases at the personalized and group level.

We used Similarity Network Fusion (SNF) by integrating transcriptomics, Olink Proteomics, and metabolomics data to stratify cohorts of COVID-19 patients as well as patients that had successfully been treated for HIV-1. Weighted co-expression network analysis was conducted to identify severity-specific gene co-expression and other associated mechanisms. We generated a context-specific genome-scale metabolic model for all individuals and patient groups, and performed flux balance analysis to characterize metabolic reactions that were altered due to the disease state. Topology analysis of the metabolic network was performed to investigate metabolic disruptions at the metabolite level and link this to infection-associated proteins identified by proteomics analysis to confirm their use as biomarkers for disease severity.

Our results demonstrate how integrative omics provide better patient clusters and disease classification. Furthermore, Olink® Explore 3072 provides biomarkers of disease severity that can easily be translated into a more targeted protein panel for further analysis.

PP03.027: Digging Deeper into Phosphoproteomes through AI-driven Deconvolution of Chimeric Spectra

Seefried F[1], Gessulat S[1], Graber M[1], Sukumar V[1], Ben Fredj S[1], Rathke-Kuhnert M[1], Samaras P[1], Schneider M[1], Schmid T[1], Zolg D[1], Frejno M[1]
[1]Msaid Gmbh

Introduction: Protein phosphorylation is a common post-translational modification in eukaryotes of high scientific interest since it plays an important role in dynamically regulating cellular signaling pathways in health and disease. During the past decade, affinity enrichment of phosphorylated peptides followed by mass spectrometry and computer-aided interpretation of mass spectra has become the method of choice to identify and quantify protein phosphorylation. Here, we utilize accurate predictions of peptide properties of phosphorylated peptides to improve the identification, correct localization, and accurate quantification of protein phosphorylation.

Methods: We extended the capabilities of our deep learning platform INFERYS by training the neural network on >1.6 M high-quality spectra of phosphorylated peptides. The resulting model is highly accurate for predictions of both fragment ion intensities and retention times of phosphorylated and unmodified peptides. A beta version of CHIMERYS utilizes these new prediction capabilities for the intensity-based scoring of experimental MS2 spectra. The algorithm aims at explaining as much experimental intensity with as few candidate peptides as possible and distributes the intensity of shared fragment ions to peptide spectrum matches given their estimated proportional contribution, resulting in the deconvolution of chimeric spectra.

Results: First, we demonstrate that intensity-based figures of merit increase the sensitivity of phosphopeptide identification and improve the localization of individual phosphorylation sites. When analyzing IMAC-based phospho-proteome enrichments, we observed a 1.4-fold increase in the number of identifiable and localizable phosphorylated sites. Next, we benchmark our prediction-based localization strategy of scoring all possible positional phosphoisomers of a given peptide against established localization tools using synthetic peptide standards. Finally, we reprocess a large base-line study of the NCI60 phospho-proteome and highlight the value of retrospective data mining to increase phospho protein and signaling pathway coverage.

Conclusions: AI-driven, intensity-based scoring of phosphopeptides enables more sensitive and reliable data analysis, unlocking biological insight though increased comprehensiveness.

PP03.028: A Bear Named ROSs: The Story of Reversible Oxidative Signaling in Tardigrade Cryptobiosis

Smythers A[1], Daughtridge M[1], Stair E[1], O'Dell H[2], Joseph K[2], Clark T[2], Flinn B[2], Kolling D[2], Hicks L[1]
[1]University Of North Carolina At Chapel Hill, [2]Marshall University

Introduction: Tardigrades, commonly known as waterbears, are microscopic invertebrates renowned for their ability to withstand extreme stressors through cryptobiosis, a metabolic stasis state enabling long term survival to adverse conditions. Tardigrades are unique in their ability to respond to a variety of cryptobiotic stressors, including freezing, extreme osmotic pressure, and anoxygenic environments, via cryobiosis, osmobiosis, and anoxybiosis, respectively. To survive extreme environments, tardigrades form what is known as a "tun," through which they retract their limbs, expel their internal water stores, and shrink to nearly a quarter of their original size. Tardigrades can survive in this stage for years, though little is known about the biochemical mechanisms that allow them to initiate, maintain, or emerge from cryptobiosis. We demonstrate that oxidative signaling plays an essential role in tardigrade survival.

Methods: Tardigrades were exposed to distinct stressors and monitored for tun formation and emergence. Stressor dose dependencies were established for all conditions for both tun formation and survival. A redox compound library was screened for tun formation inhibition. Cysteine oxidation was assessed through labeling and proteomics.

Results: Conditions for the induction and survival of tardigrade cryptobiosis were established, including the first empirical evidence of chemobiosis. Tardigrade tun formation was found to be ROS dependent, with cysteine oxidation as an essential signaling mechanism for both successful tun formation and emergence. More than 50 protein candidates post-translationally regulated for tun induction were determined using bottom-up proteomics.

Conclusions: Our work has established reproducible conditions for tardigrade cryptobiosis, including the first empirical evidence of chemobiosis. Tardigrade tun formation is reliant on reversible cysteine oxidation and can be blocked via alkylation of cysteine residues before stress induction. Future work will seek to understand how the oxidative signaling is used to generate stressor-dependent phenotypes.

PP03.029: Characterization of Cysteine-Palmitoylation using SDC Precipitation in Combination with LC-MS/MS

Toft Jensen P, Røssel Larsen M
[1]Institute of Biochemistry and Molecular Biology, University of Southern Denmark.

Introduction

Palmitoylation is the reversible modification of proteins with fatty acids attached to Cysteines via a thioester bond. The modification regulates several processes, such as folding, trafficking and interaction with other proteins and membrane structures. Characterization of protein palmitoylation commonly involves replacement of the palmitoylation modifications with affinity tags after reduction of the thioester with hydroxylamine (HA), or metabolic labelling.
Here we validate several reduction reagents (e.g., HA) using synthetic Cys-Palm peptides and combine efficient reduction with Sodium Deoxycholate (SDC) enrichment of Cys-Palm peptides.

Methods

We have made synthetic Cys-Palm peptides using TFA and palmitoyl chloride and tested several different reduction reagents for efficient release of the Palmitoyl group. We have furthermore developed a method for enrichment of palmitoylated peptides using SDC precipitation. Proteins are digested using trypsin in SDC containing 3 mM TCEP for reduction. After digestion, the SDC is precipitated using acid. Subsequently, the SDC is “washed” 4 times. After the last “wash” the SDC is buffered up to pH 8 and the Cys-Palm thioester is reduced. Subsequently, the SDC is acid-precipitated and the supernatant is analyzed using LC-MSMS.

Results

We have shown that synthetic peptides with known palmitoylation-site can be enriched using SDC precipitation and subsequently identified using LC-MS/MS after reduction. To investigate any disparity between reduction chemistry, we applied our strategy with hydroxylamine, DTT and TCEP treatment for palmitoyl cleavage. We then used our new approach to test the selective enrichment of palmitoylated peptides by combining known palmitoylated synthetic peptides with BSA peptides. Finally, we have applied our enrichment approach to complex samples such as INS-1E β-cells, cancer cells and nerve-terminals and identified several novel dynamic palmitoylation sites.

Conclusion

We have optimized a strategy for enrichment and characterization of palmitoylated peptides using SDC precipitation combined with reduction and LC-MSMS, that is applicable to complex mixtures.

PP03.030: Establishment of a Multi-PTM Workflow and Its Application on Simultaneous Large-scale Profiling of Phosphorylation, Glycosylation, Acetylation and Ubiquitination

Xu Y[1], Chen L[1], Zhang H[1]
[1]Johns Hopkins University

As the last component of bioplasticity (DNA, RNA and protein), post-translational modifications (PTMs) of protein serves as a faster responder to dynamic environmental stimuli, equipping cells with physiological functions and pathological dysfunctions greater than the coding capacity of human genome (20,000 to 25,000 genes). Studying multiple post-translational modifications (PTMs) of proteins is undoubtedly fundamental in understanding protein functions and multi-PTM crosstalk, which is gaining traction in proteomics studies. With the advent of protein mass spectrometry, the field of proteomics has witnessed major technical breakthroughs in the last two decades. Despite the importance of multi-PTM proteomics, limited studies investigate more than one PTM at a time. There are three major setbacks in multi-PTM proteomics studies, low and varying enrichment efficiencies (different input amounts) coupled with the fact that proteins are not amplifiable, lack of high-dynamic range analytical instrumentation, and low algorithm capacity to conform with the complexities of multi-PTM data. In an attempt to overcome these barriers, combined enrichment and combined mass spectrometry data acquisition were proposed for the application on clinical-relevant tissue samples, focusing on the large-scale profiling of the top four most abundant PTMs: phosphorylation, glycosylation, acetylation and ubiquitination, altogether for the first time. The combinatorial scheme identified more PTM-specific peptides compared to the typical step-by-step strategy. Additionally, with the introduction of fractionation and TMT-labeling, we are able to identify and quantify PTM-specific peptides from the same sample simultaneously. This workflow innovatively combines enrichments of PTMs of the similar enrichment conditions as well as the data acquisition of PTMs, providing a practical solution for multi-PTM proteomics studies with a low cost.

PP03.031: Phosphoproteome Profiling of 20 Breast Cancer Cell Lines Discloses Major Intrinsic Molecular Subtypes and Unique Triple-negative Breast Cancer Signaling Rewiring

Emdal K[1], Hogrebe A, Eguchi A, von Stechow L, Olsen J
[1]Novo Nordisk Foundation Center for Protein Research, Proteomics Program, University of Copenhagen

Introduction

Triple-negative breast cancer (TNBC) represents approximately 15–20% of all breast cancers. Despite extensive efforts to identify strong therapeutic targets based on the TNBC molecular landscape, targeted therapy has had little clinical success and conventional chemotherapy remains the standard of care.

Methods

In the attempt to identify novel candidates for TNBC therapy, we used a quantitative MS-based proteomics approach to profile protein expression and protein phosphorylation status across 20 breast cancer cell lines representing the major BC subtypes. Using TMT-multiplex-based quantification, we measured a 24-fraction deep phosphoproteome and 46-fraction deep proteome for each cell line.

Results

We identified and quantified >30,000 class I phosphorylation sites and >10,000 proteins across the 20 cell lines. Unsupervised hierarchical clustering of the phosphoproteome and proteome profiles recapitulated segregation into the two major molecular luminal and basal subtypes. For the group of TNBC cell lines, we found specific (phospho)proteome differences compared to non-TNBC. Gene-set enrichment analysis confirmed an overrepresentation of proteins related to mitotic spindle and epithelial-to-mesenchymal transition within the TNBC phosphoproteome and proteome, respectively. Kinase-substrate activity inference combined with functional phosphosite scoring and integration of response profiles of key pertubagens (drugs, siRNAs, CRISPR) were used to prioritize targetable kinases for functional validation. Ongoing work explores the ability to target the TNBC-specific signaling-rewiring by targeted treatment and chemotherapy (alone or in combination) to discover new avenues for effective subtype-specific therapy.

Conclusions

The phosphoproteomics data recapitulate known breast cancer signaling and hold potential to explore the repurposing of drugs for rational combination therapies.

PP03.032: Reconstructing Spatial Tissue Complexity at Single-cell and Low Input using the timsTOF SCP

Ctortecka C[1], Vashist T[1], Vaca Jacome A[1], Udeshi N[1], Carr S[1]
[1]Broad Institute

Diverse cellular identities individually and together impart tissue functionality. To understand the specific roles of those cell types we require methods that enable deepscale analysis of protein and post-translational modifications at low-multicell to single-cell input levels. An ideal solution would need to tightly integrate and optimize nanoliter sample preparation with minimal sample losses upon transfer to ultrasensitive LC-MS/MS instrumentation for data generation together with analysis tools that accurately reflect sample biology. For this we have introduced the proteoCHIP, which in conjunction with a picoliter and single-cell dispensing robot, the cellenONE® constitute a sample preparation platform for single-cell proteomics. To further minimize batch effects while improving on reproducibility and recovery, we now optimized our proteoCHIP sample preparation for label-free sample preparation with dedicated diaPASEF acquisition on the timsTOF SCP. This workflow is comprised of a dedicated label-free proteoCHIP and miniaturized sample volumes for one-step processing combining cell lysis and enzymatic digestion in less than 100 nL within the cellenONE®. The label-free proteoCHIP can be directly interfaced with multiple standard HPLC autosamplers for optimized chromatographic separation without manual sample handling and associated peptide loss. This allows to acquire on average 50 up to 100 samples per day with dedicated diaPASEF methods for efficient single cell and low input proteome profiling at improved dynamic range and analysis depth. We now complement our proteome with imaging and transcriptomics data to facilitate the reconstruction of tissue complexity to better understand spatial dynamics and cellular interactions. This combination of technologies provides greatly improved sensitivity on single samples and data completeness across large sample cohorts. Moreover, we have tailored these sensitivity driven methods to characterize post translational modifications at decreasing input requirements to expand on signaling dynamics. We are convinced that these tools will allow us to unravel heterogeneity in signaling, cancer, autoimmunity, and infectious diseases.

PP03.033: Extending Coverage in Multiplexed Single-cell Proteomics

Furtwängler B[1], Üresin N[1], McAlister G[2], Wang X[2], Goodwin M[2], Van Landuyt N[2], Op de Beeck J[2], Bergen D[2], Huang J[2], Zabrouskov V[2], Huguet R[2], Porse B[1], Schoof E[3]
[1]Copenhagen University, [2]Thermo Fisher Scientific, [3]Technical University of Denmark

Multiplexed single-cell proteomics using the SCoPE-MS approach was rapidly adopted in the field for being accessible, sensitive and enabling high single-cell throughput. This is enabled by the key concepts of isobaric multiplexing of single-cells using TMTpro and the addition of a carrier channel. However, the long ion accumulation times required for sampling enough ions from the single-cell channels limit the number of peptides that can be quantified during the LC-MS analysis. Improvements in instrument sensitivity would enable shorter ion accumulation times and thus, increase single-cell proteome coverage.

Here we evaluated a modified Orbitrap Tribrid Mass Spectrometer for the application of SCoPE-MS and real-time search assisted acquisition methods thereof. We found that improvements of ion optics and instrument architecture resulted in over two times higher signal-to-noise ratios compared to current Orbitrap MS instruments. Furthermore, in combination with the latest generation of µPAC columns for limited sample analysis, we demonstrated significant improvements in single-cell proteome coverage for high-throughput single-cell analysis.

In conclusion, we are continuing pushing the limits of high-throughput single-cell analysis thanks to state-of-the art instrumentation, consumables and LC/MS method development.

PP03.034: Microchip CE with Integrated Solid Phase Extraction Coupled to Unit Resolution MS Enables High Sensitivity Measurements for Low Input Proteomics

Heil L[1], Thompson J[2], Mellors J[2], Guerrette J[2], Remes P[3], Jacob C[3], Canterbury J[3], Yip P[3], Barshop W[3], Johnson R[1], Hsu C[1], Searle B[4], Wu C[1], MacCoss M[1]
[1]University Of Washington, [2]908 Devices Inc., [3]Thermo Fisher Scientific, [4]The Ohio State University College of Medicine

Introduction: Recent biological insights gained from single cell and spatially-resolved studies have highlighted the need for large and quantitatively accurate proteomics experiments in low input samples. However, the development of sample handling and data acquisition methods suitable for such applications remains a significant hurdle due to the sensitivity and throughput requirements inherent to low input experiments. Further, as front end separations get faster to accommodate more samples, instrument speed becomes a limiting factor. We have developed a highly sensitive method with 12 minute acquisition times to quantify 1000s of peptides from protein inputs equivalent to 10s to 100s of cells.

Methods: A prototype microchip capillary electrophoresis (µCE) device incorporating an integrated SPE bed (ref 1) (ZipChip HRP, 908 Devices) was coupled with a Fusion Lumos Tribrid (ThermoFisher Scientific) mass spectrometer, acquiring MS2 data in the linear ion trap. To accommodate the fast cycle times required for data-independent analysis with CE separations having 2-5 s peak widths, we use real time retrospective retention time alignment to run scheduled DIA and PRM acquisition methods to maximize sensitivity and coverage while maintaining reproducibility across samples.

Results: We demonstrate the ability to quantify over 2000 peptides from sub-10 ng loads in a 12 minute CE run using ultra-sensitive mass spectrometry with systematically acquired tandem MS data. Additionally, by utilizing mild detergent carrier in the samples and including DMSO in the background electrolyte, we improve CE sensitivity further. This method results in a highly linear quantitative response as well as good technical precision.

Conclusions: Novel MS acquisition methods combined with improvements to automated ZipChip SPE enable unparalleled speed and sensitivity for low input and single cell proteomics. This method is high throughput and quantitatively robust, making it ideally suited for large scale low input experiments.

Ref 1: https://pubmed.ncbi.nlm.nih.gov/35729701/

PP03.035: Parallel Measurement of Transcriptomes and Proteomes from Same Single Cells using Nanodroplet Splitting

Fulcher J[1], Markillie L[1], Williams S[1], Moore R[2], Cantlon-Bruce J[3,4], Bagnoli J[4], Seth A[4], Paša-Tolić L[1], Zhu Y[1]

[1]Environmental Molecular Sciences Laboratory , [2]Biological Sciences Division, Pacific Northwest National Laboratory, [3]3Scienion AG, [4]4Cellenion SASU

Introduction: There is a growing demand to develop high-throughput single-cell multiomics technologies to measure multiple classes of molecules from same single cells and provide integrated molecular signatures of specific cell phenotypes. To address this need, we coupled nanoPOTS sample preparation with RNA sequencing and mass spectrometry based proteomics. A new nanoSPLITS (nanodroplet SPlitting for Linked-multiomc Investigations of Trace Samples) workflow allows for division of nanodroplets containing single-cell lysates between two microchips for unbiased identification and quantitation of >5000 genes and >2000 proteins from the same single-cells.

Methods: We first optimized buffer conditions in nanoSPLITS that could accommodate downstream transcriptomic and proteomic analyses. We then evaluated the sensitivity by sorting decreasing numbers of cells onto a microchip, followed by droplet-array splitting. SMART-Seq protocol was used for single-cell transcriptomics, and label-free quantitation was used for single-cell proteomics.

Results: The median protein abundance ratios between different C10 cell populations (e.g., 11 vs. 3 vs. 1) were all within 12% of the expected theoretical values. Pearson correlations for protein abundance demonstrated high reproducibility, ranging from an average of 0.94 for single cells to 0.98 for 11 cells. RNA abundance correlations fell within expected values from 0.66 for single cells to 0.84 for 11 cells. As anticipated, the cross-correlations of protein and RNA abundances were lower with a mean coefficient of 0.49, reflective of the more dynamic nature of the transcriptome relative to the proteome. The nanoSPLITS platform was also employed for a larger scale experiment where we were able to clearly delineate different cell types (e.g., C10 and SVEC) and determine specific cell cycle states.

Conclusions: We demonstrate that the nanoSPLITS approach can enable multimodal profiling of thousands of mRNA transcripts and proteins from the same single-cells and identification of cell-type-specific markers in both modalities.

PP03.036: Single Cell Proteomics Analysis Using Spray-Capillary-Based Capillary Electrophoresis Mass Spectrometry

Wu S[1], Li J, Guo Y, Sutton K
[1]University Of Oklahoma

Introduction:
Traditional proteomics approaches typically started with bulk cells for protein expression profiling in biological samples. However, these approaches fail to account for cell-to-cell heterogeneity within the sample. Capillary electrophoresis (CE) is a promising platform for the study of limited samples such as single cells due to its ultra-low sample consumption, high separation efficiency, and ultra-low detection limit. However, offline micro-sampling processes starting with large starting sample volumes (e.g., ul-level) are often involved in current single-cell CE-MS methods, which can decrease the sampling precision and accuracy. We have developed and implemented a spray-capillary CE-MS platform that performs online micro-sampling (nL-level), CE separation, and MS analysis for ultrasensitive proteomics analysis such as single HeLa cells.

Methods:
HeLa cells (1-100) were dispensed in 10-80 nL nano-drops on a MALDI plate with droplet-based cell lysis and digestion processes. After digestion, nano-drops were dried and redissolved in 40 nL DMSO for analysis. A spray-capillary device was made using a polyethyleneimine-coated capillary was used for microsampling and online CE-MS analysis.

Results:
We have developed a spray-capillary CE-MS platform that is capable of quantitative sampling of low volumes (e.g., ~ 15 pL/s injection flow rate). Additionally, based on our previous study, the spray-capillary is capable of single-cell metabolomics via the direct coupling of CE separation and MS detection with no additional devices. Here we performed spray-capillary-based CE-MS proteomics analysis on single-cell HeLa cells (e.g., 6-12 Hela cells). An average of 3854 ± 248 mass features were detected among three replicated runs using 12 Hela cells, and 70.18% (2653 of 3780) were detected in at least 2 runs.

Conclusions:
Our results demonstrated that the spray-capillary is capable of precise microsampling and high-throughput quantitative CE-MS proteomics analysis. To our knowledge, it is the first online sample collection and CE-MS for the proteomics analysis of single cells.

PP03.037: An Ultra-high-sensitivity and High-throughput LCMS Platform for Single-cell Proteomics Analysis

Zheng R[1], Pynn C[1], Valenta A[1], Stejskal K[2,3,4], Matzinger M[2,3,4], Mechtler K[2,3,4]
[1]Thermo Fisher Scientific, [2]Institute of Molecular Pathology, [3]Institute of Molecular Biotechnology of the Austrian Academy of Sciences, [4]Gregor Mendel Institute of Molecular Plant Biology of the Austrian Academy of Sciences

State-of-the-Art low-flow liquid chromatography (LC) coupled with electrospray ionization-based mass spectrometry (MS) techniques have the capacity to probe extremely limited sample amounts, even individual cells. The increase in electrospray ionization (ESI) efficiency required is achieved by adopting narrow separation columns and reducing the LC flow rates to the “ultra-nano” (≤ 100nL/min) range. To do this consistently without a loss in separation resolution requires highly precise gradient delivery and leak-free connections, together with optimized workflows delivering sufficient throughput. Here we describe a standardized LC separation setup together with 5 novel methods for achieving the necessary sensitivity, throughput, and consistency required for routine analytics.

Experiments were performed on a Vanquish Neo UHPLC system coupled to an Orbitrap Exploris 480 mass-spectrometer. HeLa protein digest was injected onto a 50 µm I.D. column using a gradient flow rate of 100 nL/min. Contrasting data acquisition strategies, i.e., data-dependent acquisition (DDA) and data-independent acquisition (DIA), were also compared for their impact on method performance.

We developed five ultra- nano LCMS methods with gradients from 10 to 50 min. Run-to-run cycle times were optimized by utilizing the accelerated sample loading and column washing capabilities of the 1500 bar capable Vanquish Neo system, enabling a sample throughput of 24, 36, 40, 60, and 72 samples per 24 hours respectively with up to 85% MS utilization. Approximately 800 protein groups were identified using a 10-min gradient from just 250 pg HeLa digest in DDA using a standard Sequest search. These methods were subsequently validated using the advanced database searching algorithm CHIMERYS and DIA mode for the analysis of LFQ and TMT-labeled samples, demonstrating the efficacy of these novel methods for limited sample amount analysis.

PP03.038: GEM: Gold Nanoparticles Embedded Membrane for Highly Sensitive Protein Detection in Blood Plasma and Exosomes

Momenbeitollahi N[1], Aggarwal R[1], Li H[1]
[1]School of Engineering U of G

Introduction. Proteins are the functional molecules in life, performing vital physiological and pathological tasks. Quantifying protein levels in body fluids such as blood plasma can provide essential information on health and disease. For disease management, many clinically relevant proteins exist in blood at low concentrations. It is therefore important to develop highly sensitive technologies for detecting and quantifying those low-abundant proteins. 3D metal-enhanced fluorescence-based approaches such as gold nanohole arrays or nanopillar arrays have been developed for improved assay sensitivity, but require complicated micro/nanofabrication, limiting their broader applications.

Methods. In this work, we have developed a Gold nanoparticles (AuNPs)-Embedded Membrane (GEM) platform for highly sensitive protein detection in blood plasma and exosomes with 3D metal enhanced fluorescence. Sandwich immunoassays were performed on cellulose or nitrocellulose membranes for fluorescence-based protein quantification. Antibody concentrations were optimized for maximal signal-to-noise ratios. AuNPs were then applied onto the membrane and entrapped in the 3D membrane matrix (~200 μm thick) via physical adsorption, followed by signal acquisition with a fluorescence microscope. AuNPs with sizes from 5 nm to 100 nm at different particle concentrations were compared for optimal signal enhancement.

Results. Taking advantage of the high molecular loading capacity of the membranes and the fluorescent signal enhancement effects of the AuNPs, we achieved higher assay sensitivity by up to four orders of magnitude compared to conventional ELISA when measuring IgGs and cancer exosomal protein markers (EpCAM, CD24, CD9) from blood plasma samples.

Conclusions. GEM provides a useful and efficient tool for highly sensitive protein detection in blood plasma and exosomes. The high sensitivity and ease-of-implementation of GEM facilitate technology dissemination, making it suitable for a variety of applications, such as biomarker identification for early disease diagnostics.

PP03.039: Versatility of TimsTOF Platforms for 4-D High Throughput and Sensitive Proteomics Studies

Assis D[1], Speir P[1], Adams C[2], Gordon E[1], Krieger J[3], Srikumar T[3], Willetts M[1]
[1]Bruker Scientific LLC, [2]Bruker Scientific LLC, San Jose, CA, [3]Bruker Ltd.

Introduction - Significant improvements in proteomics have been achieved in the last years regarding identifications, sensitivity and high throughput. These include advances in software, hardware, a combination of chromatography, columns, and mass spectrometers. PASEF acquisition modes (DDA / DIA) for example are now consolidated and well established in the proteomics field. Here, we show some recent advances of this technology implementing the use of ion mobility prediction combined to different timsTOF instruments, column setups and chromatography improving proteomic depth. This extends to injecting samples in the order of micrograms or the level of single cell combined with distinct gradients length.

Methods - K562 tryptic digest (Promega) ranging from 60 pg to 1 ug were analyzed by coupling EVOSEP One (EVOSEP) or nanoElute (Bruker) and PepSep or IonOpticks columns systems to 3 different trapped ion mobility - QTOF mass spectrometers (timsTOF Pro2, timsTOF HT and timsTOF SCP). Data were acquired in DDA and dia-PASEF modes using several gradient lengths - 300 to 30 samples per day (SPD). Data were processed with PaSER (Bruker) using spectral libraries built with TIMScore where measured CCS values are referenced against the predicted CCS values.

Results - Using a TIMScore library, we could identify 8300 PG from 15 SPD and 3650 PG from 300 SPD method in dia-PASEF mode (200 ng on column) which represents between 10-20% more identifications when compared to previous results (no TIMScore) on timsTOF Pro 2. In addition, loadings of 1 ug in 60 minutes separation increased IDs to 10,000 protein groups on timsTOF HT. On the other side demonstrating versatility and sensitivity provided by timsTOF platforms, only 60 picograms on timsTOF SCP mass spectrometer combined to a low flow rate from the Evosep (Whisper 40 SPD) and IonOpticks 15 cm, about 900 PG and 3,000 precursors were identified.

PP03.040: Democratization of Metaproteome Analysis by Combining Fully Automated Sample Preparation and AI-driven Data Analysis

Bahten K[1], Hakimi A[1], Schauer K[1], Zheng R[2], Boychenko O[2], Horn D[1], Reeber S[1], Renuse S[1], Lopez-Ferrer D[1]
[1]Thermo Fisher Scientific, [2]Thermo Fisher Scientific
Democratization of metaproteome analysis by combining fully automated sample preparation and AI-driven data analysis

Authors
Amirmansoor Hakimi1; Kevin L. Schauer1; Runsheng Zheng2; Oleksandr Boychenko2; David M. Horn1; Steven L. Reeber1; Kristan Bahten1; Woong Kim1; Daniel Lopez Ferrer1

Institutes
1Thermo Fisher Scientific, San Jose, CA; 2Thermo Fisher Scientific, Germering, Germany

Introduction
Here we introduce a fully automated sample preparation platform for proteomics samples enabling standardized operation and robust workflows for proteomics applications. The platform was tested with metaproteomics samples that are difficult to process. Coupling to a new nLC column with extensive peak capacity hyphenated to a High-Field Asymmetric Waveform Ion Mobility Spectrometry (FAIMS TM) on a Thermo Scientific TM Orbitrap Eclipse TM Tribrid TM mass spectrometer, reduces sample preparation time, increases reproducibility and maximizes proteome coverage of metaproteome samples.

Methods
Metaproteome standards were purchased from ZymoBIOMICS and prepared using a fully automated sample preparation platform. Proteins were lysed, reduced, alkylated, and digested in the automated platform. 1µg of peptides were separated using an Easy-Spray™ PepMap™ Neo column on a Vanquish Neo UHPLC system coupled to an Orbitrap Eclipse Tribrid MS with/without FAIMS.

Preliminary Data
The automated sample preparation platform (instrument, software, reagents) provides robust and reproducible sample preparation for mass spectrometry-based proteomics analysis. We performed gas-phase fractionation using the FAIMS to gain dynamic range and improve proteome coverage. Preliminary results quantified over 12,000 proteins in the ZymoBIOMICS Microbial Community standard. Similar proteome coverage was observed in the ZymoBIOMIC Gut Microbiome standard dataset. Importantly, the system exhibited extremely high digestion efficiency for challenging samples. Zero peptide missed cleavages of 95% were achieved for plasma samples with 1-hour digestion. The sample preparation process is robust and reproducible and requires less than 20 min setup.

Novel Aspect
Fully automated proteomics sample preparation platform combined with an AI-driven search engine for comprehensive metaproteome coverage.

PP03.041: A Complete and Automated Sample Preparation Strategy for High-throughput and Standardized Proteomics Applied to a Cohort of Patient Plasma Samples

Bekker-Jensen D[1], Harking F[2], Østergaard O[2], Falkenby L[1], Iversen L[3,4,5], Bunkenborg J[1], McArdle A[1], Hørning O[1], Bache N[1], Olsen J[2]
[1]Evosep, [2]University of Copenhagen, [3]Statens Serum Institut, [4]Bispebjerg University Hospital, [5]Odense University Hospital

Introduction

For a widespread adaptation of proteomics in the clinic, the entire workflow from sample preparation through LC-MS and data analysis needs to be fast and robust to enable the required throughput. Therefore, we introduce a fully automated end-to-end sample preparation workflow starting from protein to tryptic peptides loaded on Evotips and applied to a clinical cohort of patient plasma samples.

Methods

A complete and automated sample preparation workflow was integrated on an Opentrons OT-2 robot utilizing protein aggregation capture (PAC), followed by on-bead trypsin digestion, and automatic loading of the resulting peptides onto Evotips using a specially designed pneumatic 8-channel module from Evosep (prototype). The device has a footprint of a 96 well microtiter plate, fits one position in the robot and uses positive air pressure to move liquid through Evotips.

Results

Using HeLa protein lysate, we optimized the digestion protocol for high-throughput automation on the Opentrons-2 resulting in a complete workflow for 96 samples starting from protein to digested peptides, loaded on Evotips in less than 2 hours. The entire protocol was designed for a ‘single touch’ fully automated approach, carefully considering consumption of pipette tips, buffer trays and digestion conditions to fit the Opentrons framework and finally including integrated loading of peptides on Evotips, which serve as storage until analysis.
To demonstrate clinical applicability, we digested 1 ul of plasma with our automated workflow and analyzed the samples on the Evosep One connected to an Orbitrap Exploris. We consistently quantified 340 proteins with high reproducibility covering 5 orders of magnitude in dynamic range.

Conclusions

A fully automated end-to-end workflow is fundamental to facilitate large-scale standardized proteomics. This workflow is specifically developed for integration with the Evosep One to enable analysis of large sample cohorts.

PP03.042: Pushing the Boundaries for Robust and High-throughput Single Cell Analysis with Whisper Flow Technology Powered by dia-PASEF

Bekker-Jensen D[1], Krisp C[2], Hartlmayr D[3], Seth A[3], Hoerning O[1], Heusel M[1], Huusfeldt M[1], Almeida A[4], Lubeck M[2], Kruppa G[5], Sandow J[4], Bache N[1]
[1]Evosep, [2]Bruker Daltonics GmbH & Co. KG, [3]Cellenion, [4]IonOpticks, [5]Bruker s.r.o.

Introduction

Single cell proteomics is a rapidly growing field where cutting-edge technologies are pushed to the limit. The Evosep One represents a powerful platform for high sensitivity work including single cell proteomics. Recent advancements and optimization of WhisperTM Flow technology combined with a new generation Evotip provide efficient capture and recovery of peptides from especially low sample amounts down to the single cell level.

Methods

HeLa tryptic digest (Pierce) were loaded in six replicates on Evotip Pure in concentrations ranging from 62.5 pg to 32 ng. Single HeLa cells were sorted, harvested and digested using the cellenONE in label-free proteoCHIP wells. Samples were analyzed with an IonOpticks Aurora Elite column at 50 °C using the Whisper 40 SPD method and a timsTOF SCP in dia-PASEF® mode. Data was analyzed with DIA-NN v1.8 with a predicted library.

Results

We analyzed a dilution series ranging from 62.5 pg to 32 ng HeLa digest in six replicates to establish sensitivity. From the 125 pg load, we identified 7,000 peptides leading to 1,600 proteins, whereas the high load of 32 ng resulted in just more than 55,000 peptides leading to close to 7,500 proteins. The reproducibility was excellent throughout the dilution series, so we challenged the sensitivity further by analyzing single sorted HeLa cells. Preliminary experiments showed good coverage with more than 10,000 peptides and 2,000 proteins identified from a single cell. Additionally, we developed an even faster Whisper method with a throughput of 80 SPD for scalable single cell analysis. This allowed us to analyze several hundreds of single cells to establish cell-to-cell variation and compare with image-based morphology analysis.

Conclusion

Robust and scalable workflows are needed to gain biological insight from single cell proteomics, which is efficiently achieved by combing Aurora Elite columns with the Evosep One connected to a timsTOF SCP.

PP03.043 Quantitative Proteomic Analysis of Cervical Cancer Tissues Identifies Proteins Associated With Cancer Progression

Encarnación-Guevara S[1], Ramírez Torres A[1], Gil Valdés J[2], Contreras-Martinez S[1], Salazar-Bustamante E[1]

[1]Universidad Nacional Autónoma De México, Centro de Ciencias Genómicas, [2]Department of Translational Medicine, Lund University.

Introduction: To date, several proteomics studies in cervical cancer (CC) have focused mainly on squamous cervical cancer (SCC). Our study aimed to discover and clarify differences in SCC and CAD that may provide valuable information for the identification of proteins involved in tumor progression, in CC as a whole, or specific for SCC or CAD.

Methods: Total protein extracts from 15 individual samples corresponding to 5 different CC tissue types were compared with a non-cancerous control group using bidimensional liquid chromatography-mass spectrometry (2D LC-MS/MS), isobaric tags for relative and absolute quantitation (ITRAQ), principal component analysis (PCA) and gene set enrichment analysis (GSEA).

Results: A total of 622 statistically significant different proteins were detected. Exocytosis-related proteins were the most over-represented, accounting for 25% of the identified and quantified proteins. Based on the experimental results, reticulocalbin 3 (RCN3) and Ras-related protein Rab-14 (RAB14) were chosen for further downstream in vitro and vivo analyses. RCN3 was overexpressed in all CC tissues compared to the control and RAB14 was overexpressed in squamous cervical cancer (SCC) compared to invasive cervical adenocarcinoma (CAD). In the tumor xenograft experiment, RAB14 protein expression was positively correlated with increased tumor size. In addition, RCN3-expressing HeLa cells induced a discrete size increment compared to control, at day 47 after inoculation.

Conclusion: RAB14 and RCN3 are suggested as potential biomarkers and therapeutic targets in the treatment of CC.

PP03.044: Increasing the Depth of Single Shot Proteomics with Enhanced Data Acquisition and Processing Strategies

Bergen D[1], Huang J[1], Horn D[1], Hermanson D[1], Delanghe B[2], Zolg D[3], Frejno M[3]
[1]Thermo Fisher Scientific, [2]Thermo Fisher Scientific, [3]MSAID
Introduction:

Advances in the online separation of complex proteomics samples enable a deeper mining of the proteome with single-shot methods. In addition, the CHIMERYS™ intelligent search algorithm unlocks the ability to deconvolute the chimeric spectra that still arise from the co-isolation and fragmentation of multiple peptides in tandem mass spectra.

Methods:

Gradient lengths between 30 minutes and 3 hours were used to evaluate single shot proteomics performance. Data were collected using a Thermo Scientific™ FAIMS Pro Duo™ interface and Thermo Scientific™ Orbitrap Eclipse™ Tribrid™ mass spectrometer in data-dependent acquisition mode. Quadrupole isolation widths were varied between 0.4 Th and 8 Th. Raw data files were processed with Thermo Scientific™ Proteome Discoverer™ 3.0 software using a CHIMERYS intelligent search algorithm workflow.

Results:

1 µg HeLa digest and a 1-hour gradient were used with isolation widths between 0.4 and 3 Th. Using an isolation width of 1.5 Th resulted in an average of 7,814 proteins, 59,213 peptides, and 128,647 peptide spectrum matches (PSMs) per run, increases of 13%, 31%, and 29% compared to a 0.4 Th isolation window, respectively. In contrast, processing the 1.5 Th isolation window results using a Sequest HT workflow provided an average of only 6,511 proteins, 39,549 peptides, and 53,289 PSMs. Thus, CHIMERYS provided improvements of 20% for proteins, 50% for peptides, and 141% for PSMs. 1 µg of HeLa was also run using a 2-hour and 3-hour gradient. The 2 hour runs identified an average of 8,424 proteins, 76,619 peptides, and 187,391 PSMs, while the 3 hour runs identified 8,731 proteins, 84,084 peptides, and 248,076 PSMs.

Conclusions:

Pairing optimized instrument acquisition strategies and advanced processing strategies with Proteome Discoverer software and the CHIMERYS intelligent search algorithm can synergistically improve the depth of proteome coverage and increase throughput.

PP03.045: An Automated Sample Preparation Solution for Mass Spectrometry-based Proteomics

Bomgarden R[1], Dou M[1], Reeber S[2], Robitaille A[2], Foster G[2], Snovida S[2], Barange B[2], Drew D[2], Toth J[2], Bahten K[2], Opperman K[1], Lopez-Ferrer D[2]
[1]Thermo Fisher Scientific, [2]Thermo Fisher Scientific
Introduction:

The proteomics field lacks standardized reagents and methods for sample preparation. Current methods are tedious, time-consuming, highly variable, unsuitable for processing large numbers of samples, and tend to negatively affect the robustness of the LC-MS instrumentation. Therefore, we developed an automated sample preparation solution that simplifies and standardizes mass spectrometry (MS) sample preparation. The new automated sample preparation platform offers integrated proteomics workflow solutions from designing experiments to managing the entire sample preparation process delivering high-quality samples ready for LC-MS analysis.

Methods:

Various samples including mammalian cells, plasma, tissue, purified protein, and bacteria were prepared on the AccelerOme automated sample preparation system that executes different steps including mixing, heating/cooling, reactions, sample clean-up, and peptide concentration measurement. After drying and reconstitution, the samples are ready to be directly analyzed by an LC-MS system. Both label-free and isobaric labeling (TMT11plex and TMTpro 16plex)-based protein quantification were evaluated. A nanoLC (75-µm i.d., 120-min gradient) and a Q Exactive Plus mass spectrometer were used for sample analysis. Generated data were analyzed using the Proteome Discoverer (PD) 2.4 and 2.5.

Results/Conclusions:

The automated system can process up to 36 samples containing 10-100 µg of initial protein within 4-6 hours. Reagents have been optimized to prepare cells, tissue, biofluids, and purified proteins for high-quality data acquisition. The final peptides have a minimum missed cleavage of less than 10%, complete cysteine reduction/alkylation, and most importantly, can be readily analyzed by LC-MS at a known concentration on the column determined by the systems integrated UV detector. Excellent peptide/protein identifications and quantification were achieved with less than 10% CVs between sample replicates from 3 different users with 3 different systems. Combined with the isobaric labeling (TMT11plex or TMTpro 16plex), the platform maximizes laboratory productivity with high protein/peptide quantification precision and accuracy.

PP03.046: MaxQuantAtlas Produces an Accurate Large-scale Concentration Map of Human E3 Ligases and Target Proteins for Next-generation TPD-based Precision Medicine

Ferretti D[2], Shi Y[1], Sinitcyn P[2], Tiwary S[2], Browne C[1], Rusin S[1], Kuhn E[1], Breitkopf S[1], Walther D[1], Cox J[2], Sharma K[1]
[1]Kymera, Inc., [2]Computational Systems Biochemistry, Max-Planck Institute of Biochemistry
Targeted protein degradation (TPD) has emerged as a promising new therapeutic modality. Heterobifunctional small molecule degraders degrade proteins of interest by binding an E3 ubiquitin ligase and a target protein and hijacking the body's natural cellular degradation machinery. With over 600 known E3 ligases there are possibilities to utilize their diverse tissue expression profiles for selective TPD of targets in specific tissues, cell types and subcellular compartments for which clinical applications are limited because of unwanted on-target pharmacology. Therefore, an investment in data-driven accurate mapping of E3 expression across both healthy and diseased tissues is needed to identify E3s with tissue sparing potential.

With the widespread use of MS-based shotgun proteomics, countless datasets of different human cell types and tissues with deep proteome coverage are constantly being added to public repositories providing valuable quantitative information on proteome-wide protein copy numbers. However, it remains largely underused because of technical challenges to compare protein levels across individual studies.

Here we introduce MaxQuantAtlas, a quantitative proteomics software platform designed for the integration of MaxQuant-processed proteomics datasets over samples acquired with label-free and label-based quantification strategies and instrument types.

MaxQuantAtlas enables assembly of first-in-class human protein concentration atlas over cell lines, primary cells, healthy and diseased tissues which are clustering in biologically meaningful ways, independent of quantification and acquisition technologies. The quantitative readouts of E3 expression from this software platform show good correlation with precision method results. Using comparative analyses of expression patterns from our protein concentration map, we are able to identify differentiated opportunities for selective pairings of E3 ligases with therapeutic targets of interest. We will provide examples of our LED (ligandability, expression, degradation) strategy in play as we select tissue sparing E3 ligases and showcase novel E3 ligase with broad utility for solid tumors.

PP03.047: Multiplexed Protein Assays Integrated in a Microfluidic Device for Patient-centric Clinical Biomarker Monitoring

Dale M[1], Hauser J[2], Beck O[3], Schwenk J[1], Stemme G[2], Fredolini C[1], Roxhed N[2]
[1]KTH Royal Institute of Technology, Affinity Proteomics, Science for Life Laboratory, [2]KTH Royal Institute of Technology, Micro and Nanosystems, [3]Karolinska Institute, Clinical Neuroscience

Introduction: Point-of-care assays are useful to monitor clinical biomarker over time and to detect time-critical and labile biomarkers. From a patent-centric perspective, the home-sampling procedure is convenient and easily accessible. However, it has limitations in terms of sensitivity, cost, and reliability. Advanced immunoassays carried out in centralized laboratories often requires venous blood sampling which burdens the healthcare system and is time consuming for the patient. Therefore, combining a patient-centric microfluidic sampling system with a read out at a centralized laboratory could enable a robust and sensitive method without affecting healthcare resources. A capillary-driven microfluidic system was combined with a sandwich immunoassay read out to develop a biomarker detection on chip.

Methods: The blood biomarkers CRP, MCP1, S100B, IGFBP1 and IL-6 have been selected to demonstrate the workflow and multiplexing capacity of the device. Antibodies were immobilized on color-coded magnetic beads and inserted in the microfluidic channel and dried. Blood sample was added to the channel, incubated with the capture beads, and dried. Immunocomplexes were extracted and read out was performed as a conventional sandwich immunoassay using the FlexMap3D Luminex instrument.

Results: The developed assay showed dose-response behavior of the standard curves. The limit of detection for the conventional sandwich immunoassay and the developed device for IL-6 were 10.5 pg/ml and 19.8 pg/ml respectively. The average CV of the triplicate measurements of plasma samples in the device was 6%. The multiplexing capacity of the device was tested using a panel of the five biomarkers. All showed concentration dependent standard curves and average CV of less than 6% between triplicate measurements.

Conclusions: The developed assay allows for home-sampling and quantification of biomarkers in blood with robustness and sensitivity. The assay was developed using plasma samples, for the use of whole blood samples further optimization needs to be done on the blood filter.

PP03.048: Initial Experiments on Increasing Sensitivity of Orbitrap Mass Spectrometry using Structures for Lossless Ion Manipulation

McKenzie-Coe A[1], McCabe J[1], Adamson B[2], Deng L[1], Fort K[2], Anderson G[3], Makarov A[2], DeBord D[1], Earley L[2], Wouters E[2], Dunyach J[2]
[1]MOBILion Systems, [2]Thermo Fisher Scientific, [3]GAA Custom Electronics

Introduction

Structures for Lossless Ion Manipulation (SLIM) utilizes two parallel printed circuit boards with a compact ion guide formed by traveling wave AC electrodes with confining RF voltages. Enabling high-resolution ion mobility separations via utilization of long separation lengths, SLIM has previously only been coupled with time-of-flight and triple quadrupole mass analyzers. For the first time, this presentation demonstrates the construction, and initial performance evaluation, of a SLIM device coupled to a high-resolution Orbitrap instrument.

Methods

A 13m SLIM device was coupled to a Thermo Scientific™ Orbitrap Exploris™ 480 mass spectrometer, with the SLIM device housed in an additional vacuum chamber between the high-capacity transfer tube and the injection flatapole of the Exploris instrument. Custom electronics (GAA Custom Electronics, LLC) controlled all SLIM DC, TW, and RF potentials, as well as the timing table for the on-board accumulation (OBA) region. Initial IM experiments were carried out using high-purity nitrogen as a buffer gas. Pierce™ FlexMix and Peptide Retention Time Calibration Mixture were analyzed in infusion mode to baseline performance of the SLIM-Orbitrap platform.

Results

As Orbitrap analyzers acquire mass spectra on the scale of 10s-100s of milliseconds, this usually introduces a timing mismatch with traditional drift tubes where separation occurs on 10s of milliseconds time scale. However, due to the 13m path length of the SLIM device, the separation is slowed to many 100s of milliseconds, and even seconds, thus alleviating the duty cycle mismatch. For diluted mixtures of peptides, experiments confirm that the large space charge capacity of the SLIM device enables compression of hundreds of milliseconds of incoming ion current into sequentially analyzed ion mobility packets, each well within the duration of a single Orbitrap spectrum.

Conclusions

An effective increase in sensitivity by a factor 3-10 is demonstrated, opening avenues for further improvements to proteomic depth of analysis.

PP03.049: Zeno Trap Pulsing Enables Robust Low-level Protein Quantification in Data Independent Acquisition (DIA) Workflows

Batruch I[1], Wang Z[2], Ramarathinam S[3], Campbell A[4], Deyarmin J[5], Dai K[5], Williams P[5], Ralser M[2], Purcell A[3], Unwin R[4], Wilcox B[5], Tate S[1], Bloomfield N[1], Chelur A[1], Castro-Perez J[6], Diaz N
[1]Sciex, [2]Charite, [3]Monash University, [4]The University of Manchester, [5]PrognomiQ Inc., [6]Sciex

Introduction:
DIA analysis is becoming a standard for large-scale quantification in the life science research field given the desire to be able to quantify over a wide dynamic range. The improved duty cycle provided by the Zeno trap on the ZenoTOF 7600 system enables gains in sensitivity of 5 to 20x. Zeno SWATH DIA was assessed across 0.25 ng to 200 ng of commercial digest loads and tested across multiple labs globally using similar methods with a range of chromatography systems.

Methods:
In general, a ZenoTOF 7600 system with an OptiFlow Turbo V ion source was coupled to a Waters M-Class LC system equipped with nano- and micro-flow columns. K562 cell digest (SCIEX), was loaded onto an appropriate column and analyzed using Zeno SWATH DIA. All data was processed using DIA-NN.

Results:
Using Zeno SWATH DIA with 0.25, 0.5 and 1 ng digest loads, we detected between 900 and 2300 proteins at 1% FDR with 3000 to 12000 precursors. At higher loads, 5 to 200 ng, we detected between 4200 and 7000 protein groups with 63-95% of identifications having a CV under 20%, along with 29000 to 66000 precursors. A standard result set from multiple groups was processed which showed minimal variance of protein identification rates using variable chromatography systems. We show the same trend in the detection rates across the various labs indicating the stability of the sensitivity of the technique across multiple instruments. The data shows that hardware is robust and precise at measurements in the range of single cell analysis.

Conclusions:
The high duty cycle for MS/MS on the ZenoTOF 7600 system provides a high number of identifications at single-cell level loads. The ability to determine robust quantitative measurements across multiple instruments and sites with similar results shows that reliable results are possible at low loads.

PP03.050: Computational Modeling of Protein Identification by Short-epitope Mapping (PrISM)

Egertson J[1], DiPasquo D[1], Sherman J[1], Brusniak M[1], Joly J[2], Boyce H[2], Killeen A[1], Lobanov V[1], Patel S[1], Mallick P[2]
[1]Nautilus Biotechnology, [2]Nautilus Biotechnology

Introduction: Protein Identification by Short-epitope Mapping (PrISM) is a new proteomics approach with the potential for near-comprehensive quantification of the proteome. In this work, we present computational simulations demonstrating the approach and investigate the factors influencing its performance. PrISM works by attaching single protein molecules from a sample onto a solid-support and identifying each molecule using sequential binding measurements from hundreds of non-traditional affinity reagents. This sequential measurement approach overcomes the challenges of traditional affinity reagent sensitivity and specificity. For proteome-scale measurements, billions of individual molecules are identified in parallel in as little as a day.

Methods: An implementation of PrISM was simulated where proteins are immobilized on a high-density single-molecule array and measured using up to 300 different affinity reagents each targeting a short linear epitope. The measurements are processed by a decoding algorithm to generate a protein identification for each molecule.

Results: PrISM performs optimally when low-selectivity affinity reagents are used. 150 affinity reagents each with affinity to multiple trimers are theoretically capable of identifying >90% of the human proteome. This outcome held even when the trimer targets were random and with non-human species. Using 300 affinity reagents, >95% human proteome coverage is observed with a 70% per-epitope false-negative binding rate and a 1% false-positive protein binding rate. PrISM relies on characterizing the epitopes that each reagent binds to. We found that up to 20% of epitopes may be “missed” in characterization without a significant performance impact. Simulated measurements of HeLa cell lysate indicate that an array measuring 10 billion proteins could detect >90% of the proteins in the sample up to 9 orders of magnitude dynamic range. 85% of proteins in the top 5 orders of magnitude of abundance had CV <1%.

Conclusions: PrISM is a robust approach capable of >90% species-agnostic proteome coverage.

PP03.051: Delivering Comprehensive, Single-molecule Proteomics Using Protein Identification by Short-epitope Mapping (PrISM)

Egertson J[1], Stawicki C[1], Gillies T[1], Joly J[1], Steiner N[1], Ikwa E[1], Cardenas K[1], Leano J[1], Chen K[1], Watts S[1], Boyce H[1], DiPasquo D[1], Killeen A[1], Lobanov V[1], Kapp G[1], Patel S[1], Mallick P[1]
[1]Nautilus Biotechnology

Introduction: Here we present a novel approach for single-molecule protein detection and quantification, Protein Identification by Short-epitope Mapping (PrISM). PrISM is a single-molecule analysis method where intact proteins are immobilized and analyzed massively in parallel by using non-traditional, fluorescently-labeled affinity reagents to create a pattern of binding that allows for the identification of each individual protein molecule.

Methods: PrISM uses non-traditional affinity reagents with high affinity and low specificity that bind to short epitopes in multiple proteins. Simulations using these reagents show that the accumulated information from multiple rounds of detection of short, 2-4 amino acid epitopes enables identification of more than 95% of the human proteome with just 300 different reagents. We use affinity reagents targeting short epitopes (as short as three amino acids) to identify and quantify proteins at the single molecule level.

Results: We have measured millions of single protein molecules and successfully identified a set of model proteins with a smaller number of affinity reagents, demonstrating the PrISM approach. Proteins have been identified and quantified in the presence of a complex background (a mammalian cell lysate). Defined protein mixtures have been used to determine the sensitivity and reproducibility of the quantitation. We demonstrate a prototype chip that can immobilize 10 billion individual proteins to provide up to 9 orders of magnitude dynamic range in a plasma sample.

Conclusions: Combining single-molecule analysis, intact (non-digested) proteins, and iterative affinity reagent binding cycles, PrISM provides a new tool for the discovery and quantitation of proteins. This new analytical tool will help discover novel biology, identify more effective biomarkers, and aid in understanding the molecular mechanisms of human diseases.

PP03.052: High-Throughput Proteomic Analysis of Stored Red Blood Cells from Non-Domestic Cat Species

Hakimi A[1], Schauer K[1], Reeber S[1], Bahten K[1], Renuse S[1], Lopez-Ferrer D[1], Parkinson L[2]
[1]Thermo Fisher Scientific, [2]University of Georgia

Purpose:
Blood transfusions can be a life-saving treatment for animals that have become anemic due to trauma or disease. However, adverse, often life-threatening, complications can occur if the donor and recipient blood types are not appropriately matched. In non-domesticated cat species, very little is known about the different blood types found in different species, making transfusions in most situations risky. Additionally, since non-domestic cats are often found in zoos where only a few individuals of each species are housed, there is a need to be able to either transfuse blood from one species to another or store blood for prolonged periods of times in case of future emergencies.
Methods:
Blood samples were obtained from more than 150 non-domestic cats, consisting of 18 different species, housed at AZA (Association of Zoos and Aquariums) accredited institutions across the United States. Samples from 36 of these animals were analyzed here. Fresh blood samples were aliquoted and stored in a clinical blood transfusion refrigerator for 0, 7, 14, or 28 days, after which the red blood cells were pelleted and stored at -80^{o}C until analysis. Samples were also crossmatched to determine compatibility. Pelleted red blood cells were then prepared for bottom-up proteomic analysis using the newly introduced Thermo Scientific™ AccelerOme™ automated sample preparation platform.
Results: The Thermo Scientific™ AccelerOme™ automated platform allowed for protein lysis, reduction, alkylation, digestion, and cleanup with no user intervention. Trypsin digested red blood cell samples were then analyzed using high-throughput, capillary flow LC-MS/MS analysis on a Thermo Scientific™ Orbitrap Exploris 480™ mass spectrometer, allowing for the analysis of more than 50 samples per day. Such methods allowed for the identification and label-free quantification of more than 2000 peptides per sample, mapping to approximately 500 protein groups, despite the high abundance of hemoglobin in the red blood cells.

PP03.053: DIA Performance in Discovery and Quantitation Analysis on New Orbitrap Exploris Mass Spectrometers

Hakimi A[1], Liu Y[1], Bahten K[1], Flora A[2], Bomgarden R[2], Lopez-Ferrer D[1]
[1]Thermo Fisher Scientific, [2]Thermo Fisher Scientific

Introduction

Data-independent acquisition (DIA) mass spectrometry (MS) provides deep proteome analysis without the bias from peak intensity. In addition to more comprehensive identification coverage, DIA also shows accurate label-free quantification. In this work, we evaluated the DIA performance on both Thermo Scientific™ Orbitrap Exploris™ 480 and 240 mass spectrometers for discovery and quantitative proteomics analysis.

Methods

Mixed Hela:E.coli digested peptides (1:2, 1:4, 1:8 ratio) were loaded on a 25cm Aurora column (25cm x 75um ID, 1.6um C18) with a Thermo Scientific™ Easy-nLC™ 1200 system, separated by a 90min LC gradient before being injected to Thermo Scientific™ Orbitrap Exploris™ 240 or 480 MS. Mixed Hela:Yeast peptides was analyzed using the same setup at different gradient length (15min, 30min, 90min). A chromatogram spectral library was built by matching the gas-phase fractionations (GPF) to the predicted spectral library1. Acquired DIA data were analyzed by Spectronaut™ 14.0.

Results

Spectral libraries of 1000ng Hela:E.coli mixture were built on two Orbitrap Exploris™ platforms and the dynamic ranges were demonstrated to be up to 7 orders of magnitude. By matching with each library, 9056 and 9472 proteins were identified on Orbitrap Exploris™ 240 and 480, respectively. The Hela:E.coli spike-in proteome acquired on both systems was quantified by directDIA™. Exploris™ 480 exhibited better quantification accuracy than Exploris™ 240. Furthermore, we also proved that matching with the library built at a longer gradient and a higher sample load would improve the identification coverage. Therefore, once built at ultimate setup, the spectral library can be used for universal DIA discovery of the same sample.

Conclusions

Both Thermo Scientific™ Orbitrap Exploris™ 240 and 480 mass spectrometers had excellent performance in DIA analysis in discovery and quantitation.

References

1. Searle, B.C., Swearingen, K.E., Barnes, C.A. et al. Nat Commun 11, 1548 (2020).

PP03.054: Detection of Early Prognostic Biomarkers for Metastasis of Ewing’s Sarcoma in Pediatric Patients

Ezzeldin S[1], Osama A[1], Anwar A[1], Nasr A[1], Mahgoub S[1], Ahmed E[1], Farid N[1], Elghoneimy A[1], Zamzam M[1], Magdeldin S[1]
[1]Children Cancer Hospital 57357 Egypt

Ewing’s sarcoma, a highly aggressive bone tumor, is the second most prevalent pediatric bone malignancy. Approximately 25% of Ewing’s sarcoma cases show metastasis at the time of diagnosis. The extremely aggressive metastatic nature of Ewing’s sarcoma decreases the three-year survival rate to 20% and imposes constraints on the current treatments resulting in a diminished prognosis. Detection of Ewing's sarcoma and prediction of metastatic-prone patients became vital to overcome the low survival rate and poor prognosis. Furthermore, there is currently no available data on Ewing’s sarcoma utilizing non-biopsy samples, which is mandatory for the ease of diagnosis and evaluation. In this vein, we aimed to identify early prognostic biomarkers potentially predictive for metastasis of Ewing’s sarcoma. A shotgun proteomic analysis was performed using plasma samples from Ewing's sarcoma patients. Our study revealed a set of proteins significantly associated with the diseased profile that could ultimately serve as candidate biomarkers at diagnosis, and was implicated as well in disease metastasis and tumor invasion. Aberrant proteins’ expressions were significantly detected in clusterin, transferrin, CD5 antigen-like, and ceruloplasmin. Differentially expressed proteins were found to be involved in cancer-promoting pathways required for tumor cell proliferation and survival, including PI3K/AKT, MAPK/ERK, and IL-6/STAT3 signaling pathways. This study reported for the first time the plasma proteome expression profile of Ewing's sarcoma, laying the foundation for developing potential biomarkers for disease early detection and prognosis prediction.

PP03.055: Improved Label-free Quantification using the CHIMERYS Intelligent Search Algorithm

Hermanson D[1], Horn D[1], Fritzemeier K[2], Tham K[2], Berg F[2], Schmidt T[3], Zolg D[3], Frejno M[3], Delanghe B[2]
[1]Thermo Fisher Scientific, [2]Thermo Fisher Scientific, [3]MSAID

Introduction: Current DDA-based approaches for label-free quantification match peptide identifications from a search engine to features detected independently by a quantification algorithm. Quantification algorithms can detect more than 100,000 features in a 1-2 hour run, but typical search engines may only identify half of these features. The CHIMERYS™ intelligent search algorithm deconvolutes chimeric spectra, leading to significant increases in the number of peptide identifications.

Methods: Increasing amounts of an E. coli proteome sample were mixed with a human proteome sample to make a total of 1 ug protein for each mixture. Data were analyzed using Proteome Discoverer 3.0 software using either a Sequest HT-based workflow or a CHIMERYS-based workflow connected to Minora Feature Detector to perform label-free quantification. A combined human and E. coli database was used for both searches.

Results: The Sequest HT search produced a total of 9,411 proteins and 107,742 peptide groups at 1% FDR, while the CHIMERYS search produced a total of 10,113 proteins (+7%) and 129,088 peptide groups (+20%). For the CHIMERYS search, 8,086 human proteins, 111,012 human peptide groups, 2,027 E. coli proteins, and 18,076 E. coli peptide groups were identified. The median protein ratios were similar between the two searches, with a 0.52 ratio for Sequest HT versus 0.55 for CHIMERYS for the 25 ng/50 ng ratio, a ratio of 1.92 for Sequest HT versus 1.95 for CHIMERYS for the 100 ng/50 ng ratio, and a ratio of 3.89 for Sequest HT versus 3.95 for CHIMERYS for 200 ng/50 ng ratio. The CV deviation for the ratios were similar.

Conclusions: These results demonstrate that CHIMERYS produces similar accuracy and precision as an established search engine for LFQ analysis. However, CHIMERYS also enables better sensitivity with about a 1 order of magnitude increase in dynamic range of quantified peptides compared to Sequest HT.

PP03.056: Optimizing Proteomic Sample Preparation Based on Digestion Efficiency and Specificity

Hosfield C[1], Strauss E[1], Gonzalez Rivera A[1], Rosenblatt M[1], Urh M[1]
[1]Promega Corporation
Introduction:

In bottom-up proteomic studies, proteins are proteolytically digested into peptides that are subsequently identified using LC-MS/MS. Ideally, the protease used should be both highly efficient and specific, since poor digestion due to low specificity and/or efficiency complicates LC-MS/MS analysis by increasing sample complexity and reducing sensitivity. In this study, we sought to optimize proteolytic digestion of human cell extracts by examining the relationship of enzyme:substrate ratio and digestion time on digestion efficiency and specificity for a variety of commonly used proteases.

Methods:

Human K562 extracts were digesed at a variety of digestion times and enzyme:substrate ratios. Proteases used included Trypsin, Lys-C, Arg-C, Glu-C, Asp-N and ProAlanase. Digested peptides were analyzed on an Orbitrap Exploris 240 coupled to an easy-nLC 1200 (ThermoFisher) and data were searched with Byonic (Protein Metrics) with no-enzyme specified. For “single site” proteases such as Lys-C, Arg-C, Glu-C and Asp-N, specificity was reported as the percentage of peptides observed containing the preferred amino acid at the appropriate termini, whereas with Trypsin and ProAlanase, specificity was reported as the combined percentage of cleavages at the two primary cleavage sites. Similarly, efficiency was reported as the percentage of observed amino acids that were cleaved and found at the expected terminal position on the peptide.

Results:

Specificity-Efficiency plots were utilized to visualize the relationship between specificities and efficiencies as a function of digestion conditions. Shapes of these plots varied between proteases, but generally show that observed specificity decreases as digestion efficiency increases. With trypsin, the best combination of efficiency/specificity plus total number of observed peptides was seen with a 2 hour digest at 1:5 E:S ratio. Optimal conditions for other proteases will be discussed.

Conclusions:

These results highlight the complex nature of evaluating proteolytic performance for the most commonly used proteases in bottom-up proteomics.

PP03.057: Fast microflow LC with Zeno SWATH DIA for accelerating biomarker research

Hunter C[1], Antonoplis A
[1]SCIEX

Introduction

Data independent acquisition (DIA or SWATH DIA) coupled with microflow chromatography has emerged as a powerful, higher throughput workflow for protein biomarker research. Recently, the Zeno trap was developed on a QTOF instrument that provides a 5-to-6-fold increase in peptide MS/MS sensitivity. Here, the impact of the higher sensitivity Zeno MS/MS on the DIA workflow was investigated, for protein identification and quantification workflows.

Methods:

Digested cell lysate (K562) was analyzed at various on-column loadings (12.5–400 ng) with microflow LC (150 and 300 µm ID C18 column, 1-5 µL/min). Gradient lengths ranging from 5 to 45 min were tested. Key acquisition parameters for Zeno SWATH DIA were optimized on the ZenoTOF 7600 system, including number of Q1 windows (affects S/N) and MS/MS accumulation time (impacts MS/MS sensitivity). Data were analyzed with DIA-NN software and OneOmics suite using both spectral libraries as well as library-free approaches.

Results

Using optimized conditions for each gradient length, Zeno SWATH DIA provided large gains in proteins quantified from human cell lysates across all gradient lengths. 50% more proteins were quantified at high loads and 200-400% more quantified at low loads versus SWATH DIA without Zeno trap activated. Zeno SWATH DIA is readily compatible with fast microflow gradients, enabling up to ~6,500 proteins to be identified, with ~95% of those proteins quantified at <20% CV. Using an in silico-generated library for data processing, ~2-fold more proteins were identified from a cell lysate using a 45-min gradient with Zeno SWATH DIA vs. Zeno DDA. This library free approach provided protein quantification numbers similar to using large experimentally generated libraries. Results comparing 150 µm column will also be shared.

Conclusions

Microflow Zeno SWATH DIA provides very large increases numbers of protein quantified with fast gradients for accelerating protein research.

PP03.058: High-throughput Production of Stable Isotope-labeled Recombinant Proteins for Robust MS Based Quantification

Jensen G[1], Kotol D[1,2], Hober A[1,2], Edfors F[1,2], Tegel H[1]
[1]Kth Royal Institute Of Technology, [2]Science for Life Laboratory

Introduction:
Protein Epitope Signature Tags (PrESTs) produced in the Human Protein Atlas (HPA) project have been shown to be a valuable resource of isotopic standards for quantitative Mass Spectrometry. Since the beginning of the HPA project in 2003, approximately 57,000 PrESTs have been designed and cloned, resulting in a large clonal library that now can be used as a resource to produce isotopic standards in a high-throughput manner. As the library covers almost all protein coding genes, isotopic standards can be produced for the majority of all human proteins.

Methods:
The Stable Isotope-Labeled (SIL) proteins are expressed recombinantly in the auxothrophic E.coli Rosetta (DE3) strain. Cultivation is performed in minimal auto-induction media in deep well plates or cultivation flasks depending on amount required. Heavy isotope-labeled (15N and 13C) arginine (+10Da) and lysine (+8Da) are added together with the remaining 18 unlabeled amino acids to the medium producing a product with near complete (>99%) incorporation of heavy isotopes.

Purification of the SIL proteins is performed through immobilized metal affinity chromatography (IMAC with Co2+). After purification, purity is evaluated by SDS-PAGE and to determine the molecular weight an intact protein analysis on LC-ESI-QTOF is run.

Results/Conclusion:
As the SIL proteins share a common N-terminal tag sequence this can be used for absolute quantification of the standards prior experimental deployment, assuring quantitative precision across experiments. Known amounts of SIL proteins can thereafter be spiked into biological samples as standards for determination of the endogenous levels of any target protein. As the standards are protein fragments rather than peptides it additionally allows for quantification beyond trypsin-based proteomics. Further, the SIL proteins have proven to be stable for up to one month in a vacuum dried format, allowing for streamlined preparation.

PP03.059: Matrix-Matched Calibration Curves Provide Verification of Quantitative Data-Independent Acquisition Techniques for Deep Plasma Proteomics

Just S[1], Nichols D[1], Mohtashemi I[1], Wang J[1], Ferdosi S[1], Hornburg D[1]
[1]Seer

Introduction

Deep unbiased proteomics of biofluids by DIA-MS offers significant opportunities for protein biomarker discovery, but analysis techniques vary considerably in their consistency, accuracy, and precision. Verification of the quantitative performance of acquisition and analysis techniques is essential to understanding their suitability, and may provide opportunities to increase the statistical power of differential analysis and the performance of disease classification models.

Methods

Two batches of pooled human plasma were mixed with Bovine plasma at 7 ratios and processed by the Proteograph platform using 5 distinct nanoparticles. Each biosample/NP/ratio combination was analyzed in quadruplicate by DIA-MS on a Thermo Exploris 480 employing a 30 min LC gradient. We additionally analyzed samples prepared without nanoparticle enrichment using the same technique.

All results were processed with EncyclopeDIA and DIA-NN as a single search, using a sample-specific DDA library and employing a 1% FDR threshold at the peptide or PSM level respectively. Figures of merit for each biosample/peptide/nanoparticle pair were computed by the matrix-matched calibration curve approach of Pino et al. (2020).

Results

DIA-NN and EncyclopeDIA identified 4,192/3,832 unique peptides exclusive to the human proteome (results are presented as x/y where x refers to DIA-NN and y refers to EncyclopeDIA). Of these, in one biosample, 3,646/3,448 had an LoD (meaning they were detected above noise levels) in at least one nanoparticle fraction. The proportion of peptides with an LoQ (a concentration above the LoD with bootstrap-estimated CV <20%) differed significantly, with 1,983/2,872 having an LoQ in at least one fraction. The other biosample showed a similar pattern (3,659/3,411 LoD, 2,335/2,792 LoQ).

Conclusion

Matrix-matched calibration curves provide robust quantitative validation for deep, unbiased, and scalable DIA-MS proteomics. We further demonstrate that the choice of analysis technique/tool can have a significant effect on quantitative performance.

PP03.060: Development of a Spongy-like Polymer for Subclassification of Extracellular Vesicles and Enzyme Reaction

Kanao E[1,2], Tanaka S[3], Kubo T[4], Otsuka K[4], Akiyoshi K[4], Adachi J[1,2], Ishihama Y[1,2]
[1]Graduate School of Pharmaceutical Sciences, Kyoto University, [2]National Institutes of Biomedical Innovation, Health and Nutrition, [3]Faculty of Pharmaceutical Sciences, Kyoto University, [4]Graduate School of Engineering, Kyoto University

Extracellular vesicles (EVs) are lipid-bound vesicles secreted by cells into the extracellular space. EVs represent a unique research opportunity because they are found in nearly all biological fluids, including blood, saliva, urine, semen, sputum, breast milk, and cerebrospinal fluid. Recent research into the characteristics and mechanisms involving exosomes has introduced the potential development of biomarkers for health monitoring and diagnosis of several human diseases, including cancer, neurodegenerative disease, and diseases of the kidney, liver, and placenta. EVs collected with current methods are heterogeneous in size and composition of constituent molecules, and these heterogeneities lead to uncertainties in EV research. Therefore, novel subclassification methods based on their chemical properties are required to evaluate the behavior of individual EVs.
In this study, we focused on the membrane surface glycan structure of EVs, which possibly defines the chemical properties of EVs, and develop a novel separation method of biological nanoparticles based on their surface glycan structure. lectin affinity chromatography (LAC) is a promising glycan separation method using natural glycan-recognizing proteins as stationary phases. Although agarose and/or silica particles are well known as valuable materials for lectin immobilization, their narrow interparticle volumes could promote the clogging of nanoparticles. To achieve an effective EV separation, we developed a spongy-like monolith (SPM), which consists of poly(ethylene-co-glycidyl methacrylate) (PEGM), as a separation medium for lectin affinity chromatography. Two distinct lectins with different specificity, Sambucus sieboldiana agglutinin, and concanavalin A, were effectively immobilized on SPM with remaining binding activity. Moreover, the large flow-through pores (>10 μm) of the SPM allowed high recovery rates of liposomal nanoparticles as a model of EVs. Finally, we employed lectin-immobilized SPMs for the subclassification of EVs based on their surface glycan structures and demonstrated their different subpopulations by proteome profiling.

PP03.061: Defining Tau Splicing Isoforms and Phosphoforms at the Single-Molecule Level

Tan S[1], Joly J[1], Park D[1], Nortman B[1], Robinson J[1], Guha S[1], Stawicki C[1], Egertson J[1], Boyce H[1], Gillies T[1], Inman C[1], Rouge L[2], Arnott D[2], Lipka J[2], Pandya N[2], Wiederhold T[3], Kirkpatrick D[4], Rohou A[2], Kapp G[1], Mallick P[1]
[1]Nautilus Biotechnology, [2]Genentech, [3]Cell Signaling Technology, [4]Interline Therapeutics

Introduction: Post-translational modifications (PTMs) contribute to the vast diversity of proteoforms that drive biological processes. Currently, very little is known about the molecular heterogeneity of proteoforms. This gap can be largely attributed to the lack of easy to use, high throughput methods to analyze PTMs on intact, single protein molecules at proteome-scale. Tau, for example, has a wide diversity of proteoforms and aberrant PTMs on Tau are known to lead to Tau misfolding and aggregation, which is associated with neurodegenerative disorders collectively referred to as Tauopathies. However, little is known about the precise proteoforms that contribute to disease.

Method: Here, we introduce an antibody-based single-molecule analysis platform that includes novel biochemistry for single-molecule immobilization, instrumentation for highly sensitive detection, and computation for data interpretation. In this study, we use this platform to investigate Tau proteoforms.

Results: We validate the platform by measuring defined mixtures of recombinant Tau proteins, both splicing variants and phosphorylated forms. Next, we use the platform to examine Tau protein enriched from human induced pluripotent stem cell-derived neurons and Tau-expressing cell lines. As PTMs can be assigned to individual protein molecules, the platform is able to reveal the molecular heterogeneity of Tau proteoforms. This depth of information can provide new insights missed by bulk measurements and peptide-centric proteomics.

Conclusions: Ultimately, quantitative analysis of proteoform molecular heterogeneity will lead to a more detailed view of the Tau proteoform landscape. This improved understanding of Tau proteoforms will be important for detecting, analyzing, and treating Tauopathies such as Alzheimer's disease.

PP03.062: Ion Pre-Accumulation for High Speed Orbitrap Exploris Operation

Kellmann M[1], Arrey T, Harder A, Stewart H
[1]Thermo Fisher Scientific Bremen

Introduction

Orbitrap instrumentations have played a tremendous role in advancing MS driven scientific research. However, Orbitrap instruments have been hitherto limited to maximum acquisition rates <50Hz, primarily due to time constraints imposed by the operation of the C-Trap and its conjoined Ion Routing Multipole (IRM) that prepare and inject ions into the analyser. An alteration has been implemented whereby ions are first pre-accumulated and stored ahead of the C-Trap, in the Bent Flatapole, before being transferred to the C-Trap/IRM upon its availability.

Methods

Sensitivity comparisons were made between standard and pre-accumulation mode via measurements of infused Pierce Flexmix. For high-through-put performance evaluation, HeLa digest was used.

Results

At the normal maximum Orbitrap Exploris 480 operation rate of 40Hz, the Flexmix signal intensity in the orbitrap was observed to double upon implementation of pre-accumulation, matching the understanding that the maximum inject time for this 20ms cycle time is 10ms, and duty cycle is thus normally 50%.

To reach >70Hz operation, the orbitrap transient was lowered from 16 to 8ms, corresponding to a resolution of 3750, and the maximum in-series fill time dropped to 3ms. 80Hz operation was recorded by cutting the allowed ion transfer time of the IRM into the C-Trap. It is thought that potential exists for substantial further optimization. The improvement in duty cycle / sensitivity granted by pre-accumulation was observed to explode at these higher repetition rates, to >4x greater at 70Hz.

The first results show and improvement of more than 20 percent in the number of peptides and proteins identified and quantified.

Conclusions

Standard 22 and 40Hz HeLa analyses with substantial sample load show modest improvements when pre-accumulation is used on a modified Orbitrap Exploris 480 instrument.

A new 70Hz acquisition method shows considerable analytical gains for high throughput HeLa experiments on a modified Orbitrap Exploris 480.

PP03.063: High Resolution DIA: A Workflow for Highly Accurate Relative Label-free Quantification of Microbial Proteins in Complex Cell LYSATES

Kraegenbring J[1], Arrey T[1], Op de Beeck J[2], Van Landuyt N[2], Harder A[1]
[1]Thermo Fisher Scientific, [2]Thermo Fisher Scientific

Introduction

Relative quantification of proteins in complex samples raises a demand for high sensitivity and reproducibility throughout large sample sets to gain meaningful insights into biological processes. Data-independent analysis (DIA) has emerged as a powerful technique enabling quantification of thousands of proteins because it avoids the intensity bias and missing value problem that typically limit data-dependent methods. DIA in principle interrogates all peptides that are present in a sample and therefore is especially suitable for high-throughput and large-cohort studies.

Methods

Microbial proteomes were spiked into a human proteome background at different ratios, yielding two-proteome and three-proteome mixtures with varying total protein amounts. Samples were separated on a 50 cm µPAC™ Neo HPLC column in direct injection setup on a Vanquish™ Neo system under nano-flow conditions. DIA experiments were run on an Orbitrap Exploris™ 240 mass spectrometer. Data was analyzed by Spectronaut™ 16 using a library-free approach.

Results

Using micropillar array-based column technology under nano-flow conditions for separation of peptides gives optimal peak shapes and intensities reproducibly over a long-term acquired data set with performance loss of less than 5 %. In connection with the high resolution DIA methodology, this enables for wide proteome coverage in two- and three-proteome mixtures (6200 proteins for 800 ng, and 7100 proteins for 500 ng total protein load, respectively), as well as quantification accuracy for all interrogated ratios below 10 % at high sample throughput using 30 min gradients, without the need for employing match-between-runs strategy. The uncomplicated and easily implemented library-free data analysis yields similar performance as library-based approaches.

Conclusions

Micropillar array-based separation technology and high-resolution data-independent analysis enable for a wide proteome coverage at high throughput, while maintaining excellent quantification accuracy of relative protein ratios in complex cell lysates.

PP03.064: Fast Duty Cycle Differential Mobility Separation (DMS-TOF) to Increase Selectivity in SWATH Analysis.

Le Blanc Y[1], Chernushevich I[1], Bloomfield N[1]
[1]Sciex

Introduction

Differential mobility (DMS), lime other mobility based separation technique, offers the ability to separate peptides in the gas phase prior to MS analysis. The ability to discriminate peptides based on their charge states can lead to lower risk of interference in MSMS data analysis. Here we propose to rely on differential mobility to improve the selective detection of peptide from larger SWATH windows.

Method

Cell digest from K562 was obtained from Promega. LC was performed with an Evosep One system using 60, 100, 200 and 300 samples/day (spd) workflow with the Performance column. Samepls were loaded on Evotips using the manufacturers protocol. MS analysis was performed TripleTOF 6600 system with a SelexION device for DMS separation. Ionization was performed with the OptiFlow ion source using the microflow electrodes.

Preliminary results

To ensure fast and efficient transfer of DMS selected ion at specific SV-CoV, the Q0 region of the TripleTOF 6600 analyzer was modified with tapered electrodes to provide a linear accelerator field. With this addition, the settling time between experiments can be reduced from 35 to 3ms, thus providing a 50% improvement in duty. This is particularly beneficial when combined with SWATH analysis, as precursor window may require detection at several CoV values. For tryptic peptides, transmission through the DMS cell will occur over a CoV range of 30 V, with an average width of 2-2.5 V. Therefore, to maximize coverage, at least 10 to 12 CoV steps are required for each SWATH. By optimizing the CoV range for SWATH windows of 50amu, to detect up to 70% more unique peptides and 50% more proteins at 200spd, when compared to SWATH without DMS. Details on the optimization approach and results over the various workflow will be presented.

PP03.065: Antioxidant Enzyme Expression in Carboplatin Chemoresistant Ovarian Carcinomas: A Proteomic Perspective.

Flores Palma E[1], Herrera Quiterio G[1,2], González Tolentino D[1], Torres Rojas F[1], Rodríguez Ruíz H[1], Encarnación-Guevara S[2], Hernández Ortíz M[2], Saavedra Herrera M[3], Jiménez López M[3], Illades Aguiar B[1], Leyva Hernández M[1], Garibay Cerdenares O[1,4]

[1]Lab. Biomedicina Molecular, Facultad de Ciencias Químico Biológicas, [2]Laboratorio de Proteómica, Centro de Ciencias Genómicas-UNAM, [3]Instituto Estatal de Cancerología "Dr. Arturo Beltrán Ortega", [4]CONACyT-Universidad Autónoma de Guerrero

Introduction: Ovarian cancer (OC) is the sixth most frequent tumor and malignancy after breast cancer in women over 40, with 313,959 new cases and 207,252 deaths worldwide (GLOBOCAN, 2020). Treatment of ovarian cancer begins with the simultaneous administration of platinum (cisplatin or carboplatin) and taxanes (paclitaxel or docetaxel), depending on the stage and type of cancer (American Cancer Society, 2018). However, 70% of ovarian cancer patients have recurrence associated with carboplatin, which generates large amounts of oxidative stress, so that the chemoresistance could be related to increased expression of antioxidant enzymes. Methods: Based on a label-free proteomic analysis (HPLC-MS) in nine carboplatin-resistant ovarian carcinoma tissues and four cancer-free tissues, Perseus and MaxQuant software were used. Validation analysis was performed for antioxidant enzyme expression by qPCR. Results: The proteomic analysis detected changes in 756 proteins. Interaction analysis allowed the selection of proteins with antioxidant activity (PRDX1, SOD1, GSTO1, and QSOX1) at the cytoplasmic level, where carboplatin has cytotoxic activities. PRDX1 and SOD1 were overexpressed, GSTO1 and QSOX1 were underexpressed, and the validation analysis showed PRDX1 overexpression in cancer compared with cancer-free ovarian tissues. Conclusions: Proteomic analysis performed from biopsies of patients with carboplatin chemoresistant ovarian carcinomas determined the differential expression of enzymes with antioxidant activity (PRDX1, SOD1, GSTO1, and QSOX1); the expression analysis showed a trend for PRDX1 towards overexpression in ovarian carcinomas, although not significant.

PP03.066: A Streamlined Tandem Tip-based Workflow for Sensitive Nanoscale Phosphoproteomics

Tsai C[1], Wang Y[1], Hsu C[2], Kitata R[1], Chu R[1], Velickovic M[3], Zhao R[1], Williams S[3], Chrisler W[1], Jorgensen M[4], Moore R[1], Zhu Y[3], Rodland K[1], Smith R[1], Wasserfall C[4], Shi T[1], Liu T[1]
[1]Biological Sciences Division, Pacific Northwest National Laboratory, [2]Institute of Plant and Microbial Biology, Academia Sinica, [3]Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory, [4]Department of Pathology, Immunology, and Laboratory Medicine, Diabetes Institute, College of Medicine, University of Florida

Introduction

The ability to comprehensively characterize post-translational modifications (PTMs) at a nanoscale or single-cell level is critical for better understanding of biological variability at functional levels. Effective nanoscale phosphoproteomics analysis remains a daunting task, however, primarily due to significant sample loss associated with non-specific surface adsorption during the enrichment of phosphopeptides. We developed a novel tandem tip phosphoproteomics sample preparation method that is capable of sample cleanup and enrichment without additional sample transfer, and its integration with our recently developed SOP (Surfactant-assisted One-Pot sample preparation) and iBASIL (improved Boosting to Amplify Signal with Isobaric Labeling) approaches provides a streamlined workflow enabling sensitive, high-throughput nanoscale phosphoproteome measurements.

Methods

The FACS-sorted cells or tissue voxels were first processed by SOP, followed by isobaric labeling and mixing with a much larger amount of boosting sample (e.g., 1 μg) labeled in a separate channel. After enrichment using the home-made tandem tip (C18-IMAC-C18), the samples were analyzed by LC-MS/MS applying the iBASIL settings (higher AGC target and ion injection time).

Results

This approach significantly reduces both sample loss and processing time, allowing the identification of >3,000 (>9,500) phosphopeptides from 1 (10) μg of cell lysate using the label-free method without a spectral library. It enabled precise quantification of ~600 phosphopeptides from 100 FACS-sorted MCF10A cells and ~700 phosphopeptides from 200 μm × 200 μm × 10 μm human spleen tissue voxels (~100 cells) in a high-throughput manner. It also recapitulated the dynamic changes in phosphorylation in the small-sized samples (e.g., before and after EGF treatment of the MCF10A cells), demonstrating its potential for broader applications in biological and biomedical research.

Conclusions

This streamlined workflow opens new avenues for nanoscale phosphoproteome profiling of small numbers of cells and high-resolution spatial phosphoproteome mapping of tissues, which cannot be accessed by current proteomics platforms.

PP03.067: A Toolkit for Characterization of Membrane Proteins : Rapid LC-native MS, Direct Mass Technology Mode and Electron Capture Dissociation

Liu W[1], Shaw J[2], Liko I[3], Yen J[4], Urner L[5], Vasil'ev Y[2,6], Ewbank M[7], Marty M[7], Viner R[1]
[1]Thermo Fisher Scientific, [2]e-MSion, [3]Omass Therapeutics, [4]Academia Sinica, [5]Freie Universität Berlin, [6]Linus Pauling Institute, [7]University of Arizona

Introduction:

Membrane proteins have become popular drug targets due to their diverse biological functions. Native MS characterization of G protein coupled receptors (GPCRs) is especially challenging ascribed to their instability and removal of micelles in the MS. Here, we use Aquaporin Z (AqpZ) as a standard to develop (1) a rapid online buffer exchange-MS (OBE-MS) screening method; (2) electron capture dissociation (ECD) to sequence and localize PTMs; (3) Direct Mass Technology mode analysis to elucidate proteoforms. Application of these methods to GPCRs demonstrates the potential of this toolkit for comprehensive membrane protein characterization.

Methods:

AqpZ (~99 kDa, tetramers) was provided by Michael Marty's lab. GPCRs were provided by Omass Therapeutics. OBE-MS and Direct Mass Technology mode were performed on Thermo Scientific™ Q Exactive™ UHMR equipped with an ExD cell (e-MSion, Inc.). Data were analyzed using Thermo Scientific™ BioPharma Finder™ 4.1 Software and STORIboard (Proteinaceous).

Results:

Initial OBE-MS of AqpZ at 3-min per run yields a main charge envelope deconvoluted to be 98.9kDa matching the target mass. Removal of detergent from the mobile phase reveals two soluble protein impurities. OBE-MS has been successfully applied to other transporters as well as GPCRs. Direct Mass Technology mode analysis reveals four proteoforms of AqpZ with a mass shift 28Da between each and more impurities other than the two detected in OBE-MS. Native top-down analyses using ECD supplemented with HCD of ejected monomer gives >60% sequence coverage and identified formylation site (+28 Da mass shift). By applying similar approaches to GPCRs, Direct Mass Technology mode could isotopically resolve target proteins and further uncover more low abundant species. EChcD fragments corresponding to internal fragments reflect the structure of GPCR.

Conclusions:

The results demonstrate amenability of OBE-MS for rapid membrane protein screening, ultra-high-sensitivity of Direct Mass Technology mode, and the utility of ECD for proteoform characterization.

PP03.068: PARP1 Proteoform Heterogeneity is Associated with Response to PARP1 Inhibition

Ding J[1], Park D[2], Chan W[1], Kapp G[2], Joly J[2], Wang R[2], Mallick P[2], Akbani R[1], Weinstein J[1], Lorenzi P[1]
[1]The University of Texas MD Anderson Cancer Center, [2]Nautilus Biotechnology

Introduction: Poly(ADP-ribose) polymerase 1 (PARP1) modulates DNA repair and genome stability and is associated with a number of cell death pathways (e.g., PARP1 cleavage is a biomarker of apoptosis pathway activation). PARP1 inhibitors (PARPi) have also been approved for the treatment of cancer, but the mechanisms of sensitivity and resistance remain understudied. Here we aimed to elucidate novel associations between PARP1 proteoforms and the anticancer activity of PARP1i in vitro.

Methods: We analyzed public data on the NCI-60 cell line panel for PARP1 expression and response to PARPi. We then profiled PARP1 heterogeneity at the single-molecule level in PARPi-sensitive MCF7 cells and PARPi-resistant OVCAR-8/ADR cells. Each cell type was treated with vehicle (negative control), BMN-673 (PARP1 inhibitor), methylnitronitrosoguanidine (MNNG; positive control for the induction of parthanatos), or staurosporine (positive control for induction of PARP1 cleavage). Parallel sets of samples were generated in cells treated with PARP1 siRNA or siNeg control. We used those samples to screen a collection of affinity reagents targeting PARP1 and its post-translational modifications.

Results: The NCI-60 cell line panel exhibited a range of PARP1 expression and response to PARPi. Focusing on the PARPi-sensitive MCF7 cell line and PARPi-resistant OVCAR-8/ADR cell line with matched PARP1 siRNA knockdown controls confirmed the specificity or lack thereof for the antibodies tested. Notably, BMN-673 treatment of the PARPi-sensitive MCF7 cell line resulted in no change in PARP1 levels, whereas BMN-673 treatment of the PARPi-resistant OVCAR-8/ADR line resulted in significantly decreased PARP1 levels. Further studies are underway to probe the association between PARP1 expression and response to PARPi treatment and to elucidate PARP1 proteoform expression at the single-molecule level.

Conclusions: The landscape of PARP1 proteoforms is largely unexplored, but specific PARP1 proteoforms or patterns of modification may serve as valuable biomarkers of disease progression or response to therapy with PARP1 inhibitors.

PP03.069: High-density and Scalable Protein Arrays for Single-molecule Proteomic Studies

Aksel T[1], Qian H[1], Hao P[1], Indermuhle P[1], Inman C[1], Paul S[1], Chen K[1], Seghers R[1], Robinson J[1], Garate M[1], Nortman B[1], Tan J[1], Hendricks S[1], Sankar S[1], Mallick P[1]
[1]Nautilus Biotechnology

Introduction: We have created a novel, scalable system that allows for single-molecule protein interrogation across a wide dynamic range. This system has two components: a mono-dispersed DNA origami tile with a single protein attachment site, and a nanoscale patterned surface with millions of DNA attachment sites. Protein-conjugated DNA origami tiles deposit with super-Poisson density to the nanoscale patterned binding sites, self-assembling into single-protein-molecule arrays.

Methods: Using high efficiency click chemistry, lysine residues within a protein sample are modified with a crosslinker and conjugated to a DNA origami structure containing a single reactive moiety. These origami structures are deposited onto a nanoscale patterned array created using standard nano-lithography methods. The nanoarray is comprised of DNA binding sites surrounded by hydrophobic regions of hexamethyldisilazane. To demonstrate single molecule occupancy of the nanoscale patterned array, the origami structures are labeled with one of two different dyes, mixed 1:1, and loaded onto the array for imaging. By counting the features with only one wavelength (single loading) and both wavelengths (multiple loading), the single molecule occupancy of the array can be calculated.

Results: Deploying a user friendly process for loading the origami tiles on the features of the nanoscale array, we demonstrated super-Poisson loading. In one version of this system, where we patterned approximately 200M features on a 25 mm x 25 mm array, 98% of the features of the array were loaded, with fewer than 5% showing co-localization.

Conclusions: Using a combination of DNA origami, click chemistry, and scalable, standard nano-lithography techniques we were able to create high-density single-protein arrays. These arrays have a wide range of potential uses, including iteratively probing with a variety of affinity reagents to identify and quantify the composition of complex protein mixtures.

PP03.070: High Performance 96-well Tissue and Cell Homogenization on the BeatBox Platform Improves Protein Identification, Efficiency and Reproducibility.

Mechtler K[1], Moritz C[1], Mang B[1], Hartinger K[1], Johansson S[1], Johansson J[1], Kulak N[1], Demianova Z[1]
[1]PreOmics GmbH

Introduction

The quality of the proteomic analyses is strongly affected by the quality of the sample preparation. A critical step is sample homogenization, which, if poorly performed, does not provide access to all the components of cells/tissues. Different homogenization techniques exist but have various drawbacks and are generally low throughput. The BeatBox is a new technology designed to achieve high performance lysis of up to 96 samples in 10 minutes. The instrument has also been tested in combination with the iST sample preparation kit of varying tissue and cell types for LC-MS/MS analysis.

Methods

The BeatBox technology revealed the highest protein yields against two competitors after homogenization of mouse brain, jaw, liver and quadricep tissues. In order to evaluate the gain of identified proteins and reproducibility results, BeatBox was combined with an iST kit, in addition to pre and post-fractionation homogenate analysis. Finally, the efficiency of the BeatBox-iST workflow was further evaluated using two cell counts for the following cells: HEK293, E. coli and S. cerevisiae.

Results

On tissues, BeatBox technology improved protein identification by an average of 6%, with an excellent average CV of 12.5%. With the addition of the fractionation add-on to the BeatBox-iST workflow, protein identifications increased by an average of 40%. Cell homogenates had similar protein yields and the number of identified proteins, when comparing the BeatBox and sonication methods.

Conclusions

This study highlights the versatility of the BeatBox in terms of the types of samples that can be used, from the softest to the most rigid tissues to various cell types. Additionally, the BeatBox does not generate heat that can degrade proteins and reduces the risk of contamination between samples. Overall, this easy-to-use technology can process large cohorts in just 10 minutes with high efficiency and reproducibility.

PP03.071: Evaluating the Reproducibility of Bottom-up Proteome Sample Preparation in the ProTrap XG through Quantitative Mass Spectrometry

Nickerson J[1], Doucette A[1]
[1]Dalhousie University

Introduction

Mass spectrometry-based proteomics relies on complex front-end workflows, which offer ample opportunity to introduce biases in recovery, digestion efficiency, selectivity across separations, and finally detection. Our group has recently developed a two-stage spin cartridge (the ProTrap XG) which facilitates a precipitation-based workflow. We have optimized the approach for high recovery, SDS depletion and throughput. However, even with quantitative recovery, the reproducibility from bottom-up characterization of complex biological samples remains in question. The present study aims to evaluate the precision offered by the ProTrap XG, benchmarked against conventional in-solution and in-gel digestion approaches.

Methods

Yeast was cultured and harvested under standard conditions, lysed by grinding under liquid nitrogen and extracted using either water or 5% SDS. SDS-containing samples were subject to either a conventional in-gel digest or a ProTrap XG-based workflow whereby they were reduced and alkylated with DTT and IAA, precipitated with acetone, re-solubilized in 8 M urea, and digested overnight at pH 8 with 50:1 trypsin at 37 ^{o}C. Aqueous extracts were reduced, alkylated and subject to a solution digest with 50:1 trypsin. Resulting digests were all subject to reversed phase clean-up with quantitation at A_{214}. Equal mass LC-MS/MS injections were performed in DIA mode with label-free quantitation. MS/MS spectra were searched and resulting proteome profiles were evaluated on qualitative and quantitative agreement across replicate preparations compared to replicate LC-MS/MS injections.

Results

The ProTrap XG workflows provided the greatest sample coverage, agreement in peptide and protein identifications, and peptide quantitation across preparative replicates. Specifically, peptide and protein overlap were 35% and 12% greater than that of the solution digests, respectively, while peptide quantitation was 40% more reproducible than solution digests and 10% more reproducible than in-gel preparations.

Conclusions

ProTrap XG workflows optimize qualitative and quantitative reproducibility, enabling high throughput characterization of complex samples.

PP03.072: Deep Profiling of Proteome Structural Changes with Enzymatic Reactions

Ogata K[1], Maeda A[1], Muramatsu Y[1], Sugiyama N[1], Ishihama Y[1,2]
[1]Graduate School of Pharmaceutical Sciences, Kyoto University, [2]Laboratory of Clinical and Analytical Chemistry, National Institute of Biomedical Innovation, Health and Nutrition

Introduction

The recent development of limited labeling methods with enzymatic- or chemo- reaction enabled proteome-wide analysis of protein structural changes using mass spectrometry. However, it is difficult to comprehensively identify the labeled peptides in the presence of unlabeled peptides, leading to the lower coverage of structural changes. To increase the sensitivity and the coverage, we investigated two methods: a labeling method that enables easy enrichment of labeled peptides and an analysis method using quantification of unlabeled peptides.

Methods

Heat-denatured albumin or myoglobin was incubated for 15 minutes at 37 ºC with recombinant tyrosine kinase SRC. The resulting phosphopeptides were purified by the TiO₂-based phosphopeptide enrichment protocol.
HeLa cell lysates were incubated for 15 minutes at 37 ºC with Trypsin. Partially digested samples were reduced, alkylated, and fractionated with reversed-phase HPLC. Each fraction was subjected to complete digestion by trypsin. All of the samples were analyzed by nanoLC/MS/MS.

Results

We first investigated protein structural analysis with in vitro kinase reaction. Phosphopeptides were easily purified by the well-established enrichment protocol. We found the reaction efficiencies reflect the denaturation state of the substrates.
On the other hand, we developed a new analytical workflow utilizing fractionation profile of protein fragments to detect protein conformational changes. This method enables profiling of proteome-scale structural changes without the identification of cleavage sites by limited proteolysis. In other words, it enables inferring the structural change in the regions with no detectable tryptic peptides. We applied this protocol to HeLa cell lysate with or without ATP. 255 of the 1241 proteins with fractionation profile changes were known ATP-binding proteins. It was the most strongly enriched term in DAVID functional enrichment analysis (GOMF, p-value < 10⁻²⁰).

Conclusions

We developed novel strategies for high-sensitive structural proteomics, and they will be applicable to deep profiling of the dynamics of the structural proteome.

PP03.074: The use of Linear Ion Traps in Data-independent Acquisition Methods Benefits Low-input Proteomics

Borràs E[1,2], Pastor O[1,2], Sabidó E[1,2]
[1]Center for Genomic Regulation, [2]Universitat Pompeu Fabra

Introduction

The need for a better understanding of cellular heterogeneity has pushed mass spectrometry technologies to the analysis of single-cell and single-cell-type proteome. Among the efforts towards single-cell and low-input analyses, there is the adoption of data-independent acquisition (DIA) methods to increase analytical sensitivity. Here we revisited the use of linear ion traps in DIA methods and demonstrate their benefits to improve peptide identifications in low input proteomes.

Methods

Serial dilutions of HeLa protein digest were analysed using different DIA methods with MS2 spectra acquired either in an Orbitrap (DIA-OT) or in a linear ion trap (DIA-IT, Orbitrap Fusion Lumos, EASY-nLC 1200). Acquired mass spectrometry data ranged from 500 to 900 m/z in 40 windows of 10 Da, and they were analyzed with Spectronaut v14.7 using the directDIA approach.

Results

Our results showed that linear ion trap DIA methods (DIA-IT) outcompeted Orbitrap-based DIA methods (DIA-OT) on the number of precursors identified when analyzing low amounts of sample (<10 ng). Within the DIA-IT methods, those using slower scanning rates and larger MS2 injection times resulted in higher number of peptide identifications. As expected, the set of identified peptide sequences mostly overlap between DIA-IT and DIA-OT methods, while DIA-IT shows better coefficients of variation for peptide quantification. Finally, the reduced cycle times exhibited by the DIA-IT methods increased the number sampling points per chromatographic peak and enabled the use of a large number of narrow isolation windows (100 x 4 Da) to improve the method specificity, minimize fragment ion interferences, and facilitate peptide identification.

Conclusions

Overall our results demonstrate the benefits of using linear ion trap-based DIA methods to improve the number of identified peptides in low-input proteomes. Their higher sampling rates makes them compatible not only with low-input applications, but also with ion mobility and ultra-fast chromatography separations.

PP03.075: “Structural Surfaceomics”: A Proteomics Strategy for Identification of Conformation-based Cancer Immunotherapy Targets

Mandal K[1], Yu C[2], Zalevsky A[3], Biswas D[4], Hoopmann M[5], Verma A[4], Barpanda A[4], Ihling C[6], Sinz A[6], Moritz R[5], Sali A[3], Srivastava S[4], Huang L[2], Wiita A[1]
[1]Department of Laboratory Medicine, University of California, San Francisco, CA, USA, [2]Department of Physiology & Biophysics, University of California, Irvine, CA, USA , [3]Department of Bioengineering and Therapeutic Sciences, University of California, San Francisco, CA, USA, [4]Department of Biosciences and Bioengineering, IIT Bombay, India, [5]Institute for Systems Biology, Seattle, WA, USA , [6]Department of Pharmaceutical Chemistry & Bioanalytics, Institute of Pharmacy, Martin-Luther University Halle-Wittenberg, Halle, Germany

Introduction:
Immunotherapy has brought immense excitement for cancer care. However, its clinical reach is still limited because of dearth of cancer specific markers. Intrigued by the serendipitous discovery of active integrin-β7 as a conformation specific immunotherapy target of multiple myeloma, we developed an unbiased proteomics technology platform called “structural surfaceomics” for mining more of such targets, which would otherwise be invisible to RNA-Seq or conventional quantitative proteomics.

Methods:
Cross-linking mass spectrometry (XL-MS) and glycoxidation based cell surface capture (CSC) was used for proteomics profiling of cancer cell surface proteins. MS3 based XL-MS data analysis was performed with Trans-Proteomics Pipeline using an in-house developed software called Ving, while for MS2 data pLink-2 was used. The downstream XL-MS data was processed using in-house Python scripts for structural inferences.

Results:
We developed a proteomics approach called “structural surfaceomics”, integrating XL-MS with CSC to obtain structural constraints in terms of cross-linked peptides, thereby enabling identification of protein conformation-based cancer cell surface antigen. We used two different XL-MS crosslinkers: the mass spec-cleavable DSSO and non-cleavable PhoX, the latter including a phosphonate handle for cross-peptide enrichment. In-house tool Ving was developed to facilitate DSSO based MS3 XL-MS data analysis. In application to Nomo-1 AML cells, these strategies yielded complementary information, in total resulting in 2,390 crosslinks on 250 membrane-localized proteins. These cross-links computationally mapped automatically on to the protein structures from the PDB, AlphaFold, and SwissModel databases to determine the violated crosslinks for a visual assessment of a potential conformational heterogeneity in PyMOL.

Conclusions:
We demonstrated a novel proteomics approach for identification of a new class of cancer antigen, which happens to be protein conformation based, thereby expanding the toolkit of target discovery. Our structural surfaceomics can find its application not only for immunotherapy target discovery but also basic or translational science in other fields.

PP03.076: Protein-Protein Interaction Networks Analysis, a way to Decipher the Alternative Protein's Function in Ovarian Cancer.

Garcia Del Rio D[1,2], Cardon T[1], Eyckerman S[2], Bonnefond A[3], Fournier I[1], Gevaert K[2], Salzet M[1]
[1]Laboratoire Prism - Unité Inserm 1192, [2]VIB-UGent Center for Medical Biotechnology and Department of Biomolecular Medicine, [3]Inserm UMR1283, CNRS UMR8199, European Genomic Institute for Diabetes (EGID), Institut Pasteur de Lille

Introduction: Eukaryotic mRNA has long been considered monocistronic. Nevertheless, alternative proteins (AltProts) challenge this concept. Hence, new DBs such as OpenProt, predict proteins translated from alternative ORFs (5'&3'-UTR, lncRNAs, or in-frameshift). This study aims to identify AltProts in Ovarian Cancer (OvCa) models and to decipher their functions in the pathology.
Methods: Protein extracts from Ovarian cancer cell lines were analyzed to identify the alternative and reference proteome. To identify interactions, a methodology using subcellular protein fractionation and a CID cleavable crosslinker was performed.
Results: OvCa cell lines (PEO-4 & SKOV-3) and an immortalized ovarian cell line were analyzed to identify their RefProts and AltProts. Likewise, the quantitative variation of proteins was also assessed. In total, 5,045 RefProt and 453 AltProt were identified. A significant variation of AltProt (313) and RefProt (2,894) levels between cancer and healthy cells was observed. To set a starting point to identify the function of an AltProt, crosslinking mass spectrometry was the technique of choice. In these cell lines, protein-protein interactions were analyzed using DSSO, without crosslinked peptide enrichment, but using the subcellular fractionation of a restricted number of cells (3E6), whereas one typically uses huge numbers of cells (300E6). Moreover, this fractionation provided the first insights to locate the subcellular localization of AltProts in which the majority were found at the membrane and cytoplasm. Around 20% were found in more than one location, describing an AltProt dynamism inside the cell. Interactions between AltProts and HLA-B, RALA, and ARIH2 were identified, which are important for homeostasis and pathological states.
Conclusion: AltProts are a neglected part of the human proteome which might play a role in ovarian cancer. We described our untargeted approach to identify and characterize AltProts. Our findings suggest interactions between AltProts and RefProts can be potential therapeutic targets that have to be analyzed.

PP03.077: Robust and High-throughput Analytical Flow Proteomics Assessment of a Variety of Biological Matrices with Zeno-SWATH Data Independent Acquisition

Sun W[1], Lin Y[1], Huang Y[1], Terrillon S[1], Kovacina K[1], Rosenbaum A[1], Contrepois K[1]
[1]AstraZeneca

Introduction
Recent technological advancements enable routine generation of high-quality and high-content proteomic data. However, maintaining high levels of analytical performance across numerous samples remains a challenge. In this context, we have developed, optimized and benchmarked robust and high-throughput analytical flow LC-MS methods with Zeno-SWATH DIA technique across a wide variety of biological matrices of interest in toxicological and mechanistic studies as well as for biomarker discovery.

Methods
Methods development, optimization and comparative analyses were performed with 10 cynomolgus monkey tissues, 3 human biofluids and 2 matrices from in vitro cell cultures. In addition, we profiled proteins from healthy and diseased patients in 2 different biofluids. Experiments were performed using an Exion LC coupled with a ZenoTOF 7600 MS (SCIEX). Data were acquired in SWATH DIA mode with and without prototype Zeno-pulsing activated and data processing was performed using DIA-NN (v1.8.1) and in-silico libraries.

Results
We identified optimal LC-MS methods that were slightly different for each biological matrix depending on their protein composition. Methods were then assessed across a range of peptide loads and in healthy vs diseased samples with and without Zeno-pulsing activated. We observed that Zeno-SWATH consistently improved reproducibility and protein coverage (up to 5-fold) and provided more meaningful biological information in comparison to SWATH on the same instrument. Using a 10-min gradient, >1,800 proteins were identified in liver (2 µg load) with a median CV of 4.3%. Method robustness was demonstrated by injection of 250 study samples (equivalent to ~200 µg peptides) resulting in only a 7% decrease in protein IDs and a median CV of 9.4%.

Conclusions
Zeno-SWATH allows fast and robust proteomics workflows using analytical flow and is amenable to large-scale studies. This work provides detailed method performance assessment on a variety of relevant biological matrices and serves as a valuable resource for the proteomics community.

PP03.078: Long-term Stability and Reproducibility of Nano-, Capillary- and Micro-flow LC-MS Separations: the Impact of Hardware and Separation Column

Pynn C[1], Zheng R[1], Arrey T[2], Hakimi A[3], Valenta A[1], Moyer S[1], Boychenko O[1]
[1]Thermo Fisher Scientific, [2]Thermo Fisher Scientific, [3]Thermo Fisher Scientific

Introduction

While nano-, capillary- and micro-flow LC-MS is crucial for high-sensitivity analysis of complex bio-samples, adaptation to translational research requires further proof of suitability for large sample cohorts generated across multiple laboratories. Here, we evaluated the reproducibility of proteomics data collected in three labs over several months of continuous operation and explore the impact of hardware and consumables.

Methods

Low-flow LC-MS and LC-UV data were collected in Germering (Germany), Bremen (Germany), and San Jose (USA) by different operators using identical LC and MS hardware and different batches of columns. The Vanquish Neo UHPLC system and Exploris 240 and 480 mass spectrometers operated in DDA mode were used with 15, 50, and 75 cm long 75 µm ID EASY-Spray PepMap Neo columns or 1 mm ID x 15 cm Acclaim PepMap columns. Data were processed with and without resolving of chimeric spectra and reported with 1% FDR on the peptide and protein levels.

Results

Long-term robustness: nanoLC-MS bottom-up proteomics injections of bovine serum albumin digest were continuously performed for > 6 months across two laboratories. Column backpressure, retention time precision, and peak FWHM showed suitability for long-term, uninterrupted analysis.

System-to-system reproducibility: Inter-system reproducibility was assessed by coupling multiple LC instruments to the same HRAM MS. Peak area, height, and FWHM showed <15% RSD for a peptide retention time calibration mixture. As little as 5% variation in identified peptides and proteins was observed from 200 ng HeLa digest using a 90 min gradient.

Multi-site reproducibility. Inter-laboratory reproducibility was assessed for 5 standardized, high-throughput capillary-flow LC-MS methods for proteome profiling across three laboratories. From 73% to 89% of proteins were commonly identified across all sites, independent of the cycle time.

Conclusions

A comprehensive evaluation of analytical variability in low-flow LC-MS analysis provides guidelines for achieving reproducible results across instruments and laboratories.

PP03.079: S-Trap Turbo: from Samples to Analysis Matching Analytical Time

Wilson S[1], Zougman A[1], Wilson J[1]
[1] Protifi, Llc

The ability to deliver the same results in many laboratories has made the S-Trap sample preparation system, developed in academia, widespread in proteomics analyses. However, recent advances in proteomics detection throughput, in some cases now only minutes per sample, necessitate concomitant advances in bottom-up sample preparation. This necessitates the elimination of any and all removable elements in a sample preparation workflows. One of the more tedious (and often time-variable) steps in proteomics sample processing is post-elution sample dry-down. Here we present the new S-Trap turbo: S-Traps that yield minimal elution volumes of highly concentrated peptides suited for immediate .

New snap-cap S-Trap turbo micro columns were constructed via plastic injection molding. The new columns incorporated newly developed, compressed polymeric traps derivatized with new surface modifications. The standard S-Trap protocol steps of lysis, reduction and alkylation, denaturation, binding, wash and tryptic digestion were performed both for standard and turbo S-Traps. Samples were analyzed by MS. Sample yield was compared and quantified using BCA and/or fluorescent assays. Sample quality was compared by peptide and protein identification rate and reproducibility of quantifications.

Turbo traps were compared to traditional S-Traps using samples of highly varied hydrophobicities. S-Trap turbos were found to be reproducible and similar or better than standard S-Trap digestions as judged by completeness of digestion, peptide yield and identifications at digestion times up from 1 hr at 47 C to overnight at 37 C; numbers of identified peptides and proteins were a strong function of sample type. S-Trap turbo elutions could be immediately loaded onto an autosampler with or without acidification. No significant loss of hydrophobic peptides between the standard S-Trap protocol and S-Trap turbo was noticed. S-Trap turbos allow proteomics researchers to go from samples of all kinds to ready-to-inject peptides in record time with minimal steps and equipment.

PP03.080: Understanding all of Biology: Simultaneous, High-throughput Si-Trap Multiomics Sample Preparation

Zougman A[1], Pappin D[2], Wilson J[1]
[1]ProtiFi, Llc, [2] Cold Spring Harbor Laboratory

Multiple classes of biomolecule effect life through their interactions. In typical sample preparation, all molecule classes except the one under study are removed or destroyed. This is a significant problem because we do not necessarily have a priori knowledge of which kind of biomolecule is diagnostic, prognostic, therapeutic or predictive. The integration of omics fields thus remains a significant challenge, beginning with sample preparation. We present Si-Trap technology, which enables reproducible sample preparation of multiple biomolecule classes with rapid sample processing time, high throughput sample throughput in an affordable manner.

We tested the ability of the Si-Trap method to provide simultaneous multiomics analysis through a comparative proteomics/metabolomics profiling study of cancerous and corresponding noncancerous tissues. Si-Trap sample processing was rapid – only minutes per sample in hands-on time – and could be performed in loose spin columns or in an automated 96-well format. Proteomics and metabolomics results revealed concordant changes in enzymes and substrates implicated in cancer metabolic plasticity. Proteomics comparisons showed comparable performance to S-Trap sample processing. Si-Trap processing performed equally as well for serum, FFPE blocks, cell culture and other samples.

Automation of Si-Trap sample processing and its commensurate high reproducibility could be achieved on an inexpensive Tecan A200 positive pressure workstation, a general automation platform accessible – due to its low cost – to the majority of research and clinical labs. Si-Trap sample processing is a new high-throughput solution to address all necessities for multiomics analyses including in future clinical implementations. We anticipate that the Si-Trap will become an essential omics tool in laboratory and clinical settings and will enable novel discoveries, thereby helping to usher in a new era of clinical proteomics. It provides sultaneous multiomics sample preparation for many samples including those of clinical relevance.

PP03.081: Validating the Quantitative Performance of Peptides by Multiple Proteases

Woessmann J[1,2], Kotol D[1,2], Hober A[1,2], Uhlen M[1,2], Edfors F[1,2]
[1]Department of Protein Science, KTH - Royal Institute of Technology, [2]Science for Life Laboratory

Background: Bottom-up proteomics heavily relies on the protease trypsin for the generation of peptides. A variety of alternative proteases have been introduced to the bottom-up proteomics workflow to, among others, extend proteome coverage or identify post-translational modifications. The protein quantification by multiple proteases in label-free bottom-up experiments has previously been shown to correlate poorly. We hypothesized that this quantitative bias could be overcome by using stable isotope-labeled recombinant protein standards in a targeted proteomics workflow. This approach could provide a novel way to validate the quantitative performance of peptides generated by multiple proteases.

Materials and methods: Recombinant protein standards were used to establish selected reaction monitoring (SRM) assays on a Thermo Scientific TSQ Altis for human plasma protein targets with six different proteases. We validated the identified peptides in a pool of human plasma and quantified plasma protein targets by six different proteases with stable isotope-labeled recombinant protein standards.

Results: We developed an easy-to-follow SRM workflow to establish over 500 SRM assays on ten recombinant protein standards with six different proteases. Nearly complete sequence coverage of the standards could be achieved by the combination of all proteases. We further performed absolute quantification of human plasma proteins by peptides generated by six proteases with concentrations varying from 0.02-70 µM. With a total of 60 peptides covering four proteins, we demonstrated robust absolute quantification of peptides by different proteases. The overall variance between the quantified peptides was strongly reduced in comparison to label-free data.

Conclusion: This study shows that multiple proteases have the potential to deliver a similar robust quantitative performance of proteins with targeted proteomics methods and stable isotope-labeled recombinant protein standards. Therefore, we suggest the use of multiple proteases and recombinant protein standards as a novel approach to validate the quantitative performance of targeted proteomics assays.

PP03.082: Accelerating High Throughput Proteomic Sample Preparation with 3D Design and Printing

Williams S[1]
[1]Children's Medical Research Institute

Introduction: 3D printing has recently become affordable and accessible. The additive manufacturing process enables in-house cheap and rapid development of prototypes and production of tools for custom applications, for instance, those found in research laboratory settings. This is particularly true of ProCan, where our workflow incorporates a non-standardised sample tube. Additionally, the development of data driven precision medicine requires sample counts in the hundreds to thousands, to generate accurate predictive models of disease. Sample sets of such size exist in biobanks and storage facilities, however, preparing tissue in a robust high throughput workflow in a research laboratory environment remains challenging. In proteomics, much of this challenge stems from the time involved in sample and liquid-handling, which has been addressed in part by automated robotics, though is not without its limitations. Here we describe the use of several novel 3D printed tools, designed to aid the high throughput sample preparation protocol we have developed.

Method: Models were designed in Autodesk Fusion360 and printed on a Prusa i3 MK3 using the thermo-plastic polylactic acid. Design considerations included compatibility with commercially available equipment. A set of five tools have been universally integrated into our 'Heat and Beat' protocol.

Results: Our standard sample preparation experiment consists of processing 32 samples simultaneously, for which use of printed tools saves 53 min by reducing the number of manual handling steps. This includes tube transfers, compatibility with multi-channel pipetting and number of homogenisation cycles. Not only does this reduce staff workload but also the risk of repetitive strain injuries.

Conclusions: Utilising 3D printing has reduced cost and accelerated sample preparation time, while also reducing errors and facilitating more consistent processing, consequently lowering variability and subsequent batch effects. We here present the tools and provide open-access files for download and use in other laboratories.

PP03.083: Heat and Beat (HnB): A Near Universal One-pot Rapid Sample Preparation Technique for Fresh Frozen, OCT Embedded and FFPE Tissues

Xavier D[1], Lucas N[1], Williams S[1], Koh J[1], Loudon C[1], Reddel R[1], Hains P[1], Robinson P[1]
[1]Procan, CMRI

Introduction:
Clinical proteomics is constrained by inconsistent tissue fixation, embedding and storage methods. Sample source diversity makes comparisons of biomarker trends between cohorts from different laboratories problematic. Our goal was to develop a single MS sample preparation method for tissues preserved using the 3 most common techniques, Fresh Frozen (FF), OCT embedded (FF-OCT), and formalin-fixed, paraffin-embedded (FFPE).

Methods:
Six rat organs (kidney, muscle, liver, brain, testis and lung) were fixed or stored in each of the three formats. Cut sections benchmarked the HnB method against the previous ABLE-SWATH protocol (Lucas et al 2019). The method was then scaled up to process FF-OCT preserved biobanked patient samples.

Results:
FF and OCT stored tissues achieved up to 95% increases in protein identifications with HnB. Digestion efficiency (DE) was significantly improved to 85-90% for all samples. In particular, the peptide yield from FFPE tissues improved by two to three-fold compared to ABLE-SWATH.
To demonstrate real-world high-throughput capability, HnB was scaled up for 1,171 FF-OCT samples from a cancer biobank covering over 25 human tissue types. This required only 60 hrs (2.5 days) sample preparation time and 32 hr for solid phase extraction clean-up. When run in triplicate in DIA mode, 4,000-6,000 proteins were quantified across most tissue types, with DEs of >80% being consistently achieved. Method reproducibility was demonstrated by 95 batch control samples being independently prepared by 6 lab personnel and run in triplicate. This showed a median correlation of 0.92 across 76,981 peptides.

Conclusions:
HnB is an efficient one-pot MS sample preparation procedure that reduces endogenous protease activity, improves peptide yield by up to 3-fold and delivers a DE of 85-90%. It requires significantly less processing time and thereby enables large scale, high-throughput sample preparation for proteomics with reproducible results.

References:
Lucas,N., et al (2019) J Proteome Res 18, 399-401.

PP03.084: A Novel Intelligent Data Acquisition Hybrid-DIA Mass Spectrometry Strategy: Combining Data-driven and Hypothesis-driven Approaches in one

Xuan Y[1], Huang M[2], Yang X[2], Fort K[1], Canterbury J[3], Martínez-Val A[4], Olsen J[4], Chen Y[5], Ishihama Y[6], Goetze S[7,8], Wollscheid B[7,8], Makarov A[1], Moehring T[1]
[1]Thermo Fisher Scientific (Bremen) GmbH, [2]Thermo Fisher Scientific (China) Co. Ltd, [3]Thermo Fisher Scientific, [4]Novo Nordisk Foundation Center for Protein Research, University of Copenhagen, [5]Institute of Chemistry, Academia Sinica, [6]Graduate School of Pharmaceutical Sciences, Kyoto University, [7]Institute of Translational Medicine (ITM), Dep. of Health Sciences and Technology (D-HEST), ETH Zurich, [8]Swiss Institute of Bioinformatics (SIB)
Introduction: Translational scientists face the dilemma to choose between comprehensive profiling and sensitive targeted quantitation. Though proteomic profiling has a great potential to discover prognostic and predictive biomarkers, it still misses the sensitivity to quantify all the markers of interests. Therefore, targeted quantitation experiments of the potential markers are analyzed in the validation phase. This leads to higher cost, time losses and more sample consumption. To address these challenges, we developed a novel intelligent data acquisition “Hybrid-DIA” MS strategy that combines comprehensive proteome profiling via DIA MS with on-the-fly intelligently switching to PRM acquisition mode for sensitive quantification of the markers, substantially increasing throughput and reducing sample consumption.
Methods: Hybrid-DIA MS strategy consists of a standard DIA scan cycle, where MS scan is followed by DIA scans. Based on the heavy stable isotope labelled (SIL) peptides’ signal in MS scan, PRM scans are triggered, serving as a second layer of confirmation for SIL peptides. Successful SIL peptide detection triggers the measurement of corresponding endogenous peptides multiplexed (msx) with SIL peptides through msxPRM scans acquired with narrower isolation window and maximizing ion injection time for each species.
Results: Hybrid-DIA methods are benchmarked against standard DIA methods by analyzing HeLa cell lysates and plasma digest, respectively. Comparable number of proteins (>6000 from Hela, >500 from plasma) are identified by both methods, while Hybrid-DIA improves the LOD/LOQ for the low abundant biomarkers. We then applied Hybrid-DIA on lung cancer cell lines and melanoma patient tissues, respectively. With high precision and reproducibility, Hybrid-DIA MS can quantify low abundant EGFR and KRAS mutations in lung cancer cell lines, as well as capture clinically actionable markers (PMEL and MAR1) in melanoma tissues, while digitizing their underlying proteotypes.
Conclusions: Hybrid-DIA MS presents a new capability to combine data-driven and hypothesis-driven in one experiment, accelerating biomarker study pipeline.

PP03.085: Omics Batch Correction (OBC): A Streamlined Batch Effect Correction Pipeline Pinpointing Biological Changes in the Large-scale Proteomics and Metabolomics Studies

Zheng H[1], Zhang W[1], Cheng X[1], Xuan Y[2], Zhou Y[1]
[1]Qlife Lab, [2]Thermo Fisher Scientific

Introduction: The technology advancement of mass spectrometry-based proteomics and metabolomics have enabled large-scale studies, where hundreds to thousands of samples are analyzed in batches across multiple instruments and/or across time. Therefore, technical variations and missing values are introduced as batch effect. A few batch correction methods have been proposed either for proteomics or for metabolomics data. However, the changes of biological signals caused by these methods have not been evaluated and discussed. To capture the true biological changes from the large-scale proteomics and metabolomics studies, we established an optimized web-based pipeline “Omics batch correction (OBC)”, which can evaluate and visualize the batch effects and more sensitively and accurately correct them for both proteomics and metabolomics data.

Methods: OBC consist of three main functions parts, 1) raw data quality control (QC) visualization, 2) batch effect correction, and 3) post correction data quality visualization. OBC was applied to three proteomics and metabolomics datasets1,2 to effectively correct the technical variations and accurately capture the biological changes.

Results: We first evaluated all methods on a well-controlled proteome mixture datasets acquired by eleven international labs. Combat correction showed best quantification precision and accuracy among all the methods. Combat correction was then applied on the ovarian cancer patients’ data, demonstrating a clear benefit in reproducibility, correlation, and sensitivity of detecting more differentially expressed proteins. OBC was further applied to a metabolomics dataset. It can remove intra-batch and inter-batch effects, achieve a better QC metrics than hRUV, as well as retain more biological signals.

Conclusions: A new optimized batch effect evaluating and correction pipeline for clinical proteomics and metabolomics data is established and available at http://47.114.121.217:3838/OmicsBatchCorrect/ for free.

PP03.086: Expression of Antioxidant Enzymes (PRDX1, SOD1, GSTO1, and QSOX1) and their Relation with Chemoresistance in Breast Cancer

Flores Palma E[2], González Tolentino D[2], Torres Rojas F[2], Rodríguez Ruíz H[2], Navarro Tito N[3], Cayetano Salazar L[3], Espinoza Rojo M[4], Ibarra Sierra E[5], Jiménez López M[5], Leyva Vázquez M[2], Illades Aguiar B[2], Garibay Cerdenares O[1]

[1]CONACyT-Universidad Autónoma de Guerrero, [2]Lab. Biomedicina Molecular, FCQB-UAGRO, Chilpancingo, [3]Laboratorio de Biología Celular del Cáncer, FCQB-UAGro, Chilpancingo, [4]Laboratorio de Biología Molecular y Genómica, FCQB-UAGRO, Chilpancingo, [5]Instituto Estatal de Cancerología, Dr. Arturo Beltrán Ortega, Acapulco de Juárez

Introduction: Worldwide, breast cancer (BRCA) is one of the most prevalent diagnosticated cancers and is the fifth cause of cancer-related death (Łukasiewicz et al., 2021). One of the first-line treatments for this disease is chemotherapy with carboplatin. However, a significant percentage of patients present chemoresistance, either intrinsic or acquired (Brasseur et al., 2017; Doubeni et al., 2016; Lippert et al., 2008); one of the mechanisms of intrinsic chemoresistance is drug detoxification, where the overexpression of antioxidant enzymes like peroxiredoxins and superoxide dismutases avoid the effect of carboplatin (Ghassan et al., 2017). Alternatives for BRCA treatment are needed. Therefore, this project aims to analyze the expression levels of the antioxidant enzymes PRDX1, SOD1, GSTO1, and QSOX1 at the mRNA level and relate them with chemoresistance. Methods: RNA from tissues and cell lines of breast cancer and non-tumoral were extracted, and qPCR was performed. Results: Increased levels of gene expression from PRDX1, GSTO1 ($p=0.0500$), and QSOX1 ($p=0,0466$) in breast cancer tissues were detected, and the cell line MDA-MB-231 showed overexpression of PRDX1, SOD1, and QSOX1. Conclusion: The expression levels of antioxidant enzymes PRDX1, SOD1, GSTO1, and QSOX1 increase in breast cancer and could be related to carboplatin chemoresistance.

PP03.087: Quantification of Spliceosome Proteins in Neuroblastoma Cells after Drug Treatments using Nuclear Proteome Extraction, SP3 Digestion, and Data-independent Acquisition

Gutierrez A[1], Canzani D, McEllin B, Pino L, Allen C, Broderick E, Federation A
[1]Talus Bioscience

Introduction

Dysregulated RNA splicing is a hallmark of cancer, so spliceosome proteins have become attractive targets for inhibition or elimination. We have developed a protocol for the sensitive and accurate quantification of spliceosome proteins from cells using nuclear isolation and chromatin extraction by salt separation (ChESS). ChESS-fractionated proteins are digested into peptides for data-independent acquisition mass spectrometry (DIA-MS) using an automated SP3 protein digestion on carboxyl beads. Our ChESS-SP3-DIA approach enables label-free quantification and high coverage of spliceosome proteins, which allows us to determine the effects of spliceosome-targeted drug treatments. We demonstrate our approach using the drug Indisulam, which has been proven efficacious in neuroblastoma cells as it degrades RNA Binding Motif Protein 39 (RBM39) - a gene involved in alternative splicing and regulation of transcript levels (Singh).

Methods

Cells were treated with 2uM Indisulam over several time points and fractionated via ChESS. ChESS proteins were digested by SP3 on magnetic carboxyl beads using an Opentrons 2 liquid handler. Resulting peptides were loaded onto Evotips for analysis by DIA on an Evosep One - Thermo Exploris 480 system. Samples were analyzed using EncyclopeDIA and Skyline.

Results

Cell viability was reduced after treatment with 2 uM Indisulam. Gene ontology enrichment validates that ChESS fractions contain high coverage of spliceosome proteins. Quantification of RBM39 demonstrates degradation after Indisulam treatment. Other protein markers of cancer, such as the transcription factor MycN, were monitored to assess systemic changes to the nuclear proteome.

Conclusions

Our ChESS-SP3-DIA approach answers biological questions aiding in drug discovery of spliceosome protein targets, along with other nuclear proteins such as transcription factors that operate in parallel with the spliceosome. This workflow shows that RBM39 was degraded in neuroblastoma cells treated with Indisulam. Using ChESS-SP3-DIA for these analyses allows for unbiased, sensitive, and reproducible coverage of spliceosome and other nuclear proteins.

PP03.088: Understanding Cervicovaginal Cancer Progress though Cervical Mucus: iTRAQ-Based Proteomic Approach

Hernandez-Hernandez J[1], Soto-Fuenzalida G[1], Lopez-Sanchez R[1], Hernández M[2], Contreras-Martinez S[2], Martinez-Ledesma J[1], Encarnación-Guevara S[2], Villela-Martinez L[3,4,5], Ortiz-Lopez R[6]
[1]Tecnologico De Monterrey, Escuela de Medicina y Ciencias de la Salud, [2]Universidad Nacional Autonoma de Mexico, Centro de Ciencias Genomicas , [3] ISSSTESON, Centro Medico Dr. Ignacio Chavez, [4]Universidad Autonoma de Sinaloa, Facultad de Medicina, [5]ISSSTE, Hospital Fernando Ocaranza, [6]Tecnologico de Monterrey, Instituto para la obesidad

Introduction: Cervical cancer continues to be a public health problem in Mexico and worldwide. This disease is the second place of prevalence and mortality among women aged 15-64 years. This study reports the protein present in the cervical-vaginal fluid and changes throughout non-infection to cervical cancer, in order to identify the protein evolution of the disease and that could be investigated as potential biomarkers for early diagnosis.
Methods: For this reason, we use a proteomic analysis approach-using isotope labeling with iTRAQ combined with high-resolution mass spectrometry analysis to describe the disease progression. Four groups of samples derived from patients with NIC1, NIC2, NIC3 and control (without lesion) were analyzed. Eight samples in each group were included, corresponding to two replicates of the four experimental conditions. The quantitative values were standardized based on the average abundance of all proteins in the individual sample.
Results: We identified and reported quantitative data for about 2564 different proteins quantified with high confidence, of which 1731 were common in all groups. Mass spectrometry analysis allowed for highlighting the dysregulation of several proteins associated with different stages in the transition of the disease. Two different protein patterns were identify. Forty-eight proteins were down-expressed instead, 28 were overexpressed in dysplasia groups vs control respectively. The most representative proteins down-expressed were: MLEC, KRT6A, TMED4, CILP, Meanwhile the up-expressed proteins were AGT, IGHD, OSTF1, PRDX5, PRTN3, RETN, RAB32.
Conclusions: As we expected, we did not find a complete signaling pathways possibly due to the type of sample (mucus), however, all proteins have been related with cancer development in other tissues. Additional validation of the results is necessary to establish whether proteins dysregulated are indeed indicative of disease progression and can be used as biomarkers in early screening.

PP03.089: Transmembrane Protein 160 (TMEM160) and its Role in Cancer.

Herrera Quiterio G[1], Hernández M[1], Martinez-batallar A[1], Sánchez-Castellanos N[1], Encarnación-Guevara S[1], Fernández Coto D[3], Garibay Cerdenares O[2], Salazar-Bustamante E[3]
[1]Laboratorio de Proteómica, Centro de Ciencias Genómicas, Universidad Nacional Autónoma de México, [2]Autonomous University of Guerrero, [3]National Autonomous University of Mexico
INTRODUCTION:Functional characterization of transmembrane protein 160 (TMEM160) is part of the Human Chromosome-Centered Proteome Project (C-HPP) and Human Proteome Organization (HUPO)(1). TMEM160 protein is located in the plasma membrane and is expected to be located to the mitochondrial membrane (2). Although its function in cells is unknown, it has been reported that in humans its expression in more than 200 tissues (3). We have observed TMEM160 protein expression in A549 lung adenocarcinoma and HeLa cervical adenocarcinoma cell lines, so we were interested in identifying the role that TMEM160 plays in these neoplasms.
METHODS: A549 and HeLa cell lines are used as a study model, the TMEM160 gene has been silenced by CRISPR/CAS9 in both cell lines. A proteome analysis of the A549 and HeLa wild type (WT) cell lines and A549 and HeLa TEMEM160-knockout (KO) cell lines by liquid chromatography with tandem mass spectrometry (LC-MS/MS) is currently in progress. In addition, a protein-protein interaction analysis using co-immunoprecipitation and LC-MS/MS of TMEM160 using protein extracts from A549 and HeLa cell lines is developing. With the aim of defining the role of TMEM 160 in cell function in cancer.
RESULTS: Higher expression of TMEM160 was observed by western blot in A549 cell line versus BEAS cell line (non-tumor bronchial epithelium) and in HeLa cell line, compared to HaCat (non-tumor keratinocytes). The comparison of the proteome of the cell lines A549 WT and HeLa WT versus A549 KO and HeLa KO cell lines is expected to provide relevant information on the cellular processes in which TMEM160 is involved, this information will be analyzed together with the protein-protein interaction results.
CONCLUSIONS: The results obtained from the analysis of the proteome of A549 and HeLa WT and KO cell lines, complemented with the TMEM160 interactome will allow us to suggest its probably role in cancer.

PP03.090: Histology-resolved Proteomics Identifies Unique Tumor and Stromal Proteomic Profiles Associated with Low- and High-grade Prostate Cancer

Hood B[2,3], Conrads T[1,2], Barakat W[2,3], Makohon-Moore S[2,3], Conrads K[2,3], Bateman N[2,3,4], Mani H[5], Trump D[6], Conrads T[1,2,4]

[1]Women's Health Integrated Research Center, Inova Women's Service Line, Inova Health System, [2]Gynecologic Cancer Center of Excellence and the Women's Health Integrated Research Center, Gynecologic Surgery and Obstetrics, Uniformed Services University and Walter Reed National Military Medical Center, [3]The Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc., [4]The John P. Murtha Cancer Center Research Program, Department of Surgery, Uniformed Services University, [5]Department of Pathology, Inova Fairfax Hospital, [6]Inova Schar Cancer Institute, Inova Health System

Introduction

Prostate cancer (PCa) is one of the most frequently diagnosed cancers in men. Prostate tumor staging and disease aggressiveness are evaluated based on the Gleason scoring system, which is further used to direct clinical intervention. Prostate tumor staging is determined by disease extent and distribution, serum level of prostate specific antigen (PSA), and Grade Group, determined by Gleason Grade which is based on the histologic assessment of the tumor specimen and provides an estimate of tumor aggressiveness. We performed a deep proteomic characterization of laser microdissection (LMD) enriched epithelial and stromal subpopulations from low- and high-grade tissue specimens (Gleason 6 and Gleason 9, respectively) using quantitative high-resolution liquid chromatography-tandem mass spectrometry analysis (LC-MS/MS).

Methods

Surgically resected formalin-fixed paraffin-embedded (FFPE) prostate tumor specimens (n=38) were obtained from PCa patients (n=30) with either Gleason 3+3=6 or Gleason 9 disease who underwent radical prostatectomy. Prostate tissue specimens were thin sectioned, and LMD was performed to generate samples representing enriched cellular subpopulations of tumor epithelium (n=34), benign epithelium (n=21), tumor-involved stroma (n=28), and benign stroma (n=36). LMD samples were pressure-assisted trypsin-digested labeled using tandem mass tags (TMT-11), organized into epithelial-centric and stromal-centric multiplexes, and fractionated offline prior to analysis by nanoflow LC-MS/MS.

Results

6,818 and 4,686 proteins were co-quantified across all epithelial or stromal samples, respectively. Benign epithelial and stromal populations were not inherently different between Gleason 6 versus Gleason 9 specimens. The proteomic differences between Gleason 6 versus Gleason 9 were exclusive to the tumor microenvironment, observed in both the tumor epithelium and tumor-involved stroma. Further, the molecular alterations measured in the tumor-involved stroma from Gleason 9 cases relative to the benign stroma have unique significance in disease aggressiveness, development, and/or progression.

Conclusion

Quantitative proteomic analysis identified grade-specific proteomic alterations in the tumor and stroma from Gleason 6 and Gleason 9 specimens.

PP03.091: Discover of Exosome Protein Biomarker Through Comparison of High-Grade and Low-Grade Ovarian Cancer Using In-depth Proteome Analysis.

Jeon E[1,2], Park M[1,2], Kim H[1,2], Lee G[1,2], Kim S[1,2], Cheon S[1,3], Han D[1,2]
[1]Proteomics Core Facility, Biomedical Research Institute, Seoul National University Hospital, [2]Transdisciplinary Department of Medicine and Advanced Technology, Seoul National University Hospital, [3]School of Biological Sciences and Technology, Chonnam National University

Introduction
Exosomes are 40-160nm small extracellular vesicles, which play a critical role in both physiological and pathological processes due to their exosomal production from intercellular communication in cancer. Ovarian cancer is the most lethal gynecological cancer in humans. Notably, high-grade serous carcinoma is most common type of ovarian cancer and have worst outcome in a late diagnose. Thus, early detection of ovarian cancer is important. Recent advances in cancer diagnosis and prognosis have been made possible by proteomic analysis of exosomes produced from biofluids. Furthermore, exosome released by ovarian cancer cell lines have the potential of exosome-based therapies and diagnosis. In this study, we compared exosomal proteins derived from high- and low- grade serous ovarian cancer cells by proteomic analysis based on mass spectrometry.
Methods
The human ovarian cancer cell lines including CAOV3 and SKOV3 were cultured to identify ovarian cancer specific exosome marker. Exosome were isolated from each cell lines using precipitation method. After exosomes were characterized using NTA analysis, exosomal proteins were digested with Trypsin according to S-trap protocol. Then, the peptides were desalted by stage tip and analysed for Q-Exactive LC-MS/MS. Data processing was performed by MaxQuant and statistical analysis was performed by Perseus.
Results
A total of 2019 proteins were identified from two ovarian cells-derived exosomes. Several exosomal proteins differed significantly between CAOV3 and SKOV3. Interestingly, exosome of CAOV3 were enriched to ribosome and cholesterol metabolism, while pathway associated with regulation of actin cytoskeleton and cell migration were enriched in SKOV3. Finally, exosomes contained tumor-specific proteins relevant to tumorigenesis and metastasis.
Conclusions
Exosomes derived from high-grade ovarian cancer have an impact on diagnosis and prognosis with comparison to low grade ovarian cancer. In the future, a biomarker of high-grade serous carcinoma with poor prognosis will be found and useful for therapies and early diagnosis.

PP03.092: Multiple Biomarker Identification to Diagnose Metastatic Carcinoma from Thyroid Cancer Patients Plasma Using High-precision Proteomics Approach

Jung J[1], Wang J[1], Kim Y[2], Park Y[2], Han D[1]
[1]Proteomics Core Facility, Biomedical Research Institute, Seoul National University Hospital,
[2]Department of Internal Medicine, College of Medicine, Seoul National University

Introduction: Despite the prevalence and risk of thyroid cancer, the high-survival rate has caused thyroid cancer research to be stagnated compares to other cancers as it is deemed relatively innocuous. The thyroid cancer proteome remains largely unexplored. We investigate the biomarkers to diagnose metastatic carcinoma and distinguish a differentiation status of thyroid cancer using integrative proteomics approach.

Methods: 25 plasma samples consisted with normal, non-metastasis, and metastasis group were collected from both differentiated carcinoma such as anaplastic thyroid cancer (ATC) and undifferentiated carcinoma such as papillary thyroid cancer (PTC) driven by BRAF mutation and follicular thyroid cancer (FTC) generally driven by RAS mutation. Those were investigated by qualitative LC-MS/MS analysis and identify differentially expressed proteins (DEPs) by ANOVA-test with iBAQ values. Those DEPs were validated within large cohort (n= 102) by targeted proteomic approach using multiple reaction monitoring (MRM). The optimal combination of targets for diagnosis was constructed by multivariative regression and developed multi-biomarker panel.

Results: The 107 DEPs from proteome profiling presented that EIF2 and Rac signaling pathway were found to be significantly altered as differentiation progress into ATC in RAS and BRAF, respectively. After MRM validation, 38 proteins were selected as diagnostic biomarker candidates and those were proceeded to logistic regression analysis. The constructed model equation includes 3 proteins and showed AUC value of 0.866 with statistical significance. Moreover, the 3-biomarker panel produces high discriminatory power for both metastasis and non-metastasis with 89.12% and 73.71%, respectively. The overall specificity and sensitivity of panel is also beyond 83%. Collectively, our study gained confident prediction levels with the constructed panel and will be further verified with a large independent cohort.

Conclusion: The constructed 3-biomarker panel would be able to serve as diagnostic assessment to metastatic thyroid cancer and expect to supplement plasma biomarker discovery process in the clinical field.

PP03.093: Systems-wide Analysis of CD44 Knock-down by In-depth Quantitative Proteomics in Different Subtypes of Breast Cancer Cells

Kim H[1,2], Lee G[1,2], Park M[1,2], Jeon E[1,2], Kim S[2,3], Cheon S[1,3], Ryu H[4], Han D[1,2]
[1]Proteomics Core Facility, Biomedical Research Institute, Seoul National University Hospital, [2]Transdisciplinary Department of Medicine & Advanced Technology, Seoul National University Hospital, [3]School of Biological Sciences and Technology, Chonnam National University, [4]Department of Pathology, College of Medicine, Seoul National University

Introduction:

CD44 is a transmembrane glycoprotein, which is existed as several isoforms in breast cancer cells. Binding of ligands to various CD44 isoforms affects unique intracellular signalling related to various cellular functions. The expression pattern of CD44 isoforms can be correlated with breast cancer subtypes. However, there is no comparative analysis of the comprehensive proteome effects of CD44 between two different subtype cell lines.

Methods:

To investigate the global effects of CD44 in breast cancer cell lines (Claudin-low type: MDA-MB 231, HS578T, basal A type: MDA-MB 468, BT-20), we transfected CD44 siRNA and control siRNA for 48h. A TMT- based quantitative proteomic strategy was employed. We performed well-defined proteomic methods including cell lysis, filter-aided sample preparation, peptide labelling, desalting, high pH reversed peptide fractionation high-resolution quadruple Orbitrap LC-MS/MS. Raw MS/MS files were processed with Proteome Discoverer and statistical analysis was performed using Perseus.

Results:

We identified a total of 7396, 6567, 7468 and 8788 protein groups in MDA-MB-231, HS578T, MDA-MB-468, and BT-20, respectively. In MDA-MB231, predominantly, molecule catabolism appeared to be up-regulated and nucleic acid processing appeared to be down-regulated upon CD44 knockdown. In HS578T, extracellular matrix organization seemed to be up-regulated and chromosome organization seemed to be down-regulated. In MDA-MB468, up-regulated proteins included those involved in mitochondrion organization and down-regulated proteins included those involved in cellular organelle organization. In BT-20, up-regulated proteins comprised those affected cellular transport and down-regulated proteins comprised those affected chromosome organization.

Conclusions:

In this study, we present a valuable TMT-based proteomic resource representing the response to CD44 regulation breast cancer cell lines. The results could lead to a better understanding of the biological functions of CD44 at the molecular level. These findings provide the framework for future proteomic investigations and also suggest that CD44 is a potential therapeutic target for treatment of the breast cancer.

PP03.094: Functional Study of Nucleus-localized Proteins in Temozolomide-resistant Glioblastoma

Kim C[1], Park S[1], Park J[1]
[1]Department of Cancer Biomedical Science, Graduate School of Cancer Science and Policy, National Cancer Center

Although temozolomide (TMZ) is one of the first-line therapeutic options of glioblastoma (GBM), the patients frequently suffer from TMZ-resistant recurrence. Subcellular localization of proteome is functionally more important than other molecules, such as RNA and DNA. Here, we tried to investigate nuclear localization of proteome and their functions in TMZ-resistant GBM. Using TMZ-treated patient derived xenograft (PDX) mouse model and stem cell culture, we established TMZ-resistant GBM cells. Then, we performed mass spectrometry-based (LC-MS/MS) proteome analysis with the nuclear fraction protein samples of the TMZ-resistant and control GBM cells. Analyzing the global proteome and phosphoproteome, we identified TMZ-resistance-related nuclear proteins and phospho-sites which could regulate the subcellular localization of nuclear proteins. Moreover, activity-based phosphoproteome analysis revealed the kinases which plays major role in TMZ resistance of GBM. We validated upregulation of the protein expression and phosphorylation in nucleus using immunoblot assays. By performing functional assays, such as mutant study of the phospho-sites, we tried to explain the mechanisms of protein subcellar localization and the downstream in TMZ resistance of GBM. Thus, our results show the characteristics of intracellular compartment-specific proteins of drug-resistant glioblastoma, and we find putative therapeutic targets which plays important role in nucleus.

PP03.095: Enhanced AFP-L3 Assay for Hepatocellular Carcinoma Diagnosis Using Fucose-Specific Lectins and Mass Spectrometry

Kim Y[1], Lim Y[2], Lee J[1]
[1]Seoul National University College Of Medicine, [2]Department of Gastroenterology, Asan Medical Center, University of Ulsan College of Medicine

Introduction

Glycoproteins have critical functions in various biological processes, such as receptor interactions, cell communication, immune defense, and inflammation. A well-known glycoprotein biomarker is alpha-fetoprotein (AFP), a surveillance biomarker for hepatocellular carcinoma (HCC). However, the low diagnostic sensitivity of AFP due to its upregulated level in some cases with hepatitis or liver cirrhosis led to many studies focusing on the AFP-L3. The AFP-L3 protein has a normal protein sequence as AFP but has fucosylated glycan at asparagine 251. Thus, AFP-L3 provides improved specificity for diagnosing HCC, reducing false positives. Many groups have used Lens culinaris agglutinin (LCA) lectin for detecting AFP-L3, but few studies have identified more effective lectins for capturing AFP-L3.

Methods

In this study, we aimed to find better lectin than LCA capturing AFP-L3 by comparing the amounts of enriched AFP-L3 with five fucose-specific lectins: LCA, Lotus tetragonolobus lectin (LTL), Ulex europaeus agglutinin I (UEA I), Aleuria aurantia lectin (AAL), and Aspergillus oryzae lectin (AOL). First, AFP was enriched from the serum sample pools of HCC patients and split into five fractions for fractionation with each lectin. We selected the lectin with the highest normalized amount of lectin-captured AFP in the pool of HCC patients. Finally, we compared the analytical sensitivity of the optimized workflow with the most effective lectin against the conventional LCA assay.

Results

Our results indicate that LTL was the most effective lectin for capturing AFP-L3, yielding approximately 3-fold more AFP-L3 than LCA from the same pooled serum of HCC patients. Further improvements in the fractionation efficiency for capturing AFP-L3 can be achieved by recovering the remaining proteins from the unbound fraction of LTL by subsequent use of LCA.

Conclusions

Thus, we suggest using LTL for the AFP-L3 assay to increase the diagnostic sensitivity in patient samples with limited analysis from a conventional LCA assay.

PP03.096: Proteome Changes Reveal the Cellular Resistance Mechanisms toward Proteasome Inhibitors in Multiple Myeloma

Lee C[1,2], Haertle L[3], Besse L[4], Besse A[4], Kortüm M[3], Kuster B[2,5]
[1]Young Investigator Group: Mass Spectrometry in Systems Neurosciences, School of Life Sciences, Technical University of Munich, [2]Chair of Proteomics and Bioanalytics, Technical University of Munich, [3]Department of Internal Medicine II, University Hospital Würzburg, [4]Experimental Oncology and Hematology, Department of Oncology and Hematology, Cantonal Hospital St. Gallen, [5]Bavarian Biomolecular Mass Spectrometry Center (BayBioMS), Technical University of Munich

Introduction:Proteasome inhibitors are clinical first-line treatments for multiple myeloma (MM), the second most common hematologic malignancy. Although MM patients initially receive and respond to the proteasome inhibitor Bortezomib (Btz), all patients eventually develop resistance. Second-line treatment with Carfilzomib (Cfz) or Ixazomib is effective in some Bortezomib-resistant patients even though these inhibitors supposedly share the same molecular target. However, the studies on the shared and distinct mechanisms of action of Btz and Cfz to explain the clinical observation are limited. Here, we characterise the deep proteomes of four different myeloma cell lines with acquired resistance toward Bortezomib and Carfilzomib to provide insight for re-sensitising resistant patients.

Methods:Four myeloma cell lines, AMO1, ARH77, L363, and RPMI8226, were cultured with Btz and Cfz to gradually acquire the resistance [1]. The biological triplicates of the cells were lysed, tryptic digested, and TMT11-multiplexed. The pooled peptides were fractionated by bRP-HPLC and subjected to micro-LC-Orbitrap LC-MS/MS analysis. The data was searched and analysed by MaxQuant, Perseus, and R.

Results:Our deep proteome profiling (7,200-8,300 proteins per cell line) well recapitulated the up-regulation of designated targets, proteasome subunits, of proteasome inhibitors. In addition, the known drug transporter toward Cfz resistance, ABCB1, was up-regulated in Cfz-resistant cell lines. We also found distinct differentially expressed proteins between the Btz- and Cfz- resistance and among cell lines. For example, proteins related to protein degradation were only up-regulated in Btz-resistant but not in Cfz-resistant cells. On the other hand, the proteins regulated in RPMI8226 Btz- and Cfz-resistant cells were relatively similar. These examples show the diverse resistance mechanisms toward proteasome inhibitors in myeloma.

Conclusions:The reported cellular mechanisms of action of Btz and Cfz provide helpful insight for potential clinical validation and various therapeutic targets for re-sensitising resistant MM patients.

[1] Soriano et al. (2016) Leukemia 30, 2198–2207

PP03.097: Integrated Omics Analysis to Predict Poor Prognosis in Early-stage Colorectal Cancer

Hwang H[1], Lee S[1]
[1]Korea Basic Science Institute

Introduction: Proteomics is known that accurate and intuitive analysis is possible in phenotypic analysis of diseases that directly reflect changes in genes and proteins that play a functional role. Whereas, transcriptomic is considered to be the most useful when using single omics data. Therefore, when utilizing multi-omics data, more accurate research is possible. Colorectal cancer has the third-highest incidence rate among various cancers, and its incidence rate is expected to increase continuously in the future. The prognosis of colorectal cancer also varies depending on the clinical stage, but in addition to the clinical stage, the prognosis is different according to the tumor heterogeneity of cancer. Therefore, molecular subtype analysis that can distinguish this is also being actively conducted.
Methods: In this study, TCGA transcriptome data and CPTAC proteome data were analyzed as a test set to identify colorectal cancer subtypes for predicting the prognosis of early-stage colorectal cancer patients. For predicting prognosis, we analyzed upstream regulators and the corresponding downstream target factors.
Results: Ten regulatory factors and their sub-targets commonly confirmed in transcript data and proteome data were identified. Subtypes were divided through unsupervised clustering of ten gene sets, and prognostic analyses were performed accordingly. As a result, it was confirmed that the prognosis of patients with early-stage colorectal cancer was poor when the gene sets regulated by TGFB, TNF, and IL-6 were overexpressed. In addition, proteome data confirmed that of the three protein sets, the overexpression of subfactors of TGFB can predict the prognosis of early-stage colorectal cancer patients.
Conclusions: In this study, we performed a subset-based analysis that was commonly identified in both omics data. Through this, we elucidated that overexpression of genes and proteins regulated by TGFB in patients with early-stage colorectal cancer was associated with poor progression.

PP03.098: Phosphoproteomics Reveals the Role of Constitutive KAP1 Phosphorylation by B-Cell Receptor Signaling in Chronic Lymphocytic Leukemia

Wu J[1,2], Wu H[3,4], Wu S[5], Tsai H[1,2,6], Weng S[3], Lin K[3], Lin L[7], Yao C[5], Zamanova M[3,8], Lee Y[1], Angata T[2,6], Tien H[5], Chen Y[3], Lin K[1]
[1]Genomics Research Center, Academia Sinica, [2]Institute of Biological Chemistry, Academia Sinica, [3]Institute of Chemistry, Academia Sinica, [4]Instrumentation Center, National Taiwan University, [5]Division of Hematology, Department of Internal Medicine, School of Medicine, National Taiwan University Hospital, [6]Institute of Biochemical Science and Technology, College of Life Science, National Taiwan University, [7]Department of Clinical Laboratory Science and Medical Technology, College of Medicine, National Taiwan University, [8]Sustainable Chemical Science and Technology, Taiwan International Graduate Program (TIGP), Academia Sinica

Introduction: Application of B-cell receptor (BCR) pathway inhibitor ibrutinib for chronic lymphocytic leukemia (CLL) is a major breakthrough, yet the downstream effects of BCR inhibition and during relapse await further clarification. To address this issue, we conducted quantitative phosphoproteomics to compare the healthy donor B cells, CLL B cells from treatment-naive patients and during their time course of ibrutinib therapy. The phosphoproteomics landscape and further verification revealed the significance of KAP1 S473 phosphorylation in CLL.
Methods: We conducted quantitative phosphoproteomics experiments by TMT labeling, IMAC enrichment and LC-MS/MS identification. The in vitro functional studies and network analysis are conducted for verifying the significance of targeted proteins in CLL.
Results: The quantitative phosphoproteomics analysis revealed 290 differentially expressed phosphopeptides (192 phosphoproteins) and 962 previously unreported phosphosites between 6 patients and 3 healthy donors, of which upregulation of 198 phosphopeptides and downregulation of 86 phosphopeptides were observed in CLL cells compared with healthy controls. Particularly, 192 differentially phosphorylated proteins and 176 proteins with 258 novel phosphosites are associated with several signaling pathways, including BCR in patient CLL cells. Interestingly, a constitutively elevated phosphorylation level of KAP1 S473 was found in the majority of CLL samples prior to treatment. Further functional verification showed that KAP1 S473 phosphorylation regulated by BCR is important for cell cycle progression in CLL cells. Moreover, temporal phosphoproteomics quantified 2,697 proteins and 5,412 phosphopeptides from a series ibrutinib-treated patient, confirming that the KAP1 S473 phosphorylation is also associated with the BCR-related dynamic molecular changes in the ibrutinib responsive and recurrent stages.
Conclusions: This phosphoproteomic analysis and functional validation illuminated the phosphorylation of KAP1 S473 as an important downstream BCR signaling event and a potential indicator for the success of ibrutinib treatment in CLL.
References: The results have been just published in Molecular Cancer Research (2022 May 9, doi: 10.1158/1541-7786.MCR-21-0722.).

PP03.099: Functional Analysis of Uncharacterized Protein C19orf47 of Chromosome 19 with Emphasis on the Development of Cervical Cancer.

Sánchez Castellanos N[1], Alonso Bastida R[1], Herrera Quiterio G[1], Morales Tarré O[1], Hernández M[1], Contreras-Martinez S[1], Martinez-batallar A[1], Encarnación-Guevara S[1]
[1]Centro de Ciencias Genómicas

Through the development of the Human Proteome Project focused on chromosome (C-HPP) the aims to identify, characterize, quantify and localize proteins representative of each of the chromosomes. The Mexican consortium of Chromosome 19 has the mission of defining the proteins encoded by chromosome 19. In this chromosome, 11 uPE1 proteins were identified, among which C19orf47 (Uncharacterized protein C19orf47) was found. Furthermore, our consortium identified CCDC61, TMEM160 and LENG8 proteins with unknown function in a cervical cancer context. Adifferential expression analysis in the GEPIA database showed that the mRNA of C19orf47 is overexpressed in several tumors compared to normal tissue. In addition, patients with lower expression of gene C19orf47 have lower cancer survival than those with high expression of this gene. This suggest that C19orf47 might be involved in cellular functions that contribute with cancer pathology on deregulated conditions. Comparative proteomics studies showed that the C19orf47protein increased on several cancer cell lines, mainly lung, breast, colon and cervical cancer. We currently we know about C19orf47: mRNA and protein is overexpressed in cervical cancer cell lines: SiHa (HPV 16+), Hela (HPV 18+) compared with cell lines C-33A (non -infected HPV) and HaCaT (keratinocytes transformed). Inhibition of protein C19orf47 by CRISPR-Cas sytem impact on proliferation, migration, invasion and cell adhesion of cells. We observed that the conventional treatment of cancer cells with cisplatin reduced levels of C19orf47protein, suggesting a role for this protein in the sensitivity or resistance to chemotactic reagents. In this way we propose to characterize the functions of C19orf47 in the context of cervical cancer, exploring their interactor's proteins and if there activation of survival pathways. Study C19orf47, an uPE1 chromosome 19 protein; it will allow us to generate advances in the understanding, diagnosis and treatment of this pathology.

PP03.100: Exosomal Cx43 as Key Player in BRAF/MEK Inhibitors Efficacy and Drug Resistance in Tumours with a Mutation in BRAF

Mayán Santos M[1], Varela-Vázquez A[1], Carpintero-Fernández P[1], Varela-Eirín M[1,2], Carneiro-Figueira A[1], Montes-Morado T[1], Bravo S[3], Calleja-Chuclá T[4], Quindós M[5], Fonseca E[1,6], Gomez-Duran A[7], Mayán M[1]

[1]CellCOM Group, Instituto de Investigación Biomédica de A Coruña (INIBIC), Servizo Galego de Saúde (SERGAS), Universidade da Coruña, [2]European Research Institute for the Biology of Ageing (ERIBA);, University Medical Center Groningen (UMCG), [3]Instituto de Investigación Sanitaria de Santiago de Compostela (IDIS), Proteomics laboratory, [4]Hospital Pharmacy Service. CH-Universitario A Coruña (XXIAC). Servizo Galego de Saúde (SERGAS), [5]Translational Cancer Research Group, Instituto de Investigación Biomédica de A Coruña (INIBIC). CH-Universitario A Coruña (XXIAC). Servizo Galego de Saúde (SERGAS). , [6]Dermatology Deparment, CH-Universitario A Coruña (XXIAC). Servizo Galego de Saúde (SERGAS), [7]10 MitoPhenomics Lab. Centro de Investigaciones Biológicas Margarita Salas. CSIC.

Introduction:

Mutations in the oncogenic protein kinase BRAF are involved in the onset and progression of several tumours, including more than the 50% of cases of melanoma. BRAF/MEK inhibitors (BRAF/MEKi) in monotherapy or in combined therapies with immune checkpoint inhibitors have become the standard therapy in BRAF mutated melanoma. However, resistance to targeted therapies is a frequent cause of therapy failure. We have recently found that the channel protein connexin43 (Cx43), a growth and differentiation regulator, increases the efficacy of BRAF/MEKi in different tumour types with a BRAF driver mutation. Yet, the molecular mechanism underlying these observations remains unknown.

Methods:

LC-MS/MS analysis of tumour-derived extracellular vesicles (EVs) and EVs derived from Cx43-enriched melanoma cells revealed Cx43 as a key regulator in EVs function and in the recruitment of proteins to the EVs.

Results:

Our results have shown that Cx43 leads to an enrichment in proteins implicated in cell senescence (PPP1CC, TGFB1), mitochondria-mediated apoptosis (APAF1, AIFM1), cell cycle regulation (PSME2, CDK1), DNA repair (H2AFX, RAD50), pol-II transcription regulation (POLR2, NELFA) or immune response (HLA-DPB1, PPP6C) among others. Indeed, CSTB, a pro-apoptotic and cell cycle regulator protein, is not only enriched in EVs-Cx43, but also directly interacts with Cx43 in the EVs. We have investigated the potential use of EVs as drug/protein delivery system to restore Cx43 in BRAF-mutant tumour cells. Our results show that exosomal Cx43 increases the efficacy of BRAF/MEKi and avoids drug resistance by enhancing cellular senescence and activating cell death by apoptosis.

Conclusions:

Our results demonstrated that Cx43 changes the protein cargo and function of tumour-derived EVs and the engineered EVs can be used as a drug/protein delivery vehicles to restore transmembrane proteins in target cells in order to develop new therapeutic strategies to combat metastatic tumours and to improve the potency of BRAF/MEKi in BRAF-mutated melanoma patients.

PP03.101: In-depth Label Free Proteomic Analysis of Medulloblastoma for the Elucidation of Differential Drivers Between Group 3 and Group 4

Nair D[1], J Pai M[1], Moiyadi A[2], Epari S[3], Shirsat N[4], Srivastava S[1]
[1]Indian Institute Of Technology, [2]Department of Neurosurgery, Tata Memorial Center's-Advanced Center for Treatment, Research and education in Cancer, [3]Department of Pathology, Tata Memorial Center's-Advanced Center for Treatment, Research and education in Cancer, Navi Mumbai, India and Homi Bhabha National Institute, [4]Advanced Center for Treatment, Research and education in Cancer

Introduction
Medulloblastoma (MB) is the most common malignant brain tumor in children. Out of the four subgroups, Group 3 has the worst prognosed and the pathobiology is less understood. Here we used mass spectrometry-based proteomic characterization of fresh frozen (FF) and formalin-fixed paraffin-embedded (FFPE) samples from the Indian cohort to study the pathobiology of the Group 3 tumor. This study highlighted the metabolic pathways, and DNA repair mechanism to be altered significantly. The proteins mapped to these pathways were validated using MRM assay providing an insight into new therapeutic targets.
Methods
FF and FFPE samples were collected from Tata Memorial Hospital, Mumbai, Maharashtra. The FF samples were processed directly, the FFPE samples were deparaffinized, and the tissue samples were lysed, followed by filter-aided sample preparation (FASP) method to perform protein digestion. High resolution label free proteomic (LFQ) analysis of the samples was performed. Gene set enrichment analysis of the differentially expressed proteins was done. The key proteins belonging to pathways enriched in Group 3 were taken for MRM -based validation. The pathological role of the targeted proteins was validated through various cell-culture-based assays.
Results
The number of proteins identified from FF and FFPE had a considerable difference with more unique proteins identified from FFPE tumor samples. The differentially expressed proteins identified in Group 3 mapped to metabolic pathways, including TCA cycle gluconeogenesis and carbohydrate metabolism as well as DNA repair mechanism, and MAP2K and MAPK. We have found Dihydropyrimidinase-related protein 4 to be significantly downregulated indicating alteration in the DNA damage response and cell proliferation.
Conclusion
Extensive metabolic rewiring in Group 3 cells plays a role in the aggressive nature of this subgroup. Although molecules targeting these pathways need to be tested, this novel understanding can be exploited for therapeutic benefit of Group 3 MB patients.

PP03.102: Development of a Large-scale Targeted Proteomics Assay for Potential Salivary Biomarkers Associated with Oral Cancer Initiation

Normando A[1], Granato D[1], Carnielli C[1], Pauletti B[1], Paes Leme A[1]
[1]CNPEM

Introduction: Aiming accurate quantification of salivary targets associated with oral cancer initiation, we developed a parallel reaction monitoring mass spectrometry (PRM-MS) assay to achieve highest sensitivity, quantification accuracy, and reproducibility.
Methods: Targeted selection was based on salivary proteome DDA data, and literature review. Three proteotypic peptides were selected for each target and quantified in a concentration range. Liquid chromatography was optimized by testing gradient lengths of 30, 60, and 110 minutes, 0-30% acetonitrile and 0.1% formic acid. Gradient selection was based on peptide identification/quantitation, peak quality, and maximum points across peak. Normalized collision energy (NCE) was optimized in a scheduled PRM method (6 min-window, 1.2 m/z isolation width, 80 ms maximum injection time) and set to 18, 21, 24, 27, and 30. Optimal NCE was that yielding highest total peak area. Optimizations were performed on Acclaim PepMapTM and PepMap RSLC columns, using an Easy-nLC-1200, coupled to Orbitrap Exploris 240 Mass Spectrometer. Data analysis was performed in Skyline software.
Results: 123 protein targets were selected, and 349 heavy peptides were assessed. Most peptides (n=285, 82%) were identified by injecting 12.5 fmol, and the remaining peptides were identified by column injection between 25 and 150 fmol. Heavy peptides met the defined criteria in a 60-minute gradient, with 96.6% of built library match and 80.5% presenting > 10 points across peak. NCE optimization performed on a 60-minute gradient, revealed that the average total peak area was higher with a NCE of 21 compared to 24, 27, and 30 ($p<0.05$). Nevertheless, optimized NCE was selected for each peptide individually based on highest value of total peak area among all assessed NCEs.
Conclusions: Large-scale PRM optimizations indicated that most peptides met the quantitation criteria, requiring distinct amounts in column and specific NCEs to reach the maximum fragment ion intensity response, in the selected gradient length.

PP03.103: CCDC97 Protein Modulates Migration, Proliferation, and Anticancer Drug Treatment Sensitivity in a Cervical Cancer Context

Osio V[1], Encarnación-Guevara S[1]
[1]UNAM, CCG

Introduction: Cancer disease is caused by proliferation, metastasis, apoptosis, and angiogenesis alterations. Nowadays, proteomic approaches are used to understand cancer pathogenesis; and biomarkers and potential treatment targets identification (1). Proteomic analysis indicated that deacetylase sirtuin 1 inhibition changed protein expression in many cellular processes. Also, changes in protein expression without a known biological function were detected. One of these is CCDC97 (Coiled-Coil Domain-Containing Protein 97) (2). CCDC97 protein has a protein domain known as coiled-coil (CC); this CC is present in approximately 2-3% of the human proteome. CCs are present in proteins that support cellular homeostasis. Likewise, CC-containing proteins are associated with cancer development (3). Because CC-containing proteins promote cellular homeostasis or cancer growth, we evaluate the CCDC97 protein role in cervical cancer development.

Methods: HaCaT, immortalized keratinocytes negative for Human Papilloma Virus infection (HPV-), C-33A, SiHa, and HeLa tumorigenic cells (HPV-, HPV-16+, HPV-18+, respectively) were used for analysis. The CCDC97 coding region was deleted through CRISPR/Cas9 system. CCDC97-knockout cells were subjected to in vitro cancer assays.

Results: CCDC97 protein probably acts as a tumor suppressor in a cervical cancer context. Wound healing assays showed an increased migration capacity in CCDC97 protein absence. Also, MTT assays indicated that CCDC97-deficient cells enhanced cell proliferation capability. Furthermore, CCDC97-knockout cells showed differences in anticancer drug treatment sensitivity, related to control cells.

Conclusions: Function associated with CCDC97 protein, in a normal or carcinogenic context is not documented. Cell-based assays in cancer research suggest that the CCDC97 protein has suppressive effects on cervical cancer development. However, the way by which CCDC97 protein exerts these effects is under investigation.

References:

1) Kwon, Y. et al. (2021). Front Med. 8: 747333.
2) Gil, J. et al. (2017). J. Biol. Chem. 292: 18129-18144.
3) Burkhard, P. et al. (2001). Trends Cell Biol. 11: 82-88.

PP03.104: Mass Spectrometry Identifies Novel Interactors of zDHHC23 and MROH6 in Neuroblastoma

Oswald S[1,2], See V[3], Pizer B[1,4], Prior I[5], Eyers C[1,2]
[1]Biochemistry and Systems Biology Department, University of Liverpool, [2]Centre for Proteome Research, University of Liverpool, [3]Laboratory of Tissue Biology and Therapeutic Engineering, CNRS UMR5305, [4]Department of Haematology and Oncology, Alder Hey Children's Hospital, [5]Molecular Physiology and Cell Signalling Department, University of Liverpool

Introduction

Despite rigorous multimodal therapies, neuroblastoma has the lowest survival rates of all childhood cancers with high risk survival at 40%. There are currently no mutational/expression criteria that can be used to determine prognosis or identify a therapeutic target for patients that do not have a MYCN amplification (75%). In this study, we will investigate the role of two proteins shown to be upregulated at the transcriptional level in non-MYCN neuroblastoma tumours obtained from the chick embryo model: Zinc finger DHHC – type containing 23 (zDHHC23) and Maestro heat like repeat family member 6 (MROH6). Both have been identified to be upregulated in hypoxia (1% O2 – mimicking an aggressive metastatic phenotype) and correlate with a significantly poorer prognosis.

Methods

SK-N-AS cells were cultured for 72 hours in either normoxia (21% O2) or hypoxia (1% O2) following transfection of the HA-tagged protein of interest. Proteins were immunoprecipitated via their HA-tag, proteolysed and analysed by LC-MS/MS to identify binding partners and post-translational modifications. Additionally for zDHHC23, click reaction chemistry was utilised with palmitoyl-azide/alkyne IP to identify specific palmitoylation targets of this palmitoyltransferase following trypsin digestion and LC-MS/MS analysis. All data were analysed using Proteome Discoverer (PD) PD 2.4 and subject to bioinformatics analysis.

Results

We identify extensive interaction networks for both zDHHC23 and MROH6 that change in response to hypoxia, allowing us to hypothesise on the broader physiological roles of these two novel neuroblastoma biomarkers. Amongst those interactors induced under low oxygen conditions, we also identify well known/characterised markers of cancer including BCAR1/3, GLUT1 and VCAN, suggesting how these proteins may function to drive neuroblastoma progression.

Conclusion

This comprehensive biochemical investigation of neuroblastoma biomarkers will hopefully aid in identifying potential mechanisms of action and future therapeutic targets in key signalling pathways within neuroblastoma, providing a much needed new clinical avenue.

PP03.106: Longitudinal Phosphoproteomics and Chemicogenomics Screening to Study Entrectinib Resistance Mechanism

Pham V[1], Lee H[1], Lin E[1], Yu K[2], Choi M[1], Yu P[1], Wilson T[1], Lill J[1]
[1]Genentech Inc., [2]Fuhong Therapeutics

Introduction

Entrectinib, a selective tyrosine kinase inhibitor of neurotrophic tyrosine receptor kinase (NTRK) 1/2/3, ROS1, and ALK kinases, is a medication for the treatment of ROS1 positive and NTRK-fusion positive solid tumors. Clinical trial on NTRK-fusion positive solid tumor patients has proven that Entrectinib induced durable response. However, overtime patients experienced resistance. In this experiment, we employed longitudinal phosphoproteomics and chemicogenomics screening to study drug resistance mechanism and proposed the combo treatment in order to overcome resistance.

Methods

Phosphoproteomics involves the enrichment of phosphorylated species using pTyr motif antibody (licensed with Cell signaling Technology) and TiO2 resin. In this experiment, we monitored the phosphorylation level in KM12 cells treated with DMSO or Entrectinib for 1h, 5 days, and 4 weeks using Tandem Mass Tag (TMT) quantitation approach. In addition, we also performed chemicogenomics screening in KM12 cells against a library of well- curated compounds to identify those that re-sensitized the resistant cells.

Results

Phosphoproteomics data revealed several phosphotyrosine sites on NTRK1 being downregulated upon Entrectinib treatment confirming drug-target engagement. Interestingly, from day 5 we observed a significant increase in phosphorylation level on ERK1/ERK2 activation loop indicating the reactivation of the MAPK pathway. Noticeably, multiple receptor tyrosine kinases and protein X were also activated at 4 weeks. Chemicogenomics screening further discovered that the inhibitors of MEK1/2, ERK1/2, and Protein X could re-sensitize Entrectinib resistant cells. To this end, we proposed the upfront Entrectinib/ProteinXi combo treatment in order to overcome drug resistance.

Conclusions

Phosphoproteomics and chemicogenomics screening uncovered Entrectinib resistance mechanism and the Entrectinib/ProteinXi combo treatment could overcome drug resistance.

PP03.107: Proteomics and Mass Spectrometry Imaging of Three-dimensional Cell Culture Models of Breast Cancer

Raffo Romero A, Duhamel M, Fournier I, Salzet M
[1]Laboratoire de Protéomique, Réponse Inflammatoire et Spectrométrie de Masse (PRISM), INSERM U1192, Université de Lille

Introduction: Breast cancer (BC) is the most common cancer among women causing the highest number of cancer-related deaths. Currently, BC research is mainly conducted using 2D cultures or mouse models. Both have proven to have limitations: 2D culture lacks cell-cell or cell-matrix interactions and mice are associated with ethical problems and poorly mimic the human diseases. There is an unmet need to develop novel more accurate, realistic, standardisable and robust experimentation models.

Methodology: We have developed two 3D culture models: the first one is BC organoids made with a mixture of tumor cells isolated from human and canine BC tissues; the second is a bioprinting-assisted 3D culture in which we print different BC cell lines in a bio-ink. To complexify the model, we add autologous macrophages isolated from patient blood and we study their dynamic distribution. Proteomic as well as mass spectrometry imaging studies were performed to compare the tumor of origin with the organoids coming from it and to verify whether cryopreservation does not modify the proteomic identity of the organoids.

Results: Our results reveal that the organoids are very close to the original tumor and that fresh or frozen organoids do not significantly modify their proteomic profiles. Moreover, thanks to mass spectrometry imaging, we were able to show the intratumoral heterogeneity is maintained in the organoid model, which is very important to recapitulate in vivo drug responses. Thanks to these results, we became interested in studying the different sub-clones within the organoids that represent the intratumoral heterogeneity. We recovered different subpopulations and showed by mass spectrometry imaging their similarities with the organoid of origin.

Conclusion: Our knowledge in biology together with proteomics and mass spectrometry imaging skills helped us to better understand the BC organoids and study the different intratumoral populations that are so problematic today.

PP03.108: Omic Characterization of the Cell Membrane for Understanding the Correlation between Neoplasia, Chronic Inflammation and Membrane Remodeling

Rojas Cervantes K[1], Velázquez-Fernández J[1], Verdugo Molinares M[1], Flores-Hernández F[1], Muñiz-Hernández S[2], Padilla-Camberos E[1], Salgado I[3], Chairez J[4], Prado Montes de Oca E[1], Figueroa-Yañez L[1], Vallejo-Cardona A[1]
[1]Centro de Investigación en Asistencia y Tecnología del Estado de Jalisco, México, [2]Centro de Investigación Básica, Instituto Nacional de Cancerología, Méxic, [3]Centro de Innovación y Desarrollo Tecnológico en Cómputo, [4]School of enginering, Tecnológico de Monterrey, Campus Guadalajara.
Introduction: Pancreatic cancer is one of the most lethal and aggressive neoplasms, its study is a constant challenge due to the molecular heterogeneity it presents, causing a diagnosis in advanced stages and an alarming survival. Therefore, continuing the study is an important piece to elucidate the changes in the progression of carcinogenesis.
Cell line cultures have been shown to be effective for the study of cancer development and progression, monolayer and spheroid models provide an important strategy to mimic in vivo conditions and analyze potential changes in the tumor membrane microenvironment at the microenvironment level omic. And subsequently compare them with an in-silico strategy of artificial intelligence through already established databases, where the omic correlation between the changes typical of neoplasia and chronic inflammation at the membrane level would be found.
This correlation will provide important information on cancer progression and may be useful in the search for therapeutic targets.

Methods: Pancreatic cancer cell lines will be cultured in monolayer and spheroids, obtaining the membrane proteome and lipidome of pancreatic cancer cell lines for subsequent analysis by mass spectrometry with a top-down label free method.

Results: It was observed that the cultured cells in both models expressed different omic compounds in the cell membrane and some proteins were found that could be useful by artificial intelligence software. The cell culture in spheroids showed a greater amount of proteins, this because it better mimics the tumor environment.
Membrane proteins from both cell lines were analyzed on the spectrometer and compared to the database to identify changes in the proteome under both conditions. Statistical differences in abundance were analyzed taking $P<0.05$ by ANOVA using Progenesis software.

Conclusions: The culture conditions of pancreatic cancer cell lines express different proteins that provide relevant information for the study of this neoplasm.

PP03.109: Molecular Understanding of the Palmitic Acid-induced Hepatocellular Carcinoma Cell Death; a Proteomics Approach

Saha S[1], Verma R[2], Kumar C[1], Kumar B[1], Dey A[1], Surjit M[2], Mylavarapu S[1], Maiti T[1]
[1]Regional Centre For Biotechnology, [2]Translational Health Science and Technology Institute

Introduction:
The accumulation of free fatty acids (FFAs) in the liver is a histological and biochemical hallmark of obesity-associated hepato-steatosis. Growing evidence indicates that higher FFAs level in hepatocytes affects a myriad of biological processes leading to excessive metabolic imbalance, increased reactive oxygen species (ROS), deregulated autophagy, and impairment of mitochondrial and ER function, which collectively drives cell death. Despite many studies, the mechanism associated with lipotoxicity and subsequent cell death still remains poorly understood.

Methods:
In the present study, we have used a high-resolution proteomics approach to circumvent the mechanism for lipotoxicity (0.5mM palmitic acid) using hepatocellular carcinoma cells (HepG2) as a model.

Results:
Quantitative proteomics data revealed that ectopic lipids accumulation in cells severely affected the ubiquitin-proteasomal system. Eventually, MS-based activity-based deubiquitinating enzymes (DUBs) enrichment and their temporal quantification revealed the perturbed ubiquitin homeostasis due to altered DUBs expression during palmitic acid toxicity. In addition, a landscape profile of ubiquitinylated and phosphorylated proteome distinguishes a metabolic load that may facilitate increased organelle stress and an imbalance of hepatocellular proliferation and death.

Conclusions:
Our integrated analysis provides strong evidence of an altered expression of cell cycle checkpoint proteins that abrogates early G2/M checkpoints recovery with damaged DNA and induces mitotic catastrophe leading to hepatocyte death.

PP03.110: Predictive Modeling of Pan-cancer Proteotranscriptomic Data Reveals Functional Organization of the Human Cancer Proteome

Shi Z[1], Lei J[1], Moon C[1], Zhang B[1]
[1]Baylor College Of Medicine

Introduction: Protein functional association networks are essential for understanding the function and organization of proteins at the systems level. The Clinical Proteomic Tumor Analysis Consortium (CPTAC) has generated mRNA and protein profiling data on tumor and matched normal adjacent tissues from >1,000 patients spanning 10 cancer types, providing an exceptional opportunity to globally infer protein functional association based on mRNA and protein co-expression. Here we applied machine learning to CPTAC pan-cancer proteotranscriptomic data and built a proteome wide functional association network, which was shown to provide fundamental functional insights into human cancer biology.
Methods: Harmonized mRNA and protein profiling datasets from 10 CPTAC cancer types were used to compute pair-wise mRNA and protein correlations, which served as features to train an XGBoost classifier to distinguish functionally associated and unrelated gene pairs in a gold-standard training set. The classifier was then applied to all gene pairs to construct a functional association network named FunMap. Hierarchical modular organization of FunMap was revealed by NetSAM. FunMap-based gene function prediction was performed using random walk with restart. A graph neural network was used to predict cancer drivers based on FunMap and somatic mutations.
Results: FunMap covered 10,525 proteins and 196,800 functional associations. It was 50 times more likely to connect known functionally associated than unrelated protein pairs, and the ratio was higher than those of the human reference interactome (10 times) and the BioPlex interactome (28 times). Hierarchical network modules showed high functional and spatial homogeneity, and they provided a functional context linking genomic aberrations to various cancer phenotypes. FunMap-based gene function prediction assigned functions to hundreds of understudied genes. Graph neural network modeling prioritized new cancer drivers.
Conclusions: The pan-cancer proteotranscriptomic data-driven FunMap outperforms physical protein interaction networks with regard to functional coherence and provides a new framework for human cancer research.

PP03.111: Do Specific Autoantibodies Participate in Pathophysiological Processes Associated with Tumor Regression?

Skultety L[1,2], Jankovicova B[3], Krocova E[3], Bilkova Z[3], Minichova L[1], Chalova P[1,4], Lakota J[5,6]
[1]Biomedical Research Center of the SAS, [2]Institute of Microbiology of the CAS, [3]Department of Biological and Biochemical Sciences, University of Pardubice, [4]Faculty of Pharmacy, Comenius University, [5]Center of Experimental Medicine of the SAS, [6]Faculty of Management, Comenius University

Introduction: Our team has observed spontaneous tumor regression in four patients (breast cancer, Hodgkin's disease, non-Hodgkin's lymphoma, and Ewing's sarcoma) who had relapsed/progressed after high-dose chemotherapy and autologous hematopoietic stem cell transplantation. Patients' blood counts strongly resembled the blood counts in aplastic anemia (AA-like syndrome). Moreover, some of these patients' morphology of the bone marrow trephine biopsies was identical to the AA picture. Nissen & Stern, in their paper, proposed that the autoimmune (anti-tumor) activity against present malignancy also operates against hematopoietic stem cells. The final clinical and laboratory pattern in these patients is present as acquired aplastic anemia (AA). This pathophysiological mechanism could explain pancytopenia, which frequently occurs in patients with hematological and non-hematological malignancies.

Methods: Comprehensive proteomic and metabolomic studies were performed to identify protein-antibody and protein-protein interactions using tandem mass spectrometry

Results: To understand the interconnected processes occurring in vivo, we detected, identified, and characterized clinically essential molecules involved in the depletion of tumor cells or tumors as a whole. At first, sera of patients in long-lasting remission were applied for detailed serological proteome analysis to screen their reactivity against antigens isolated from representative cancer cell lines. In addition to anti-carbonic anhydrase I antibodies, the patient's serum also contained a significantly higher titer of autoantibodies against α-enolase. We defined the immunodominant epitopes of these antigens and showed that treatment with these antibodies might result in the down-regulation of structural proteins of the basal lamina, the cytoskeleton, and some other extracellular matrix proteins.

Conclusions: This study revealed that autoantibodies against specific antigens could be an excellent prognostic marker and an essential regulator of pathophysiological processes associated with tumor regression.

Acknowledgments: This study was supported by a grant from the Slovak Research and Development Agency (APVV-18-0340).

PP03.112: Six Potential Immune-checkpoint Predictors are Associated with Immunotherapy Response in Three Independent Metastatic Melanoma Cohorts

Szadai L[1], Bartha Á[2], Pla Parada I[3], Balázs Németh I[1], B Domont G[4], Marko-Varga G[5], Sanchez A[3]
[1]Department of Dermatology and Allergology, University of Szeged, [2]Department of Bioinformatics, Semmelweis University, [3]Section for Clinical Chemistry, Department of Translational Medicine, Lund University, Skåne University Hospital Malmö, [4]Chemistry Institute, Federal University of Rio de Janeiro, [5]Clinical Protein Science & Imaging, Biomedical Centre, Department of Biomedical Engineering, Lund University

Introduction: What is really problematic in melanoma care that there is unmet need for novel protein discovery with prognostic and predictive implications regarding therapy response. In our published study (1), 392 potential proteins associated with immunotherapy response provided new results and opportunities to understand the progression of melanoma. To continue the predictor research, we validated these proteins in two independent metastatic melanoma cohorts for selecting the best immunotherapy responder proteins.

Methods: A machine-based learning score system tool (EaSIeR) (2) was used to test the proteins and distinguish responders and non-responders by quantifying the elements of tumor microenvironment. The proteins were ranked based on their association (Pearson correlations-adjusted p-values) with the score system and we also compared their abundances in patients ordered by score system quartiles (Q1 (non-responders) - Q4 (responders)). We added supplemental information using survival analysis (Cox regression adjusted by age-sex-clinical stage) at transcriptomic (TCGA dataset) and protein levels. RNA expression levels in melanoma cells were also investigated (nTMP values from single-cell (3)).

Results: Due to the testing in two different metastatic cohorts with machine-based learning method, 6 proteins were overlapped with the best association scores to immunotherapy response (Q1Q4Test $p < 0.05$; Pearson $p < 0.05$), namely ITGAX, TNFAIP2, SAMSN1 (Cox, $p < 0.05$), PSMB5 (Cox $p = 0.065$), CD163 (Cox, $p = 0.067$) and MTSS1L (Cox, $p=0.961$). CD163 and MTSS1L were not observed in melanoma cells based on transcriptomic level (nTMP). The expression of PSMB5 and MTSS1L were downregulated in parallel with better immunotherapy response.

Conclusions: Overall, in our study, we have seen potential biomarkers predicting immunotherapy response in different metastatic melanoma cohorts. These proteins can be basis for further initiatives in treating melanoma patients.

1. Szadai, L., et al. 2021 Cancers 3;13(23):6105
2. Lapuente-Santana, Ó., et al. Cell 2; 100293
3. The Human Protein Atlas, https://www.proteinatlas.org/

PP03.113: Proteomics Investigation of High Grade Gliomas to Understand the Role of Oligosaccharyltransferase in Tumor Progression¬¬

Verma A[1], Bhat A[2], Srivastava S[3]
[1]Indian Institute of Technology Bombay, Mumbai, [2]Indian Institute of Sciences, [3]Indian Institute of Technology Bombay, Mumbai

Introduction: High grade gliomas (HGGs), which also include glioblastomas, are the most malignant brain tumors. These are highly invasive and difficult to treat with a median survival time of less than 15 months. Extracellular matrix (ECM) remodelling helps in tumor growth and invasion in HGGs. Despite several studies aimed at understanding tumor aggressiveness in HGGs, the role of oligosaccharyltransferase (OST) mediated N-linked glycosylation remains unexplored. We propose OST mediated N-linked glycosylation plays a major role in tumor invasiveness and accelerates epithelial to mesenchymal transition (EMT) in HGGs.

Method: In total, 5 low grade and 11 high grade samples were processed for label free quantification using HR-LC MS/MS. The statistical data analysis was performed using Perseus (v2.0.6.0) and protein-protein interaction (PPI) analysis was performed using STRING along with gene set enrichment analysis (GSEA). Further, multiple reaction monitoring (MRM) was performed to validate the expression levels of the identified proteins from global proteomics data.

Results: Several proteins of ECM including Fibronectin, Fibrinogens, collagens along with mesenchymal markers like Vimentin and TGF-β were found to be significantly upregulated in HGGs. We also observed enhanced expression of OST subunits such as RPN1, RPN2, DAD1 and OST48 in HGGs compared to low grade gliomas. PPI analysis indicated a role of these proteins in N-glycan biosynthesis. Gene set enrichment analysis (GSEA) showed matrisome pathways to be highly enriched.

Conclusions: We hypothesize that an upregulation of OST mediated N-linked glycosylation of membrane proteins affects EMT thereby increasing tumor invasiveness in HGGs. Studies also indicate that cancer cells utilize glycosylation to escape immune response by activating the immune checkpoint inhibitor PDL1. Cell-culture based experiments are currently in progress to better understand the role of OST mediated N-linked glycosylation in progression and increased invasiveness of HGGs.

PP03.114: Aggressive Prostate Cancer Associated Urinary Glycoproteins Detection in both Pre- and Post-Digital Rectal Examination Urine Samples

Wang Y[1], Lih T[1], Li M[1], Höti N[1], Sokoll L[1], Petrovics G[2], Kohaar I[2], Zhang H[1]
[1]Johns Hopkins University, [2]Uniformed Services University of the Health Sciences

Nonaggressive prostate cancer (NAG-PCa) with Gleason score of 6 is considered as a low-risk disease and does not require clinical interventions. However, current assessment of aggressiveness of PCa is invasive using needle biopsies. Urine is an appealing biospecimen for noninvasive detection of aggressive PCa. Using urine, particularly from easily obtainable from pre-DRE urine specimens, to identify aggressive prostate cancer (AG-PCa) associated molecular markers is critical for clinical risk stratification. Herein, we utilized data-independent acquisition mass spectrometry (DIA-MS) to quantitatively analyzed urinary glycopeptides, which were acquired from 154 pre-DRE and 292 post-DRE urine specimens from patients underwent PCa diagnosis with biopsies. Compared to glycopeptides between pre- and post-DRE urine data, humoral immunity-related proteins were enriched in pre-DRE urine samples, whereas immune cell-related proteins were enriched in post-DRE urine samples. We discovered several urinary glycoproteomic signatures in post-DRE urine specimens, including prostate-specific antigen (PSA), prostatic acid phosphatase (ACPP), clusterin (CLU), and CD97, showing good performance in distinguishing aggressive and non-aggressive PCa individually and/or in combination via receiver operating characteristic (ROC) analysis (area under the curve, AUC ranged from 0.63 to 0.8). Compared with urinary glycoproteins identified from post-DRE urine specimens, we confirmed that three AG-PCa-associated glycoproteins, urinary PSA, ACPP and CD97 identified in post-DRE urine specimens were also found to be significantly associated with patients with Gleason 7 and above urine. We also identified three new glycoproteins, Fibrillin 1 (FBN1), Vitronectin (VTN) and Hemicentin 2 (HMCN2), to be potentially associated with AG-PCa in pre-DRE urine specimens (AUC ranged from 0.66 to 0.76). In summary, glycoprotein profiles differ between pre- and post-DRE urine specimens. Using the ROC analysis, we identified three glycoproteins that were associated with AG-PCa in both pre- and post-DRE urine specimens. Our study provides a foundation for further studies of AG-PCa biomarkers using pre-DRE urine specimens.

PP03.115: Proteomic Analysis of Primary Tumors and Paired Brain Metastases in Lung Adenocarcinoma Patients Identifies Survival-related Protein Candidates

Woldmar N[1,2], Kuras M[3], Szeitz B[4], László V[5,6], Moldvay J[6,7], Szász A[4,6], Malm J[3], Horvatovich P[8], Pizzatti L[1], Domont G[9], Marko-Varga G[2], Schelch K[5], Megyesfalvi Z[5,6,10], Döme B[5,6,10], Rezeli M[2]
[1]Laboratory of Molecular Biology and Proteomics of Blood/LADETEC, Institute of Chemistry, Federal University of Rio de Janeiro, [2]Department of Biomedical Engineering, Lund University, [3]Section for Clinical Chemistry, Department of Translational Medicine, Lund University, Skåne University Hospital Malmö, [4]Department of Bioinformatics, Semmelweis University, [5]Department of Thoracic Surgery, Medical University Vienna, [6]I. Department of Pulmonology, National Korányi Institute of Pulmonology, [7]Department of Pathology, Forensic and Insurance Medicine, MTA-SE NAP, Brain Metastasis Research Group, Hungarian Academy of Sciences, [8]Department of Analytical Biochemistry, Groningen Research Institute of Pharmacy, University of Groningen, [9]Department of Biochemistry, Institute of Chemistry, Federal University of Rio de Janeiro, [10]Department of Thoracic Surgery, National Institute of Oncology-Semmelweis University

Introduction
Approximately 50% of all lung cancer patients have existing metastases at the time of diagnosis. In lung adenocarcinoma (LADC), brain metastases are the most common and have a significant negative impact on patients' outcome. We aimed to identify prognosis- and survival-related proteins by proteomic profiling of LADC patients using a unique cohort of 58 primary tumors and their corresponding brain metastases.

Methods
The 116 FFPE samples were processed according to our previously described method (10.1021/acs.jproteome.0c00850). Proteomic analysis was performed by isobaric labeling using TMTpro 16-plex. Peptide fractions were analyzed on an Ultimate 3000 pump coupled to a QExactive HF-X following high pH fractionation. Raw data were searched using PD 2.4. Further data analysis was performed using Perseus, RStudio, Graphpad prism and QuPath programs.

Results
A total of 7,418 proteins were quantified across all samples. Unsupervised clustering on the primary samples, in which only proteins with the highest variation were used and samples with low tumor content were filtered out, defined four patient groups. These four patient groups showed differences in both clinical and histopathological parameters. Comparison of the short- and long-survival patient groups identified several differentially expressed proteins within the primary and metastatic tumors, separately. Datasets associated with these proteins were subjected to ROC curve and Kaplan-Meier analyses. In primary tumors, we identified 19 proteins (e.g. SLC25A44 and SUN2) associated with "Disease Specific Survival", while in the metastatic samples 51 proteins (e.g. TBRG4 and PPT1) related to "Survival after Brain Metastasis Surgery" were found.

Conclusions
This study is based on a large and unique cohort of paired primary and metastatic tumors from LADC patients. Our findings provide insight into the biology of brain metastasis development in LADC and, through the identification of new biomarkers, may contribute to the improvement of patient stratification and personalized treatment.

PP03.116: High-throughput Cancer Proteomic Characterization using Data-independent Mass Spectrometry (DIA-MS)

Yeh C[1,2], Perner S[1,2], Ji Y[3], Münch S[2], Enßle J[2], Urlaub H[3], Oellerich T[1,2,4], Pan K[1,2]
[1]Frankfurt Cancer Institute, [2]University Hospital Frankfurt, Department of Hematology/Oncology, [3]Max-Planck-Institute of Biophysical Chemistry, [4]German Cancer Consortium/German Cancer Research Center

Introduction: Multiplexed proteomics using data-dependent acquisition (DDA) has become one of the primary tools for tumor characterization and drug target discovery. Although the emerging DIA approaches have shown advantages over DDA for improved data completeness, proteome coverage, and overall throughput, their applications in FFPE tumor tissues are less reported. Therefore, we developed DIA workflows and evaluated their performance for high-throughput cancer proteome characterization. Besides, we established DIA-based thermal proteome profiling for drug target identification.
Methods: We used the standard HeLa digest for establishing the DIA workflow. For a side-by-side comparison, we prepared and analyzed diffuse large B-cell lymphoma (DLBCL) FFPE tissues using TMT-labeled DDA and label-free DIA (with or without employing ion mobility). In addition, ibrutinib-treated DLBCL cell lines were analyzed after heat-treatment and determined the protein thermal stability shift as compared to the untreated cells.
Results: Our library-free-DIA pipeline outperformed DDA, identifying and quantifying more than 7000 and 5000 HeLa proteins using a one-hour or 30-min run, respectively. We further demonstrated that label-free DIA reached comparable proteome coverage of DLBCL FFPE tissues than multiplexed TMT-labeled DDA. Using field asymmetric ion mobility spectrometry (FAIMS) with multiple compensation-voltage steps in a single DIA run reduced sample complexity and increased proteome coverage. Compared to multiplexed DDA with prior peptide prefractionation, single-shot FAIMS-DIA analysis identified 17% more proteins with comparable LC-MS instrument time per sample. Thermal proteome profiling showed the thermal shift of the drug binding proteins and assist Ibrutinib binding proteins and their related proteins identification.
Conclusions: Compared to the multiplexed DDA analysis, DIA-MS methods simplified sample preparation workflow, lowered instrument time, and decreased total cost, resulting in better data quality and reduced missing values. Our data demonstrated DIA-MS's capability for high-throughput cancer characterization and biomarker and drug target discovery.

PP03.117: Exploration Towards the Cancer Protein Biomarkers with Mutations upon Omics Data

Zhang X[1,2], Liu J[1], Zhu Y[1], Ren Z[1], Liu S[1]
[1]Department Of Proteomics, Beijing Genomics Instituion (bgi)-shenzhen, [2]College of Life Science, University of Chinese Academy of Sciences, Beijing, China

Introduction: The Cancer Genome Atlas (TCGA) to colorectal cancer (CRC) has shown approximately 93% of single nucleotide variants (SNVs), whereas most of them have been not verified at transcriptional or translational level. A primary question for the missed detection of SNVs is lack of an appropriate approach that accurately traces the SNVs delivery from genome to transcriptome. An integrated pipeline was postulated, which combines the current RNA-seq techniques and focuses on how to accurately find the transcript SNVs through iterative treatment then facilitates identification of mutant proteins.
Methods: HCT116, a typical CRC cell line, was taken and its omics was measured in parallel, including genome, transcriptome and proteome. Next generation sequencing (NGS) and third generation sequencing (TGS), were employed to gain RNAseq data. The TGS-based transcripts were used as a genomic ruler for NGS to define the relatively true SNVs which treated as a training set in machine learning. A prediction model thus was established to discriminate true and false SNVs in the NGS transcripts data, which then was generated to a SNVs library for identification of mutant proteins.
Results: A total of 5548 SNVs were identified from HCT116 transcriptome made from NGS, in which 4879 were directly delivered from genomic level and 669 were only detected at transcriptional level. More importantly, 349 single amino acid variants (SAVs) were verified by LC MS/MS based on peptide search against the SNVs dataset that was built by the pipeline developed in this study. In contrast to the rate of SAVs identification upon TCGA SNVs dataset with 3.4%, it obtained from the pipeline was significantly improved to 6.29%.
Conclusions: The new pipeline, therefore, enables more accurate identification of SNVs and facilitates exploration of cancer biomarkers related to SAVs.

PP03.118: Deep Multi-omics Analysis Reveals the Impact of Blood Collection Methods on Molecular Profile of Plasma Samples

Ahadi S[1], Szoke B, Lohr S, Braithwaite S, Lehallier B
[1]Alkahest

Introduction

Recent advances in omics technologies have revealed deeper understanding of biological molecules in different types of samples. However, each omic assay individually, although powerful, cannot provide a whole picture of mechanisms and pathways of complex biological events. Integrative analysis of multi-omics data can empower pathways identified in diseases with identification of molecules from different classes such as metabolites, lipids, proteins and genes. Although there are multiple reports on multi-omics analysis of biological samples, technical variation of omics measurements in different specimens has not been well-investigated.

Methods

Plasma as one the best resources for biomarker discovery has been studied extensively in different omics analysis. To better understand technical variability of omics' measurements in plasma samples, we have analyzed plasma samples collected through 3 different methods: plasmapheresis, EDTA and citrate recovery. Each plasma sample was analyzed with two proteomics platforms, metabolomics and lipidomics.

Results

Our data on proteomics analysis of three types of plasma collected from 82 individuals suggests that proteins measured in plasmapheresis samples are more correlated with the ones in plasma recovered from citrate tubes. The lower correlation is observed mainly between less-abundant proteins. Based on principal component analysis proteins measured in Olink and SomaLogic assays, the method of plasma collection explains most of the variance in the proteome. Linear modeling of proteomics data and variables that are known to affect the plasma proteome such as age, sex, BMI and race suggests that samples collected with plasmapheresis explain more biological variance in the data compared to EDTA and citrate samples. Variance decomposition of other omes resulted in similar patterns in all three collections.

Conclusions

Altogether, our results demonstrate profound effects of plasma collection methods on the omics signal and a better understanding of these effects will help to shed light on unknown biological processes previously masked by technical noise.

PP03.119: Discovery of Plasma Modulators of Brain Ageing as a Novel Therapeutic Target for Alzheimer's Disease using DIA Proteomics

Anastasi F[1,2], González Escalante A[1,2], Cumplido-Mayoral I[1,3], Ortiz-Romero P[1,2], Vilor-Tejedor N[1,3,4,5], Gispert J[1,2,6,7], Borràs E[3,4], Sabidó E[3,4], Suárez-Calvet M[1,2,3,8]
[1]BarcelonaBeta Brain Research Center (BBRC), Pasqual Maragall Foundation, [2]Hospital del Mar Medical Research Institute (IMIM), [3]Universitat Pompeu Fabra, [4]Centre for Genomic Regulation (CRG), The Barcelona Institute of Science and Technology, [5]Department of Clinical Genetics, Erasmus University Medical Center, [6]Centro de Investigación Biomédica en Red de Fragilidad y Envejecimiento Saludable, [7]Centro de Investigación Biomédica en Red de Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN), [8]Servei de Neurologia, Hospital del Mar

Introduction. Parabiosis experiments in mice identified circulating blood proteins that have an effect on brain ageing and thus exhibit a great potential as Alzheimer's disease therapeutic target in humans. We aim to identify human circulating proteins able to modulate brain ageing.

Methods. Using magnetic resonance imaging data from cognitively unimpaired individuals of the ALFA cohort (N=1414) we computed the brain-age of these subjects. Individual brain-age is estimated and subtracted from chronological age (DeltaAge) to identify individuals with extreme phenotypes (accelerated/decelerated brain aging) stratified by sex (n=85 each group). We also recruited healthy individuals with extreme chronological age [30 young (18-25yo) and 30 old (70-84yo)]. We then optimized a high-throughput proteomics workflow for the analysis of plasma based on StageTip protein digestion followed by a 60 minute LC gradient in a 15 cm column and data-independent-acquisition (DIA) in a tribrid mass spectrometer (Orbitrap Eclipse, Thermo). Data analysis was performed with DIA-NN. To define the differences in the plasma proteome related to age we initially applied the optimized DIA workflow to the analysis of plasma samples of the extreme chronological age individuals. Proteins whose expression is linked to ageing are identified by comparing their relative abundances among the extreme groups.

Results. We quantified around 3500 precursors and 300 unique proteins consistently in all samples and identified the proteins that significantly change with chronological age. Extreme brain-age groups plasma samples are also being analysed to identify proteins exhibiting brain age related changes.

Conclusions. We are establishing the plasma proteome that changes according to chronological and brain ageing by applying a DIA proteomics workflow. To achieve this, we used both an extreme chronological and an extreme brain-age groups. These analyses will be integrated with plasma metabolomics of the same subjects to better unravel the mechanisms associated with variability in the rate of brain aging.

PP03.120: Internal TMT-Based Standard Curve for the Absolute Quantitation of Plasma Proteins by Parallel Reaction Monitoring Mass Spectrometry (PRM-MS)

Gaither C[1], Popp R[1], Mitsa G[2,3], Zahedi R[4,5], Borchers C[2,3,6]
[1]MRM Proteomics Inc., [2]Division of Experimental Medicine, McGill University, [3]Segal Cancer Proteomics Centre, Lady Davis Institute, Jewish General Hospital, McGill University, [4]Department of Internal Medicine, University of Manitoba, [5]Manitoba Centre for Proteomics and Systems Biology, [6]Gerald Bronfman Department of Oncology, Jewish General Hospital, McGill University

Introduction

We investigated the use of TMT-labeled light (natural) peptides to replace stable-isotope labeled internal standards for absolute plasma-protein quantitation. The isobaric nature of TMT allows multiplexed analysis while microflow LC requires low sample label amounts, thus decreasing analysis costs. Several channels of the multiplexed TMT reagent are used to generate calibration curves, while the remaining channels are used for sample analysis.

Methods

We used 125 synthetic light peptide surrogates to quantify 125 mouse-plasma proteins. Using an 11-plex reagent, eight aliquots of the synthetic light peptide mixture at different concentrations were labeled with 8 different TMT labels of the TMT 11-plex reagent. Mouse plasma digests were labeled with the remaining three TMT labels. The standard-curve peptides and the TMT-labeled plasma digests were combined and analyzed by PRM on an Evosep Q Exactive Plus in a single run. Data were quality-controlled using Proteome Discoverer. Reporter-ion peak areas were evaluated and exported from Skyline. Peptide concentrations were calculated using regression lines from Microsoft Excel.

Results

Standard curves were generated for the quantitation of 113 of the 125 targeted mouse-plasma proteins using this novel method which combines the accuracy and precision of internal standards with the multiplexing capability of isobaric labeling. Reconstructed-ion-chromatogram peak areas from five of the six calibration channels allowed the generation of standard curves with R2 of >0.9. Ninety-nine endogenous proteins were within the linear ranges of the calibration curves.

Conclusion

Absolute plasma protein concentrations were determined by LC/PRM-MS using synthetic peptides labeled with isobaric TMT-labeled peptides as internal-standards. This novel TMT-based calibration curve approach provides absolute “bottom-up” protein calibration without stable isotope-labeled standard peptides and external calibration curves. This method also reduces LC-MS run time by 8 to 10-fold compared to conventional strategies and absolute quantitation can be performed on hundreds of different peptides within the same LC-PRM run.

PP03.121: The Study of Plasma Proteome as a Measure of Personalized Human Health and Fitness

Borràs E[1,2], Mallol M[3], Rodas G[3], Sabidó E[1,2]
[1]University Pompeu Fabra, [2]Centre for Genomic Regulation, Barcelona Institute of Science and Technology, [3]Barça Innovation Hub, Medical and Performance Department, Futbol Club Barcelona

Introduction

Plasma proteome is a distinctive measure of each person, it defines its health status and it deviates as individuals abandon this state. Disruption of health state can be caused by disease and life style-related factors like exercise. Professional athletes represent a unique cohort to closely monitor the fitness progression. In this work, we obtained plasma proteome individual profiles for a professional team of men's futsal in response to acute and long-term exercise throughout a sport season.

Methods

Plasma from 11 athletes was collected at the beginning and at the end of the season at three points: pre-match, post-match, and after 24-hour rest. Samples were precipitated and digested previous to mass spectrometry analysis (Orbitrap Fusion Lumos, EASY-nLC 1200). Samples were analyzed in data-independent acquisition (DIA) mode (40 windows of 10 Da, 500-900 m/z) using a library-free strategy with DIA-NN. Athletes were also monitored with GPS, and external load, heart rate and rated perceived exertion (RPE) data has been collected.

Results

Individual plasma proteome profiles were obtained for a professional team of men's futsal from Futbol Club Barcelona (Barça) in response to acute and long-term exercise throughout a sport season. We show that athletes present an individualized profile of their plasma proteome distinct from the rest and identifiable throughout the season. Some collective proteome responses to acute exercise are common to the whole cohort, however, not all athletes respond equally to a given training load and plasma proteome profiles exhibit different recovery trajectories for each individual. Finally, we evaluated how the response to acute exercise evolved along the season as a measure of an athlete fitness.

Conclusions

Plasma proteome profiles from professional athletes is a good read-out to acute exercise response and recovery, and they enable to closely monitor health status and fitness.

PP03.122: Discovery of Protein Signature of Early-stage Recurrent Lung Adenocarcinoma by Plasma Proteome Profiling

Chen Y[1], Lin K[1], Hsiao Y[1,2], Chang G[3], Chen J[4], Yu S[2], Chen Y[1]
[1]Institute of Chemistry, Academia Sinica, [2]Department of Clinical Laboratory Sciences and Medical Biotechnology, College of Medicine, National Taiwan University, [3]Institute of Medicine of Chung Shan Medical University, [4]Department of Surgical Oncology, National Taiwan University Cancer Center

Introduction: Lung cancer is the leading cause of cancer mortality worldwide and proximately 30% of early-stage patients still experience recurrence and distant metastasis. A proteome-based classification revealed a new subtype, “late-like” in early-stage patients, characterized by proteome profile resembling that of late-stage patients and aggressive clinical feature and further confirmed with high recurrence (1). To verify the potential protein biomarkers in identification of high-risk early-stage patients for early treatment, we establish plasma proteome profiles to define a panel of biomarker candidates to discriminate early late-like patients.

Methods: Plasma samples from 26 early-stage IA and IB of lung adenocarcinoma patients were collected from hospitals. The top 12 high abundant proteins were depleted and the supernatant were performed by in-solution tryptic digestion. The peptides were desalted and analyzed by data-independent acquisition (DIA) mass spectrometry. Two IB-late-like plasma samples were performed by data-dependent acquisition (DDA) and processed as the spectra library for DIA.

Results: The preliminary result showed that 3326 proteins were quantified by library-based DIA analysis from 26 plasma samples of individual lung adenocarcinoma patients with early-stage IA and IB. The refined stage, IB late-like, among these patients was referred from our previous proteogenomic study (1). Among 138 differentially expressed proteins significantly enriched by ANOVA ($p<0.05$), 18 proteins show dramatical up-regulation in IB late-like cohort compared to stage IB (fold change >2 and Student un-paired T-test $p<0.05$). These protein candidates will be further validated by ELISA assay in another independent cohort. In addition, we will also design a targeted assay for protein candidate panel for early detection and treatment of recurrent lung cancer patients.

Conclusions: DIA-MS-based proteomics facilitates discovery and validation of plasma biomarker candidates for high-risk early-stage lung cancer patients.

(1) Chen, et al. Cell. 2020. 182, 226-244.

PP03.123: Proteolytic Signatures in Plasma Associated with Activation of the Coagulation Cascade

Del Castillo Alferez J[1], van Alphen F[1], van der Zwaan C[1], Bakhtiari K[1], van den Biggelaar M[1], Hoogendijk A[1], Meijers J[1], Meijer A[1]
[1]Sanquin

Introduction:
Regulation of the hemostatic system is accomplished by activation and inhibition of pro and anticoagulant processes. This interplay is largely mediated by limited proteolysis of cofactors and serine proteases. Proteolytic events result in the formation and dissolution of a fibrin clot. Although many individual reactions within hemostasis have been well characterized, comprehensive insight into their combined action is lacking. Here, we aim to assess the proteolytic events in plasma that are associated with hemostasis.

Methods:
Citrated plasma from healthy individuals was recalcified and the extrinsic pathway of coagulation was initiated with 7pM Tissue Factor in the presence and absence of platelets. Resulting endogenous peptides were extracted using differential solubilization[1] and analyzed by nano-LC coupled to an orbitrap mass spectrometer (Fusion ThermoFisher) operating in data-dependant mode. LC-MS/MS spectra were interpreted with PEAKSX De Novo algorithm. Identified peptides were mapped in silico against the Human UniProt database.

Results:
Upon activation of the coagulation cascade by tissue factor, we observed the formation of peptides which included (truncated) variants of activation peptides of amongst other fibrinogen and factor XIII. In addition, we identified fibrinogen-derived peptides that could be mapped to cleavages by thrombin and plasmin. Activation of coagulation's extrinsic pathway lead to proteolytic processing of protease inhibitors such as SerpinA1, SerpinC1 and Alpha-2-macroglobulin. In the presence of platelets, we observed an increased number of proteolytic peptides of prothrombin and members of the complement system including CO4A, CO4B,CO7 and VTNC.

Conclusions:
Our data reveal the general impact of coagulation on plasma proteins. We found expected and novel proteolytic events upon activation of the coagulation cascade. Our results demonstrate that peptidomics can be leveraged to obtain functional insights of processes where proteolysis is involved and may be a useful approach to identify modulators of hemostasis.

References:
1. Kawashima Y., et al., J Proteome Research., 2010.

PP03.124: Fully Automated Proteomics Workflow for Liquid Biopsies

Ellis C[1], Moritz C[1], Abreha M[1], Ellis C[1], Demianova Z[1]
[1]PreOmics GmbH

Introduction

Liquid biopsy (LB) is a convenient, non-invasive alternative to surgical biopsies. LB, such as plasma, serum, and cerebrospinal fluid, have been increasingly used for genetic and epigenetic studies in oncology and other clinical applications. Notably, LB analyses with mass spectrometry-based tools could offer a valuable, easily accessible source of biomarkers and evidence for elucidating pathological processes. To unleash this potential, robust sample preparation tools and continuous improvements in LC-MS and data analysis are needed. Here we introduce a proteomics workflow for liquid biopsies integrated into a fully automated sample preparation platform.

Methods

Biological fluids from human, mouse, and rat were obtained from BioIVT. Samples (100 µg total protein per sample) were processed using the iST-BCT kit on the PreON automation platform as per PreON software v10.7 instructions. 300ng of peptides were analyzed on EASY nLC 1200 coupled with timsTOF using a 45 minutes gradient. Data were processed using the MaxQuant and Perseus software.

Results

The workflow here presented includes biological fluids lysis, reduction, alkylation, digestion, and peptide cleanup steps automated on the PreON.

Preliminary results showed that over 350 protein groups were identified in undeleted plasma with minimum missed cleavage sites, an excellent alkylation rate of cysteines, and minimal artificial modifications.

Samples processed with the PreON platform showed intra-day and inter-day great reproducibility. Hands-on time was reduced to less than 5 minutes, compared to the ~60 minutes required for the manual preparation with the iST-BCT kit. This solution is ideal for low- to mid-throughput analyses, with up to 36 samples potentially processed per working day.

Conclusions

Here we introduced a fully automated solution tailored to various liquid biopsy types, such as plasma, serum, and CSF. The presented low- to the mid-throughput solution can be seamlessly integrated into laboratories working on biomarker discovery in oncology and other relevant clinical fields.

PP03.125: A Highly Scaled Proteomic Discovery Study for Prostate Cancer Diagnostic Signatures Using Proteograph Technology with Trapped Ion Mobility Mass Spectrometry

Flory M, Chang M, Cartier J, Lange J, Kopp R, Liss M, Leach R, Albracht B, Dai J, Krawitzky M, Elgierari E, Chu J, Cuevas J, Pease P, Mahoney M, Ferdosi S, Benz R, Siddiqui A, Zamanighomi M, Guturu H, Hornburg D, Batzoglou S

Introduction: The low cancer specificity of Prostate-Specific Antigen (PSA), the principal blood biomarker for prostate cancer detection, leads to a high frequency of unwarranted prostate biopsies. To alleviate this deficit, we initiated a proteomic discovery study seeking PSA reflex signatures using the Proteograph™ platform, a multi-nanoparticle-based deep plasma proteomics workflow, with timsTOF Pro mass spectrometry (Bruker) to interrogate over 900 serum specimens from patients referred for biopsy based on elevated PSA and/or abnormal digital rectal exam.

Methods: Our study followed rigorous design principles including separate training and validation sets and specimen randomization and blinding. Specimens were processed with the Proteograph Product Suite. Liquid chromatography-mass spectrometry (LC-MS) analyses leveraged the Bruker timsTOF Pro MS platform utilizing 30-minute reversed-phase chromatography and a label-free dia-PASEF (data independent acquisition - parallel accumulation serial fragmentation) data acquisition method. Peptides deriving from a chosen subset of specimens were pooled, fractionated and analyzed using DDA (data-dependent acquisition)-PASEF to build a spectral reference library.

Results: The DIA-NN algorithm was employed through the cloud-based Proteograph Analysis Suite (PAS) to search all LC-MS data. Proteomic depth achieved was approximately 2500 protein groups per specimen and more than 5500 across the study. Following dataset normalization, machine learning approaches will be applied to identify both single markers and multi-marker signatures with analytic thresholds set to retain the high sensitivity of PSA while improving the ability to specifically detect all and/or higher-grade prostate cancer.

Conclusions: Our data demonstrate remarkable proteomic depth achievable in a highly scaled patient serum specimen discovery study using a combination of Proteograph and timsTOF platforms. This significant effort has the potential to uncover new diagnostic signatures, in particular among the low-abundance serum proteome, with improved specificity for prostate cancer and thus reduce the current high frequency of unnecessary prostate biopsy.

PP03.126: Proteomic Study on IMMODIN, a Commercially Available Dialysable Leukocytes Extract Prepared from Disintegrated White Blood Cells of Healthy Human Donors

Zavala Meneses S[1,2], Zuniga Navarrete F[3], Zelnik V[3], Kopacek J[3], Fucikova A[4], Skultety L[1,3]
[1]Institute of Microbiology of the CAS, [2]Charles University, Faculty of Science, [3]Biomedical Research Center of the SAS, [4]Military Health Institute, Military Medical Agency
Introduction: One of the most significant public health challenges of the 21st century is the prevention and treatment of infectious diseases caused by various microorganisms, including resistant bacteria and viruses. With aging, there is a progressive alteration of the immune system and its responses that may result in immune senescence and immunodepression. Nevertheless, immune modulators can speed up recovery. IMMODIN® is a commercially available dialysable leukocyte extract (DLE) prepared from disintegrated white blood cells of healthy human donors. It is used clinically as a prophylactic or therapeutic agent when cell-mediated immunity plays a key role.
Methods: In our study, the DLE was prepared by corporate pharmaceutical companies Sevapharma (Prague, Czech Republic) and IMUNA Pharm (Sarisske Michalany, Slovakia) according to the protocol described by Cardoso (1). Each ampoule was dissolved in 2% acetonitrile and then protein identification was performed on a tandem mass spectrometer coupled with nano-Acquity UHPLC (Waters).
Results: We identified dozens of unique proteins associated with blood cells or plasma. The highest number of proteins was related to innate immunity. There were identified receptors for parasites or microbes that have the ability to recognize the invaders and initiate their inhibition or elimination. We also detected proteins associated with inflammatory response, including those having the potential to inhibit cytokines and treat the cytokine storm. There were found even proteins that can speed up recovery by regulating cell growth and repair.
Conclusions: This proteomic analysis revealed that IMMODIN® is a cocktail of small proteins, peptides, and other previously described components with therapeutic and prophylactic potential for patients suffering from infectious or malignant diseases in which cell-mediated immunity may play a critical role.
Acknowledgments: This study was supported by a grant from the Slovak Research and Development Agency (APVV-15-0720 and APVV-19-0519).

1. Cardoso, FM., et al. 2017 J Pharm Biomed Anal 138:100–108.

PP03.127: dia-PASEF for Targeted Proteomics: Development of Large-scale Assay for Quantitation of more than 500 Proteins in Human Plasma Sample

Ghose S[1], Kaspar-Schoenefeld S[1], Gandhi T[2], Mueller S[2], Echevarria-Zomeno S[2], Lubeck M[1], Raether O[1], Kruppa G[1]
[1]Bruker Daltonik GmbH & Co KG, [2]Biognosys

Introduction:
dia-PASEF merges the benefits of DIA with the advantages of ion mobility in proteomics experiments making it an advantageous method to be integrated in a platform for large-scale biomarker studies without the need for in-depth method optimization. Here, we use dia-PASEF in combination with the PQ500 kit to develop a targeted quantitation assay for peptides in human plasma sample.

Methods:
Plasma samples were digested using the iST kit from PreOmics. The PQ500™ kit (Biognosys) was spiked into the prepared digests. Tryptic peptides were separated on a nanoElute system coupled to a timsTOF HT mass spectrometer via a CaptiveSpray source using a 30-min ACN gradient. For the dia-PASEF acquisition, a window placement scheme consisting of 6 TIMS ramps with 3 mass ranges per ramp was applied. Data was processed in Spectronaut (v16, Biognosys) using an ion mobility annotated PQ500 library.

Results:
Here, we developed a targeted quantitation assay for human plasma proteins using dia-PASEF. The major advantage of the approach is that there is no need for tedious method development as is typically required for targeted approaches like SRM and MRM.
The assay was applied to a proof-of-concept study of non-depleted plasma samples from patients diagnosed with lung cancer. In total, 663 peptides and 463 protein groups were identified and quantified, covering around 80% of the PQ500 panel. Of those, 55 proteins were found to be significantly regulated with three of them (Fibronectin, Immunoglobulin lambda-like polypeptide 1, Immunoglobulin lambda-like 1 light chain) detected to be higher abundant in healthy donors.

Conclusions:
We developed a workflow for targeted quantitation in non-depleted human plasma using dia-PASEF and applied it to a biologically relevant lung cancer study. Significantly regulated peptides are known to be associated with cancer, which confirms the potential of applying the presented approach to clinical research studies.

PP03.128: Models to Predict Pre-Analytical Variation

Astling D[1], Drolet D[1], Gogain J[1], Hagar Y[1], Sampson L[1], Soucie K[1], Trinder K[1], VonCarlowitz I[1], Westacott M[1]
[1]Somalogic Operating Co., Inc.

Introduction: In biomarker discovery, it is critical to assess any pre-analytical variation (PAV) in order to avoid artificial bias in the intended measurements. PAV may arise from both avoidable and unavoidable factors, resulting in misleading data and incorrect conclusions. Proteins, in particular, are vulnerable to variation in collection methods, storage temperatures, and processing protocols. It is vitally important to understand this PAV when analyzing samples using protein assays.

Methods: Human EDTA plasma and serum samples, subjected to standardized sample processing methods, with distinct excursions from ideal collection, were assayed on the SomaScan Platform measuring ~7,000 analytes. Using machine-learning methods, these quantitative protein measurements were compared to sample processing truth standards (eg, time-to-spin) to create predictive models. These models, termed SomaSignal tests, were developed to enable the assessment of PAV related to processing methods.

Results: SomaSignal tests have been developed to predict time-to-spin, time-to-decant and time-to-freeze, reported in the number of hours, for both plasma and serum. Models that predict the number for freeze/thaws a sample has been subjected to, have also been developed. All eight models had Lin's CCC and R2 values greater than 0.90 in hold-out validation datasets. In addition to these sample handling predictions, effect size calculations for all ~7,000 measurements have been determined for multiple time points, or freeze-thaw cycles, for each model.

Conclusions: SomaLogic has developed a unique class of PAV models that are able to assess variation related to processing methods. Results from these predictions can be used during biomarker evaluations to exclude samples due to apparent excessive delay in processing, identify collection site bias for current and future analysis and identify sample groupings that may impact analysis. Further, knowing the effect size metrics for all measurements could also enable the removal of specific analytes from modeling and/or be used as covariates in model development.

PP03.129: Evaluation of Sample Preparation Steps in an Automated Plasma Proteomics Workflow for the PrHADAA Study

Guerrette J[1], Choi M[1], Arul A[1], Oliver N[1], Patterson K[1], Tindal J[1], Hansen S[2], Libby J[2], Dumistrescu L[2,3], Hohman T[2,3], Jefferson A[2,4], Zhang K[5], Du L[5,6], Barnes L[7], Manly J[8], Beckman J[9], Robinson R[1,2,4,10,11]
[1]Department of Chemistry, Vanderbilt University, [2]Vanderbilt Memory and Alzheimer's Center, Vanderbilt University Medical Center, [3]Vanderbilt Genetics Institute, Vanderbilt University Medical Center, [4]Department of Neurology, Vanderbilt University Medical Center, [5]Department of Biostatistics, Vanderbilt University Medical Center, [6]Vanderbilt Center for Quantitative Sciences, Vanderbilt University Medical Center, [7]Department of Neurological Sciences, Rush University Medical Center, [8]Department of Neurology, Columbia University Medical Center, [9]Department of Medicine, Vanderbilt University Medical Center, [10]Vanderbilt Institute of Chemical Biology, Vanderbilt University Medical Center, [11]Vanderbilt Brain Institute, Vanderbilt University Medical Center

Introduction: Large-scale proteomic studies can help clarify underlying biochemical pathways that link hypertension and Alzheimer's disease (AD) in African Americans. We initiated the Proteomics of Hypertension and Alzheimer's Disease in African Americans (PrHADAA) study to establish the molecular signatures of both conditions. An automated plasma proteomic sample preparation workflow was applied to four large-scale (N>100) cohorts to summarize aspects of the study design, the variation of each preparation step, and assess robust quality control (QC) metrics.

Methods: Plasma samples from the Religious Orders Study and Rush Memory and Aging Project, Minority Aging Research Study, Rush African American Clinical Core (N=859), Southern Community Cohort Study (SCSS) (N=808), and Vanderbilt Memory and Aging Project (N=35) were randomized and double-blinded into batches of 16-18 samples, with embedded QCs. Samples were subjected to immunodepletion, digestion, tandem mass tag (TMTpro) labeling, and fractionation using an automated robotic liquid handler. QC metrics were assessed at different stages in the sample preparation workflow.

Results: To ensure consistent analysis of the 1702 plasma samples throughout the entire project, design of the proteomics workflow across sample cohorts was considered. QC samples were included throughout to monitor established QC metrics such as: immunodepletion efficiencies (>93%); reproducibility of chromatographic retention times on a MARS-14 column (<5% coefficient of variation [CV]); protein amounts (0.19-5.32 µg/µL); digestion and TMTpro labeling efficiencies; and reproducibility of high pH reversed phase fractionation. These efficiencies were calculated using liquid-chromatography tandem mass spectrometry data of ~300 proteins routinely measured in plasma.

Conclusions: The establishment of a robust platform for sample preparation has enabled lower variation across participant plasma samples in diverse cohorts, allowing samples to aid in the understanding of the molecular contributors that link hypertension and AD.

PP03.130: Enhanced Assay and Computational Workflows Enabling Next Generation Nanoparticle-based Plasma Proteomics with Improved Detection of Low Abundance Chemokines

Hornburg D[1], Ferdosi S[1], Stukalov A[1], Hasan M[1], Lee-McMullen B[1], Siddiqui A[1], Farokhzad O[1], Batzoglou S[1]
[1]Seer

Introduction

To overcome limitations for accessing the complex, high dynamic range of proteomes in samples like blood plasma in large cohorts, we have developed panels of functionalized nanoparticles (NPs) engineered to interrogate the entire dynamic range of proteomes in an automated process. Introduced into biological matrix, NPs form selective, specific, and reproducible protein coronas at the nano-bio interface driven by proteoform-NP affinity, protein abundance, and protein-protein interactions. A combination of NP-engineering, optimized LC-MS based workflows, and machine learning provides new avenues to tailor NP designs for deeper interrogation of proteomes, low abundance chemokines, and proteins containing posttranslational modifications.

Methods

We investigated 135 distinct protein coronas from functionalized NPs using a Bruker timsTOF Pro LC-MS with diaPASEF method. To explore the adsorption-desorption dynamics, we tested different combinations of corona formation equilibration times and increasingly limited binding surfaces. Based on the intensity distributions of 3,184 proteins we evaluated precision of corona formation for all assay conditions and modeled protein corona dynamics.

Results

We demonstrate that NP capture posttranslationally modified peptides and an improved dynamic range, at high precision of quantification. Specifically, thousands of proteins, including low abundance cytokines and chemokines are increasingly and robustly captured under competitive assay conditions. Using UMAP and annotation enrichment, we probed the physicochemical and functional proteome space quantitatively captured at different corona formation assay conditions identifying protein families and proteins with distinct physicochemically characteristics.

Conclusions

Combining machine learning with deep quantitative characterizations of protein coronas provides a new avenue for deeper NP-based plasma proteomics workflows capturing proteins and proteoforms broadly and tailored strategies to sample subsets of the proteome like posttranslational modifications.

PP03.131: Aptamer-based Analysis of Plasma Proteome of Growing Tumours

Janjic N[1], Gupta S, Westacott M, Ayers D, Schneider D, Drolet D, Karimpour-Fard A, Hunter L
[1]Somalogic, Inc.

In most cases, detecting malignancies at an early-stage increases treatment options, minimizes the risk of metastases and dramatically improves long-term survival. Nevertheless, tumours are typically discovered at more advanced stages, either with onset of symptoms or as part of unrelated procedures. Non-invasive means of detecting tumours in systemic circulation has long held considerable appeal, but also enormous challenges. A major difficulty is related to the need to detect a small volume of a malignancy remotely, after massive dilution of tumour-associated analytes in the total circulating volume of blood. To date, such “liquid biopsies” have mainly focused on the identification of genetic material unique to transformed cells, typically derived from circulating tumor cells, or cell-free DNA. With proteins, the presence of a tumour is more often accompanied with changes in the levels of endogenous, unmutated proteins in circulation. In this context, knowing which proteins represent the earliest markers or tumour presence would be enormously useful. So what are the first tumor-associated proteins that can be detected in blood as the initial transformed cells establish residence in their tissues of origin? To answer this question, we used aptamer-based SomaScan assay to monitor the time course of changes in the plasma proteome of mice carrying transplanted human tumours. Our study shows that tumour cells representing human lung, ovarian, breast and colon cancer produce both unique and common sentinel proteomic signatures in plasma that could be used to identify both the early presence as well as the identity of tumours growing remotely. These proteins provide the basis for early detection as well as insights into the basic biology of tumour growth.

PP03.132: An Automated End to End Sample Preparation Platform for Human Plasma Proteomics Analysis

Renuse S[1], Hakimi A[1], Reeber S[1], Bahten K[1], Zheng R[2], Boychenko O[2], Horn D[1], Lopez-Ferrer D[1]
[1]Thermo Fisher Scientific, [2]Thermo Fisher Scientific

INTRODUCTION

Plasma continues to be one of the major biological fluid used for diagnostics and prognosis of diseases, and health monitoring of various physiological aspect of human health. As the throughput of data analysis is increasing, a comprehensive hands-off solution for sample preparation of human plasma is becoming essential to gain deeper insight into the biological information contained in the human plasma. Here we describe an automated end to end and hands-off workflow reducing the potential for human error and increasing the reproducibility and quality of plasma processing for bottom-up mass spectrometry analysis.

Methods

A plate containing plasma samples was placed into the liquid handler. Custom reagents were pre-made as a batch to ensure batch to batch consistency and ready-for-use format including all chemicals required for lysis, reduction/alkylation, digest, peptide measurement, and cleanup. The processed samples were analyzed in an Orbitrap Exploris™ 480 mass spectrometer coupled to Ultimate 3000 UHPLC system. Proteome Discoverer™ software was used for data analysis.

Results

Plasma from one source was used to evaluate the variation introduced through the liquid handler, new chemistry, and the automated processing steps. The characteristics evaluated included digestion efficiency, alkylation efficiency, in vitro artifactual modification introduced during the sample processing, peptide concentration, peak characteristics peptides. Additionally, the ease of use and the user experience were also evaluated to assess the value of automating sample preparation. Digestion efficiency was >90%, reduction/alkylation efficiency was >90%, and non-specific modification <1%. Manual tasks required <20min (aliquoting samples into the sample input plate and resuspension of the clean peptides). More importantly, the CV among the 34 samples was below 10%.

Conclusions

This analysis demonstrates a robust platform for plasma analysis. The platform also offers expandability into other more complex sample preparation steps for plasma analysis, such as protein-protein interaction and PTM analysis.

PP03.133: Quantitative Multiplexed Comparison of Colon Cancer Cell Line Through Automated TMT Sample Preparation and Improved Tribrid Analysis

Renuse S[1], Reeber S[1], Bahten K[1], Zheng R[2], Horn D[1], Huguet R[1], Bergen D[1], Huang J[1], Zabrouskov V[1], Lopez-Ferrer D[1]
[1]Thermo Fisher Scientific, [2]Thermo Fisher Scientific

INTRODUCTION

Cancer cell lines have played a key role in understanding the biology underlying various cancers and as screening tools to identify drug targets. As technologies continue to improve to study cellular mechanisms from bulk cell analysis to single cell to single molecules, mass spectrometry based proteomics technologies have also continued to increase its sensitivity and speed. As the throughput for analysis is increasing, method for high quality samples preparation have also become essential. Here we described an end to end solution for quantitative MS analysis combining automated sample preparation with a new mass spectrometer.

Methods

Sixteen different colon cancer cell lines were grown in four different batches and harvested in four different dates as replicates. AccelerOme TMTproTM kit was used together with the AccelerOme system. Each sample was lysed using 50µl of lysis buffer and transferred to a 96 well plate. The 96 well plate was placed in AccelerOme where the samples were reduced/alkylated, digested, TMT labeled, and cleaned up completely hands-free. Analysis was performed in Orbitrap Ascend Tribrid Mass Spectrometer coupled to VanquishTM Neo UHPLC system. Acquired data was analyzed using Proteome DiscovererTM software.

Results

In quantitative analysis of large numbers of samples, quality of sample preparation, high confidence analysis with high throughput analysis are key aspects to address. AccelerOme system where the reaction chemistry and workflow have been optimized. Digestion efficiency >90% and TMT labeling efficiency >98% were achieved. The system reduced sample preparation time from 3 days of manual hands-on time to seven hour of hands-off time. The samples were analyzed in Orbitrap Ascend where architecture improvement has increased the ion management resulting in approximately 20% increase in total peptides quantified while reducing the analysis time 30%.

Conclusions

Above workflow provides a confident multiplexed quantitative solution for high throughput cellular analysis.

PP03.134: Towards a Human Disease Blood Atlas – A Pan-cancer Plasma Proteome Profiling using SIS Recombinant Protein Fragments and Targeted Proteomics

Kotol D[1]
[1]Science For Life Laboratory, KTH Royal Institute of Technology, [2]KTH Royal Institute of Technology, Division of Systems Biology, Department of Protein Science, School of Chemistry, Biotechnology and Health

Introduction

Proteins are biological molecules representing physiological state of an organism. Therefore, they are crucial to study in relation to health and disease. Even though, there are great efforts to map the protein content of blood plasma in relation to various disorders, a comprehensive disease map is lacking. Here, we present an endeavor with aim to quantify plasma proteome across 100 different diseases.

Methods

Up to 100 blood samples per disease have been collected in clinical setting. The protein quantification is performed using targeted proteomics powered by stable isotope standard protein epitope signature tags (SIS-PrESTs). The SIS-PrESTs are heavy labeled polypeptides each covering a single protein target while being individually purified and absolutely quantified. The standards can be pooled to cover thousands of tryptic peptides and stored at room temperature for long time in vacuum dried format. The plasma samples are added onto the dried standards as the first step followed by trypsin digestion and MS analysis which enhances the robustness and quantitative precision over time.

Results

The workflow quantified over 200 proteins across thousands of plasma samples with great analytical reproducibility over time. Potential biomarkers for various cancer types could be identified using machine learning (AUC = 0.96) and 56 already known FDA approved and other clinical biomarkers were part of the panel. The proteome profiles of all diseases are being integrated into a single dataset, which can be used to generate and evaluate prediction models for specific diseases.

Conclusions

The strategy using vacuum dried SIS-PrESTs for proteomics quantification is a great tool for robust protein quantification. This strategy allows for identification of high to medium abundant protein profiles in all major human diseases in the current Human Disease Blood Atlas endeavor. The pan-disease analysis strategy can have a major impact on how diseases are seen and diagnosed today.

PP03.136: Peptide-centric Analysis Identifies Defining Plasma Proteomic Signatures of Ischemic and Non-ischemic Cardiomyopathy Patients

McFadden M[1,2], Lee D[1,3,4], Lawler P[1,4,5], Ross H[1,4], Gramolini A[1,6], Billia F[1,4,6]
[1]Ted Rogers Centre for Heart Research, [2]Institute of Biomedical Engineering, University of Toronto, [3]ICES, University of Toronto, [4]Peter Munk Cardiac Centre, University Health Network, [5]Interdepartmental Division of Critical Care Medicine, University of Toronto, [6]Department of Physiology, Faculty of Medicine, University of Toronto

Introduction: Unpredictable trajectory of heart failure (HF) and subsequent timing of advanced therapies (e.g. transplant, mechanical circulatory support) limits clinical strategies to symptom response and management [1-3]. Biomarkers to anticipate impending decompensation allow for proactive initiation of interventions to improve quality of life and patient outcomes [4,5]. We propose applying a peptide-centric analysis workflow to bottom-up proteomics, instead of classic overall protein abundance, to preserve greater detail about the plasma proteome, and generate a larger pool of candidates for biomarker discovery.

Methods: Plasma was obtained from nonischemic (NICM, n=4) and ischemic (ICM, n=5) cardiomyopathy patients at two time points reflecting disease progression (decompensation) and when clinically stable. After albumin and IgG depletion, reduction, alkylation, and digestion, samples were fractionated by alkaline C18 SPE tips with an 8-step elution gradient, and analyzed on a Thermo EASY 1200 nLC and Q Exactive Plus.

Results: Using a classic protein-level approach, 328 proteins were present in at least 50% of samples, with 60 differential between etiologies ($p<0.05$). In contrast, by utilizing intensities for tryptic sequences there were 3232 peptides identified in at least 70% of samples, with 1237 differential ($p<0.05$). The peptide-level dataset better resolved patient groups by PCA, and provided a greater number of differential candidates. Comparison of peptide proteomes for each patient over time showed 182/4267 differential peptides for NICM and 302/3931 for ICM ($p<0.05$). Validation cohorts are currently being analyzed.

Conclusions: In addition to identification of etiology-specific indicators of HF trajectory, this work demonstrates advantages of peptide-centric analyses for monitoring changes in the plasma proteome.

References: [1] Ezekowitz et al. (2017). Can J Cardiol. 33:1342-1433; [2] Ponikowski et al. (2016). Eur J of Heart Failure. 18:891-975; [3] Yancy et al. (2013). J Am Coll Cardiol. 62:e147-239; [4] Chow et al. (2017). Circulation. 135(22):e1054-e1091; [5] Ibrahim et al. (2018). Circ Res. 123(5):614-629

PP03.137: Integrated Plasma Multi-omics using Nano-particle Technology and Single-shot Capillary MS

Moss C[1], He Y[1], Shishkova E[1], Overmyer K[2], Hill D[3], Williamson L[3], Coon J[1]
[1]University of Wisconsin-Madison, [2]Morgridge Institute for Research, [3]Seer

Introduction: Recently we described an integrated approach to combine the chromatographic and mass spectrometric analysis of complex peptide and lipid mixtures – nano Multi-Omic Single Shot Technology (nMOST). Here we describe the use of this method for the analysis of human plasma – the richest and easiest to obtain clinically relevant tissue. Preliminary data using nMOST has already allowed for the detection of over 1,600 proteins and lipids in a single, integrated two-hour LC-MS/MS experiment. Here we build on these results by leveraging the Seer nanoparticle technology that offers considerably increased proteomic sampling depth.

Methods: Undepleted, tryptically digested plasma peptides were loaded onto C18 reverse-phase (RP) BEH columns and separated at using nano-flow (0.075 mm I.D. column; 0.28 uL/min). Eluting analytes were ionized using electrospray and analyzed using an Orbitrap Eclipse mass spectrometer (Thermo). Two scan functions were used to achieve optimal data acquisition for peptides and lipids, which switched at 70% mobile phase B.
Building on this novel development of the MOST platform, we have extracted plasma proteins using the Seer Proteograph Product Suite into a single peptide sample. Lipids were extracted from the matched undepleted plasma using n-butanol. Peptides and lipids were then analyzed using nano-flow capillary LC with the MOST gradient.

Results: Using a single-shot, multi-omic method (nMOST) we have identified 1,604 molecules - 463 proteins and 1141 lipids - from a single plasma sample following a two-hour experiment. We are presently developing a method that should afford the detection of over 3,000 molecules in the same time-frame by combining the Seer nanoparticle technology, nMOST, and FAIMS.

Conclusions: We present a new technology that offers the ability to rapidly and deeply monitor human plasma across two critical molecular planes in a single format.

PP03.138: High-throughput Quantitative Peptidomics in Human Serum for Analysis of 1000 Samples per Day

Nakai A[1], Tomioka R[1], Ogata K[1], Ishihama Y[1,2]
[1]Graduate School of Pharmaceutical Sciences, Kyoto University, [2]National Institutes of Biomedical Innovation, Health and Nutrition

Introduction: Since serum or plasma is the most common clinical specimen and contains many molecules that reflect various disease states, it is required to obtain not only quantification targeting known molecules but also unbiased molecular profiles on an omics scale with high specimen throughput. However, serum proteomics requires selective depletion of proteins at high concentrations and has yet to produce outputs that meet expectations. On the other hand, peptidome fractions with MW below 10,000 also contain molecular profiles that reflect various disease states, and bioactive peptides have been identified in a variety of proteoforms. In this study, with the aim of developing a platform for analysis of 1000 samples per day, we investigated conditions that maximize peptide identification efficiency and quantification accuracy under gradient times of 1 minute or less, and applied these conditions to the quantification of endogenous peptides in human serum.

Methods: A Bruker timsTOF Pro 2 mass spectrometer and a C18 monolithic silica capillary column (100 µm i.d.) were used. HeLa extracts were digested by trypsin and used to optimize LC/MS/MS conditions. Human serum samples from healthy subjects or patients were analyzed with approval by the Kyoto University Ethics Committee.

Results: From 250 ng of HeLa digest, more than 10,000 precursors were quantified with a high reproducibility of 9% RSD (median) in a 1-minute gradient elution. When human serum was deproteinized with acetonitrile and assayed under optimal conditions, the targeted endogenous peptide (MW 4800) was successfully quantified in a throughput of 100 samples per less than 4 hours with the spiked heavy-labeled internal standard peptide. Untargeted quantitation was also performed for other endogenous peptides simultaneously.

Conclusions: We have successfully developed a reproducible and high-speed system for serum peptidomics, overcoming the trend-off between the throughput and the depth of analysis.

PP03.139: Quality Control of High-Throughput Proteomics Workflow of CSF Samples from Parkinson's Disease Patients for DIA-MS Analysis

Washington K[1], McArdle A[1], Manalo D[1], Rivas A[1], Phebus C[1], Ayres M[1], Raedschelders K[1], Sundararaman N[1], Venkatraman V[1], Van Eyk J[1], Pandey R[1]
[1]Cedars Sinai Medical Center

Introduction

As part of the Accelerating Medicines Partnerships in Parkinson's Disease (PD), we undertook thorough, multi-level quality control (QC) to ensure reproducible LC-MS performance and consistent sample digestion for 2284 human cerebrospinal fluid samples from PD patients

Objectives

•Reproducible bottom-up proteomic measurements in 2284 of cerebrospinal fluid samples over 39 weeks.
• Digestion QC was done by processing replicate CSF samples during automation
•LC QC was done by indexed retention time (iRT) peptide standards (Biognosys), HeLa Weekly run and Technical control reference (TCR) pooled

Materials and Methods

2284 human cerebrospinal fluid (CSF) samples from Parkinson's Disease (PD) patients were digested using an automated workflow and were analyzed via DIA-MS along with 624 Digestion and LC-MS QC samples

•39 Batches of 75 patient samples with 4 digestion control reference (DCR) samples were processed in 96-well-plate format on the Beckman i7 automated workstation for protein denaturation (TFE), reduction (DTT), alkylation (IAA), and digestion (1:10 trypsin:protein; 4hrs @42°C)
•Weekly MS-system suitability was carried out using triplicate injections of HeLa digests and nine injections of MS-Technical control reference (TCR) pooled sample for each 96-well plate of patient samples
•Data were acquired using an EVOSEP-One LC with Orbitrap-Exploris-480 MS
•Data were processed using an automated data analysis pipeline using OpenSwath software and mapDIA algorithm.
•For each batch of patient samples, automated QC reports provided total peptides and proteins with CV% <20% and <40%.

Conclusions

On average DCR reference samples quantified >1300 peptides corresponding to 456 proteins, while TRC samples quantified >600 peptides and 392 proteins with 70% of proteins with CV%<40%.
•Principal Component Analysis of the DRC sample data showed PC1 contributed to 12.38% and PC2 contributed to 5.85% of the data variance.
•Principal Component Analysis of the TRC sample data showed PC1 contributed to 11.31% and PC2 contributed to 5.93% of the data variance.

PP03.140: Deep and Unbiased Proteomics Analysis Reveals Differences between Serum and Plasma Proteome in Matched Donors.

Pease P[1], Jiang W[1], Zhao X[1], Williamson L[1], Chu J[1], Gajadhar A, Motamedchaboki K[1]
[1]Seer, Inc.

Introduction:
Serum and plasma are common sample types used for biomarker discovery studies. While these sample types are similar, the abundance of many proteins could be altered from the original source material (i.e. blood) during their collection and processing. To better characterize the differences between serum and plasma proteomes and their suitability for large scale unbiased protein biomarker discovery studies, we collected matched serum and plasma from 15 individual donors, and processed them with the Seer ProteographTM Platform, and identified peptides and protein groups using LC-MS analysis.

Methods:
Blood was collected from 15 individual donors. Three separate sample types, serum, EDTA-plasma and citrate-plasma, were prepared from each donor from a single blood draw, yielding 45 samples in total.
Samples were processed using the Proteograph Platform. Samples were analyzed with three Proteograph runs. Peptides were dried and reconstituted before DIA LC-MS analysis with a 30 min gradient using a Bruker TimsTOF Pro2.

Results:
Across the 45-sample cohort we identified 4,079 protein groups (PGs) in total, with an average of 2,306 PGs per sample. Each of the three sample types had a different average of PGs per sample, with citrate-plasma having the fewest at 1,965 PGs, EDTA-plasma averaging 2,339 PGs per sample, and serum having the highest average of 2,613 PGs per sample.

The three sample types formed three well-separated clusters by principal component analysis, and hierarchical clustering was able to correctly identify serum as distinct from plasma, and also could distinguish citrate-plasma from EDTA-plasma.

Proteins like fibrinogen were in fact measured to be at much lower levels in serum relative to plasma.

Conclusion:
Seer's Proteograph Platform allowed us to rapidly measure over 4,000 protein groups across a 45-sample cohort consisting of serum and plasma from matched donors. PCA, hierarchical clustering and differential expression analysis demonstrate clear differences between sample types.

PP03.141: Deep Blood Plasma Profiling using Single Shot Data Independent Acquisition Mass Spectrometry

Tognetti M[1], Below C[1], Müller S[1], Bruderer R[1], Reiter L[1]
[1]Biognosys

INTRODUCTION

Blood flows through all organs and has the potential to be informative of the state of the whole human body. Further, it is readily available. Therefore, it is a highly relevant sample type. Even though one can speculate that blood carries all human proteins to a certain extent, deep plasma profiling was rather limited in the past due to the immense dynamic range of plasma of more than ten orders of magnitude. Improvements in sample preparation and total peak capacity of mass spectrometry based workflows have lead to substantial increases of proteome coverage in recent years. We show evidence that this is related to the protein abundance distribution of blood. Finally, we show some data how state of the art mass spectrometry workflows can quantify 4000 plasma proteins using single shot acquisition.

METHODS

Plasma samples were measured using Biognosys' TrueDiscovery workflow. Samples were depleted of high abundant proteins in a first step, and analyzed using liquid chromatography ion mobility separation (FAIMS) mass spectrometry (Orbitrap Exploris 480). DIA data was either directly searched using directDIA or analyzed with a library using Spectronaut (Biognosys).

RESULTS

Using a workflow with a high total peak capacity we could quantify more than 4000 proteins using single shot acquisition. This corresponds to a 800% improvement compared to the roughly 500 proteins which were achieved few years ago.

The data suggests that these substantial improvements are due to the particular protein abundance distribution of human plasma.

CONCLUSIONS

Due to the particular protein abundance distribution in human plasma substantial improvements in proteome depth could be achieved allowing to profile more than 4000 proteins using a single shot mass spectrometry workflow.

PP03.142: Experimental and Computational Approach to Profile Nanoparticle-Protein Interactions for Deep Plasma Proteomics

Stukalov A[1], Ferdosi S[1], Hasan M[1], Lee B[1], Batzoglou S[1], Siddiqui A[1], Farokhzad O[1,2,3], Hornburg D[1]
[1]Seer, Inc., [2]Center for Nanomedicine, Brigham and Women's Hospital, Harvard Medical School, [3]Department of Anesthesiology, Brigham and Women's Hospital, Harvard Medical School

Introduction

Seer's nanoparticles combined with mass spectrometry enable unbiased analysis of the plasma proteome at scale and depth(1,2). This is facilitated through the adsorption of proteins onto the nanoparticle surface, which boosts the signal of high-affinity low-abundance proteins and suppresses the low-affinity high-abundance ones, the process known as Vroman effect(3). High diversity of structural and chemical properties across plasma proteome as well as the wide dynamic range of protein concentrations determine a highly complex nanoparticle adsorption-desorption dynamics across individual plasma proteins.

Here we propose a combination of experimental and computational approaches to characterize this landscape of nanoparticle–protein interaction dynamics.

Methods

For the analysis of human plasma proteome, we have used 3 distinctly functionalized nanoparticles, and measured the protein coronae adsorbed on nanoparticles using a Bruker timsTOF Pro mass spectrometer in diaPASEF mode. To explore the adsorption-desorption dynamics, we tested 45 different combinations of nanoparticle incubation times and concentrations. By modulating these conditions, we shift the dynamic equilibrium of nanoparticle–protein interactions, and that affects protein intensities measured by LC-MS. The resulting 534 raw LC-MS files were analyzed with DIA-NN software(4) yielding intensity profiles for 3200 protein groups.

Results

UMAP visualization of the protein intensity profiles revealed a complex landscape of protein-nanoparticle dynamic modes. Using these data, we developed models that predict protein characteristics relevant for nanoparticle binding.

Conclusions

Understanding of the nanoparticle–protein interactions on the quantitative level provides considerable benefits for the nanoparticle-enabled LC-MS analysis. It allows to streamline the design of nanoparticles interrogating specific physicochemical fraction of the proteome occupied by hundreds to thousands of proteoforms and to improve the processing of the retrieved data, including large cohort studies.

References

1. Blume et al, Nat Comm 2020,11(1)
2. Ferdosi et al, PNAS 2022,119(11)
3. Vroman et al, Blood 1980, 55(1)
4. Demichev et al, Nat Meth 2020, 17(1)

PP03.143: Characterizing Blood Plasma and Organ Tissue Proteins in Rainbow Trout (Oncorhynchus Mykiss) using a Non-targeted Proteomics Approach

Tannouri N[1], Simmons D[1]
[1]OntarioTech University

Introduction: the word “proteome” refers to the entire set of proteins expressed by a cell, tissue, or organism. The proteome is not static. Instead, protein expression patterns fluctuate and adapt to internal or external cues to meet the needs of an organism. The dynamicity of an organism’s proteome can therefore reveal significant information about an organism’s health, such as early detection of disease and environmental exposure. Our study analyzed the plasma proteome using fish because we can perform lethal research on their tissues, they are easy to house, are vertebrates, and share many common proteins with other vertebrates, including mammals. By taking this approach with fish, we can learn more about the tissue origin of proteins in plasma, and understand what information plasma can provide when looking for biomarkers of tissue pathology. The current study aimed to discover tissue-specific information on the rainbow trout proteome using a systems-biology approach.

Methods: Blood and tissue from male and female rainbow trout were collected, plasma and tissue proteins were analyzed using liquid chromatography tandem mass spectrometry with data dependent acquisition, peptide sequences were identified and matched to the Uniprot rainbow trout reference proteome. Blood plasma and tissue proteins (brain, liver, gill, heart, kidney) were identified and plasma proteins were compared to tissue proteomes. Sex differences were also considered.

Results: over 10,000 proteins were identified across all groups. The majority of the plasma proteome was shared with multiple tissue types, though 4-7% of the plasma proteome is uniquely shared with each individual tissue (gill > heart > liver > kidney > brain). There were 576 proteins that were unique to only plasma.

Conclusion: tissue-specific plasma proteins held a substantial amount of functional information specific to each tissue type examined, signifying the capability of plasma to serve as a useful biofluid in biomarker discovery.

PP03.144: A Fully Automated and Rapid Platform for Biomarker Discovery using Deep-plasma Proteomic Workflow

Martelli C[1], Wendt F[2], Bauer M[2], Kruppa G[3], Kulak N[4]
[1]Bruker Switzerland AG, [2]Tecan Group Ltd., [3]Bruker Daltonics GmbH, [4]PreOmics GmbH

Introduction

Plasma represents a rich source of disease biomarkers while being the most available clinical specimen with minimal collection invasiveness. However, discovering protein biomarker candidates in human plasma is exceptionally challenging. The high complexity and wide dynamic range of proteins complicate the deep profiling of the plasma proteome at the throughput required for the study of large cohorts in a reasonable time. To address these challenges, we developed a pipeline of automated sample preparation using a novel deep-plasma workflow and the next-generation data-independent MS acquisition. It is designed for high-throughput and profound characterization of the plasma proteome.

Methods

The automation platform consists of the Fluent® liquid-handling system with the positive pressure module Resolvex® A200 (Tecan). The platform has an eight-channel Flexible Channel Arm™ mounting Disposable Tips (DiTis), a Robotic Gripper Arm™, and on-deck accessories such as magnetic separation and heating/shaking. The automated sample processing covers every step from enrichment of low abundant proteins to LC-MS ready peptides (PreOmics). A total of 96 samples/run is prepared for LC-MS analysis in ~5 hours starting with 20 µL of plasma. Peptides (300 ng) were analyzed with a 45-min gradient on the nanoElute system using dia-PASEF® acquisition on the timsTOF Pro 2 with online data analysis by the DIA-NN algorithm implemented in the PaSER software (Bruker).

Results and Discussion

The fully automated pipeline processed plasma samples from healthy donors in a 96-well plate format. The complete pipeline from sample to peptides takes less than 5 hours. It is a robust, high-throughput proteomic workflow with minimal user intervention. From 300 ng of peptides, over 1800 unique protein groups were quantified with superb reproducibility. It brings simplicity into the challenging field of plasma biomarkers.

Conclusions

Here, we present a fully automated platform for precise deep plasma analysis from patient samples to results with minimal hands-on time.

PP03.145: A Large Scale Multi-cancer, Multi-omics Biomarker Study of >1,800 Subjects Incorporating Deep Unbiased Plasma Proteomics

Wilcox B[1], Belthangady C[1], Blume J[1], Chan J[1], Chao-Shern C[1], Choi J[1], Chou W[1], Cuaresma R[1], Dai K[1], Deyarmin J[1], Doherty M[1], Flores-Campuzano G[1], Heok Jang J[1], Karimjee R[1], Khadka M[1], Khaledian E[1], Khin T[1], Kodama Y[1], Lee J[1], Liu M[1], Marispini M[1], Mora M[1], Mudaliar N[1], Nouri Golmoei S[1], Prasad P[1], Ramaiah M[1], Ramaswamy S[1], Swaminathan K[1], Vitko D[1], Williams P[1], Yang M[1], Young B[1], Zawada R[1], Zhang S[1], Ma P[1]
[1]Prognomiq

Introduction: Cancer is a leading cause of death worldwide. Many cancers are diagnosed in the late stage of disease progression with poor prognosis, highlighting the need for early detection. Cancers of high unmet diagnostic need include Non-Small Cell Lung Cancer (NSCLC), Colorectal Cancer (CRC) and Pancreatic Cancer. Recently, multi-omic approaches have gained attention due to their potential to identify putative cancer biomarkers from complementary 'omic' data from each patient. To date, we have utilized our proprietary multi-omic platform to deeply profile the proteome, lipidome and metabolome of over 1800 patient samples from our ongoing large-scale cancers study representing NSCLC, CRC and pancreatic cancer.

Methods: Over 1800 proteomic samples from within our larger scale multi-cancer study were processed though the Seer Proteograph assay suite and subsequently analyzed via LC-MS/MS. Metabolomic and lipidomic measurements were performed via LC-MS/MS after analyte extraction. LC-MS/MS data were analyzed utilizing Proteograph Analysis Software (PAS) and Sciex OS, with statistical analysis performed utilizing R and Python

Results: Our multi-omic multi-cancer investigation of NSCLC, CRC and pancreatic cancer cohorts resulted in deep, unbiased measurements of cancer and control subjects by identifying an average of ~2700 protein groups per subject sample in the proteomic workflow with median QC samples CV's <25%. In total, almost 4000 proteins were found in at least one subject sample. We also profile numerous glycoproteins, including at least 40 cancer-related glycoproteins. Furthermore, our lipid and metabolite panels measured 906 and 377, analytes, respectively, with average identifications per subject of 657 lipids and 300 metabolites.

Conclusions: We report one of the largest multi-cancer multi-omic plasma biomarker discovery studies to date. We demonstrate unparalleled protein detection by LCMS on 1,000's of subjects with the necessary reproducibility and statistical power to address historical challenges in plasma biomarker discovery and facilitate translation of putative biomarkers to the clinic.

PP03.146: Development of Biochemical and Analytical Pipelines for Human Plasma Peptidomes

Zellner A[1], Tiedt S[2], Lee C[1]
[1]Young Investigator Group: Mass Spectrometry in Systems Neurosciences, School of Life Sciences, Technical University of Munich, [2]Institute for Stroke and Dementia Research, University Hospital, LMU Munich

Introduction: Endogenous peptides in body fluids perform various functions and are widely used for medical diagnosis or therapy. However, knowledge about endogenous peptides is still limited due to considerable challenges faced by the analytical pipeline, including sample preparation, MS data acquisition, and data analysis. The main analytical challenge in sample preparation is the presence of a few highly abundant proteins that dominate the protein content in plasma. This high dynamic range causes a “masking” effect, thus detecting low-abundant peptide biomarkers is very difficult. In addition, the percentage of identified mass spectra collected from body fluids is very low compared to e. g. tissues. Nevertheless, the quality of many of the unidentified spectra is very high, implying the presence of (endogenous non-tryptic or modified) peptides that are not identified by standard data analysis approaches. This study aims to develop a robust workflow to facilitate the identification of endogenous peptides in plasma.

Methods: We have performed a systematic investigation of various biochemical techniques to remove high abundant proteins, such as antibody-based depletion, separation according to size (e.g. ultrafiltration or size exclusion chromatography), precipitation by organic solvents and mixed-mode solid-phase extraction prior to liquid chromatography and MS/MS analysis.

Results: Comparing different biochemical protein separation techniques, we have achieved an optimized workflow for detecting endogenous peptides applicable for large clinical cohorts. The peptide identification was improved using artificial intelligence-based rescoring algorithms[a].

Conclusion: We have optimized a biochemical and analytical pipeline for detecting peptidomes in plasma with the potential to apply to other body fluids. These results will aid in studying disease mechanisms and progression and discovering novel biomarkers.

[a] Gessulat, S. et al. Prosit: proteome-wide prediction of peptide tandem mass spectra by deep learning. Nat Methods 16, 509–518 (2019)

PP03.147: A Deep Dive into Phosphopeptide Positional Isomers Leveraging CCS Information and Novel Software Tools

Adams C[1], Michna T[2], Distler U[2], Krieger J[1], Tenzer S[2]
[1]Bruker, [2]Institute for Immunology, University Medical Center of the Johannes Gutenberg University

Introduction

Phosphopeptides are a key signaling molecule where mass spectrometry plays a central role in identifying, localizing and quantifying these signals. Given many proteolytic peptide sequences have multiple serine, threonine and tyrosine amino acids site localization and positional isomer deconvolution is challenging. Peptides phosphorylated at different sites yet existing with the same primary amino acid sequence can co-elute in the chromatographic phase. Trapped ion mobility (TIMS) first partitions and concentrates ions by size, shape and charge and from this generates a mobility term. In this work we exploit differential mobilities of phosphopeptide positional isomers to 1) identify 25% more phosphopetides 2) identify and quantify hundreds of positional isomers that with traditional analysis remain hidden 3) demonstrate a graphical software tool to visualize and capture phosphopeptide positional isomers in treated and untreated Human Osteosarcoma samples.

Methods

Human osteosarcoma cells were cultured with and without drug treatment, where the cells were collected, lysed, digested using trypsin followed by phosphopeptide enrichment. The enriched phosphopeptides were run by nanoLC MS where the mass spectrometer was a timsTOF Pro 2 run in dda-PASEF and dia-PASEF modes. A custom polygon to exclude singly charged ions was used. Data analysis was performed using PEAKS, PaSER and PaSER + TIMScore. TIMS Viz was used to assign, visualize and extract information specific to co-eluting isomers.

Results

We compared the number of unique phosphopeptides identified with and without TIMScore (33,953 v. 23,881) showing a marked improvement of over 25% increase where >99% of the peptides are shared. The data was also evaluated to understand the advantages of detecting phosphopeptide positional isomers where without TIMScore some 221 positional isomers were identified improving to 327 with TIMScore. Of the additional 100 isomers we show by TIMS Viz the capability to visualize, extract and quantify based upon the mobility, m/z and RT domains.

PP03.148: Interrelationship Analysis between Acetylation and Ubiquitination in Lysine Residues in Human Cervical Cancer Cells

Alonso R[1], Morales Tarré O[1], Gomez L, Hernández M[1], Encarnación-Guevara S[1]
[1]CCG

Introduction: Cervical-uterine cancer is the fourth leading cause of death in women. Cancer is a group of diseases with a high level of complexity, which means that it is necessary to know more fully the details of this in order to reach a possible solution. One approach is proteomics, which lets us analyze all proteins expressed in a cell at a given time. Post-translational modifications (PTMs) of proteins, such as ubiquitination and acetylation, are a way to increase the complexity and variety of functions of their proteins target. This project aims to know the differences in the stoichiometry of ubiquitination and acetylation in cancer cells concerning healthy cells.
Methods: Cervical cancer (HeLa and SiHa), non-viral (C33a) cell lines and a non-cancer control line (HaCat) are used. Following our previous successful experience in stoichiometry analysis of lysine acetylation, we designed a quantification strategy for stoichiometry analysis of ubiquitination. Subsequently by liquid chromatography coupled to mass spectrometer is possible to identify these proteins.
Results: We identified 1621 proteins of which 333 ubiquitinated proteins were detected. The peptides had stoichiometry ranging from 0.25 to 100%. We analyzed expression patterns and ontology to characterize these proteins, which allowed us to know more about processes where ubiquitination is present, such as redox homeostasis and regulation of apoptotic signaling pathways.
Conclusions: With our approach we are able to determine the stoichiometry of a modification such as ubiquitination, which let us know more about its targets and their roles in different pathways, which plays vital roles in cellular physiology and protein homeostasis in cancerous and healthy cells. The results of this work will contribute to opening new investigation lines about ubiquitination in new study cases. Adding acetylation stoichiometry of these cell lines as a perspective to increase the understanding of cancer.

PP03.149: The Role of Mitochondrial Deacetylase SIRT3 in Mitochondrial Function in Lung Cancer y Breast Cancer

Ayala M[1], Encarnación-Guevara S[1], Fernández Coto D[1], Sánchez Castellanos N[1]
[1]UNAM

Introduction: Mitochondrial deacetylase sirtuin 3 (SIRT3) is the sirtuin with the highest deacetylation activity in mitochondria. Studies suggest that may act as an oncogene or as a tumor suppressor depending on the type of metabolism of cancer cells. However, the study of SIRT3 in mitochondrial protein acetylation has been poorly explored. This is why this study aims to establish through acetylation stoichiometry and the evaluation of mitochondrial functions, the role that SIRT3 plays in lung and breast cancer, seen from the mitochondrial perspective. Methods: SIRT3 was inhibited and overexpressed in lung and breast cancer cell lines, A549 and MCF7, where SIRT3 has been reported to act as an oncogene and tumor suppressor, respectively. The mitochondria of these cell lines will be enriched to obtain mitochondrial proteins and subsequent chemical acetylation of the endogenously non-acetylated lysine residues (1), the acetylation stoichiometry of these samples will be analyzed by liquid chromatography with tandem mass spectrometry (LC-MS-MS). Mitochondrial function will also be evaluated through the following studies: production of reactive oxygen species (ROS), activity of oxidative phosphorylation system complexes (OXPHOS), production of NAD+/NADH and ATP. Results: Western blot confirmed the upregulated and downregulated expression of SIRT3 in cell lines A549 and MCF7, respectively. Similarly, inhibition and overexpression of the generated clones were verified. A differential acetylation/deacetylation pattern is expected in these two types of cancer, and we suspect that mainly proteins responsible for regulating ROS in the mitochondria will be targeted for this post-translational modification. Conclusions: SIRT3 plays a role as an oncogene and as a tumor suppressor, knowing the acetylation stoichiometry of mitochondrial proteins will broaden the current knowledge on acetylation/deacetylation regulation in the mitochondria and its relationship with cancer.

1. Gil, J., & Encarnación-Guevara, S. (2022). Methods in molecular biology (Clifton, N.J.), 2420, 73–86.

PP03.150: Characterisation of Peptide and Protein Isomeric Post-translational Modification by Multi-pass Cyclic Ion Mobility

Carroll L[1], Zenaidee M[1], Fitzhenry M[1], Hart-Smith G[1]
[1]Australian Proteome Analysis Facility

Introduction: Proteins and peptides can be altered post-translation in a myriad of different ways with multiple functional groups and variation in modification localisation. These post-translational modifications (PTMs) can alter protein structure and hence function, which affects protein action in biological systems. The presence of modifications can usually be detected easily by mass spectrometry due to a change in peptide mass, though this does not always yield site specific information. As the same modification at different sites can cause drastic differences in activity, the ability to specifically identify PTM's sites is crucial to understanding how these modifications affect protein function.

Method: Post-translationally modified peptides and proteins were isolated from simple and complex standards and analysed using a Waters cyclic ion mobility instrument (cIMS) by direct infusion and LC-MS methods. Site specific isomers were separated and isolated by ion mobility (IM) and identified by MS/MS experiments.

Results: IM enables the separation of isomeric peptides and proteins that may be otherwise indistinguishable by traditional LC-MS. Furthermore, the cIMS allows for unique MSn experiments, where species can be separated by IM, selected and re-injected for further IM separation. This enables very selective experiments, speciation of PTMs and in-depth characterisation of proteins and peptides. Here, we demonstrate the capacity for cIMS to separate, characterise and identify a range of common PTMs, including phosphorylation and glycosylation, in both simple and complex mixtures.

Conclusion: The ability to more clearly separate and characterise PTMs will enable greater insight into the role these moieties play in biological systems.

PP03.151: Method Development for the Investigation of the Understudied (essential) PTM - Tyrosine-O-Sulfation

Daly L[1,2], Byrne D[1], Perkins S[1,3], Brownridge P[1,2], Jones A[1,3], Eyers P[1], Eyers C[1,2]
[1]Biochemistry and Systems Biology Department, University of Liverpool, [2]Centre for Proteome Research, University of Liverpool, [3]Computational Biology Facility, University of Liverpool

Introduction

Tyrosine-O-sulfation is a post translational modification similar in nature to phosphorylation. While phosphorylation has been extensively investigated, largely due to multiple analytical strategies developed, only ~50 human proteins have been identified as sulfated. Phosphorylation is known to be catalysed by over 500 kinases (~90 tyrosine kinases), in contrast to two known tyrosine protein sulfotransferases (TPSTs) encoded within vertebrates. Single isoform knock-out mice display extensive phenotypic abnormalities (including growth, visual and reproductive issues) and double isoform knock-outs result in postpartum death; suggesting sulfation is essential for cellular physiology. Tyrosine-O-sulfation is also essential for host-pathogen interactions. Here we develop a high-throughput mass spectrometry (MS)-based workflow for the analysis of sulfopeptides.

Methods

Tryptic peptides of known sulfation sites (12 total) were synthesised and sulfated in-vitro, generating a sulfopeptide standard panel. Equivalent phospho-tyrosine peptides were purchased. IMAC and TiO2 strategies (used for phosphopeptide enrichment) were evaluated/optimised for sulfopeptides prior to LC-MS/MS on a ThermoFisher Fusion Lumos Tribrid instrument; investigating comparative enrichment efficiency and multiple fragmentation methods for site-localisation ability.

Results

TiO2 resin is inefficient for the enrichment of sulfopeptides, explaining the lack of identified tyrosine-O-sulfation in re-searched public datasets. IMAC, testing 13 different counterions, identified Zirconium (Zr4+) capable of the preferential capture of sulfopeptides. However, localisation of sulfation remains significantly challenging due to the high lability of the O-sulfate bond (resulting in substantial neutral loss), inherent charge reduction (versus phosphopeptides) due to the significantly lower pKa of sulfate, and deficiencies in current UVPD fragmentation capabilities on commercial instrumentation. Negative ion MS holds promise for site-localisation, however encompasses its own drawbacks due to sensitivity and lack of suitable data analysis pipelines.

Conclusion

We have developed a pipeline for the enrichment and analysis of tyrosine-O-sulfated peptides and their discrimination from phosphopeptides. However, sulfation localisation remains a significant challenge using commercially available instrumentation.

PP03.152: Influence of Propagation Time on Yeast Metabolism during Bottle Refermentation at an Industrial Scale

Dilmetz B[1], Desire C, Meneses J, klingler-Hoffmann M, Young C, Hoffmann P
[1]University Of South Australia

Bottle refermentation is a process that involves secondary fermentation through the addition of fermentable extract and yeast to the bottle. The ability of yeast to maintain a healthy state for an extended time after the refermentation is complete is vital as yeast autolysis results in changes to the sensory profile of the beer, poor foam stability, and a decrease in shelf-life. Bottle refermentation is largely dependent on the yeast strain, fermentable extract, and the propagation process utilised. Despite bottle refermentation being a mainstay in many craft breweries, the underlying biological changes that occur during bottle refermentation remain elusive. Here, we investigated the metabolism of yeast during bottle refermentation and the impact of propagation time on yeast performance.

Yeast cells were grown in an industrial-scale propagator (30 hL) for 24h or 72h in wort. Yeast was seeded into the green beer at ~0.5x10^6 cells/mL containing fermentable extract. Samples were collected regularly from day 0 to day 30 post-refermentation. Proteomics and phosphoproteomics using Zr-IMAC enrichment were performed, and samples were analysed using DDA on an Exploris480 Orbitrap.

Yeast propagated for 72h was able to maintain a higher viability for a prolonged time compared to yeast propagated for 24h during bottle refermentation. A similar profile of glycogen accumulation and degradation was observed during bottle refermentation irrespective of the propagation time. The proteomic profile of yeast during bottle refermentation shared similarities to that observed in primary fermentation. The main pathways in yeast influenced by the conditions of bottle refermentation were related to aerobic respiration, respiratory metabolism, and oxidative stress.

The seeding of yeast for bottle refermentation after propagation for 24h resulted in a more rapid loss in viability compared to yeast seeded after propagation for 72h. Proteomic and phosphoproteomics analysis facilitated a more in-depth understanding of yeast metabolism during bottle refermentation.

PP03.153: The Nanoparticle-based Plasma Proteomics Workflow Enables the Investigation of Glycoproteome

Ferdosi S[1], Hasan M[1], Mohtashemi I[1], O'Brien E[1], Xia H[1], Wang J[1], Motamed K[1], Zamanighomi M[1], Guturu H[1], Hornburg D[1]
[1]Seer Inc

Introduction

To overcome limitation of deep plasma proteomics in large cohorts, we have developed a fast and scalable technology that employs intricate protein-nano interactions. Introducing a nanoparticle (NP) into a biofluid such as blood plasma leads to the formation of a selective, specific, and reproducible protein corona at the nano-bio interface driven by the relationship between protein-NP affinity, protein abundance and protein-protein interactions. Since nanoparticle coronas differentially interrogate complex samples at the proteoform level and plasma proteins are often glycosylated, here we investigate whether an automated NP-corona based proteomics workflow can interrogate the plasma glycoproteome. Protein glycosylation states can provide diagnostic evidence where total protein abundance is uninformative. Improved methods for profiling the plasma glycoproteome, specially at the lower abundance level thus can potentially have a major impact in biomarker discovery.

Methods

We have investigated the utility of NPs in enrichment of low abundant glycoproteins. Samples were analyzed with timsTOF Pro mass spectrometry and UltiMate3000 Dionex LC system using 60min DDA and 30 min DIA runs. We evaluated depth, dynamic range, coverage, and precision of quantification at a wide range of concentrations for each NP.

Results

By compressing the dynamic range and making the low abundance ions more visible to the downstream LC-MS, NPs facilitate the detection of low abundant proteins and corresponding peptides even without subsequent enrichment of glycopeptides. Based on the physicochemical properties of NPs, they capture distinct sets of glycoproteins at the lower abundance range, enhancing the coverage of these proteins in blood plasma.

Conclusions

In summary, these data provide evidence that nanoparticle protein coronas have a potential to provide the ability to analyze subpopulations of the glycoproteome without subsequent, glycopeptide-specific enrichment. Furthermore, the different nanoparticles offer complementary views of the plasma glycoproteome due to their specificities for different proteins, and likely, different glycosylated proteoforms.

PP03.154: Identification of SIRT3 Deacetylation Targets in Glioblastoma Cancer Cells with Different Energy Metabolism

Fernández Coto D[1], Encarnación-Guevara S[1], Ayala Aguilar G[2], Gil Valdés J[3]
[1]Universidad Nacional Autónoma De México, [2]Instituto Nacional de Salud Pública, [3]Lund University
Acetylation in lysines of proteins influence many cellular processes. It has attracted the attention of researchers since its consequences in cell biology, metabolism, interactions between proteins, subcellular localization of proteins, and their involvement in some pathologies like cancer. Stoichiometry information on acetylation is essential to interpret the biological significance. SIRT3 removes the acetyl group of modified proteins in mitochondria. It plays a significant role in cancer cell metabolism since it is an NAD+ dependent enzyme. Our study focused on finding the different proteins regulated by SIRT3 in two glioblastoma cell lines with different energy metabolism.
Methods: Two glioblastoma cell lines were used; an oxidative (T98G) and a glycolytic (U87MG). We used mass spectrometry-based proteomics strategy to mapped acetylation sites. SIRT3 was first inhibited, total protein extract and mitochondrial extract were obtained, and chemically acetylated with a reagent that adds a heavier acetyl group. The acetylation stoichiometry of all proteins was obtained by mass spectrometry thanks to the distinction in the spectrum of endogenously acetylated proteins and those chemically acetylated with the heaviest reagent.
Results: We describe the differences in protein expression and corroborate some oxidative and glycolytic metabolism markers. In addition to being metabolically different, the cell lines studied also have a different acetylation profile. Our results show these differences. In addition, we show that SIRT3 deacetylation sites also differ depending on the cell's predominant metabolism.
Conclusions: Acetylation profile can potentially define the cell´s metabolic state. In the glycolytic cell line, the targets of SIRT3 are mainly proteins of the electron transport chain, however, the low expression of this enzyme is not enough to keep this pathway working, therefore, a treatment based on overexpression of SIRT3 or on molecules that eliminate acetyl groups in specific proteins in glycolytic tumors could be a good therapeutic target.

PP03.155: Deciphering (Phospho)proteomics Brain and Spinal Cord Signatures of an in vivo Model of Multiple Sclerosis

Fucito M[1], Ojha B[2], Khorooshi R[2], Owens T[2], Nawrocki A[2], R. Larsen M[3], De Laurenzi V[1], Del Boccio P[1], Pieragostino D[1]
[1]University "G. D'Annunzio" of Chieti-Pescara, Center For Advanced Studies And Technology, Analytical Biochemistry and Proteomics Unit, [2]University of Southern Denmark, Institute of Molecular Medicine, Neurobiology, [3]University of Southern Denmark, Department of Biochemistry and Molecular Biology, Protein Research Group

Introduction: Proteomics is widely used to investigate molecular pathways and to find biomarkers for complex and heterogeneous neurodegenerative diseases such as multiple sclerosis [1]. As a matter of fact, proteins have several important biological functions and play a crucial role as signaling pathways mediators. Moreover, proteins are regulated by reversible phosphorylation needed for cell life, while abnormal phosphorylation causes or results from diseases [2].

Methods: In this context, we used an elegant analytical strategy consisting in a high pH (HpH) reversed-phase fractionation approach coupled to tandem mass tag (TMT) mass spectrometry-based proteomics to quantify non-modified and phosphorylated proteins. We performed such approach on extracted spinal cords and on the corpus callosum region of extracted brains from a focal experimental autoimmune encephalomyelitis (EAE) in vivo mouse model. Five conditions were chosen namely non-immunized, immunized, immunized with a focal injection of cytokines, immunized with a focal injection of phosphate-buffered saline and treated with minocycline. All experiments were conducted in triplicates.

Results: Regarding the extracted brains, data allowed to shed light on non-modified and phosphorylated proteins altered in the different conditions, followed by a functional analysis using Ingenuity Pathway Analysis highlighting molecular pathways involved in the inhibition of the nervous system development, in the activation of the inflammatory response and in the activation of the cellular movement and migration. Regarding the extracted spinal cords, data is being investigated and the comparison with the results from the extracted brains could open new doors to the understanding of the EAE model.

Conclusion: Investigating (phospho)proteomics brain and spinal cord signatures of an in vivo model of multiple sclerosis using such approach leads to a better understanding of the molecular mechanisms and pathways involved in the model.

[1] Singh, V., Tripathi, A., & Dutta, R.; Proteomics, (2019).
[2] Cohen, P.; European journal of biochemistry vol. 268, (2001).

PP03.156: From Ligand Affinity to Signal Potency: Phospho-regulations and Protein-protein Interactions Underlying Early Antigen Discrimination by the T-cell Receptor

Locard-paulet M[1], Voisinne G[2], Froment C[1], Maturin E[2], Goncalves Menoita M[2], Girard L[2], Mellado V[2], Burlet-Schiltz O[1], Malissen B[2], Roncagalli R[2], Gonzalez De Peredo A[1]
[1]Institut de Pharmacologie et de Biologie Structurale, Université de Toulouse, CNRS, [2]Centre d'Immunologie de Marseille-Luminy, INSERM, CNRS

Introduction

T lymphocytes recognize a few high-affinity antigens amongst a vast majority of lower affinity antigens. According to the kinetic proofreading model, this discrimination could be explained by the gradual amplification of small binding differences along the T-cell receptor (TCR) signaling cascade. How and which early molecular events are affected by ligand affinity is still unclear. By using time-resolved proteomic analyses, we characterized the phosphorylation events and protein-protein interactions encoding T cell ligand discrimination.

Methods

Mouse OT-I T cells were stimulated with their specific agonist (ovalbumin 257-264 peptide), or with altered forms of this peptide, for different time length. Phosphoproteomic characterization was performed through TMT labelling, TiO2 or phospho-Tyrosine antibodies enrichment, and SPS-MS3 analysis on an Orbitrap Fusion instrument. In addition, OT-I mice were crossed onto mice expressing a tagged version of either the CD3z, ZAP70 or SLP76 signalling molecule, and complexes formed dynamically around these nodes were purified and analyzed by MS.

Results

Among more than 600 phospho-sites significantly regulated during the course of TCR stimulation, some displayed an amplitude of phosphorylation unaffected by the affinity of the antigen, while others exhibited a response either scaling with ligand affinity, or specific to strong stimulation. Particularly, weak antigens were as efficient as the high-affinity ligand to promote phosphorylation of the TCR chains and recruitment of the kinase ZAP70, but failed to efficiently activate ZAP70, leading to strong defects in the LAT signalosome.

Conclusions

This study integrated phosphoproteomic and interactomic analyses to provide a comprehensive picture of the impact of ligand affinity on the molecular events associated with early TCR signaling, and specifically highlighted the key roles of ZAP70 and LAT in implementing TCR ligand discrimination.

References

Voisinne, Locard-Paulet et al, Kinetic proofreading through multi-step activation of ZAP70 underlies early T cell ligand discrimination, Nat. Immunol, in press

PP03.157: Multidimensional Proteomics Reveals that Loss of N-terminal Acetyltransferase A Activity Induces Thermally Unstable Ribosomal Proteins and Increases their Turnover

H. Guzman U[1,2], Aksnes H[3], Ree R[3], E Jakobsson M[1,4], J. Jensen L[1], Arnesen T[5,6], V. Olsen J[1,2]
[1]Novo Nordisk Foundation Center for Protein Research, [2]Faculty of Health and Medical Sciences, University of Copenhagen, [3]Department of Biomedicine, University of Bergen, [4]Department of Immunotechnology, Lund University, [5]Department of Biosciences, University of Bergen, [6]Department of Surgery, Haukeland University Hospital

Introduction

Protein modifications are essential to modulate cellular protein activity, stability, subcellular localization and interactions. Within the PTM universe, the Nt-acetylation is arguably the most abundant protein modification in all eukaryotic proteomes, however its function remains poorly understood. The main contributor to the eukaryotic proteome Nt-acetylation is the Nt-acetyl transferse A. The effects of NatA-mediated Nt-acetylation reported so far suggest a relationship to protein folding, aggregation and turnover. However, a proteome-wide understanding of how NAT activity may steer proteostasis remains unclear. For that reason, we developed and applied mass spectrometry strategies to explore the effect of the Nt-acetylation catalyzed by NAT A complex on protein turnover and its relation with protein stability by measuring multiple dimensions of the proteome on Saccharomyces cerevisiae +/- NatA model.

Methods

Saccharomyces cerevisiae +/- NatA strains were generated and analized by LC-MS/MS, DIA-TPP and pSILAC chase.

Results

We generated a comprehensive yeast proteome containing 4,113 and 3,943 protein-coding genes for WT and naa10Δ model, respectively. The differential proteome expression analysis between the WT and the naa10Δ highlighted that proteins related to autophagy and the UPS system were up-regulated, whereas some cytosolic ribosomal proteins from the small and large subunit in addition to mitochondrial proteins related to the electron transport chain were down-regulated in naa10Δ. As the proteome is a dynamic system where multiple protein properties contribute to the cell phenotype, we decided to investigate the protein thermostability and degradation rate of the WT and naa10Δ model by DIA-TPP and pSILAC chase.

Conclusion

Conjointly, our results suggest that the lack of Nt-acetylation carried out by the NatA complex in Saccharomyces cerevisiae promotes the fast turnover of cytosolic ribosomal proteins. This finding is in agreement with recent studies in other species supporting the idea that Nt-acetylation promotes the proteome stability rather than its degradation across eukaryotic kingdoms.

PP03.158: Potential Molecular Transducer of Exercise-induced Cardiac Adaptation

Park N[1,2,3], Marquize J[1,2,3], Kim H[1,2,3], Han J[1,2,2]
[1]Department of Physiology, College of Medicine, Inje University, [2]Cardiovascular and Metabolic Disease Center, Inje University, [3]Smart Marine Therapeutic Center, Inje University

The heart is the primary pump that circulates blood through the entire cardiovascular system, serving many important functions in the body. Exercise training provides favorable anatomical and physiological changes that reduce the risk of heart disease and failure. Compared with pathological cardiac hypertrophy, exercise-induced physiological cardiac hypertrophy leads to an improvement in heart function. Exercise-induced cardiac remodeling is associated with gene regulatory mechanisms and cellular signaling pathways underlying cellular, molecular, and metabolic adaptations. We found that aerobic exercise training decreased cereblon (CRBN), a substrate recognition protein in the E3-ligase ubiquitin complex. The binding target of CRBN varies according to tissues and cells, and the protein regulates various biological functions by regulating tissue-specific targets. As new endogenous targets of CRBN have been identified over the past decade, the physiological and pathological functions of CRBN and its potential as a therapeutic target in various diseases have greatly expanded. Here, I will present a cellular and molecular signaling pathway of CRBN to understand the exercise-induced cardiac adaptation.

PP03.159: Expanded Characterization of Protease Activity by using Combined Terminomics Approaches.

Hanna R[1], Rosenberg A[1], Hershkovic A[1], Lavy T[1], Fishman A[1], Kleifeld O[1]
[1]Technion Israel Institute Of Technology

Introduction: Proteases are critical players in many cellular processes, and extensive studies are being done to characterize their substrates. Mass-spectrometry based proteomics is vastly used, yet, given the current technology it is impossible to identify the whole pool in a single experimental approach. To enable a deeper characterization of proteolysis, we developed two novel methodologies which we termed LATE and CAPE for both N- and C- terminal peptides enrichment respectively. We combined our methodologies with existing ones to study Caspase-3 function during apoptosis, and Adam-10 sheddase activity.

Methods: Caspase-3; cells were transfected with caspase-3 and induced apoptosis using ABT199. Adam-10; differentiated neuronal cell line were treated with Adam-10 in a serum-free media, then the media proteins were collected. All samples were subjected for N-terminomics and C-terminomics.

Results: We uniquely identified 193 and 131 caspase-3 cleavage sites by in CAPE and LATE respectively, we also applied existing N-terminomics called HUNTER giving 271 unique identification, additionally, 85 sites that were identified in at least two out of three methods. About 25% of cleavages were not reported previously in the literature. Furthermore, we found a direct cross-talk between Caspase-3 proteolysis and post translational n-acetylation which we also validated in-vitro. In the case of Adam-10 having only one neo terminus released into the media urges the use of combined termini approach. The identification filtered to include only outer-membrane or secreted proteins only. We identified 372 and 286 using CAPE and HUNTER, with less than 10% overlap between them. Several proteins show ectodomain cleavage flanking the transmembrane sequence, underlining soluble Adam-10 sheddase activity.

Conclusions: Using two terminomics methods that we developed alongside existing ones, we managed to expand our knowledge in different biological systems. We identified previously unreported substrates of Caspase-3 and Adam-10, and we revealed related biological mechanisms related to their activity.

PP03.160: Identification of a Membrane-integral N-terminal Histidine Methyltransferase by CRISPR and Proteomics Screening

Hernandez Rollan C[1], Batth T[1], Simonsen J[2], Morth J[2], Ipsen J[3], Brander S[3], Johansen K[3], Nørholm M[4], Hoof J[2], Olsen J[1]
[1]The Novo Nordisk Foundation Center for Protein Research, University of Copenhaguen, [2]Department of Biotechnology and Biomedicine, Technical University of Denmark, [3]Department of Geosciences and Natural Resource Management, University of Copenhagen, [4]The Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark
Introduction: Lytic Polysaccharide Monooxygenases (LPMOs) are a relatively new kind of enzymes with members in all kingdoms of life and are involved in roles ranging from carbon recycling to plant and animal virulence. One intriguing characteristic found in LPMOs of fungal origin is the presence of a rare posttranslational modification at their N-terminal amino acid, histidine, which is methylated and part of their catalytic domain. However, the methyltransferase (MTase) responsible for this modification has not yet been identified. Here, we report the identification and characterization of the enzyme responsible for this specific histidine methylation along with the engineering of a yeast production strain for heterologous methylation of N-terminal histidine targets.
Methods: The filamentous fungi Aspergillus nidulans was differentially grown on different carbon sources (glucose vs. cellulose) to identify differentially expressed methyltransferase candidates and substrates by quantitative proteomics of whole extracts. Differential candidate MTases were prioritized based on bioinformatics and selected targets were individually knocked down using CRISPR/Cas9, and a targeted MS analysis was used to examine the methylation status of co-expressed LPMOs.
Results: A single MTase gene was identified solely responsible for the N-terminal histidine methylation of LPMOs. Functional analysis revealed that this is a 7-transmembrane domain protein located in the ER, where it catalyzes the histidine methylation reaction upon encountering its targets. We also demonstrate that we can eliminate its methylation activity by site-directed mutagenesis. Finally, we recombinantly methylate LPMO targets (of both bacterial and fungal origin) by co-expression in the industrially relevant yeast K. phaffii.
Conclusions: We report the identification and characterization of the elusive N-terminal histidine methyltransferase and demonstrate that it is possible to engineer production yeast to produce N-terminal methylated LPMOs.

PP03.161: A Proteomics Approach for Profiling Redox-Sensitive Changes in Mesothelial Cells during Experimental Peritoneal Dialysis

Herzog R[1], Wagner A[1], Kratochwill K[1]
[1]Medical University of Vienna

Peritoneal dialysis (PD) is a life-saving renal replacement therapy, which uses the peritoneum as semi-permeable membrane to remove uremic toxins and water from the patient by a glucose-based PD-fluid. The special composition of these hyperosmotic fluids triggers morphological and functional changes in the peritoneum. The high glucose concentration, low pH, non-physiological buffer and glucose degradation products lead to generation of reactive oxygen species (ROS) in the peritoneal cavity, leading to impaired cell viability and progression of PD-related vasculopathy, hyper-vascularization, and diabetes-like damage of vessels, eventually leading to failure of the technique. Here, we aim to analyse the specific targets of ROS during PD and the impact and of a potentially anti-oxidative pharmacological intervention with glutamine-supplementation.

To establish a redox-proteomics workflow for studying the targets and mechanisms of oxidative stress in peritoneal mesothelial cells we used a gold-standard model of redox-stress (H2O2) and PD-induced stress. Elevated levels of oxidative stress were validated by increased abundance of intracellular ROS and increased superoxide dismutase activity with PD and H2O2 treatment; a reduction of these parameters was observed with added glutamine. To detect alterations of the redox-proteome, cysteine residues were either directly or indirectly labelled with isobaric tags (iodo-TMT). The LC/MS-based workflow was optimized regarding cell lysis and labelling conditions, blocking steps, affinity tag-enrichment, elution and reproducibility. The optimized protocol enabled identification of ~7600 proteins and ~2400 proteins affected by redox-stress. Mesothelial cells exposed to H2O2, PD-fluid or PD-fluid with glutamine show specific differences in their oxidation status. With the established workflow, we were able to detect a higher oxidation status of PD treated cells compared to controls and a lower oxidation status of the samples supplemented with glutamine compared to PD-fluid without. Redox-proteomics of peritoneal cells may represent a novel approach for the identification of PD-pathomechanisms and evaluation of clinically relevant anti-oxidative interventions.

PP03.162: Assessing PAK1/PAK2 Redundancy using Targeted Protein Degradation, an Allosteric PAK1/PAK2 Inhibitor, and High throughput LC-MS/MS and Quantitative Proteomics

Howarth C[1], Gerber S[1]
[1]Dartmouth College

Introduction: PAK1, a group 1 PAK kinase along with PAK2 and PAK3, is a known regulator of cytoskeletal dynamics with implications in breast and ovarian cancer. However, the complete list of PAK1 substrates has not been characterized and the extent of substrate overlap with PAK2 is poorly understood. Here, we used targeted protein degradation, an allosteric group 1 PAK inhibitor (NVS-PAK1-1), and quantitative LC-MS/MS to address these knowledge gaps.

Methods: We used CRISPR/Cas9 to introduce components of the auxin-inducible degron (AID) system, allowing for rapid and precise degradation of PAK1. We directly compared phosphopeptide abundance after PAK1 degradation to inhibition with NVS-PAK1-1, an allosteric PAK1/PAK2 inhibitor, using isobaric labeling and LC-MS/MS, to assess changes in phosphopeptide abundance. In parallel, we treated SU.86.86 cells, a pancreatic cancer cell line with high PAK1/PAK2 expression, with low-dose NVS-PAK1-1 to inhibit PAK1 alone, or high-dose NVS-PAK1-1 to inhibit PAK1 and PAK2, and measured changes in phosphosite abundance by quantitative LC-MS/MS.

Results: Phosphoproteomics experiments comparing PAK1 degradation to PAK1/PAK2 inhibition identified auto-activation sites in PAK1/PAK2 kinases, in addition to other candidate substrates. Correlation analysis comparing phosphopeptide abundances with PAK1 degradation to PAK1/PAK2 inhibition provides evidence of partial redundancy between PAK1 and PAK2 and the presence of PAK1/PAK2-specific substrates. However, few sites are downregulated with PAK1 degradation or PAK1 inhibition alone, when compared to PAK1/PAK2 inhibition.

Conclusions: The low number of significantly downregulated phosphosites with PAK1-specific degradation or inhibition suggests that PAK2 phosphorylates most group 1 PAK substrates, PAK1-specific substrates are stable over longer time periods than PAK2 substrates, or that there is a high degree of overlap between PAK1 and PAK2 substrates. Ongoing experiments are addressing these possibilities.

PP03.163: High-throughput Profiling of Histone Post-translational Modifications and Chromatin Modifying Proteins by Reverse Phase Protein Array

Huang S, Wang X, Shi Z, Kim J, Lu H, Bu W, Villalobos J, Perera D, Jung S, Wang T, Grimm S, Taylor B, Wulfkuhle J, Young N, Li Y, Coarfa C, Edwards D
[1]Baylor College of Medicine, [2]George Mason University

Introduction: Epigenetic variation plays a significant role in normal development and human diseases including cancer, in part through post-translational modifications (PTMs) of histones. Identification and profiling of changes in histone PTMs, and in proteins regulating PTMs, are crucial to understanding diseases, and for discovery of epigenetic therapeutic agents.

Methods: In this study, we have adapted and validated an antibody-based reverse phase protein array (RPPA) platform for profiling 20 histone PTMs and expression of 40 proteins that modify histones and other epigenomic regulators.

Results: The specificity of the RPPA assay for histone PTMs was validated with synthetic peptides corresponding to histone PTMs and by detection of histone PTM changes in response to inhibitors of histone modifier proteins in cell cultures. The useful application of the RPPA platform was demonstrated with two models: induction of pluripotent stem cells and a mouse mammary tumor progression model. Described here is a robust platform that includes a rapid microscale method for histone isolation and partially automated workflows for analysis of histone PTMs and histone modifiers that can be performed in a high-throughput manner with hundreds of samples. Our study also showed the complementary feature of RPPA and middle-down mass spectrometry in histone PTM profiling, and established RPPA as a valuable and a reliable tool for profiling changes in histone PTMs.

Conclusions: This RPPA platform has potential for translational applications through the discovery and validation of epigenetic states as therapeutic targets and biomarkers.

Reference:
Wang X, Shi Z, Lu HY, Kim JJ, Bu W, Villalobos JA, Perera DN, Jung SY, Wang T, Grimm SL, Taylor BC, Rajapakshe K, Park H, Wulfkuhle J, Young NL, Li Y, Coarfa C, Edwards DP, Huang S. High-throughput profiling of histone post-translational modifications and chromatin modifying proteins by reverse phase protein array. J Proteomics. 2022 Jun 30;262:104596

PP03.164: Phosphoproteomic Mining of Formalin-fixed Paraffin-embedded Oropharyngeal Cancer using SWATH Proteomic Data

Humphries E[1], Hains P[1], Hecker M[1], Dausmann M[1], Williams S[1], Jackson C[2], Porceddu S[3], Liu H[3], Ladwa R[3,4], Whitfield J[5], Simpson F[6], Panizza B[4,7], Robinson P[1]
[1]Children's Medical Research Institute, [2]Department of Otolaryngology, Head and Neck Surgery, Princess Alexandra Hospital, [3]Department of Cancer Services, Princess Alexandra Hospital , [4]School of Medicine, University of Queensland , [5]Pathology Queensland, Princess Alexandra Hospital , [6]The University of Queensland Diamantina Institute , [7]The Kinghorn Cancer Centre, St Vincent's Hospital
Introduction: Moderately large numbers of phosphopeptides have been reported in formalin-fixed, paraffin-embedded (FFPE) tissues using time-consuming sample fractionation combined with phosphopeptide enrichment strategies. Identification of phosphopeptides from FFPE samples without enrichment has not been investigated. We used high performance computing and new computational tools to investigate phosphoproteomic signatures in oropharyngeal cancer using proteomic data that was neither fractionated nor phosphopeptide-enriched. Instead, the data was taken from whole FFPE tissue digests.
Methods: Over 600 SWATH files from tryptic digests of 124 oropharyngeal cancer patients containing both tumour and adjacent normal tissue were acquired across four QTOF instruments. An Intel Xeon Platinum 8168 Processor was used to run FragPipe/DIA-NN via the DIA_SpecLib_Quant workflow[1] over 25 days. Default settings were used, aside from adding phosphorylation of serine, threonine and tyrosine residues as a variable modification.
Results: The spectral reference library contained 3,000 protein groups and 74,000 precursors after 1% FDR filtering. Cyclic loess normalisation on the raw quant value from the DIA-NN output matrix containing 50,000 non-phosphopeptide precursors and 20,000 phosphopeptide precursors revealed no batch effects. Technical replicates were averaged, charge states were summed and phosphopeptides with multiple phosphorylation sites in the same peptide were summed. A total of 80 phosphopeptides, involved in cell cycle regulation and glycolysis, were upregulated in tumour tissues compared to adjacent normal tissue.
Conclusions: The findings suggest that some phosphopeptides are preserved through the creation of FFPE blocks and that their identification can be used for biological insight. More investigations are needed to validate these peptides and determine whether a phosphoproteomic signature can predict patient outcomes and recurrence-free survival.
1. Demichev, V., et al. DOI:10.1101/2021.03.08.434385

PP03.165: Systematic Characterization of Proline Hydroxylation using Hydrophilic Interaction Chromatography (HILIC) and MS-based Proteomic Analysis to Reveal Novel PHDs Substrates

Jiang H[1], Druker J[1], Swedlow J[1], Rocha S[2], Lamond A[1]
[1]University Of Dundee, [2]University of Liverpool

Proline hydroxylation is one of the most important post-translational modifications (PTMs) in the control of hypoxia-inducible factor (HIF) levels and activity in normoxia. This PTM is catalysed by the prolyl hydroxylase domain proteins (PHDs) enzymes, while thus far no proline de-hydroxylase has been identified. The analysis of proline hydroxylation has been filled with technical difficulties, resulting in confusion and dogmas in the field. To understand the role of hydroxylation in controlling the function of proteins with hydroxyprolines, it is necessary to develop a comprehensive and robust method to identify proline hydroxylation sites.

Here we have developed a systematic work-flow for the global profiling of proline hydroxylation sites in proteins. Based on the hydrophilicity difference of adding the hydroxyl group to proline, classical HILIC can be used to distinguish and enrich peptides with hydroxyproline. By combining HILIC fractionation with high resolution LC-MS analysis, together with several refining and filtering parameters during data analysis, more than 1700 hydroxylated peptides were reliably identified from HEK293 cell samples. By comparing the hydroxylated peptides to the unmodified or oxidated peptides, as expected, hydroxylated peptides were significantly enriched in the more hydrophilic fractions using HILIC, and also clear difference of charge and mass distribution were found. Furthermore, we proved that the appearance and intensity of the hydroxyproline immonium ion, which was also considered as the diagnostic ion and the golden standard for the MS identification of hydroxyproline, was greatly influenced by the amount of the parent peptide ion, the position of prolines and the collision parameter setting in MS.

Moreover, combining SILAC-based quantitative proteomic analysis, we applied the work-flow to monitor the hydroxyproline sites changes in cells treated with the pan-PHDs inhibitor FG-4592 and to explore potential PHDs substrates other than HIF. This screen revealed a novel PHD dependent hydroxyl modification on RepoMan (CDCA2).

PP03.166: Phosphoproteomic Profiling of K-Ras G12C Inhibiting Drugs using decryptM

Kabella N[1], Bayer F[1], Kuster B[1]
[1]Technical University Of Munich

Introduction

K-Ras is the most frequently mutated proto-oncogene in cancer. The development of the first clinical K-Ras G12C inhibitors provide a new treatment option for cancer patients that harbor a K-Ras G12C mutation. Because K-Ras functions in the MAPK pathway which is mediated through a phosphorylation cascade, it is important to understand changes in the phosphoproteome as a result of K-Ras inhibition.

Methods

The clinical KRAS inhibitors Sotorasib and Adagrasib, as well as Mrtx1257, Ars853 and Ars1620, were investigated in the cell lines MiaPaCa2 (pancreatic cancer) and NCI-H23 (lung cancer) using the decryPTM approach. Cells were treated in a dose-dependent manner, lyzed in SDS, detergent was removed using the SP3 method, peptides were stable isotope encoded by tandem mass tags (TMT) and subsequently pooled following separation into 12 fractions by high-pH reversed-phase liquid chromatography and IMAC enrichment. Peptides were analysed by LC-MS3 on an Orbitrap Eclipse and quantified using MaxQuant.

Results

We identified around 20,000 phospho-peptides (p-peptides) for each decryPTM experiment of which 200 were regulated. For each p-peptide, EC50s values were derived that represent the in cellulo potency of the drug acting on the targeted protein. Adagrasib and Sotorasib displayed on target potencies of 6 nM and 20 nM in MiaPaCa2, respectively.
Regulated sites included p-sites on MAPK1/3 as well as other MAPK-pathway related proteins showing that all inhibitors engaged their K-Ras G12C target. Additionally, several further proteins with various described biological functions were regulated. Among these are p-sites on proteins that regulate mRNA transport, protein dephosphorylation or GTPase binding. This not only indicates that K-Ras activity regulates various cellular functions that are important for cell proliferation, but also places hitherto functionally unannotated p-peptides into the functional context of KRAS-MAPK signaling.

Conclusion

This study provides information on the mode of action of KRAS G12C inhibitors and cellular drug response.

PP03.167: Screening of Post-Translational Modifications (PTMs) of Raw and Thermally Treated Invertebrates and Vertebrates Digesta

Khulal U[1,2], Djukic T[3], Vasovic T[3], Rajkovic A[2], Velickovic T[1,2,3,4]
[1]Ghent University Global Campus, [2]Ghent University-Faculty of Bioscience Engineering, [3]University of Belgrade-Faculty of Chemistry, [4]Serbian Academy of Sciences and Arts

Introduction: Peptidyl PTMs could influence not only the final meat quality but also could exert adverse effects in vivo such as allergenicity and/or toxicity among several functional properties [1]. The complex real food matrix, processing treatment, and gastro-intestinal (GI) digestion can lead to major peptidyl PTMs via structural changes which consequently affect the protein functionalities, bioavailability at GI tract, immunoreactivity, allergenicity, toxicity and so on. Therefore, we have attempted to screen seven of the protein PTMs by probing the commercially available PTM specific antibodies (Ab) to the crude soluble proteins of meat samples upon thermal processing combined with simulated in vitro GI digestion.

Methods: Thermal treatment of samples (abalone, oyster, shrimp and chicken, pork, beef) followed by standardized static simulated in vitro gastrointestinal digestion [2], reducing SDS-PAGE of digesta supernatants, Western blot (WB) based on 7 PTM-specific pAb (4 types of lysine modifications, deamidation, methionine oxidation, and proline hydroxylation), nano Liquid Chromatography coupled to tandem mass spectrometry (nLC-MS/MS).

Results: Based on our WB PTM screening, subsequent nLC-MS/MS identification and PTM profiling, specific proteins at ≈20 (identified as sarcoplasmic calcium binding protein, SCP), ≈37 (tropomyosin, TM), ≈50, and ≈65 kDa were co-identified among invertebrates and vertebrates alike. These peptides (≈37 and ≈65 kDa corresponds to the shrimp allergen TM, ≈20 kDa SCP to oyster allergen, and ≈50 kDa to an abalone allergen) revealed strong Ab-signal to more than 3 PTM specific pAb out of 7. Accordingly, the most prominent PTM was observed to be lysine methylation and methionine oxidation. Ab-signal was not observed in samples treated with combined thermal treatment and digestion.

Conclusions: PTMs screening revealed specific peptides (allergenic) demonstrate certain PTM during real-life simulation of food processing and human digestion, thus facilitating a basic and relevant framework for further research into the relationship between peptidyl PTMs and their allergenicity.

PP03.168: Characterization of Pan-citrullination Antibodies for Proteome-wide Citrullination Studies

Laposchan S[1], Meelker Gonzalez R[1], Lee C[1]
[1]Young Investigator Group: Mass Spectrometry in Systems Neurosciences, School of Life Sciences, Technical University of Munich

Introduction: Citrullination is an irreversible post-translational modification (PTM) catalyzed by the enzyme family of peptidylarginine deiminases. This modification is concomitant with a mass increase of 0.98 Da and the loss of one positive charge. While citrullination occurs in healthy tissue to a low extent, its upregulation has been connected to various diseases including rheumatoid arthritis, multiple sclerosis, Alzheimer's disease, and amytrophic lateral sclerosis. To better understand the biological role of this PTM, selective enrichment of citrullination integrated into a proteomics workflow is aspired. However, suitable antibodies are not readily available, as most commercial products lack specificity or target particular proteins with a defined citrullination site. Here, we generate and evaluate potential antibodies for an effective immunoprecipitation workflow on the peptide level to systematically study the citrullination proteome.

Methods: Custom pan-citrullination antibodies were produced by immunization of rats with a peptide mixture containing the most commonly found sequences around the citrullination site in the human proteome (1). Performance and selectivity of these antibodies were examined along with a range of commercially available anti-citrulline antibodies. Artificial samples generated by in-vitro citrullination of cell lysate were used to develop an adequate immunoprecipitation protocol at peptide level. Enriched samples were measured using LC-MS/MS and data analysis was carried out using MaxQuant and a customized Python script for facilitated identification of citrullination events.

Results: The immunization of rats with the mixture of citrullinated peptides resulted in two positive clones. The specificity and efficiency of five (two customized and three commercial) antibodies to enrich citrullinated peptides were examined. Preliminary results show that these antibodies differ in their selectivity and enrichment efficiency towards citrullinated peptides.

Conclusions: The results show that selective enrichment of citrullinated peptides for proteome-wide citrullination studies using pan anti-citrullination antibodies is feasible with further optimization.

(1) Lee, et al. 2015 Mol Cell Proteomics 17;1378-1391.

PP03.169: Differential Protein Expression in Subjects with TBI with and without Neurological Deterioration

Hinson H[1], Jacobs J[2], McWeeney S[1], McCoy H[2], Rodland K[1]
[1]Oregon Health & Science University, [2]Environmental and Molecular Sciences Laboratory, Pacific Northwest National Laboratories

Introduction: Traumatic brain injury (TBI) is a common disease producing significant morbidity. Gaps in identifying subtypes of TBI have led to the intense search for fluid-based biomarkers. We sought to use global discovery mass spectrometry (MS) techniques to identify differential protein expression in subjects with TBI with and without neurological deterioration from intracranial hematoma expansion in the intensive care unit.

Methods: A representative subset (n=30) of subjects with TBI were analyzed from prospective observational trial. Baseline blood samples were collected within 3 hours of the antecedent trauma and aliquoted for plasma. Neurological Deterioration (ND) was defined as a decreased in Glasgow Coma Scale (GCS) score by 2 points or more sustained over 2 hourly measurements attributable to intracranial hematoma expansion in the first 48 hours after injury. Plasma was analyzed via a global proteomic approach which included depletion of high abundant proteins and utilized a high mass accuracy liquid chromatography MS/MS platform that encompassed TMT labeling and off-line HPLC fractionation for quantification and sensitivity of coverage. Descriptive statistics (mean+/-SD or median[IQR] for continuous variables; frequencies and percentages for categorical variables) were used to describe clinical data by group. Significance was set at $P<0.05$. All analyses were conducted in R.

Results: Twelve subjects (40%) deteriorated from hematoma expansion. Comparing those with and without deterioration, there were no differences in sex (75% v. 72% male), age (51+/-20 v. 44+/-18 years) or admission GCS scores (9 [6-10] v. 5 [3-8], p=0.196). Blood was obtained an average of 1.36 (+/-0.65) hours from the antecedent trauma. We confidently identified 3147 proteins in overall cohort, with a false discovery rate of 0.05. The results of complete computational analysis will be presented.

Conclusions: Computational analysis of the differentially abundant proteins should provide information about the biological processes underpinning ND that are differentially enriched in patients with ND.