See you in Busan!
BEXCO, Busan, Korea
2023.hupo.org
#HUPO2023

Together Through Proteomics

PROGRAM

Session Date/Time: Sunday, September 17, 2023 - 08:00 AM - 10:00 AM

PS01: The Non-canonical Proteome - A Novel Class of Clinically Targetable T Cell Antigens (Ticketed Session)

08:00   **PS01.01: Welcome Address**
Juan Antonio Vizcaino, United Kingdom

08:00   **PS01.01: Welcome Address**
Nicola Ternette, United Kingdom

08:05   **PS01.02: An Introduction to Riboseq**
Jorge Ruiz-Orera, Germany

08:15   **PS01.03: An Introduction to Immunopeptidomics**
Michal Bassani-sternberg, Switzerland

08:30   **PS01.04: Annotation of the Non-canonical Proteome - Current Requirements and Guidelines**
Sandra Orchard, United Kingdom

08:45   **PS01.05: Short Talks: Advances, Challenges, and Validation Strategies in Riboseq Annotations**
James Wright, United Kingdom

09:15   **PS01.06: Short Talks: Advances, Challenges, and Validation Strategies in HLA-associated Peptide Annotations**
Wei Wu, Singapore

09:15   **PS01.06: Short Talks: Advances, Challenges, and Validation strategies in HLA-associated Peptide Annotations**
Pouya Faridi, Australia

09:15   **PS01.06: Short talks: Advances, Challenges, and Validation Strategies in HLA-associated Peptide Annotations**
Anthony Purcell, Australia

Session Date/Time: Sunday, September 17, 2023 - 09:00 AM - 12:00 PM

PCT01: Computational Proteomics/Bioinformatics (Ticketed Session)

See you in Busan!
BEXCO, Busan, Korea
2023.hupo.org
#HUPO2023

Together Through Proteomics

PROGRAM

Chair

Wout Bittremieux, Belgium

09:00   **PCT01.01: Introduction to Statistical Design and Analysis of MS-based Proteomics Experiments**

Meena Choi, United States

09:45   **PCT01.02: An Overview of the MaxQuant Platform for Proteomics Data Analysis**

Dmitry Alexeev, Germany

10:30   **PCT01.03: Demystifying Post-translational Modification Analyses: Best Practices and Pitfalls**

Eunok Paek, Republic of Korea

11:15   **PCT01.04: An Overview of Machine Learning for Mass Spectrometry-based Proteomics**

Wout Bittremieux, Belgium

Session Date/Time: Sunday, September 17, 2023 - 09:00 AM - 12:00 PM

PCT02: Protein Interactions/Spatial Proteomics (Ticketed Session)

09:00   **PCT02.01: An Overview of Protein Interactions and their Location**

Kathryn Lilley, United Kingdom

Proteins are typically part of protein complexes that function in discreet locations within a cell. Many diseases such as cancers and dementias are associated with mislocalization of proteins and aberrant protein interactions. To fully understand subcellular mechanisms and how they are subverted in disease states, it is essential to create dynamic maps of interactions within and spatial location of the proteome. In this talk I will give an overview of methods to determine the components of proteins complexes and their protein subcellular location and how they are evolving.

09:45   **PCT02.02: Spatial Proteomics for Exploring the Tissue Heterogeneity**

Ruijun Tian, China

This lecture will cover the key steps for performing laser capture microdissection (LCM)-based spatial proteomic analysis of H&E- and IHC-stained fresh frozen or FFPE tissue slice. In addition, spatial proteomic analysis of tumor microenvironment by using multi-color IHC staining and automated imaging navigation-based LCM will be covered.

10:30   **PCT02.03: Mapping Functional Associations of Proteins**

Christoph Messner, Switzerland

Biological systems are organized in networks of functionally connected proteins that coordinately respond to perturbations or signals. Understanding these functional interactions can be crucial to interpret proteomics results and to understand phenotypes and protein functions. In this lecture, we will discuss how covariation analysis and systematic knock-out screens can be used to infer functional associations among proteins.

PROGRAM

11:15   **PCT02.04: How to Catch your Protein's Friends: Practical Tips for Designing Pull-Down Experiments**

Terence Chuen Wai Poon, Macao

Pull-down experiments are a powerful technique to identify and characterize the protein interaction partners of your protein of interest. However, designing and performing a successful pull-down experiment can be challenging, especially if you are new to the field. In this seminar, we will cover some tips and tricks to help you optimize your pull-down experiments and avoid common pitfalls.

Session Date/Time: Sunday, September 17, 2023 - 10:15 AM - 12:15 PM

PS02: Advancing Biomedical Research and Healthcare: Proteomics and Multi-Omics Integration for Precision Medicine (Ticketed Session)

10:17   **PS02:01: Emerging Opportunities in Multi-omics Research towards Precision Medicine**

Michael Snyder, United States

10:37   **PS02.02: Biology through a Multi-omics Prism: Challenges and Pathways for Synergistic Integration**

Sara Ahadi, United States

10:37   **PS02.02: Biology through a Multi-omics Prism: Challenges and Pathways for Synergistic Integration**

Daniel Hornburg, United States

10:59   **PS02.03: Bringing Multi-Omics to Clinics**

Julia Wang, United States

10:59   **PS02.03: Bringing Multi-Omics to Clinics**

Michael Roehrl, United States

11:39   **PS02.04: Moderator**

Khatereh Motamedchaboki, United States

11:39   **PS02.04: Panelist**

Michael Snyder, United States

11:39   **PS02.04: Panelist**

Sara Ahadi, United States

11:39   **PS02.04: Panelist**

Daniel Hornburg, United States

11:39   **PS02.04: Panelist**

Julia Wang, United States

PROGRAM

11:39   **PS02.04: Panelist**

Michael Roehrl, United States

Session Date/Time: Sunday, September 17, 2023 - 10:15 AM - 12:15 PM

PS03: The π-HuB Project: The Proteomic Navigator of the Human Body (Ticketed Session)

10:15   **PS03.01: Opening Introduction, π-HuB Progress**

Fuchu He, China

10:25   **PS03.02: ProteomicsDB**

Bernhard Küster, Germany

10:35   **PS03.03: Huiyan Progress, Application Guidelines**

Ruijun Tian, China

10:45   **PS03.04: Progress in the Liver Project**

Ying Jiang, China

10:55   **PS03.05 - ProtTalks**

Tiannan Guo, China

11:05   **PS03.06: Panelist**

Neil Kelleher, United States

11:05   **PS03.06: Panelist**

Jennifer Van Eyk, United States

11:05   **PS03.06: Panelist**

Juan Antonio Vizcaino, United Kingdom

Session Date/Time: Sunday, September 17, 2023 - 12:30 PM - 03:30 PM

PCT03: Single Cell Proteomics (Ticketed Session)

12:30   **PCT03.01: Setting the Stage for this Interactive Training Course**

Aleksandra Binek, United States

PROGRAM

12:35   **PCT03.02: Single-cell Proteomics Creates Opportunities for Unique Research in Heart Disease**
Aleksandra Binek, United States

13:05   **PCT03.03: Preparation Workflows for Single-cell Proteomics Analysis**
Bogdan Budnik, United States

13:35   **PCT03.04: Separation Methods in Single-cell Proteomics: Liquid Chromatography (LC) or Capillary Electrophoresis (CE)?**
Si Wu, United States

14:30   **PCT03.05: From MS Acquisition to Biological Insights into Single-cell Heterogeneity**
Florian A Rosenberger, Germany

Session Date/Time: Sunday, September 17, 2023 - 12:30 PM - 03:30 PM
PCT04: Proteomics 101 (Ticketed Session)

Chair
Je Yoel Cho, Republic of Korea

Chair
Min-sik Kim, Republic of Korea

12:30   **PCT04.01 - Review of the History of How We Came to Use NanoLC-MS for Bottom-Up Proteomics**
David Goodlett, Canada

We will review the technical developments leading to what we now refer to as shotgun proteomics or bottom-up proteomics as most often practiced for qualitiative description of the proteins in a sample. This history covers the time span from the early 1980s to the late 1990s demonstrating how the basics of nanoLC-ESI-MS were ready and waiting when the term proteomics was coined in 1994. It will highlight how being clever is critical to success in science. We will also teach how to assign b- and y-ions present in a peptide tandem mass spectrum, which is the currency of proteomics, to a known sequence. Finally we will touch on the very basics of how one uses a so-called search engine to query a peptide tandem mass spectrum against a sequence database.

13:15   **PCT04.02: Quantitative Proteomics: Measuring Protein Abundance by MS and How We Use It for Clinical Questions**
Yeoun Jin Kim, United States

This lecture will review the basic principles of protein quantification using LC-MS, and the major strategies in quantitative proteomics including SRM, PRM and DIA. We will compare each method for strength and limitations; critical steps in workflows for generating accurate readouts; and instrumentation settings. Finally, we will introduce the recent advances of quantitative proteomics in clinical applications.

See you in Busan!
BEXCO, Busan, Korea
2023.hupo.org
#HUPO2023

Together Through Proteomics

PROGRAM

14:00   **PCT04.03: Enrichment Strategies for Sub-Cellular Proteomics and Post-Translational Modifications**

Stuart Cordwell, Australia

This presentation will discuss the reasons for breaking complex proteomes into more, manageable, yet potentially more informative, parts. We will introduce the concept of 'looking at less, to see more' by examining the scientific questions that require enrichment of specific sub-proteomes, describing various approaches for achieving sub-proteome enrichment and the analysis of enriched sub-proteomes by mass spectrometry. Specifically, we will examine membrane and organelle enrichment, as well as several specific post-translational modifications (PTMs), including phosphorylation, glycosylation, lysine acetylation and redox PTMs. Finally, we will discuss what the expectations should be following an enrichment proteomics approach, sources of contamination, levels of purity and interpretation of the resulting data.

14:45   **PCT04.04: Affinity and Interaction Proteomics to Understand Functional Biology**

Wei Wu, Singapore

Targeting both MS novice and experienced biologists, this section will explain the basic need, rationale and strategies for affinity and interaction proteomics, using recently published examples. Key questions that will be addressed include how to design focused affinity and interaction proteomics experiments to aid in the study of functional biology, and conversely how biologists should interpret affinity and interaction proteomics output for conceptual advance in biology.

Session Date/Time: Sunday, September 17, 2023 - 01:15 PM - 03:15 PM

PS04: Implementing Proteomics for Clinical Translation – Implementation and Future Promise (Ticketed Session)

Chair

Yu-Ju Chen, Taiwan

13:17   **PS04.01: Bridging Proteomics to the Clinic – A Multivariate Blood Test for Disease Activity in Multiple Sclerosis**

Ferhan Qureshi, United States

01:32   **PS04.02: From Pre-Clinical Research to Clinical Settings: Development of a Biomarker Panel for Ovarian Cancer**

Stefan Enroth, Sweden

13:47   **PS04.03: Proteomics-Based Diagnostics, How to Overcome Obstacles in the Development of Commercial Service**

Myeong-Hee Yu, Republic of Korea

Despite protein markers dominating traditional clinical diagnosis, proteomics (LC/MS)-based diagnosis has not grown as significantly as genomics-based diagnosis. Clinical validation in the development of diagnostic agents is the most difficult process regardless of which method is used technically. Today's talk will consider what additional technical obstacles exist in the development of LC/MS-based diagnostics from a business standpoint, and discuss their solutions.

14:02   **PS04.04: Using the Immune Response to Detect Disease**

Joshua Labaer, United States

14:20   **PS04.05: Panelist**

Ferhan Qureshi, United States

14:20   PS04.05: Panelist
Stefan Enroth, Sweden

14:20   PS04.05: Panelist
Myeong-Hee Yu, Republic of Korea

14:20   PS04.05: Panelist
Joshua Labaer, United States

14:20   PS04.05: Panelist
Henry Rodriguez, United States

14:20   PS04.05: Panelist
Yeoun Jin Kim, United States

14:20   PS04.05: Panelist
Parag Mallick, United States

Session Date/Time: Sunday, September 17, 2023 - 01:15 PM - 03:15 PM

PS05: Science, Ethics, and Equity: Considering all Aspects of the Human Proteome (Ticketed Session)

13:15   PS05.01: Beyond the Genome: Ethical Issues in Large-Scale Proteomics Research
Kristien Hens, Belgium

13:15   PS05.01: Beyond the Genome: Ethical Issues in Large-Scale Proteomics Research
Ina Devos, Belgium

13:15   PS05.01: Beyond the Genome: Ethical Issues in Large-Scale Proteomics Research
Daan Kenis, Belgium

13:15   PS05.02: Enhancing equity, diversity, and inclusion in STEM
Jennifer Geddes-McAlister, Canada

14:15   PS05.03: Panelist
Jennifer Geddes-McAlister, Canada

14:15   **PS05.03: Panelist**

Kristien Hens, Belgium

14:15   **PS05.03: Panelist**

Daan Kenis, Belgium

14:15   **PS05.03: Panelist**

Ina Devos, Belgium

Session Date/Time: Sunday, September 17, 2023 - 04:00 PM - 05:00 PM

MS01: Mentoring Session 1 - AI Tools in Grant Writing Applications, Research Articles, CVs (Academic, Journal Editor)

Chair

Emily Hashimoto-Roth, Canada

Chair

Ruth Huttenhain, United States

MS01.01: Panelist

Laura Elo, Finland

MS01.02: Panelist

Min-sik Kim, Republic of Korea

MS01.03: Panelist

Jack Washington, United Kingdom

Session Date/Time: Sunday, September 17, 2023 - 05:45 PM - 07:30 PM

PL01: Opening Plenary: Jeremy Nicholson, Australia - Metabolic Systems Medicine and Phenomic Approaches to Understand the Natural History of COVID-19 and Long COVID

Chair

Jennifer Van Eyk, United States

18:00   **PL01.01: Opening Remarks KHUPO**

Jin Han, Republic of Korea

PROGRAM

18:00   **PL01.01 Opening Remarks**
Je Yoel Cho, Republic of Korea

18:10   **PL01.02: Opening Remarks HUPO**
Jennifer Van Eyk, United States

18:15   **PL01.03: Introduction to the Program**
Jennifer Van Eyk, United States

18:20   **PL01.04: ECR Update**
Ruth Huttenhain, United States

18:20   **PL01.04: ECR Update**
Mathieu Lavallée-Adam, Canada

18:32   **PL01.05: Plenary Speaker - Metabolic Systems Medicine and Phenomic Approaches to Understand the Natural History of COVID-19 and Long COVID**
Jeremy Nicholson, Australia

When a new zoonotic disease emerges in human populations, we engage in a desperate race to understand the complex disease biology in order to create new diagnostic and prognostic technologies and interventions to prevent or mitigate the effects of the pathogen. The emergence of the SARS COV-2 virus in late 2019 provided a spectacular example of this process in action. Diseases such as COVID-19 have complex systemic effects that lend themselves to exploratory studies using metabolic systems medicine and phenomic approaches that have been developed to study many diseases over the last few years. We and others have shown that molecular phenomic approaches using NMR and mass spectrometric generated multi-level metabolic data reveal the severity and complexity of the multi-organ impacts and the systemic biochemical perturbations caused by COVID-19. These methods also provide new multivariate quantitative metrics for assessing the functional recovery and systemic long-term risks from the disease. The use of standardised exploratory and targeted metabolic phenotyping linked to immunology has also proved to be a powerful way of exploring the patient journey from the "normal" population physiological state through the acute phase of the disease and into the "long COVID" and "recovery" states from the disease. In particular, the immuno-metabolic drivers of Long COVID are still poorly understood and require new diagnostic, prognostic and predictive tool development. In this lecture I will illustrate the integrative use of multiple phenomic technologies for studying the COVID-19 patient journey using collections of samples from multiple populations round the world and demonstrate a translational strategy for population level monitoring and assessment of PACS and functional biochemical recovery from the disease.

19:20   **PL01:06: Invite Delegates to Welcome Reception in Exhibit Hall**
Jennifer Van Eyk, United States

Session Date/Time: Monday, September 18, 2023 - 08:00 AM - 09:00 AM

ISS01: Standardization is Key – Proteomics for Everyone

See you in Busan!
BEXCO, Busan, Korea
2023.hupo.org
#HUPO2023

Together Through Proteomics

PROGRAM

08:00 **Single Cell and Plasma Proteomics on the Evosep: Bringing Proteomics to the Clinic**
Matthias Mann, Germany

08:30 **Slice-PASEF and Evosep One for Sensitive High-Throughput Proteomics**
Vadim Demichev, Germany

Session Date/Time: Monday, September 18, 2023 - 08:00 AM - 09:00 AM
ISS02: New Generation of PEAKS: Deep Learning Enabled Software Solutions for Immunopeptidomics, Glycoproteomics, and Post-translational Modifications

08:00 **The Non-canonical Immunopeptidome - What Contributes to Antigenic Diversity in Cancer and Autoimmune Disease**
Anthony Purcell, Australia

08:20 **Mining Widespread Lactylation in the Human Proteome with Cyclic Immonium Ion**
Hui Ye, China

08:40 **Glycan de Novo Sequencing and Label Free Quantification in PEAKS GlycanFinder**
Kyle Hoffman, Canada

Session Date/Time: Monday, September 18, 2023 - 08:00 AM - 09:00 AM
ISS03: Confidence from Discovery to Clinical Research: Harnessing the Versatility of the ZenoTOF 7600

08:00 **Multiple Applications of the ZenoTOF 7600**
Peter Hoffmann, Australia

08:30 **TBD**
Phil Robinson, Australia

Session Date/Time: Monday, September 18, 2023 - 09:15 AM - 10:00 AM
PL02: Plenary Session: Yu-Ju Chen, Taiwan - Evolution of Micro-to-nano Proteomics toward Precision Oncology

Chair
Youngsoo Kim, Republic of Korea

PROGRAM

09:17   **PL02.01: Plenary Speaker - Evolution of Micro-to-nano Proteomics toward Precision Oncology**

Yu-Ju Chen, Taiwan

Personalized proteomics starts to inspire new strategies for precision medicine by facilitating discovery of protein biomarkers, molecular signature of disease subtype and response to treatment of individual patients. Since the launch of Taiwan Cancer Moonshot under the collaboration framework of the International Cancer Proteogenome Consortium (ICPC), we have established the proteogenomics landscape of early stage lung adenocarcinomas and breast cancer in Taiwan, providing new insight on the molecular signature and pathogenesis Most excitingly, proteomics-informed classification demonstrated unprecedent resolution to resolve the diverse clinical trajectories of patients within and enables the path for pursuing precision oncology for early stage management. Nevertheless, the translation of proteomic discovery faces multiple challenges in both technical robustness and sensitivity, as well as large-scale validation and assay validation. Though these works provide rich information on the molecular cancer landscape, genome-wide (phosphor)proteome profiling is still limited for microscale clinical specimens that are most accessible for diagnosis. To implement its practical utility for the most accessible specimen such as needle biopsy, we recently developed a global phosphoproteomics system (GPS) strategy based on DIA-MS with a high-quality hybrid spectral library for rapid and deep profiling. With an ultra-streamlined workflow combining integrated proteomics chip (iProChip) and DIA, we also demonstrated ultra-sensitive analysis at the resolution of nano-to-single cell proteomics and phosphoproteomic and their application to reveal the high heterogeneity of patient with acquired resistance to third-generation EGFR therapy.

Session Date/Time: Monday, September 18, 2023 - 10:15 AM - 11:45 AM

CS01: Multi-omics

Chair

Phil Robinson, Australia

Chair

Sun-young Kong, Republic of Korea

10:16   **CS01.01: Keynote Speaker - Transforming Health with Deep Data and Remote Profiling**

Michael Snyder, United States

10:41   **CS01.02: Keynote Speaker: Population Proteomics: A Path to Precision Medicine**

Chris Whelan, United States

11:06 **CS01.03: Molecular Responses During Bacterial Filamentation Reveal Inhibition Methods of Drug Resistant Bacteria**

Dongxue Zhang, China

Bacterial antimicrobial resistance (AMR) is among the most significant challenges to current human society. Exposing bacteria to antibiotics can activate their self-saving responses, e.g. filamentation, leading to the development of bacterial AMR. Understanding the molecular changes during the self-saving responses can reveal new inhibition methods of drug-resistant bacteria. Herein, we used an online microfluidics mass spectrometry system for real-time characterization of metabolic changes of bacteria during filamentation under the stimulus of antibiotics. Significant pathways, e.g. nucleotide metabolism and coenzyme A biosynthesis, correlated to the filamentation of extended spectrum beta-lactamase producing E. coli (ESBL-E. coli) were identified. A cyclic dinucleotide, c-di-GMP, which is derived from nucleotide metabolism and reported closely related to bacterial resistance and tolerance, was observed significantly upregulated during the bacterial filamentation. By using a chemical inhibitor, ebselen, to inhibit diguanylate cylases which catalyzes the synthesis of c-di-GMP, the minimum inhibitory concentration of ceftriaxone against ESBL-E. coli was significantly decreased. This inhibitory effect was also verified with other ESBL-E. coli strains, and other beta-lactam antibiotics, i.e. ampicillin. A mutant strain of ESBL-E. coli by knocking-out the dgcM gene was used to demonstrate that the inhibition of the antibiotic resistance to beta-lactams by ebselen was mediated through the inhibition of the diguanylate cyclase DgcM and the modulation of c-di-GMP levels. Our study uncovers the molecular changes during bacterial filamentation, and proposes a new method to inhibit antibiotic-resistant bacteria by combining traditional antibiotics and chemical inhibitors against the enzymes involved in bacterial self-saving responses.

11:19 **CS01.04: Proteogenomic Analysis of Human Pancreatic Ductal Adenocarcinoma**

Do Young Hyeon, Republic of Korea

Background: Pancreatic ductal adenocarcinoma (PDAC) is a devastating disease with poor prognosis, and the situation has not improved despite extensive clinical and scientific efforts. To better optimize therapeutic strategies based on molecular signatures associated with cancer pathogenesis, we performed a proteogenomic analysis of PDAC.
Methods: We collected tumor and matched blood samples from 196 patients with PDAC. We performed whole exome sequencing (WES) and mRNA sequencing (mRNA-seq) for the tumor and blood samples. We selected 150 of 196 tumor samples with high tumor cellularity for global proteome and phosphoproteome profiling using liquid chromatography-tandem mass spectrometry (LC-MS/MS).
Results: By integrating these WES, mRNA-seq, and LC-MS/MS data, we found: i) mutation-signaling interplays in PDAC by mutation-phosphorylation correlation analysis, ii) potential prognostic biomarkers in PDAC by mRNA-protein correlation analysis, iii) six proteogenomic PDAC subtypes by integrative clustering analysis, and iv) potential therapeutic target molecules and pathways associated with the six PDAC subtypes. Notably, we subdivided conventional squamous subtype of PDAC, most treatment-resistant subtype of PDAC, into 3 subtypes (activated stroma-enriched, invasive, and proliferative) based on proteome and phosphoproteome data and suggested combined therapeutic strategies for each subtype.
Conclusions: Our study shows the importance of proteogenomic analysis in unveiling the potential biomarkers and subtype-specific targetable pathways of PDAC that are beyond reach by genomic data alone. The identified subtypes could be helpful in tailoring therapeutic options and would also be a valuable launching point for further mechanistic and translational studies.

PROGRAM

11:32   **CS01.05: Integrative Proteogenomics Profiling Uncovers Non-histone Protein Methyltransferases as Novel Therapeutic Targets in Diffuse Midline Glioma**
Arun Kumaran Anguraj Vadivel, Canada

Diffuse midline glioma (DMG) is an aggressive and devastating pediatric brain tumor that poses significant challenges in terms of treatment and prognosis. The prognosis for DMG is extremely poor, with a median survival rate of less than a year. Despite significant advancements in genomics, DMG remains incurable with conventional treatment modalities. Recognizing the urgent need for effective therapeutic strategies and targets, we embarked on a comprehensive proteomics profiling study to unravel the molecular mechanisms driving DMG tumorigenesis. We performed an extensive mass spectrometry-based deep proteomic analysis of DMG tissues, including total proteome, phosphoproteome, and methylproteome. Our findings revealed that DIPG tissues have lower global protein phosphorylation and higher global protein methylation compared to normal brains, implying that DMG may use methyl-signaling rather than phospho-signaling for tumour growth. Integrating proteomics data with DNA methylation and transcriptomics (bulk RNAseq and single-cell RNAseq) using unsupervised and supervised machine learning (ML) provided new insights into DMG tumorigenesis. Our ML analysis identified EEF1A1, EEF1A2, METTL13, and METTL21B as top features across multi-omics datasets in DMG. The translation elongation proteins EEF1A1 and EEF1A2 are the most highly methylated proteins in DMG. Methylation modifications such as K55me2, K79me3, and K165me2 of EEF1A1 are significantly higher in DMG tissues compared to normal brains. These enriched methylpeptides are substrates of the non-histone methyltransferases METTL13 and METTL21B. Knocking down these methyltransferases in DMG cells significantly decreases their target protein methylation, reduces global protein synthesis, and inhibits cell growth in vitro. Thus, proteogenomic analysis of DMG revealed tumour-enriched non-histone methyltransferases, METTL13 and METTL21B as new therapeutic targets. These novel findings present previously unexplored avenues for the development of targeted therapies in the treatment of DMG and underscore the importance of integrative proteogenomic investigations in uncovering specific targets for incurable cancers like DMG.

Session Date/Time: Monday, September 18, 2023 - 10:15 AM - 11:45 AM

CS02: Precision Medicine

Chair
Stefan Tenzer, Germany

Chair
Sang Hoon Song, Republic of Korea

10:16   **CS02.01: Keynote Speaker - Unlocking Food Composition Data and Cracking the Safe to Better Health**
Jennifer Hall, United States

See you in Busan!
BEXCO, Busan, Korea
2023.hupo.org
#HUPO2023

Together Through Proteomics

PROGRAM

10:41   **CS02.02: Keynote Speaker - Unlocking the Potential of Precision Medicine (Genotype-To-Proteotype Analysis)**

Henry Rodriguez, United States

Precision medicine, the practice of providing appropriate treatments to specific patients at the right time, has emerged as the preferred approach for effective healthcare. Genomic information has played a crucial role in advancing precision medicine by aiding in the identification of various tumor types based on gene transcript abundance. It has also facilitated the selection of therapeutic drugs for patients, resulting in remarkable responses, including complete remissions.

However, precision medicine is currently limited to specific clinical issues due to the complex regulatory processes involved in gene activity and protein expression. These complexities make it challenging to identify significant biological differences based solely on gene activity, which hinders optimal interventions and patient care. As a result, precision medicine has not been widely successful in treating most cancer patients.

To overcome these challenges, integrating proteomics data with genomics data has emerged as a valuable approach. Proteogenomics enhances our understanding of the causes of cancer and enables the selection of more precise targets for potential therapeutic interventions by providing detailed insights into cancer-related pathways. Integrating genomics, transcriptomics, and proteomics has the potential to become an essential part of laboratory medicine. This seminar will explore how these three areas are being combined in basic and translational research, including clinical trials.

11:06   **CS02.03: Ex Vivo Drug Response Heterogeneity Combined With Cell-Population-Specific Proteotyping Reveals Personalized Therapeutic Strategies for Patients With Multiple Myeloma**

Sandra Goetze, Switzerland

Background
Multiple myeloma (MM) is a complex disease characterized by the proliferation of abnormal plasma cells in the bone marrow. In recent years, treatment options for MM patients have increased substantially: instead of standard chemotherapy, patients receive proteasome inhibitors frequently containing immunomodulatory drugs as initial treatment. However, patients respond differently and invariably relapse, which requires successive therapies to control the disease. To understand clinical treatment response and variability within primary patient-derived cells, we combined pharmacoscopy, an image-based ex-vivo drug-screening strategy, with cellular proteotyping.

Methods
In parallel to a pharmacoscopy screen to assess the ex-vivo drug responsiveness of malignant MM cells to 61 drugs and immunotherapies, the proteotype of sub-sorted cell populations from the patient-derived samples was analyzed. CD138-positive plasma cells, CD3-positive T-cells and CD14-positive monocytes which support malignant plasma cell growth and survival, were isolated from MM patient bone marrow aspirates. Using a standardized and harmonized DIA-based proteotyping strategy, we generated a data matrix of 5723 human proteins across 294 samples of 102 patients with MM.

Results
The MM patient cohort was stratified into three phenogroups (PGs) based on the cellular composition of the bone marrow biopsies. These PGs corresponded to disease progression, inflammation, and clonality and were strong predictors for ex-vivo drug sensitivity. Proteotype profiles from CD138-positive plasma cells differed significantly between the different PGs, which was also reflected in CD3-/CD14-positive cell populations. Correlation of proteotype patterns across the 61 drugs and combinations revealed well-defined functionally related protein sub-networks, centered around a ribosomal and translational core network. Amongst others, prototyping identified CDK5 and HLA-DRB5 as potential new drug targets.

Conclusions
The integration of proteotyping with pharmacoscopy enabled the identification of protein signaling networks involved in MM drug responsiveness. The proteotyping data provide a rational basis to better understand the molecular mode of action of selected MM drugs and combinations.

PROGRAM

11:19

CS02.04: Precision Proteomic Landscape of Health-To-Disease Transitions of Rheumatic Musculoskeletal Disorders and Links to Cancer Associate irAEs

Allan Stensballe, Denmark

Background
Rheumatic musculoskeletal disorders (RMDs) comprise over 200 diseases and syndromes, which are usually associated by autoantibody emergence, progressive and associated with pain. Early diagnosis and long-term remission efforts necessitate routine clinical assessment. Immune checkpoint inhibitors (ICI) associate with a wide range of immune-related adverse events (IrAEs), including musculoskeletal manifestations. Our long-standing research focus have addressed serological and synovial histological heterogeneity in key RMDs including rheumatoid arthritis (RA) and cancer irAEs. Identifying determinants of synovial pathotypes and associated complexity-titer of autoantibodies we pursue new insights into RMD's pathological mechanisms, identify new therapeutic targets, and pave the way for personalized medicine options.

Methods
Patient cohorts were based on patient heterogeneity, clinical data and disease trajectory (e.g. naïve, early, late; irAE/-). Precision proteomic analysis were performed by deep discovery proteomics profiling of biofluids and synovial needle biopsies (ST) (EvosepOne;timsTOF PRO2) as well as autoantigen array technology (Sengenics) followed by data integration in R-environment.

Results
For RA we have determine novel molecular and cellular determinants of synovial histological pathotypes were identified in untreated ERA patients by integrating the ST proteome, plasma proteome, Krenn synovitis score, and synovial immune infiltration. 335 plasma proteins and 3416 ST proteins were identified and quantified. Data integration identified mainly tissue proteomic and cellular signatures underlying synovial pathotypes, that suggests the difference between lymphoid, myeloid, and fibroid pathotypes is a continuous spectrum that is linearly modellable. A discovery autoantibody profiling of 1600 targets determined unique disease stage signatures. The latter autoantibody signatures were simultaneously associated with irAEs in non-small cell lung cancer patients and associated treatment outcome.

Conclusions
Our findings in multiple studies reveal novel insight into the proteomic landscape of the synovium in RA, elucidating the pathological mechanisms occurring in the synovium at different stages of disease. Autoantibody complexity and dynamics pave the way for personalized medicine options.

11:32   **CS02.05: Circulating Proteins in Breast Cancer Risk and Etiology**

Jochen Schwenk, Sweden

Background:
Breast cancer is the most diagnosed malignancy in women in the Western world and the second leading cause of death. Despite the progress in diagnosing and treating those with the disease, improved treatment outcomes, life quality, and lower cost burden for healthcare systems will ultimately be facilitated through earlier cancer detection. Hence, more focus should be on identifying the causal processes and protecting those women at risk of developing breast cancer.

Methods:
We profiled > 1600 women from Sweden's most comprehensive breast cancer screening cohort (https://karmastudy.org), including 800 women sampled within three years before diagnosis. First, we studied cancer risk using 190 cancer- and metabolism-related proteins using Olink's proximity extension assays. Next, we studied 3000 proteins using the Olink Explore assay in 600 women by genome-wide association studies (GWAS) and Mendelian randomization (MR) to identify proteins with a causal role in the disease. Lastly, we examined these 3000 proteins in a low-dose tamoxifen pilot belonging to a preventive treatment trial.

Results:
Our investigations identified dominant associations of blood proteins with basic clinical parameters (BMI, age, menopause, smoking, or alcohol use). Risk analysis indicated some but only marginally significant links between protein levels and future disease development. The genome-wide discovery of variants revealed > 700 cis-acting protein quantitative trait loci (pQTL) associated with plasma proteins. Using the pQTLs as instruments in Mendelian randomization (MR) analysis shortlisted five proteins with a possible causal role in breast cancer. We found the the effects of low-dose tamoxifen to affect a variety of system-wide processes and more extensive than currently anticipated.

Conclusion:
Deep and integrative analyses of circulating proteome identified proteins associated with breast cancer etiology and preventive treatment. The identified proteins can assist the development of new drugs and monitor their therapeutic effects.

Session Date/Time: Monday, September 18, 2023 - 10:15 AM - 11:45 AM

CS03: Interactomes/Protein Networks

Chair

Christoph Messner, Switzerland

Chair

Mikhail Savitski, Germany

10:16   **CS03.01: Keynote Speaker - MitoAtlas, a Super-resolution Proteome Map of Mitochondria**

Hyun-Woo Rhee, South Korea

10:41   **CS03.02: Keynote Speaker - Mapping the Diversity in Spatiotemporal Regulation of G Protein-Coupled Receptors**

Ruth Huttenhain, United States

11:06

CS03.03: Disease Representation in the Reactome Pathways Database

Henning Hermjakob, United Kingdom

Reactome (https://reactome.org) is a free, open-source, open-data, curated and peer-reviewed knowledge base of biomolecular pathways, currently covering 11,371 proteins in 14,516 reactions supported by 36,444 literature references (Release 84, March 2023).

Pathways are arranged in a hierarchical structure, allowing the user to navigate from high level concepts like immune system to detailed pathway diagrams showing biomolecular events like membrane transport or phosphorylation.

For the higher levels of the hierarchy, Reactome provides scalable, interactive textbook-style diagrams in SVG format, which are also freely downloadable and editable. Repeated diagram elements like mitochondrion or receptor are freely available as a library of graphic elements at https://reactome.org/icon-lib.

Pathway analysis capabilities range from simple gene enrichment to quantitative multi-omics analysis with ReactomeGSA. Higher confidence interactors from the IntAct database can be integrated in the analysis. Analysis can be submitted through an R interface, and results are presented interactively in a visually attractive genome-wide results overview based on Voronoi maps.

Reactome curates consensus pathways in the context of a generic human cell. Based on these "normal" pathways, we curate disease information following three key paradigms: Loss of function (often metabolic diseases), gain of function (often cancer related), and host-pathogen interactions. A recent curation focus are sequence variants underlying disease and their mechanistic impact, as well mode of action for drugs as disease modulators. For 372 disease genes, we annotate the mechanistic impact of 4,832 unique disease variants. Known disease associations from DisGeNET can now be overlayed on the pathway visualisations.

In the context of pandemic preparedness and our collaboration with the Disease Maps Community, we have developed a concept of curating detailed host-pathogen interactions for representatives of pathogen classes.

Reactome follows an open source, open data policy, all data and source code are available under permissive licences.

11:19

CS03.04: Mapping Evolutionarily Conserved Protein-Protein Interactions in the Vertebrate Brain

Vy Dang, United States

Much of the complexity of the vertebrate brain arises from intricate networks of proteins that regulate neuronal functions, signaling, and plasticity. Protein-protein interactions (PPIs) are critical for proper neural functioning and mapping PPIs offers a promising avenue for addressing long-standing questions in molecular neuroscience relating to protein's detailed molecular and cellular roles in the brain. However, characterizing PPIs directly from primary brain tissues in a high-throughput manner remains a significant challenge. We report a systematic survey of evolutionarily conserved brain-specific protein-protein interactions across 5 vertebrates (pig, mouse, chicken, rabbit, and dolphin), spanning >300 million years of evolutionary distance. We applied co-fractionation/mass spectrometry (CF-MS), a tag- and label-free protein interaction mapping approach, to identify neuronal PPIs and "protein neighborhoods" shared across the vertebrate brains. The CF/MS involved non-denaturing extraction of endogenous proteins, chromatographic fractionation of native protein assemblies, and mass spectrometry to identify co-eluting proteins. By interpreting data from >2,000 mass spectrometry experiments in an evolutionary framework, we observed >6,000 conserved proteins participating in multi-protein assemblies and defined >80,000 pairwise PPIs (8% FDR) in total. Our data capture known neuronal complexes as well as potential novel interactions, with many independently supported by AlphaFold structure prediction or independent validation. This work lay a foundation for more detailed explorations of molecular mechanisms underlying neuronal activities and for future investigations of neurological disorders.

11:32 **CS03.05: The Social and Structural Architecture of an In-Depth Cellular Protein Interactome**

André Michaelis, Germany

Nearly all cellular functions are mediated by protein-protein interactions and mapping the interactome provides fundamental insights into the regulation and structure of biological systems. In principle, affinity purification coupled to mass spectrometry (AP-MS) is an ideal and scalable tool, however, it has been difficult to identify low copy number complexes, membrane complexes and those disturbed by protein-tagging. As a result, our current knowledge of the interactome is far from complete, and assessing the reliability of reported interactions is challenging. Here we develop a sensitive, high-throughput, and highly reproducible affinity enrichment coupled to mass spectrometry (AE-MS) technology combined with a quantitative two-dimensional analysis strategy for comprehensive interactome mapping of Saccharomyces cerevisiae. We reduced required cell culture volumes thousand-fold and employed 96-well formats throughout, allowing replicate analysis of the endogenous green fluorescent protein (GFP) tagged library covering the entire expressed yeast proteome. The 4159 pull-downs generated a highly structured network of 3,927 proteins connected by 31,004 interactions. Compared to previous large-scale studies, we double the number of proteins (nodes in the network) and triple the number of reliable interactions (edges), including very low abundant epigenetic complexes, organellar membrane complexes and non-taggable complexes interfered by abundance correlation. This nearly saturated interactome reveals that the vast majority of yeast proteins are highly connected, with an average of 16 interactors, the majority of them unreported so far. Similar to social networks between humans, the average shortest distance is 4.2 interactions. Taking advantage of global structure prediction using AlphaFold-Multimer, we gained novel insights into the functional roles of previously uncharacterized proteins in complexes. A web portal (www.yeast-interactome.org) enables exploration of our dataset by the network and biological communities and variations of our AE-MS technology can be employed in any organism or dynamic conditions.

Session Date/Time: Monday, September 18, 2023 - 10:15 AM - 11:45 AM

CS04: Proteomics of Brain Diseases

Chair

Jong Bae Park, Republic of Korea

Chair

Chien-Yun Lee, Germany

10:16 **CS04.01: Keynote Speaker - Antibody-based Neuroproteomics**

Peter Nilsson, Sweden

10:41 **CS04.02: Keynote Speaker -Molecular Pathogenesis Based on Proteomic and Genomic Analysis in Alzheimer's Disease**

Inhee Mook-Jung, Republic of Korea

PROGRAM

11:06 CS04.03: Draft Human Brain Proteome Atlas for Understanding the Molecular Basis of Brain Functions

Qi Xiao, China

A comprehensive understanding of the molecular basis of brain functions requires the proteomic characterization of the entire human brain. Here, we present a draft human brain proteome atlas that characterizes 12,768 proteins, including 3780 protein isoforms, expressed in 1430 functional brain regions from the cerebral cortex, subcortical nuclei, and cerebellum of four Chinese individuals. The cerebral cortex samples were segmented into white and gray matter and analyzed separately. After sampling, the MRI scanning was performed to obtain the spatial coordinates of all the sampling locations. Furthermore, we also analyzed the proteome of 86 epilepsy brain tissues from 49 patients. Totally we acquired 2291 proteomes. Compared with transcript expression, we observed greater heterogeneity in protein expression across cerebral cortex regions. The regions in cerebral cortex have seven times as many cumulative elevated proteins compared to transcripts, suggesting a high level of post-transcriptional regulation in the cortex. Next, we exposed 328 proteins in associated with specific psychological processes in the cortex. Specifically, the olfaction and gustation show association with the highest number of proteins, suggesting that they are tightly modulated. Additionally, by comparing the proteomes of normal and epileptic brain tissue, we found 1560 of dysregulated proteins as potential drug targets. Among them, 58 proteins are targets of existing antiepileptic drugs. Notably, we observed distinct patterns of protein alterations in different cell types within the epileptic brain. Specifically, epileptic neurons and astrocytes exhibited prominent changes in mitochondrial function and EIF2 signaling, while oligodendrocytes were primarily associated with inflammatory responses. Our atlas provides a valuable resource for the neuroscience community to explore the molecular basis of brain functions, and identify potential drug targets for neurological disorders.

11:19 CS04.04: Paired CSF Proteins With Enhanced Potential to Monitor Neurodegeneration

Sara Mravinacova, Sweden

Background: Amyloid and tau aggregates are considered to cause neurodegeneration and consequently cognitive decline in individuals with Alzheimer's disease. To combat this, new treatments are emerging targeting these aggregates, which increases the need for biomarkers to evaluate their efficacy. Here, we explore the potential of paired CSF proteins matched based on their association to CSF amyloid, tau and nFL levels to reflect neurodegeneration and hence to monitor treatment outcomes.

Methods: We used a multiplex antibody-based suspension bead array technology to measure the levels of 49 pre-selected CSF proteins in a Swedish cohort from Karolinska University Hospital comprising of 241 individuals with AD or mild/subjective cognitive decline (MCI/SCD). A cohort of 52 AD/SCD individuals from VUMC, Netherlands was further used for validation.

Results: Correlation analysis of the measured proteins to CSF amyloid and tau levels revealed two protein clusters. One cluster with proteins strongly correlated to t-tau and p-tau, these were mainly synaptic proteins previously found elevated in AD including GAP43, SNCB or NRGN; and one cluster with aβ42 correlated proteins including PTPRN2, NCAN or CHL1. Further analysis using support vector machine modelling revealed that protein pairs combined from the two clusters discriminate AD-affected from unaffected individuals with high accuracy (AUC = 0.98 for GAP43+PTPRN2) compared to single proteins (AUC = 0.81 for GAP43). Moreover, GAP43/PTPRN2 ratio demonstrated negative correlation to cognitive scores ranging between R = -0.38 (MMSE) and -0.56 (RAVLT), considerably higher compared to GAP43 alone (R = -0.15 for both). The results were further validated with an independent cohort.

Conclusions: Combining the levels of CSF synaptic proteins in pairs largely enhance their correlation to cognitive decline, potentially through adjustment for inter-individual variability in protein levels. With these results, we highlight the potential of CSF synaptic protein pairs to monitor neurodegeneration and hence the efficacy of AD causative therapies.

11:32   **CS04.05: Plasma-Multiprotein-Biomarker Models for Screening of Brain Amyloid Status and Early Diagnosis of Alzheimer's Dementia Using Multiple Reaction Monitoring-Mass Spectrometry**
Youngsoo Kim, Republic of Korea

Introduction: Globally, the number of individuals with Alzheimer's Dementia(AD), the most leading cause of dementia, has increased sharply with the trend of population aging. Brain amyloid plaques and cerebrospinal fluid biomarkers acknowledged as pathologic features of AD are examined by positron emission tomography (PET)-imaging regarded as gold standards for the clinical diagnosis of AD. However, the invasiveness and high costs of PET-imaging confine their application in large-scale clinical use and society screening. Conversely, blood-based biomarkers have the potential to mitigate these obstacles that impede efficient and accurate early diagnosis of AD. In this study, plasma-multiprotein-biomarker(PMB) models were developed to screen brain amyloid status and early diagnosis of AD using a highly selective and sensitive Multiple Reaction Monitoring-Mass Spectrometry(MRM-MS) in targeted proteomics approach.
Methods: The study population included total 1393 patients(607 PET-positive and 775 PET-negative amyloid status) with 232 AD, 775 amnestic mild cognitive impairment(aMCI), and 386 normal control(NC), aged 42 to 92 years. The MRM-MS analysis was performed to quantify our lab-developed biomarker candidates in plasma samples from each individual. Through machine learning-based regression analysis, the best optimized PMB models were developed by combination of these candidates. Discriminatory and diagnostic performances of the models were evaluated by AUROC analysis and optimal cutoff-based diagnostic parameters—sensitivity, specificity, and accuracy. Furthermore, improved PMB models were developed by adding plasma beta-amyloid 42 levels and demographical information as features.
Results: The 15-plasma protein PMB model was developed and discriminated amyloid beta status with good performances(e.g. AUC>0.90). When beta-amyloid 42 levels, age, and gender as features were added to the PMB model, the performances were improved(e.g. AUC>0.94).
Conclusions: Our study demonstrated the potential of a blood multiprotein biomarker-based screening method with MRM-MS in discrimination and early diagnosis of brain amyloid status and AD, which is likely to be comparable to the PET image-based golden standard.

Session Date/Time: Monday, September 18, 2023 - 11:45 AM - 01:00 PM

PP01: Odd Numbered Posters - Poster Viewing with Coffee Break

    PP01.01: Ecklonia Cava Extracellular Vesicles Mitigated UV-B Induced Skin Pigmentation and Basement Membrane by Modulating NRF2
Kyung-A Byun, Republic of Korea

Excessive exposure to ultraviolet B (UV-B) radiation is a major cause of skin pigmentation and basement membrane damage, which can lead to a variety of skin diseases. Echlonia cava, a brown alga abundant in marine ecosystems, has gained attention for its potential health benefits. With the increasing focus on appearance, exosomes are being utilized in cosmetics and medical devices. As natural nanocarriers, exosomes are gaining attention as the next generation of biotherapeutics and drug delivery vehicles. Recently, plant exosomes have attracted much attention due to their suitability for large-scale isolation. In this study, we investigated the protective effects of Echlonia cava extracellular vesicles (EVs) against UV-B-induced skin pigmentation and basement membrane damage and elucidated the underlying molecular mechanisms involving the nuclear factor erythropoietin 2 (NRF2).
UV-B irradiation significantly increased melanin production and induced basement membrane disruption in human keratinocytes and a three-dimensional human skin model; however, pretreatment with Echlonia cava EV effectively mitigated these UV-B-induced effects. Echlonia cava EV reduced melanin synthesis by inhibiting the expression of key melanogenic enzymes, such as tyrosinase and tyrosinase-related protein 1 (TRP-1), and restored basement membrane integrity by promoting the expression of basement membrane components, including nidogen and laminin. In addition, Echlonia cava EV activated the NRF2 signaling pathway in UV-B-irradiated HaCaTs. Activation of NRF2 increased the expression of antioxidant enzymes, including heme oxygenase-1 (HO-1), and decreased intracellular reactive oxygen species levels.
In conclusion, our findings demonstrate that Echlonia cava EV effectively mitigates UV-B-induced skin pigmentation and basement membrane damage. These protective effects are mediated by modulation of NRF2 signaling, which inhibits melanogenesis and promotes basement membrane integrity. Echlonia cava EV is a potential natural therapeutic strategy for the prevention and treatment of UV-B-induced skin diseases and has emerged as a promising application in the field of dermatology and skin health.

PROGRAM

PP01.03: OMICS Analysis for the Discovery and Verification of Novel Brain-specific Extracellular Vesicles for the Diagnosis of Brain Diseases

Yuri Choi, Republic of Korea

The utilization of blood-based extracellular vesicles (EVs) for the diagnosis of brain disorders has gained significant attention due to its potential for early detection, disease progression tracking, non-invasiveness, and cost-effectiveness, allowing for repeated measurements. Numerous studies have reported a association between molecules within blood-based EVs and various diseases, including brain disorders, confirming their potential for disease diagnosis. However, the identification of brain-specific EVs as biomarkers in blood for the diagnosis of brain disorders is still lacking.
In this study, based on human protein data, proteomics, and small sequencing data, we aim to employ OMICS analysis to propose and validate new markers of brain-specific EVs.

By utilizing human protein data, we can identify potential candidate markers that exhibit specificity for brain-related processes. These candidate markers are then subjected to further experimental validation using proteomics, which allows for the identification and quantification of proteins present in the EVs. Additionally, small RNA sequencing can provide insights into the specific RNA molecules present in these vesicles, further enhancing the brain specificity assessment.

To validate the brain specificity of these markers, we compare their expression in samples derived from various brain disorders and healthy controls. By analyzing the differential expression patterns, we can assess the potential of these markers to distinguish between diseased and healthy conditions, thereby demonstrating their relevance in the diagnosis or monitoring of brain disorders.
Overall, the strategy involves integrating human protein data, proteomics, and small RNA sequencing to explore, validate, and compare the expression of brain-specific EV markers in clinical samples of brain disorders. These findings demonstrate that the newly discovered brain-specific EV marker can capture brain-derived EVs in human plasma. This holds potential for non-invasive diagnosis and monitoring of brain disorders.

PP01.05: The Orbitrap Astral Mass Spectrometer Enables the Quantification of >5000 Proteins from a Plasma Extracellular Vesicle Sample

Lilian Heil, United States

Despite its clinical and biological importance, accurate quantification across the plasma proteome remains a major analytical challenge due to the wide dynamic range and complexity. Proteome-wide measurements can generally be improved by decreasing the dynamic range of the sample before measuring it or increasing the dynamic range of the measurements themselves. Here, we combine both of these strategies to generate high quality measurements of over 5,000 plasma proteins in a single 60-minute run and over 4,700 proteins in a 30-minute gradient.
We use Mag-Net, a simple and robust strategy that simultaneously enriches extracellular vesicles (EVs) and depletes highly abundant proteins from plasma (Wu et al, 2023). This protocol is automated on the KingFisher Apex (Thermo Scientific) uses MagResyn strong-anion exchange magnetic particles that are hyper-porous polymer matrix and functions as a molecular sieve (or net) to capture membrane-bound particles based on size and charge. Unlike antibody-based depletion methods, Mag-Net is species agnostic, inexpensive, compatible with hemolyzed plasma samples, and requires only 10-100 uL plasma input. Additionally, it is more easily scalable and reproducible than fractionation based approaches. Overall, the Mag-Net protocol developed by Wu et al. (2023) is simple, scalable, and effective, allowing for the quantification of over 3000-4000 proteins in a 90-minute gradient on an Orbitrap Fusion Eclipse.
To ensure systematic sampling and maximize quantitative performance, we acquire data in data independent acquisition mode on the Thermo Scientific Orbitrap Astral Mass Spectrometer. We have previously demonstrated the quantitative merits of the Astral analyzer, demonstrating that it is significantly faster and more sensitive than state of the art methods (Heil et al., 2023). By combining innovative sample preparation and instrumentation, we are able to generate deep coverage of the plasma proteome in a single LC-MS/MS run.

See you in Busan!
BEXCO, Busan, Korea
2023.hupo.org
#HUPO2023

Together Through Proteomics

PROGRAM

PP01.07: Construction of Lipid Component Analysis Platform for Assessment of Extracellular Vesicles (Exosome) Therapy Products
Eun Ji Jang, Republic of Korea

Extracellular vesicles (EVs), including exosomes and microvesicles (MVs), are explored for use in diagnostics, therapeutics and drug delivery and exosome is recently in the spotlight as a therapy products. However, little is known about assessment method of its quality. So it is urgent to construct an evaluation method for quality control. In this study, we constructed the lipid component analysis platform for assessment of exosome therapy products. To this end, we first developed the optimal lipid extraction method (Liquid-liquid extraction method). Second, we optimized the LC-MS (Q-Exactive mass spectrometer) conditions using the data-dependent acquisition (DDA) method and optimized the parameters in the Lipid search program (ver.4.2.21). Third, we evaluated the lipid search data set from the control sample (14 lipid standards) to reduce the false positive ratio. Finally analyzed the component of lipid species in exosome derived from X-cell. In this study, a total of 12 lipid classes, 88 lipid species were identified in exosome derived from X-cell. Of the lipid class composition, Cholesterol ester occupied the highest proportion, which accounted for about 88%. Among lipid species, CE 18:3 accounted for the highest concentration followed by CE 18:2, CE 20:5.

PP01.09: Shotgun Proteomics Reveals Senomorphic Targets Based on SASP-mediated by Small Extracellular Vesicles
Jesus Mateos, España

Introduction: Cells have the capacity to modulate the microenvironment through the secretion of molecules (cytokines, chemokines, matrix proteins…) and vesicles, which vary according to the pathology involved, such as cancer or natural process such as senescence. Cellular senescence is a process that enhance with ageing and its association with the onset of age-related diseases. The process is characterized by less proliferation, increased β-galactosidase activity and specific secretory phenotype known as SASP. SASP lead the microenvironment to a more pro-inflammatory one and has the capacity to induce paracrine senescence in neighbouring cells. The regulation of small extracellular vesicles (sEV), which are part of SASP, have a high potential to develop drugs that modulate the senescence transmission. Our aim is to find a proteomic signature of the SASP mediated by sEV to reveal pathways associated with the senescence transmission.
Methods: We knocked-down, in mesenchymal stem cells, RELA or RAB27A, genes implicated in the paracrine senescence and sEV biogenesis respectively, using CRISPR-Cas9 methodology. After their treatment with senescent or non-senescent sEV, we evaluated the senescence phenotype (proliferation and β-galactosidase activity) achieving the inhibition of senescence transmission. Finally, we performed a shotgun study by Tandem Mass Tag (TMT10-plex) to identify, quantify and compare the proteome of senescent-sEV recipient cells with or without the knocked-down genes.
Results: This quantitative and comparative proteomic analysis identified 4099 and quantify 4044 proteins, which of 28 were differentially regulated by the SASP mediated by sEV. Further bioinformatic analysis of the dysregulated proteins show the implication of the Golgi traffic and network which evidencing that is involved in the SASP mediated by sEV.
Conclusion: This data will be useful to design new therapeutic strategies or support the actual ones against age-related diseases.

See you in Busan!
BEXCO, Busan, Korea
2023.hupo.org
#HUPO2023

Together Through Proteomics

PROGRAM

PP01.11: Proteomic Profiling of Circulating Extracellular Vesicles from β-thalassemia/HbE Reveals Association of Immunoglobulin Inducing Membrane Vesiculation

Kunwadee Phongpao, Thailand

Increased levels of medium extracellular vesicles (mEVs) have been reported in splenectomized β-thalassemia/HbE patients. Thrombosis, a significant complication leading to morbidity and mortality in β-thalassemia, is increased in splenectomized patients and correlated with the increased levels of circulating mEVs. The mEVs from β-thalassemia/HbE patients can induce platelet activation and endothelial cell dysfunction, key factors leading to thrombosis, with more pronounced effect by splenectomized mEVs. It is hypothesized that β-thalassemic mEVs are generated from oxidative stress response of red blood cells and platelets, which was supported by the altered proteome with a higher level of oxidative stress response proteins compared to normal subjects. However, oxidative stress might not be the only mechanism involved in mEV generation. Additionally, there is no document about the different mechanism of mEV generation between non-splenectomized and splenectomized patients though the molecular pathology is different. Herein, proteomic analysis of mEVs from β-thalassemia/HbE patients (splenectomy and non-splenectomy) and normal subjects was performed by liquid chromatography with tandem mass spectrometry (LC-MS/MS). A total of 172 proteins were identified among mEVs, with 72 proteins found only in splenectomized mEVs. Interestingly, an increased level of immunoglobulin (Ig) subunits was found, especially in splenectomized mEVs. Consistency with proteomic analysis, flow cytometry showed significantly higher IgG bearing mEVs in splenectomized patients compared to non-splenectomized patients and normal subjects. Importantly, the levels of IgG bearing red blood cells and platelets were not significantly different among groups. Protein-protein interaction analysis using the STRING database demonstrated a protein cluster of cytoskeleton proteins, adaptor proteins and stabilizing proteins unique to splenectomized mEVs. These finding suggests releasing of splenectomized mEVs could be a mechanism to remove coated Ig on cell surface to eliminate the subsequent binding of complements leading to cell lysis. This could indicate a new perspective on mEVs release in βthalassemia.

PP01.13: Standardized and Fully Automated Profiling of Plasma and Extracellular Vesicles Integrated with Evosep One Enables Large Scale Clinical Cohort Analysis

Joel Vej-nielsen, Denmark

Plasma is a rich source of potential biomarkers for monitoring disease onset and progression. However, the lack of reproducible, robust, and high-throughput proteomic workflows is a major limiting factor in the transition from biomarker discovery to verification and ultimately clinical application. To enable these efforts, we have implemented end-to-end sample preparation methods, integrated with the Evosep One, for profiling of undepleted plasma and extracellular vesicles (EVs).
For undepleted plasma we utilized protein aggregation capture (PAC) whilst an additional enrichment step was added for profiling of plasma EVs through the use of quarternary ammonium functionalized magnetic beads. Both methods were fully automated on an Opentrons OT-2 robot, covering all steps from raw plasma to mass spectrometer including: EV enrichment with parallel, abundant plasma protein depletion, on-bead protein capture, clean-up, digestion and finally Evotip loading, based on a unique layered sandwich approach. The two workflows were evaluated against the standard Evosep One methods (30SPD – 500 SPD) and later utilized to profile a clinical cohort (n   1000 patients).
The automated workflows are capable of processing 96 samples in parallel from only 1 μl (undepleted) and 20 μl (EVs) plasma. Preliminary results indicated that in combination with library free Data Independent-Acquisition (DIA) up to 400 protein groups could be reliably quantified from undepleted plasma using the 60SPD method whilst   2,000 proteins were detected with the EV profiling workflow using the 30SPD method.
The two fully integrated Evosep One workflows enable large-scale clinical cohort analysis with deep, robust, high-throughput and cost-effective profiling of plasma, from minimal amounts of starting material.

PROGRAM

PP01.15: Determining the Antigen Processing Requirements for Spliced Peptide Presentation

Ritchlynn Aranha, Australia

In the HLA-I pathway, the antigen presentation and processing machinery (APPM) degrades endogenous antigens (including cancer-associated antigens) into peptides that are presented to T cells for immunosurveillance. Collectively, these cell surface-presented peptide-HLA-I (pHLA-I) make up the cell's immunopeptidome. Cancer-associated pHLA-I make good candidates for CD8+ T-cell immunotherapy, however, clinical trials with peptide vaccines have shown poor overall response rates. This creates an urgent need to expand the repertoire of novel peptide antigen targets. Spliced pHLA-I are an attractive inclusion in peptide-based vaccines as they broaden the repertoire of available targets. Whilst peptide splicing has been attributed to proteasomal-catalysed processes, it is unknown what effect other components of the antigen processing and presentation machinery (APPM) play. To gain a better understanding of the mechanism involved in spliced peptide formation, we used a haploid cell line (HAP1) with various components of the APPM knocked out. Cells were also treated with interferon-γ leading to a significant upregulation in pHLA-I presentation. Preliminary analysis of a melanoma cell line treated with an ERAP1 inhibitor showed no abrogation of spliced peptide presentation. The HAP1 pHLA-I data revealed that interferon-γ led to an upregulation in pHLA-I with ~50% of the peptides being unique to this treatment. This was observed in the wild-type cells and the APPM knockout cells. Further analysis showed that knocking out APPM components modulated the repertoire of peptides. ERAP1 knockout led to an increase in the percentage and abundance of peptides derived from cancer-associated antigens with a ~10% increase in the total number of these pHLA-I. TAP1/2 knockout on the other hand led to a decrease in the available repertoire of linear and spliced peptides but interferon-γ stimulation led to a recapture of some of the pHLA-I. We anticipate that this study will improve our understanding of the mechanism of spliced peptide presentation.

PP01.17: Identification and Validation the of HLA-I Proteasomal Spliced Epitopes by Immunopeptidomics

Pouya Faridi, Australia

CD8+ T cells recognize antigens primarily through the presentation of peptide antigens on the cell surface in conjunction with Human Leukocyte Antigens class I (HLA-I) complexes. Recent studies have shed light on a fraction of these presented peptides that are generated through proteasome-mediated splicing, wherein non-contiguous regions of proteins are fused together to form novel peptide antigens. Although the exact proportion of spliced peptides in HLA-I immunopeptidomics is a subject of debate, emerging evidence demonstrates their presence and contribution to immune recognition in various contexts such as tumour immunity, viral infections, and autoimmune diseases.

In this presentation, I will focus on recent advancements in the identification of spliced peptides using immunopeptidomics and bioinformatics workflows. I will discuss different methods employed to validate the authenticity of identified sequences and the source antigens for spliced peptides. Additionally, I will address the evaluation of the immunogenicity of these identified spliced peptides. Furthermore, I will introduce a novel set of spliced peptides derived from tumour-associated antigens, which exhibit shared characteristics across different types of tumours. These spliced peptides were identified through immunopeptidomic analysis of nine different HLA-A*02:01 patient-derived cell lines representing diverse cancers such as melanoma, glioblastoma, triple-negative breast cancer, and colorectal cancer. We have validated the presence of this unique set of spliced peptides in patients' tumour biopsies and confirmed their immunogenicity using peripheral blood mononuclear cells derived from both patients and healthy donors. Notably, a subset of these spliced peptides is currently undergoing Phase-I clinical trials for melanoma vaccination.

See you in Busan!
BEXCO, Busan, Korea
🌐 2023.hupo.org
🐦 📘 📷 in #HUPO2023

Together Through Proteomics

PROGRAM

PP01.19: A Comparison of Sputum Proteome Analysis in Asthma Patients Receiving Different Biologic Treatments

Jeong-yeon Hong, Republic of Korea

Asthma is a chronic respiratory disease in which the bronchi are repeatedly narrowed by allergic inflammation. Sputum is a sticky mucous liquid produced by the bronchi and normal person secrete about 100 ml/day. It consists of 95% water and the remaining 5% protein, lipids and minerals. Sputum, a protein-rich airway biofluid, was sampled using a non-invasive method. 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, alpha-amylase being the top-ranked protein. It also the saliva-elevated protein was present at high concentration. 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.

PP01.21: Correlation between CD4 T Cells and Lymphotoxin Beta Expression in Breast Cancer: A Comparative Medicine Approach.

Haeju Lee, Republic of Korea

Breast cancer has long been the leading type of cancer among women. the incidence of breast cancer continues to rise, and its prognosis is known to be poor. To advance our understanding of breast cancer, we employed a comparative medicine perspective, comparing canine mammary tumors to human breast cancer.

Analysis of PBMC RNA sequencing data from previous studies revealed a decrease in CD4 T cell population and function in dogs with mammary tumors compared to healthy dogs. Furthermore, we observed a specific decrease in lymphotoxin beta (LTB) expression within CD4 T cells. LTB, a key member of the TNF superfamily, plays critical roles in lymphoid tissue development, maintenance, and immune system function. Based on these findings, we hypothesized a correlation between the decrease in CD4 T cells and LTB expression and approached the topic from a comparative medicine perspective.

Using an in vitro system, we conducted experiments with human primary T cells. Direct co-culture experiments revealed a significant decrease in LTB expression in T cells with human breast cancer cells compared to T cells with human breast normal cells, as confirmed by qRT-PCR. Additionally, conditioned media (CM) culture experiments demonstrated a significant reduction in LTB expression in T cells treated with cancer CM compared to T cells treated with normal cell CM, as assessed by qRT-PCR. These findings suggest that the alterations in T cells are induced by cancer-secreted factors, resulting in the downregulation of LTB expression. Moreover, our ongoing research aims to identify the specific factors secreted by cancer cells and elucidate the consequential changes in T cells. Ultimately, our objective is to unravel the mechanism by which cancer modulates the immune system to ensure its survival.

PP01.23: MARS: a Novel Antigen Discovery Pipeline by Improved LCMS De-Novo Peptide Identification for Characterizing Non-Canonical Immunopeptidome

Hanqing Liao, United Kingdom

Background: Successful immunotherapy against cancer requires accurate characterization of human leukocyte antigen class 1 (HLA-1) binding peptides to find and develop target antigens. Liquid-Chromatography tandem Mass-spectrometry (LCMS) based immunopeptidomics provides high throughput and accuracy HLA-1 binding peptide identification for canonical protein degradation products, but when it comes to identifying peptides originated from non-canonical, aberrant or unknown translations (such as lncRNAs or transposable elements), there is no well-curated protein database for search, and de-novo algorithms are considered of poor specificity.

Method: We propose a novel MARS score, constructed by combining the average local confidence (ALC) score from Peaks de-novo sequencing output with HLA eluted ligand likelihood rank (ELR) from netMHCpan 4.1 and difference of observed-predicted retention time (DRT) from DeepRTplus, to re-rank the de-novo peptide candidates. After re-ranking, for each spectrum the top MARS score peptide candidate is selected as the identified peptide sequence. The new top-hit peptides were matched to large non-canonical databases such as 6-frame translated Gencode reference genome, 3-frame translated reference transcriptome, etc. to find their possible origins.

Results: We compared Peaks ALC top-hit based peptide identification against MARS score top-hit based peptide identification using synthetic peptide standard data and real immunopeptidomics data. Measured by fractional rank (F-Rank) of true peptides and full sequence recall (FSR), MARS score outperformed ALC score in identifying peptides in all data sets under study. An exact-match-only strategy can also match a large percentage of MARS identified peptides to unique gene origins.

Conclusion: Incorporating HLA binding affinity and LC retention time information can improve the specificity of selecting de-novo peptide candidates, which in turn can help characterize non-canonical HLA-1 immunopeptidome, and thus advance the antigen discovery for immunotherapy development.

PP01.25: Exploring the Antigenic Landscape in Head and Neck Cancer

Miriam Melake, United Kingdom

Immunotherapy potentiates the eradication of cancer cells by the immune system and has thus emerged as a promising cancer treatment. However, in many cancer types, including head and neck cancer, lack of treatment response has driven research to better understand the tumour-immune crosstalk aiming at augmenting immunotherapy. Tumour specific antigens are central to successful anti-tumour immune responses, which is why their characterisation has become a major research focus for targeted immunotherapy. Here we characterise the antigen landscape of human papilloma virus (HPV) positive and negative head and neck squamous cell carcinoma (HNSCC) by integrating quantitative proteomics and immunopepitidomics. Using these MS methods, we investigate the immunopeptidomic and underlying proteomic repertoire in model cell lines at baseline as well as following clinically relevant treatments including irradiation and cisplatin. Our goal is to uncover how these treatments modulate mechanisms of antigen presentation and to thereby identify signatures that will inform combination approaches of standard therapy with immunotherapy. In addition, we extend our method to patient blood plasma samples to investigate the emergence of treatment-induced antigens that, passing verification in clinical samples, could guide novel targeted immunotherapy strategies and improve head and neck cancer patients' survival.

PROGRAM

PP01.27: Beyond Discovery: Streamlining Immunopeptidomics Methods for Drug Development.

Arun Tailor, United States

Background: Immunopeptidomics methods are rapidly becoming incorporated beyond the realm of antigen discovery as a pivotal tool for drug development. This includes the assessment of MHC II antigens originating from biotherapeutics which may contribute to immunogenicity, also known in industry as MHC-Associated-Peptide-Proteomics (MAPPs), but also for the pharmacodynamics evaluation of MHC-I antigens as part of the bioanalytical strategy for clinical trials in cell-based therapies. For these applications, the high sensitivity required for the detection of low abundant neo-antigens is coupled with the need for assay robustness where sample to sample, operator to operator and lab to lab consistency is of the utmost importance. This is especially true with increased outsourcing to CRO's with minimal immunopeptidomics experience. Furthermore, current methodologies are tedious, complex and require a large quantity of clinical material despite the increasing sensitivity of high resolution instruments.

Methods & Results: Here, we communicate the implementation of the AssayMAP-Bravo platform combined with a biotin-streptavidin antibody coupling strategy for the assessment of adalimumab-associated MHC-II peptides presented by 2-million dendritic cells. The preparation of samples using the AssayMAP-Bravo substantially reduced sample preparation time from multiple days to only a few hours, and increased throughput enabling the sequential capture and stratification of DR, DQ and DP presented peptides. Furthermore, the omission of Protein A resulted in cleaner MS-ready samples removing the necessity for post-IP clean-up or contamination-prone drying steps. We detected consistent clusters of biotherapeutic-associated peptides across replicate preparations irrespective of operator or instrument calibration cycle demonstrating the reproducibility of our MAPPs workflow. Finally, we demonstrated how the robustness of this method can be translated to improve the quantitation of low-abundant MHC-I epitopes.

Conclusion: Streamlining the immunopeptidomics workflow can increase the consistency of immunopeptidomics applications across the pharmaceutical industry increasing the ability to assess the safety and efficacy of potentially life-saving modalities.

PP01.29: Enhanced Proteomic Coverage in Tissue Microenvironment by Immune Cell library-assisted DIA-MS

Jhih-Ci Yang, Taiwan

Introduction: Immune cells infiltrating the tumor microenvironment (TME) have an important impact on shaping cancer development and determining the clinical outcomes and therapeutic responses. However, a comprehensive snapshot of the composition of the tumor-infiltrating immunity profile in clinical specimens is often limited by the small volume of the sample as well as the low proportion of the immune infiltrating cells in the TME. To achieve highly sensitive quantitative immunoproteomics profiling for micro-scale tissues, we established a data-independent acquisition (DIA) method based on immune cell-enriched spectral libraries. Methods: As a proof of concept, six immune subtype-specific spectral libraries were generated with samples from FACS-sorted immune cells in the murine draining lymph nodes and applied to MC38 murine colorectal cancer (CRC) models.
Results: We generated 6 spectral libraries from common immune cell subtypes including CD8+, CD4+ T lymphocytes, B lymphocytes, natural killer cells, dendritic cells, and macrophages with data-dependent acquisition (DDA) measurements of small-scale fractionation. Compared to the direct tissue profiling of 5,650 proteins using single shot direct-DIA analysis, the immune library enhanced the coverage to quantify over 6,600 proteins from 1 mg colon tumor, including 757 proteins annotated to the immune system process. Moreover, low abundant immunophenotyping markers were exclusively identified from the tissue by library-based DIA analysis. Further application to tumor tissue and their corresponding mesenteric lymph nodes (MLN) in CRC animal models demonstrated increased coverage of immune cell markers, confirming their infiltration in the CRC TME.
Conclusions: Our immune-enriched library approach demonstrated enhanced proteome coverage of the immune landscape for micro-scale samples with deep coverage and high sensitivity.

See you in Busan!
BEXCO, Busan, Korea
2023.hupo.org
#HUPO2023

Together Through Proteomics

PROGRAM

PP01.31: Boosting DIA Immunopeptidomics with Personalized Libraries Generated by a Deep Learning -based Workflow

Wen-Feng Zeng, Germany

Mass-spectrometry (MS)-based immunopeptidomics has become a crucial tool to identify tumor specific antigens for immunotherapies. Equally to the field of proteomics, immunopeptidomics profits greatly from data-independent acquisition (DIA)-MS due to its increasing reproducibility and sensitivity. However, there are ~70M non-specific in-silico peptides generatable from reviewed human proteome sequences, and only tens of thousands of them may be relevant as immunopeptides presented by major histocompatibility complex (MHC) in an individual. Therefore, sensitive immunopeptide identification with DIA-MS is very computationally challenging.

Here we presented a sensitive immunopeptidomics workflow with personalized spectral libraries predicted from the MHC-binding peptide model for personalized alleles using the AlphaPeptDeep framework. The data-dependent acquisition (DDA) experiment of the donor was used to identify a donor-specific immunopeptide list which is also obtainable by searching the DIA data with library-free mode or with public immunopeptidomics spectral libraries. After removing the peptides with low binding affinity to the donor's alleles, these peptides were used to refine the pretrained model for personalized immunopeptide prediction, which was in turn used to predict all potential MHC-binding peptides from proteome sequences. The spectral library of the predicted peptides was generated using fragment, retention time and mobility models in AlphaPeptDeep. At last, the DIA-MS data were searched against the personalized library to boost identifications with relatively high accuracy.

We tested our workflow with MHC-I DIA datasets from different donors. Overall, the refined MHC-I-binding model achieved 92% recall with only ~3% false positives rates. This greatly reduced background peptides while retaining most of the relevant immunopeptides in the personalized library. Searching the DIA data with the personalized library identified >50% more unique peptide sequences and only obtained <2% trash sequences.

Overall, personalized library prediction can boost the identification of immunopeptides by dramatically reducing the search space of irrelevant peptides. This will be useful for personalized immunotherapies.

PP01.33: Phosphoproteomics Reveals Alternative Roles of PknG in Mycobacterial Pathogenesis

Seanantha Baros-Steyl, South Africa

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. Gene knock-out or 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. Here, to further investigate the role of mycobacterial PknG during early events of macrophage infection, alveolar-like macrophage cell lines were infected with M. bovis BCG wild-type and PknG knock-out mutant strains. After proteolysis, phosphopeptides were enriched using Zr-IMAC HP magnetic microparticles and subjected to LC-MS/MS to identify differentially phosphorylated peptides between the wild-type and PknG mutant infected macrophages. A total of 5898 phosphosites on 2285 unique proteins were identified. Following phosphoproteome normalisation and differential expression analysis, a total of 145 phosphosites were differentially phosphorylated in the treated versus untreated infected macrophages. A subset of 65 phosphosites was differentially upregulated in the presence of PknG activity. Functional enrichment 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.

See you in Busan!
BEXCO, Busan, Korea
2023.hupo.org
#HUPO2023

Together Through Proteomics

PROGRAM

PP01.35: Towards a New Generation of Infectious Diseases Diagnosis Methods Using LC-MSMS and Artificial Intelligence

Florence Roux-Dalvai, Canada

Antimicrobial resistance (AMR) is predicted to be the leading cause of death around the world by 2050. Among the main causes contributing to AMR are the over-prescription of antibiotics in clinics and the over-use of antibiotics in farming. Therefore, it is imperative to explore new strategies to slow down and even reverse the evolution of AMR and preserve our ability to fight infectious diseases. A critical area for intervention includes the rapid diagnosis of microbes in biological samples. The gold-standard method (microbial culturing followed by MALDI-TOF analysis) takes approximately 24-48 h, lacks of specificity for close-related species and is not quantitative. Since several years, our team develops new approaches for microbial diagnosis using high-sensitive/ high-specific LC-MSMS combined to Artificial Intelligence in order to provide a microbial identification in <4 hours. We applied this strategy to two problems related to microbial infections: the urinary tract infection in humans (UTI), accounting for 14% of all antibiotic prescriptions in Canada and the bovine mastitis (BM) which causes significant losses to the dairy industry. We demonstrated that combining LC-MSMS data to machine learning algorithms allows to extract a peptide signature able to distinguish between 15 bacterial species and we improved this method using crude signal from ultrafast LC-MSMS runs (< 5 min) without peptide identification. We also demonstrated the transfer of the method to cost-effective triple quadrupole instruments capable to operate in routine. In overall, our work paves the way towards the new generation of diagnosis methods for infectious diseases that could in the end have an important contribution to reduce the use antibiotics.

PP01.37: Comparison of Olink and NULISAseq Proteomic Technologies Applied to a COVID-19 Case-control Cohort Showed High Similarity Between the Two Technologies.

Frank Schmidt, Qatar

Background: In the wake of the global COVID-19 outbreak, it has become critical to precisely characterize infection using high throughput proteomics. Rapid and reliable quantification of low-abundance proteins in small volumes require highly sophisticated technologies to bring them into the analytical window. The Olink approach, which combines antibodies with RT-PCR, is one such technology that can produce significant results. This goal can also be achieved with the newly developed NULISAseq technology. Here, we assess the detectability of infection-related proteins of the two technologies and compare them with already existing Olink COVID-19 case-control study [Suhre et al., 2022].

Methods: We compared targeted Olink-PEA and NULISAseq-based high-throughput proteomics data collected from controls (Con=44) and COVID-19 patients (Case=83). Plasma samples were measured with three different Olink Inflammation, CVDII and CVDIII panels (300 proteins) and with NULISAseq (200 proteins). Both technologies have 94 proteins in common. Statistical comparisons were performed using FDR adjusted linear model to construct volcano and correlation plots.

Results: UMAP PCA shows a clear pattern with the Con group clustering distinctly from the Case group in PC1 in both techniques. When the 94 common targets were compared, most of the analytes showed a positive correlation (spearman $r2=0.767$), except a few (IL4, IL5, and IL13) that displayed a negative association. Volcano plots from Olink and NULISAseq showed similar patterns with high significance, for up- and down-regulated proteins, such as IL6, CCL7, CXCL8, and CXCL9. 62 were significant changed ($p<0.05$) in both platforms; of which 55 showed concordant effect directionality, while 7 showed opposite directionality (IL6R, IL1R2, IL15RA, KITLG, TSLP, IL17C and IL4R). In addition, Olink and NULISAseq data yielded 14 and 16 unique significant proteins, respectively. Data from Olink and NULISAseq revealed a comparable expression trend for 11 and 12 proteins, respectively, which were previously discussed in Suhre et al. [2022].

PP01.39: Auto-Immunoproteomics Analysis of COVID-19 ICU Patients Revealed Increased Levels of Autoantibodies Related to Male Reproductive System

Muhammad Umar Sohail, Qatar

Background: COVID-19 manifests many clinical symptoms, including an exacerbated immune response and cytokine storm. Autoantibodies in COVID-19 may have severe prodromal systemic consequences that are poorly understood. The interaction between these autoantibodies and self-antigens can result in systemic inflammation and organ dysfunction.

Methods: The current investigation screened two independent cohorts of 97 COVID-19 patients (Discovery (Disc) cohort from Qatar (n = 49) and Replication (Rep) cohort from New York (n = 48)) utilizing high-throughput KoRectly Expressed (KREX) immunome protein-array technology. We screened total IgG autoantibody responses against 1,318 correctly folded and full-length human proteins displaying an entire repertoire of continuous and discontinuous epitopes for autoantibody binding. Differentially expressed proteins were used for KEGG- and WIKI-Pathways annotation to determine pathways in which the proteins of interest were significantly over-represented.

Results and conclusions: Autoantibody responses to 57 proteins were significantly altered in the COVID-19 Disc cohort compared to healthy controls (P ≤ 0.05). The Rep cohort had altered autoantibody responses against 26 proteins compared to non-COVID-19 ICU patients that served as controls. Both cohorts showed substantial similarities (r2 = 0.73) and exhibited higher autoantibodies responses to numerous transcription factors, immunomodulatory proteins, and human disease markers. Analysis of the combined cohorts revealed elevated autoantibody responses against SPANXN4, STK25, ATF4, PRKD2, and CHMP3 proteins in COVID-19 patients. KREX analysis of the specific IgG autoantibody responses indicates that the targeted host proteins are supposedly increased in COVID-19 patients. The sequences for SPANXN4 and STK25 were cross-validated using sequence alignment tools, and ELISA and Western blot further verified the autoantigen-autoantibody response of SPANXN4. Pathways associated with infection, Th cells differentiation, interferon signaling, and prostate cancer were activated. SPANXN4 is essential for spermiogenesis and male fertility, which may predict a potential role for this protein in COVID-19-associated male reproductive tract complications and warrants further research.

PP01.41: Proteome Analysis of BP1092 – a Potential New Virulence Factor Regulator in Bordetella Pertussis

Kristin Surmann, Germany

Background
Virulence factors play a key role in Bordetella pertussis (Bp) persistence inside host cells. Virulence factor expression in Bp is controlled by the interrelated two-component systems (TCS) BvgAS and RisAK. There are hints that further, yet unknown, regulatory systems might be involved, too. Thus, BP1092, a TCS histidine kinase, which displays increased protein levels upon internalization by a human macrophage cell line (Lamberti et al., JProteomics, 2016), is an interesting candidate for more detailed analysis.

Methods
We first characterized the impact of BP1092 by proteome profiling. To this end, we performed mass spectrometric analysis of Bp Tohama I wildtype (wt), an isogenic BP1092 deficient mutant (ΔBP1092), and ΔBP1092 trans-complemented with BP1092 (ΔBP1092 pBBR-BP1092) under control (SS) and virulence-modulating (SS + 40 mM MgSO4) conditions. Further, we used the three strains to infect THP-1 macrophages and determined bacterial survival as well as host proteome adaptation after 24 h.

Results
In total, we found eleven proteins with altered abundance between ΔBP1092 and wt or the trans-complemented strain, respectively. Among these eleven proteins, nine were related to virulence. Proteins with adhesion function FhaB, FhaC, and CyaA toxin had lower levels in the mutant compared to the wt under control conditions. Response regulator BvgAS and BvgR as well as FimC and toxins PtxAB showed lower levels in the mutant under modulating conditions. In addition, the wt and the complemented strain displayed a higher survival rate within THP-1 macrophages over 48 h. Twenty host proteins displayed distinctively different levels (fold change > |1.5|; q < 0.05) after infection with wt or complemented strain compared to infection with a mutant lacking BP1092.

Conclusion
Our data of B. pertussis infection-mimicking models suggest that BP1092 plays a crucial role in fine-tuning of virulence factor levels after infection with an impact on intracellular survival.

PP01.43: Landscape of the RBD-Specific Antibody Responses Triggered by the Inactivated Virus Vaccine Against the Omicron Variant Using Protein Microarray

Junbiao Xue, China

The COVID-19 pandemic caused by the SARS-CoV-2 virus has been a global health threat for over three years. The emergence of variant strains, particularly the Omicron variant, has posed a great challenge to global pandemic prevention. While vaccination is one of the most effective ways to deal with infectious diseases like COVID-19, there are concerns about the effectiveness of vaccines against emerging variant strains. In China, inactivated vaccines were primarily administered to the population. To explore the effectiveness of inactivated vaccines against the Omicron variant, we printed a protein microarray contained RBD proteins for both the wild-type and Omicron-variants. The study aimed to detect the titer of RBD-specific antibodies in the serum after inoculation with the inactivated vaccine (BBIBP-CorV) and map the changes in antibody levels within one year following vaccination. The results showed that the IgG response to RBD-Omicron was significantly lower, approximately 1/3-1/5 that of the RBD-wild type. The booster dose produced a response approximately six times higher than the second dose for RBD-Omicron and reached a plateau within two weeks after administration. However, the antibody levels dropped about 1/5 within two weeks after reaching the plateau. Similar results were also obtained for IgM and IgA. The high correlation between the RBD-specific antibody response and neutralization activity against the authentic virus indirectly revealed the landscape of antibody protection against the Omicron variant throughout the vaccination stages. The study revealed the effectiveness of the inactivated vaccine against the Omicron variant and the importance of booster vaccinations. However, the results also suggest that post-booster vaccinations may also be necessary. Furthermore, the study revealed the use of protein microarrays to efficiently and accurately detect the level of viral protein-related antibodies in serum and indirectly revealed vaccine effectiveness. This method could be of great value in the future to other related diseases.

PP01.43A: Multi-omics Approach Reveals Putative Serum Biomarkers for Congenital Zika Syndrome

Fábio Nogueira, Brazil

During pregnancy, the Zika virus (ZIKV) can be vertically transmitted, causing Congenital Zika Syndrome (CZS) in fetuses. ZIKV infection in early gestational trimesters increases the chances to develop CZS. This syndrome involves several pathologies with a difficult diagnosis, which usually occurs in the postnatal stage. In this work, we aim to identify biological processes and molecular pathways related to CZS development and propose a series of putative protein and metabolite biomarkers for CZS prognosis in early pregnancy trimesters. Twenty-five serum samples of pregnant women were analyzed. For biological analysis, samples were separated into 3 biological groups composed of a control group of healthy pregnant women and two groups of ZIKV-infected pregnant women bearing non-microcephalic and microcephalic fetuses. Control and ZIKV-infected groups - without microcephalic fetuses - were subdivided into healthy and Cognitive Developmental Delay (CDD) newborns for biomarker analysis. We detected over 1,000 proteins and 500 metabolites by bottom-up proteomics and untargeted metabolomics, respectively. Statistical approaches - (t-Student, Limma, ANOVA, and DIABLO) - were applied to find CZS differentially abundant proteins (DAP) and metabolites (DAM). Enrichment analysis (i.e., biological processes and molecular pathways) of the DAP and the DAM allowed us to identify the ECM organization and proteoglycans, amino acid metabolism, and arachidonic acid metabolism as signatures in the CZS development. Five proteins and four metabolites were selected as CZS biomarkers candidates. The protein-based model indicated superior performance values for the Vitamin K-dependent protein S, Selenoprotein P, Inter-alpha-trypsin inhibitor heavy chain H2, Kallistatin, and Protein Z-dependent protease inhibitor proteins. Furthermore, the metabolite-based model was able to predict CZS with a probability of 90%. Serum multi-omics analysis led us to propose for further studies seven potential biomarkers for CZS early prognosis with high sensitivity and specificity.

PP01.45: To Predict how Proteins Bind RNA: RBS-ID & pRBS-ID

Jong Woo Bae, Republic of Korea

Introduction
RNA-binding proteins (RBPs) interact with RNA to affect every step of RNA metabolism. RNA binding sites (RBSs) constitute the molecular basis of RNA-protein interaction. The RBSs can be identified by liquid chromatography and tandem mass spectrometry (LC-MS/MS) analyses of the protein–RNA conjugates created by crosslinking. However, RBS mapping remains highly challenging due to the complexity of the formed RNA adducts.

Methods
Here, we introduce RBS-ID, a method that uses hydrofluoride (HF) to fully cleave RNA into mono-nucleosides, thereby drastically enhancing the coverage and reaching single amino acid resolution. Moreover, the simple mono-nucleoside adducts offer a confident and quantitative measure of direct RNA–protein interaction.

Results
We structurally verified RBS-ID in Streptococcus pyogenes Cas9 in complex with its guide RNA. Using RBS-ID, we profiled ~2,000 human RBSs to discover novel RNA binding residues and potential roles of RBS post-translational modification in the modulation of RNA binding activity.
In addition, we present a recently developed method called pRBS-ID. Here, RBSs are more comprehensively profiled using photoactivatable ribonucleosides. Labeling RNA with 4-thiouridine or 6-thioguanosine allowed specific capture of the U or G-interacting RBSs in human RBPs, respectively.

Discussion
Overall, RBS-ID and pRBS-ID are valuable platforms for investigating the dynamic RNA-protein interactions. In particular, the RBS datasets can be integrated with recent 'game-changing' de novo single protein structure prediction tools (e.g. AlphaFold, RoseTTAFold) to enhance the accuracy and extent of RNP conformational prediction. Also, RBSs identified on disordered regions of proteins can serve as a starting point to investigate their roles in RNA binding.

PP01.47: FAX-RIC In Peptide Level Enables Robust Mapping of RNA-Binding Regions of RNA Interactome

Seonmin Ju, Republic of Korea

RNA and proteins dynamically interact to regulate a variety of biological processes. In order to map these interactions, a potent approach typically involves inducing cross-linking between RNA and proteins using either UVC light or formaldehyde, followed by an RNA-centric enrichment and an exhaustive proteomic analysis via LC-MS/MS. In this study, we propose pepFAX-RIC, a strategy based on formaldehyde crosslinking (FAX), that identifies RNA binding domains (RBDs) at the peptide level. Leveraging the property that crosslinking induced by formaldehyde can be readily reversed by heat treatment, our approach involves proteolytic digestion of ribonucleoproteins (RNPs) into peptides, succeeded by RNA enrichment, to precisely delineate the RNA-interacting regions of proteins. In HeLa cells, we successfully identified approximately 900 proteins, a tally comparable to protein-level capture, as well as pinpointing their respective RNA-binding domains. Compared to the known RNA binding domains identified through RBDmap and RBS-ID methodologies, more than 400 of these were not previously reported as RNA binding domains. Moreover, we conducted poly A-tailed RNA interactome profiling in mouse liver, transcending the constraints of previous methods and thereby enabling in-depth RBP profiling within the tissue. We expect that this method can be utilized as a tool for structural interpretation and tracking changes in RNA-protein interactions by identifying directly interacting regions. By enabling a deeper understanding of the dynamic interactions between RNA and proteins, our approach offers significant potential to significantly advance the study of RNA biology.

See you in Busan!
BEXCO, Busan, Korea
2023.hupo.org
#HUPO2023

Together Through Proteomics

PROGRAM

PP01.49: ARID3C Acts as a Regulator of Monocytic Differentiation Interacting with NPM1

Hui-su Kim, Republic of Korea

The journey to map the entire human proteome is currently underway, driving numerous studies aimed at elucidating the biological and molecular functions of missing proteins. In this study, we aimed to investigate the cellular function of ARID3C, which is located on chromosome 9 and expressed in various organs with low levels. Here, we utilized affinity purification and mass spectrometry to identify the interaction partner of ARID3C. Based on the interactome of ARID3C, we focused on NPM1 among the candidate proteins and found that NPM1 was colocalized in the nucleus. Overexpression of ARID3C facilitated monocyte-to-macrophage differentiation by acting as a transcription factor for STAT3, STAT1, and JUNB. In contrast, mutated ARID3C did not have an impact on the monocytic differentiation. Mechanically, AIRD3C that mutated the binding site between ARID3C and NPM1, resulted in ARID3C being retained in the cytoplasm and unable to enter the nucleus, leading to the inability to bind to the promoters of target genes such as STAT3, STAT1, and JUNB. Collectively, these findings indicate that ARID3C acts as a transcription factor promoting monocytic differentiation by forming a complex with NPM1 for the nuclear shuttle.

PP01.51: Comprehensive Profiling of Human Brain Protein Complexes Using Co-fractionation Mass Spectrometry

DongGeun Lee, United States

Introduction
Proteins are essential regulators of biological processes and can have diverse functions depending on their interactions with other proteins. The organization of proteins into macromolecular complexes and their quantitative distribution within these complexes holds great biological and clinical significance. Despite the great biological significance, the profiling of protein complexes in the human brain has remained inadequate. In recent years, co-fractionation mass spectrometry (CF-MS) has emerged as a promising proteomics technique for globally identifying protein-protein interactions and complexes in a single operation. In this study, Ion Exchange Chromatography (IEX) were employed to separate native protein complexes. Data independent acquisition (DIA) mass spectrometry was utilized to accurately quantify proteins and profile coeluting patterns in each fraction. The resulting data is then analyzed using computational tools using both CCprofiler and PCprophet to detect and measure protein complexes.

Methods
10 pooled human Alzheimer's disease (AD) brain tissues were used for CF-MS analysis, Brain samples were weighed and homogenized using Dounce homogenizer in the lysis buffer (10mM HEPES, 250 mM sucrose, 5 mM MgCl2, 0.1% (v/v) dodecyl-b-D-maltopyranoside (DDM), protein:buffer = 1:10, v/v). Using two different phase of ion-exchange chromatography columns, the lysate was extensively fractionated, and all fractions were collected every 30 sec. 192 fractions were collected and BSA proteins were added in each fraction. After acidification with TFA, the resulting peptides were desalted. The Exploris 480 (Thermo) was used to run MS (30 min gradient) and DIA mode was used

Results
More than 4,600 proteins were identified and interestingly, more than 700 unique proteins complex and 10,000 protein-protein interaction were identified from the human brain samples, with a protein false discovery rate (FDR) less than 5%.

Conclusions
The novel protein complex and their protein-protein interaction can help a comprehensive understanding of AD molecular mechanisms which provide potential diagnostic and therapeutic biomarkers for AD.

PP01.55: Systematic Proteomic Analysis of MUDENG Interactome Using TurboID Proximity Labeling

Jung Soo Hyun, Republic of Korea

MUDENG, or Mu-2-related death-inducing gene, encodes a protein that plays a vital role in the cellular process of apoptosis. Remarkably, the MUDENG protein contains the adaptin domain typically found in the μ subunits of adaptor protein complexes, which is not commonly observed in proteins associated with apoptosis. As a result, the specific contribution of this domain to apoptosis signaling remains uncertain. To shed light on this matter, we conducted a systematic study aimed at identifying the partner proteins that interact with each domain of the MUDENG protein. To achieve this, we divided the MUDENG protein into three distinct segments, designated as N, M, and C. Subsequently, we employed MUDENG segment proteins comprising the N segment, the N and M segments, and the wild type MUDENG protein. Additionally, we generated TurboID constructs to target each of the three segments individually. In this study, we performed LC-MS/MS experiments peptide samples obtained after streptavidin immunoprecipitation from the three TurboID constructs. The samples were separated by using dual online ultrahigh-pressure liquid chromatography (DO-UHPLC) with a 3 hr gradient. To obtain confident identification of biotinylated peptides, the resultant peptide spectrum matches (PSMs) were inspected manually before reporting their partner proteins for each of three MUDEUNG segments. Consequently, the partner proteins related to chromosome organization pathway were highly enriched in the N segment, while the proteins related to positive regulation of viral genome replication pathway were notably enriched in the M segment. In addition, the proteins involved in the cellular macromolecule localization pathway are highly enriched in the C segment. Therefore, by conducting systematic investigations into the partner proteins that interact with MUDENG in each domain, we will be able to obtain information on MUDENG functions in apoptosis signaling.

PP01.57: The First Profile of Steroid Hormones in Human Aqueous Humor is Generated from the LC MS/MS Approach

Hongkai Xu, China

Introduction
Steroid hormones (SHs) in body fluids have been generally accepted by clinical diagnosis, whereas their distribution in aqueous humor (AH) is still unknown. Since the involvement of some SHs in eye diseases has been reported, global profiling and quantification of AH SHs are urgently required in ophthalmologic medicine.

Methods
A total of 171 AH and 107 plasma samples were collected, and the SHs in AH and plasma were extracted and delivered to liquid chromatography coupled with tandem mass spectrometry (LC MS/MS). The corresponding ions of steroid hormones were acquired in multiple reaction monitor mode for identification and quantification.

Preliminary data
Six kinds of SHs were quantitatively identified in the AH samples, ranking at: cortisol (F, 2.102±.225 ng/mL), cortisone (COR, 1.82±0.481 ng/mL), corticosterone (CORT, 0.455±0.147 ng/mL), aldosterone (ALD, 0.073±0.032 ng/mL), androstenedione (A2, 0.073±0.039 ng/mL) and 11-deoxycortisol (11DOC, 0.068±0.016 ng/mL). Compared with the SH profile in plasma, the AH SH types and the corresponding abundance were significantly lower. Moreover, the abundance of AH SHs generally lacked a tight correlation with the abundance of plasma SHs. Interestingly, dehydroepiandrosterone sulfate (DHEAS), the most abundant plasma SH, was undetectable in AH, implying that it is specifically blocked by the blood aqueous barrier. Four kinds of SHs (COR, CORT, ALD and A2) were found to have increased abundance in the AH with axial myopia. Based upon a discriminator model, the panel of F, COR, CORT, A2 and 11DOC in AH was able to discriminate myopia from normal.

Novel aspect
The steroid hormones possess a specific distribution in human AH and can potentially contribute to axial myopia.

PP01.61: The Expectation and Reality of the HepG2 Core Metabolic Portrait

Olga Kiseleva, Russian Federation

To represent the composition of small molecules circulating in HepG2 cells and the formation of the "core" of characteristic metabolites that often attract researchers' attention, we conducted a meta-analysis of over 50 published datasets obtained through metabolomic profiling by mass spectrometry and NMR.
We highlighted a fraction of HepG2 metabolome routinely registered by mass spectrometric techniques and nuclear magnetic resonance analysis. Our study collected information on 15,161 metabolites ever detected in HepG2 cells. Meta-analysis of published data showed that even in panoramic studies, scientists focus on specific metabolites, ignoring the rest of the metabolomic profile. Interestingly, these "deja vu" metabolites are repeated from study to study (in our case - 288 metabolites of diverse chemical nature), which on the one hand, may indirectly confirm their crucial role in the metabolism of hepatocytes, and on the other hand - indicate significant limitations of technologies that allow high-reliability identification only for these compounds.
Our research has shed light on an important issue - the comprehensive list of identified metabolites is often not fully disclosed. Despite the existing paradigm of data policy leading to a streetlight effect, we remain optimistic about the potential for future discoveries in metabolomics. By embracing transparency and widely accepted standards for processing and presenting data, we can unlock many molecular secrets and advance our understanding of cellular metabolism. While building a complete map of the metabolome of a cell is a challenging task, our research design aimed to reveal crucial metabolites for the Hepg2 cell line, regardless of any impact factors.
This research was funded by the Ministry of Science and Higher Education of the Russian Federation within the framework of state support for the creation and development of World-Class Research Centers "Digital biodesign and personalized healthcare" No. 75-15-2020-91.

PP01.63: MSMP: an Integrated Mass Spectrometry Database of Medicinal Plants for Natural Products Identification

Ziyi Li, China

In the past decade, numerous medicinal plant (MP) databases have been developed to offer valuable information on MP ingredients, including structural annotations and related herbs, targets, or diseases. However, these databases face challenges due to redundant entries, non-standardized data, and insufficient mass spectrometry, which hinder the accurate identification of ingredients from medicinal plants. To overcome these limitations, we conducted Ultra-Performance Liquid Chromatography-High-Resolution Mass Spectrometry (UPLC-HRMS) experiments on medicinal plants and constructed MSMP (integrated Mass Spectrometry database of Medicinal Plants) for natural products identification.
We integrated multiple existing MP databases and eliminated redundant MP ingredients to ensure data consistency. The chemical information of each compound was calibrated using several authoritative chemistry databases. Furthermore, we trained a deep learning model using in-house UPLC-HRMS data of MP standard substances, enabling accurate prediction of retention time (RT) for small molecules under specific chromatographic conditions. Additionally, we enriched the database with experimental and predicted UPLC-HRMS related information on each compound, including RT, collision cross-section (CCS), ms1 profile, and ms2 profile. We also included comprehensive classifications and metabolism information on MP compounds.
MSMP is a comprehensive mass spectrometry database for natural products, which comprises a collection of 35,445 unique ingredients related to over 8000 medicinal plants. It serves as a valuable resource for researchers, providing comprehensively accurate chemical information of MP ingredients, and enhancing the identification of natural products through LC-MS techniques. As an illustration of its utility, we conducted UPLC-HRMS analysis on Jueyin granules (JYG), a traditional Chinese medicine formula, and successfully identified a total of 726 compounds in both positive and negative ion modes. Remarkably, MSMP played a crucial role in the validation and accurate identification of 11 compounds from the analysis result.

PP01.65: The Role of AC9 in Glucagon Secretion in Islet α Cell Specific AC9 Knockout Mice

Yanping Zhu, China

The glucose homeostasis is regulated by insulin from the β-cells and glucagon from α-cells, and these two hormones show the opposite effects in almost all respects. In addition to insulin, abnormal glucagon secretion has also been found to play an important role in the process of diabetes, but the mechanisms of glucagon secretion remain unclear. Our previous study found that adenylyl cyclase 9 (AC9) was involved in insulin secretion, suggesting that AC9 may also be associated with glucagon secretion. In this study, we constructed the islet α-cell-specific AC9 knockout mice (Gcg-AC9loxp/loxp), isolated the mouse primary islet tissue and performed unlabeled quantitative proteomic analysis. Among the 1356 proteins detected, 16 proteins were differentially expressed in Gcg-AC9loxp/loxp mice. Further bioinformatic analysis showed that Endocrine and other factor-regulated calcium reabsorption and synaptic vesicle cycle were dysregulated. Lpl, Glg1, Psmd14, Rbm8a, Cad, Mtch2 were down-regulated, and Eif3g, Rab18, Ap2b1, Lamp1, Idh3g were up-regulated. We also found that the serum glucagon contents were decreased in Gcg-AC9loxp/loxp mice. These results indicated that AC9 in α cells was involved in the regulation of glucagon secretion. It may provide a new target for treating dysglycemia in diabetes.

PP01.67: Adaptive Focused Acoustics(R) Technology for Confident, Robust, and Reliable Sample Preparation in Protein Analysis

Debadeep Bhattacharyya, United States

Growing complexities of samples, matrices, and the need to get more data out of smaller sample volume continues to be a big challenge for every Omics laboratory. Covaris' Adaptive Focused Acoustics® (AFA®) Technology can address a wide variety of sample types (FFPE, LCM, fresh tissue, cells, bacteria, yeast) for protein extraction, purification, digestion with higher speed, reproducibility, and reliability.

Samples obtained from different matrices were evaluated. For FFPE samples, an active deparaffinization (without any organic solvent/mineral oil) with AFA followed by SP3 was used for protein extraction, depletion, digestion, and post-digestion clean-up. The workflow can be easily implemented in an 8-strip or 96-well plate depending on throughput requirements.

Significant benefits were observed for proteomics studies with Tandem Mass Tag (TMT) based labeling. The TMT based labeling were found to be significantly faster (reduction from traditional workflows of ~48 hours to 1.5-3 hours) and simpler. A 50 step process was reduced to 24 step process. In addition, increased number of proteins and peptides were identified and quantified using for every bottom-up assay. In addition, enhanced binding and digestion efficiencies were observed with AFA for some of the assays.

The data in this study demonstrates the ability of AFA in achieving increased confidence in protein analysis by starting with complex biological matrices, regardless of the nature and type of proteins (phosphoproteins, membrane proteins, hydrophobic peptides, etc.).

References
Hughes et al, Nat Protoc. 2019 Jan;14(1):68-85; Hughes et al, Mol Syst Biol. 2014 Oct 30;10(10):757; Mueller et al, Mol Syst Biol. 2020, 16:e9111; Schweitzer et al, Covaris Application Note M020141; Covaris Application Note M020154; Staeber et al, Covaris Application Note M020155; Herrera et al, Clin. Proteom. 2020, 17:24; Mueller et al, Mol. Syst. Biol. 2020,16:e9111; Herrera et al, Clin. Proteom. 2020, 17:24; Stukalov et al, bioRxiv 2020
Niu et al, bioRxiv 2020

PP01.69: Broad-scale Multiomics Reveals Protein Signature Suggestive of Microbiome Dysbiosis and Inflammatory Bowel Disease Risk

Ray Chen, United States

Understanding the dynamics of the human proteome is crucial for identifying biomarkers to be used as measurable indicators for disease severity and progression, patient stratification, and drug development. We highlight the use of a broad-scale proteomics approach, including coverage of the low abundant plasma proteome, to reveal biomarkers and insight into the mechanism of dysbiosis and its potential role as an early driver in the pathogenesis of inflammatory bowel disease (IBD) in a lifestyle coaching cohort of 2872 healthy participants. The work includes multi-omic analyses including linking protein levels and rare loss of function variants linked to increased plasma proteomic levels, and cite evidence from IBD patient mucosal biopsy data and mouse model data that suggest the same IL17C pathway along mucosal expansion of specific gram negative Proteobacteria. The body of work identifies an association of deleterious DUOX2 variants with a preclinical hallmark of disturbed micro-biota immune homeostasis that appears to precede the manifextation of IBD.

References:
Grasberger et al 2021 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8087203/
Wik et al 2021 https://www.mcponline.org/article/S1535-9476(21)00140-7/fulltext

PP01.71: Exploring the Synergy of A-to-I RNA Editing and Splicing: Unveiling a Promising Target for Cancer Therapeutics

Anton Goncharov, Russian Federation

Signaling pathways responsible for the recognition and elimination of foreign nucleic acids play a significant role in activating innate antitumor immunity. Furthermore, these pathways have the potential to be activated not only in the presence of foreign nucleic acids but also in response to various other stimuli. It is known that one of the mechanisms through which low molecular splicing modulators exert their antitumor effect is by activating type I interferon-mediated innate immunity.

Through analysis of proteomic and transcriptomic data, we discovered that exposing tumor cells to splicing modulators, such as Pladienolide B and its synthetic analog H3B-8800, resulted in the upregulation of interferon-stimulated genes (ISGs) responsible for antiviral immunity. Notably, one of the prominent proteins induced by these modulators is double-stranded RNA-specific adenosine deaminase (ADAR), which acts as a safeguard against excessive activation of innate immunity. Additionally, our findings demonstrated that cells with disrupted splicing exhibited a significant increase in ADAR-mediated RNA editing. This further supports our hypothesis that splicing perturbations caused by Pladienolide B and H3B-8800 lead to the accumulation of aberrant RNA molecules containing double-stranded structures within the cytoplasm. These structures, resembling viral RNA, trigger the activation of type I interferon-mediated immunity. However, the presence of ADAR mitigates the cytotoxic effects of such RNAs by deaminating adenosine nucleosides and disrupting the double-stranded structure.

In summary, our study sheds light on the interplay between splicing modulators, ADAR-mediated RNA editing, and innate immune responses in the context of cancer. These findings contribute to our understanding of the potential therapeutic implications of splicing modulation and highlight the intricate mechanisms underlying the activation of antitumor immunity.

PP01.73: Integrated Omics Analysis of the HEPG2 Cell Line: Unraveling Molecular Insights through Transcriptomics, Translatomics, and Proteomics

Ekaterina Ilgisonis, Russian Federation

In our research, we adopted a multi-omics approach to comprehensively explore the molecular landscape of HEPG2 cells. Our study focused on transcriptomics, translatomics, and proteomics analyses, utilizing state-of-the-art techniques to provide an integrated perspective on cellular processes.

Previously, Wang et al. (2021) demonstrated significant disparities in gene expression patterns between the transcriptome and translatome layers in mammalian organs, with the translatome layer exhibiting lower expression divergence due to widespread compensatory co-evolution.

To unravel the transcriptional dynamics of HEPG2 cells, we employed Oxford Nanopore Technologies (ONT) for transcriptomics analysis. Additionally, we conducted translatomics analysis using polyseq profiling followed by ONT sequencing. Furthermore, we performed proteomics analysis (shotgun LC-MS-MS and SRM) on the HEPG2 cell line to identify and quantify the protein abundances.

Integrating these omics datasets allowed us to identify regulatory networks and uncover potential associations between transcriptional dynamics, translation efficiency, and protein abundance. We observed a correlation of ~90% between the results of transcriptomic and translatomic profiling. Additionally, the correlation between transcriptomic/translatomic and proteomic profiling was ~61%.

The differences between the number of detected proteins and transcripts/translats (actively translated transcripts) can be attributed to various factors related to their lifespans and regulation. Proteins generally have longer lifespans compared to transcripts/translats. Transcripts, particularly mRNA molecules, exhibit more transient characteristics with shorter half-lives due to active degradation processes, such as mRNA decay pathways. The number of detected proteins may not directly correlate with the abundance of transcripts/translats due to differences in the translation efficiency of specific transcripts. Integrating multi-omics data and considering the interplay among these regulatory mechanisms is pivotal for achieving a comprehensive understanding of cellular processes.

PP01.75: Proteomic Profiles Associated with Early Life Exposome in Asthma and Atopic Dermatitis : ECHO-COCOA Study.

Yeonwoo Jo, Republic of Korea

Asthma and atopic dermatitis are common childhood environmental diseases, with the onset of these conditions significantly influenced by early life exposome. However, the biological significance of environmental factors in these conditions remains poorly understood. In this study, we employed proteomic approaches to investigate the influence of the exposome on asthma and atopic dermatitis.

Plasma samples were collected from 7-year-olds , comprising healthy (n=118), asthma (n=21), atopic dermatitis (n=86) and both conditions (n=11). Quantitative proteomics analysis using Zeno SWATH-MS was performed on the samples. To establish a link between the proteomic signatures and the exposome, paired urine samples obtained at aged 3 and 7 were measured for 74 environmental exposures. These exposures included 26 heavy metals, 22 volatile organic compounds, 9 types of environmental phenols and parabens, 9 PHTs metabolites, 9 PAHs metabolites, 1 type of pyrethroid pesticide, and the metabolite related to tobacco smoking .

Correlation analysis between expression levels of these 170 proteins that were commonly identified across all four groups and the individual's level of 74 exposures revealed 5 proteins with moderate correlations ($|r| \geq 0.3$). Gene ontology analysis indicated that these proteins were enriched in the biological processes of glycolysis/gluconeogenesis, biosynthesis of amino acids and carbon metabolism. Moreover, these proteins effectively differentiated between the disease groups.

To further investigate the interaction between the exposome and plasma proteome in asthma and atopic dermatitis, we will conduct subgroup-level correlation analyses between the expression levels of the 5 identified proteins and specific environmental exposures, based on their presence or absence . This approach will provide insights into the underlying features of asthma and atopic dermatitis development.

See you in Busan!
BEXCO, Busan, Korea
🌐 2023.hupo.org
🐦 f 📷 in #HUPO2023

Together Through Proteomics

PROGRAM

PP01.77: Multi-omics Analysis for Characterization of Extracellular Vesicle

Hye-Jung Kim, Republic of Korea

Extracellular vesicles (EVs), or more frequently known as exosomes, are membrane-surrounded vesicles released by numerous cell types into the extracellular microenvironment. EVs contain multiple classes of molecules, including nucleic acids, proteins, and lipids. EVs are involved in cell–cell communication, coagulation, inflammation, immune response modulation, and disease progression. EV have recently emerged as novel therapeutics in various clinical applications. Despite the growing number of evidences demonstrating that EVs are a crucial mediator of intercellular communication, challenges to commercialize EVs in clinical applications remain largely unresolved. Here we report the multi-omics characterization of vesicles and provide evidence for highly conserved features of EV structure and function. Our multi-omics platform with Liquid chromatography-mass spectrometry (LC-MS/MS) can be used in a number of ways in the quality control (QC) of EVs as a new therapeutic and carrier.

PP01.79: The Combination of Quantitative Proteomics And Systems Genetics Analysis Reveals TNR is Associated With Novelty Seeking

Hui Li, China

Objective: Novelty-seeking refers to the tendency of humans and animals to explore novel and unfamiliar stimuli and environments. High novelty-seeking behavior is correlated with substance abuse. It has been reported novelty-seeking is modulated by the central reward system in the brain. However, the details of the mechanisms are poorly understood.

Methods: High and low novelty-seeking mice are categorized on the basis of behavioral reactivity to the novel objects. A Label free quantitative proteomics analysis was performed with the hippocampus from high and low novelty-seeking mice. The mechanism of novelty seeking was investigated by combining bioinformatics analysis of proteomics profile and transcriptome-wide association screening of BXD mouse panels.

Results: A total of 165 differently expressed proteins were identified, of which 31 proteins were significantly upregulated in highly novel seeking mice and 68 proteins were significantly downregulated. Systematic genetic analysis showed that the upregulated protein Tnr was significantly associated with the addiction phenotype. Gene co-expression analysis further revealed Tnr as a potential mechanism for regulating novel seeking by mediating the dopaminergic prominence pathway. Increased Tnr secretion in high novelty seekers further affects dopaminergic by inhibiting DRD2 through the PI3K/AKT/GSK3β pathway.

Conclusion: In summary, we propose that Tnr expression is significantly elevated in high novelty seeking mice, which in turn affects synaptic dopaminergic release via the PI3K/AKT/GSK3β/DRD2 signaling pathway, leading to susceptibility to addiction.

PP01.81: Unexpectedly Large Plasma Proteome Variation and its Genetic Determinants in Children and Adolescents

Lili Niu, Denmark

Background:
Understanding the genetic underpinning of the human plasma proteome is crucial for biomarker research and drug development. A few proof-of-concept studies using mass spectrometry (MS)-based proteomics have identified dozens of protein quantitative trait loci (pQTLs) for plasma proteins. However, these studies are limited by small sample sizes and/or proteome depths. Existing large-scale studies using affinity-based proteomics have focused on adult populations. We aim to investigate the impact of genetic variation on the human plasma proteome in children and adolescents using high-throughput MS-based proteomics.

Methods:
Employing a streamlined and highly quantitative MS-based proteomics workflow, we measured the plasma proteome of 2,147 children and adolescents aged 5–20 years. We performed genome-wide association analysis between 420 proteins and 5.2 million single nucleotide polymorphisms using a linear mixed model. We then replicated the pQTLs in an independent cohort of 558 adults aged 18-82 years. We developed a framework to directly eliminate artefactual pQTLs due to protein-altering variants using peptide-level information.

Results:
The levels of a striking 90% of these proteins were associated with age, sex, body mass index or genetics, which were in turn predictable by protein levels. For half of the quantified proteins, we found pQTLs that regulated levels between a few percent and up to 30-fold. Approximately 80% of the pQTLs were successfully replicated in the independent replication cohort (p<0.05), with highly concordant effect sizes in direction and strength (searchable results available at http:/proteomevariation.org).

Conclusions:
This study provides the largest set of pQTLs for plasma proteins identified by MS-based proteomics. Together with the excellent replication in the independent cohort, our data reveals unexpectedly extensive genetic impacts on plasma protein levels, consistent from childhood into adulthood. These findings provide new insights into the molecular underpinnings of the human plasma proteome with potential implications for biomarker research and drug development.

PROGRAM

PP01.83: The Combination of Quantitative Proteomics And Systems Genetics Analysis Reveals PTN is Associated With Sleep Loss Induced Cognitive Impairment

Shuijing Pan, China

Objective: Sleep plays a critical role in maintaining physical and mental health. Acute sleep deprivation significantly affects short-term cognitive dysfunction. However, the detailed mechanisms remain unknown. In our research, we investigated the potential mechanism of acute sleep deprivation-induced cognitive impairment with a combination of quantitative proteomics and systems genetics approach.

Methods: We constructed a mouse model of acute sleep deprivation with intraperitoneal injection of chlorophenylalanine (300 mg/kg). A Label free quantitative proteomics analysis was performed with the hippocampus from wild type and acute sleep deprivation mice. The mechanism of cognitive dysfunction caused by sleep deprivation was investigated by combining bioinformatics analysis of proteomics profile and transcriptome-wide association screening on BXD mouse panels.

Results: A total of 164 differently expressed proteins were identified, of which 71 proteins were significantly upregulated in acute sleep deprivation mice and 93 proteins were significantly downregulated. Systematic genetic analysis showed that the downregulated protein Ptn was significantly associated with cognitive function. Further Western Blotting and Immunnohistochemical staining confirmed PTN expression is associated with sleep length. Gene co-expression analysis further revealed the potential mechanism of Ptn-mediated insomnia induced cognitive impairment via MAPK signaling pathway. The decreased secretion of Ptn by insomnia leads to reduced binding to Ptprz1 on the postsynaptic membrane with the activation of the MAPK pathway via C-fos and Nr4a1, and further leads to apoptosis of neuron.

Conclusion: In summary, we propose that acute sleep deprivation significantly reduces the expression of Ptn in the hippocampus, which results in cognitive impairment through Ptn-Ptprz1-MAPK signaling. Our study provides a novel biomarker for insomnia-induced cognitive impairment and a new strategy for seeking neurological biomarkers by the integration of proteomics and systems genetics.

PP01.85: Multi-Omics Integration of Thousands of Plasma Proteins: Unveiling Type 2 Diabetes Signatures and Clinical Associations in Large-Scale Study

Willy Pena Buttner, United States

Background
Despite the functional insights proteins can provide, the large dynamic range of the plasma proteome has historically limited comprehensive discovery studies at scale and depth. We recently introduced a novel plasma proteomics workflow enabling deep and unbiased proteomics at the scale of large cohorts. Here we explored the synergy of these novel deep proteomics insights for multi-omics studies, integrating thousands of proteins with other molecular modalities including lipids and metabolites in a cohort of 388 Type 2 Diabetes (T2D) cases and controls from Qatar Metabolomics Study of Diabetes (QMDiab, Halama et al. 2022).

Methods
Multi-omics data was collected on 11 platforms surveying various molecular layers in different biospecimen including circulating micro-RNAs, proteins, metabolites and lipids. Plasma proteomics was performed on a grand scale using Seer's Proteograph™ Product Suite, deploying a panel of five distinctly functionalized nanoparticles. We applied mixed linear regression models to discern biological signatures for T2D and various clinical health measures. Integration of all omics was achieved using Multi-Omics Factor Analysis (Argelaguet et al. 2018), investigating the variance across omes in correlation with phenotypes. We also utilized functional enrichments to detect shifts in pathway activities.

Results
Single-ome analysis confidently pinpointed T2D biomarkers, such as increased sugar metabolites, a decrease in 1,5-anhydroglucitol, and variations in low-abundance plasma proteins after adjusting for age, sex, and body mass index. Multi-omics data integration highlighted medication usage, facilitating analytical approaches to differentiate T2D-related changes and external factors, while also revealing multi-omics signatures linked to specific clinical health measures.

Conclusions
Comprehensive integration of large-scale, deep proteomics data with other molecular modalities using methods like MOFA offers unique insights into the molecular landscape of health and disease, underscoring its potential for improved disease diagnosis and management.

PP01.87: Splicing Inhibitors Sensitise Cancer Cells to DNA Damage

Victoria Shender, Russian Federation

Despite the growing interest in mRNA alternative splicing (AS) as a therapeutic anticancer target, the potential of splicing inhibitors (SI) in treating solid tumors remains largely unexplored. Moreover, the exact mechanism of cytotoxic and antiproliferative activity of SI remains unclear. We conducted a meta-analysis of transcriptome data from six different tumor types and revealed that splicing inhibitors induced similar patterns of AS, resulting in widespread exon-skipping events that underwent nonsense-mediated decay. These changes significantly downregulated one-third of essential DNA repair genes, thereby creating a therapeutic vulnerability that can be exploited for cancer treatment. To harness this vulnerability, we proposed a new approach to cancer treatment consisting of sequential addition of a SI (Pl-B or H3B-8800) followed by a DNA-damaging agent. Using FACS analysis and MTT assay, we found that each of the five tested DNA-damaging agents can be used sequentially with the splicing inhibitor, opening up a massive field for their future practical applications for tumor treatment. Our proteomic and RNA-seq analyses revealed that the combination of Pl-B and cisplatin disrupts signaling pathways important for the response of tumor cells to DNA damage compared to cells treated with cisplatin alone. Using different immunofluorescence assays, we noticed a decrease in the number of γH2AX foci (which is the early marker of DNA damage) while observing a greater number of DNA breaks in cells treated with the drug combination compared to cisplatin alone. This effect indicated that Pl-B either disrupts sensing of DNA breaks due to suppression of DNA repair process or overloads the DNA repair machinery due to excessive replicative stress, which makes cells more susceptible to subsequent cisplatin treatment. Our in vitro and in vivo experiments demonstrated that this approach exhibits promising therapeutic potential for a wide range of tumors. The work was supported by the grant 22-15-00462 of RSF.

PROGRAM

PP01.89: Higher and Higher N: SimpliFi Data Analysis for the Masses Now Takes Masses of Samples

John Wilson, United States

In the era of ever-increasing omics data, understanding and making actionable decisions have become challenging. To address this, we developed SimpliFi, a cloud-based data-to-meaning engine that accepts all omics types, allowing easy sharing, exploration, and publication via URL. Now, SimpliFi has undergone extensive upgrades to handle hundreds to thousands of samples.

SimpliFi employs nonparametric statistics and defines distributions based on biological replicates, enabling p-values and fold-changes to account for biological variation, sample size, observations, and measurement error. Confidence intervals are provided for all values, including p-values. It preserves data integrity, accommodating increased variance at low or high intensities. The user interface is intuitive, making it accessible even for new-to-omics users. The backend analysis speed has been dramatically improved, while the frontend offers summary displays for large sample sizes (>100s).

Using nonparametric statistics, SimpliFi captures the non-Gaussian nature of biology and omics data, yielding significantly different p-values compared to T-tests. It handles oversampling and undersampling of biological variability, preventing false negatives and false positives. SimpliFi facilitates analysis of mono- or multiomics data, presenting interactive displays of pathways, tissue states, diseases, cells, and molecular-level classifications.

The front-end displays have been completely revamped for population-scale analyses. Quality control flags unusual samples for manual assessment. Data visualizations accommodate hundreds to thousands of samples, allowing comprehensive exploration. Importantly, differential expression analysis is now available at a population-wide level, featuring distributions, violin plots, box-and-whisker plots, and other visualizations for immediate understanding of large-scale experiments.

These enhancements make SimpliFi an invaluable tool for analyzing and interpreting omics data, empowering researchers to gain insights and make informed decisions.

See you in Busan!
BEXCO, Busan, Korea
🌐 2023.hupo.org
🐦 f 📷 in #HUPO2023

Together Through Proteomics

PROGRAM

PP01.91: GlyPAQ Kit: Easy, Fast, and Standardized Sample Preparation for Simultaneous Processing of Deglycosylated Peptides and N-glycans for Structural Analysis.

Melinda Wojtkiewicz, United States

Introduction

Structure-based glycan analyses play a vital role in comprehending physiology and characterizing biologic drugs, offering the potential to unveil new biomarkers for various human diseases. However, the widespread adoption of structure-based glycomics analyses has been hindered by the absence of user-friendly methods for high-throughput sample preparation. We have successfully developed GlyPAQ, a sample preparation kit that automates the process of generating MS-ready samples for structure-based glycomic and proteomic analysis, all from a single starting material. GlyPAQ is suitable for preparing a wide range of sample complexities, including monoclonal antibodies, cells, tissues, serum, plasma, and urine.

Methods

Samples were solubilized and non-mammalian glycoprotein standard was added. Samples were added to the Biomolecular Sorting Plate. N-glycans were enzymatically released and collected. Proteins were digested with trypsin and peptides collected separately. Glycans were reduced, and contaminants and salts were removed with the Glycan Clean-Up Plate. Sample processing was complete within 6-24 hours. Reduced native N-glycans were separated by PGC chromatography and analyzed using an Orbitrap Eclipse. Peptides were separated by C18 and analyzed using an Exploris 480.

Preliminary Data

From 0.5 µg RNAseB, all expected glycan structures (high mannose type and complex type glycans) were observed. From 100 µg of protein from cells, homogenized tissue or plasma, hundreds of N-linked glycan structures are identified over 5 orders of magnitude of abundance. From 1 million human cells (150 µg total protein), >200 glycan structures and >5,000 proteins were identified in a single sample preparation.

Conclusions

GlyPAQ is the first standardized preparation kit for simultaneous preparation of proteomics and reduced native structure-based glycomics samples. Studying deglycosylated peptides can provide valuable information about the functional role of specific peptide sequences. Glycans can modulate protein-protein interactions, cellular targeting, and molecular recognition events. Removing the glycans enables a more direct investigation of the peptide's functional properties.

![HUPO Busan 2023 logo]

See you in Busan!
BEXCO, Busan, Korea

🌐 2023.hupo.org

🐦 f 📷 in #HUPO2023

Together Through Proteomics

PROGRAM

PP01.93: De Novo Assembly of RNA-sequencing Reads Defines Non-canonical Viral-human Fusion Transcripts as a Source for Antigen Presentation in Cervical Cancer

Qian Yang, United Kingdom

Background: Cervical cancer is the fourth leading cancer for female worldwide, it is mainly caused by human papillomavirus (HPV) and HPV16 is the most prevalent high-risk subtype. We exploited the power of RNA-sequencing to aid the analysis of HPV transcriptomic profiles and the associated antigenic landscape in HPV16 positive cancer cell lines to understand targetable immunopeptides presented to T cells.

Methods: We performed RNA sequencing on human cervical cancer cell line CaSki (HPV16+). Reads were pre-processed with Trim_Galore and FastQC. Processed reads were mapped onto a customised genome created with hg38.p13 and HPV16 (K02718.1) using STAR, sorted and indexed by Samtools. Unmapped reads were used for de novo assembly with Trinity. Assembled transcripts underwent 6-frame translation with Seqkit and appended to Human UniProt protein database for search with PEAKS. CTAT-VIF was used to identify sites of viral insertion into the human genome and IVG was used for visualization.

Results: We confirmed the presentation of the known HLA-A*02:01 peptide (YMLDLQPET) derived from HPV16 E7. Importantly, we identified a peptide derived from an alternative reading frame (ARF) in the HPV16 E1 transcript (MLYQMTRTK) and a hybrid fusion transcript spanning the HPV16 E6 and E7 genes and continuing into a non-coding area upstream of gene RUNX2 on human chromosome 6 (chr6). We mapped a key HLA-presented peptide (RLFSTLITV) encoded by this region. We showed the immunogenicity of these peptides in HPV-specific T cell lines from PBMC from women with cervical intraepithelial neoplasia.

Conclusions: Using our unbiased proteogenomic approach combining de novo assembly of Illumina short reads from RNA-sequencing and immunopeptidomics, we report for the first time the direct evidence of non-canonical HPV16-human fusion transcript, which forms the source for an immunogenic HLA-presented peptide in CaSki cells. These findings offer a new source for antigens that may assist immunotherapeutic approaches to treat cervical cancer.

PP01.95: Data-independent Acquisition (DIA) Performance on Proteomic Samples Using a Zeno Trap

Ihor Batruch, Canada

Background:
Identification and quantification of proteins is important for the characterization of biological systems and DIA analysis is becoming a method of choice for these types of workflows. A ZenoTOF 7600 system is equipped with a Zeno trap that improves duty cycle and enables gains in sensitivity of 5 to 20x. Zeno SWATH DIA protein identification across 0.25 ng to 200 ng of commercial digest loads was evaluated and consistency in performance was assessed.

Methods:
A ZenoTOF 7600 with an OptiFlow Turbo V ion source was coupled to a Waters M-Class LC system with nano- or micro-flow columns. K562 cell digest (SCIEX) at 0.25-200 loads was analyzed using Zeno SWATH DIA methods and long-term consistency in system performance was assessed with quality control (QC) K562 samples. Data was processed with DIA-NN (1.8.1) software using a spectral library.

Results:
Zeno SWATH DIA methods on a ZenoTOF 7600 system for 0.25, 0.5 and 1 ng digest loads detected between 1900 (6900 with MBR activated) and 3500 protein groups at 1% FDR with 8200 (25000 with MBR) to 17000 precursors. At loads 5 to 200 ng, between 5800 and 7900 protein groups were detected with 89-95% of protein groups having a CV under 20%, along with 44000 to 74000 precursors. Using K562 QC samples, we show that the system delivers reproducible protein group and precursor identifications with single digit CVs over an extended period of instrument usage.

Conclusions:
A ZenoTOF 7600 system with Zeno trap enabled identifies and quantifies a high number of proteins at single-cell level loads. Long term system performance evaluation shows that the system is reliable in terms of protein identification and quantification.

PROGRAM

PP01.97: A Comprehensive Spectral Library of Chicken RPE Proteome by Novel Ion Mobility Fractionation Coupled to dia-PASEF Technology

Jingfang Bian, Hong Kong

Chickens were a well-accepted model for studying ocular growth. The retinal pigment epithelium (RPE) plays a crucial role in relaying signals from the retina to the sclera and driving structural changes underlying ocular growth. Despite the barrier and homeostatic roles of the RPE are well recognized, there is no publicly available spectral library for the chicken RPE proteome. Hence, the purpose of the study was to establish a comprehensive spectral library of chicken RPE proteome using novel Trapped Ion Mobility Spectrometry (TIMS) and parallel accumulation serial fragmentation (PASEF®) technology.

Six RPE tissues were collected from normal chickens at postnatal 18 and digested with EasyPep MS Sample Prep Kit following the manufacturer's instructions. 100ng peptides from each eye were analyzed by Ion Mobility Fractionation coupled to dia-PASEF on a TIMS-QTOF Pro2 system using nanoflow LC with 60-min gradient. DIA-NN and Spectronaut® were used to analyze the dia-PASEF data searched against the UniProt Chicken protein database (Gallus gallus, 51486 proteins). Functional analysis of Gene Ontology (GO) annotations was performed using the PANTHER classification system.

A total of 7016 proteins (45765 peptides) and 6168 proteins (44087 peptides) were identified by DIA-NN and Spectronaut® respectively (at 1% FDR) after combining search. Although different analysis strategies, 4706 common proteins were found. The GO analysis revealed that the frequent molecular functions were binding and catalytic activity as well as cellular and metabolic process for biological process. The top-ranked enriched pathways were Integrin, Inflammation mediated by chemokine and cytokine, and Wnt signaling pathways.

This study provides the largest spectral library of chick RPE, forming a fundamental repository for the understanding of ocular growth at the cross-tissue level and other proteomic studies in the visual field. Data are available via ProteomeXchange with identifier PXD042426.

PP01.99: Functionalized Nanoparticles Provide Quantitative Large-Scale, Unbiased, and Deep Plasma Proteomics

Lee Cantrell, United States

The large dynamic range and diversity of proteoforms in plasma have limited the depth of protein coverage in large-scale proteomics studies. However, the utilization of automated nanoparticle (NP) protein corona-based proteomics workflows can effectively compress the dynamic range of protein abundances into a mass spectrometry (MS)-compatible range. This enhancement of the depth and scalability of quantitative MS-based methods holds significant potential to reveal the molecular mechanisms of biological processes.

This study assesses the quantitative performance of NP corona-based plasma proteomics workflow by examining fold change accuracy, linearity, and precision at proteome-wide scale. A mixed-species experiment involving spiking bovine plasma into human plasma at varying dilution ratios was conducted to evaluate quantification performance. Both NP protein corona-based workflow, known as Proteograph™, and neat digestion workflow were used for sample processing, followed by DIA LC-MS analysis.

The NP-protein corona-based workflow provided superior plasma proteome coverage (2360 vs 744 proteins identified) and reproducibly quantified 2.7x more proteins at ≤20% CV (1058 vs 395). The NP workflow is also able to quantify more proteins at given accuracy threshold (e.g. 1.6x more proteins at ≤25% error for fold change = 2) and maintain high linearity in quantification with a median Pearson correlation 0.995 for NP workflow and 0.998 for neat workflow.

To further validate the quantitative performance, protein quantifications from NP workflow were compared to those generated by a corresponding 23-protein ELISA panel in a plasma cohort of 30 biosamples.   For proteins that had at least two-fold variations within the cohort, median correlation between two methods is 0.6.   Additionally, upon closer examination of peptide-level quantification, we discovered indications of potential proteoform signals in cases where weak or anti-correlation was observed.

In summary, the Proteograph workflow enables scalable and unbiased biomarker discovery in plasma by reproducibly quantifying plasma proteins with higher protein coverage.

PP01.101: A Pioneering Deep Proteomics Data Analytics Pipeline: Illuminating a New Functional Breast Cancer Subtype

Senuri De Silva, Singapore

Background
Recent advancements in mass spectrometry enable system-wide identification and quantification of thousands of proteins. However, small sample sizes in published studies limit mining and identification of precise biological information. To this end, we devised a computational proteomics pipeline to systematically integrate publicly available large proteomics data sets followed by novel feature extraction strategy and demonstrate its utility using breast cancer (BC) as a case study.

Methods
In this study, eight public BC proteomic datasets obtained from the analysis of 700 patients' samples evenly distributed across defined microarray-based subtypes (PAM50) were curated. All the data sets (>3K raw files) were reprocessed using FragPipe pipeline and the outputs of relatively quantified 7,000 proteins were subjected to state-of-the-art statistical and clustering analysis followed by bioinformatics.

Results
Our curated dataset reproduced known BC subtypes with associated protein markers, affirming the validity of our pipeline. Unbiased clustering revealed five distinct clusters: HER2-like, Basal-like, Luminal A-like, Luminal B-like, and a mixed type. While three clusters aligned with PAM50 subtypes (HER2, basal, Luminal A), we identified a novel cluster combining luminal and HER2 features, with increased mitochondrial activity and mRNA splicing regulation. Differential expression analysis pinpointed characteristic markers for each proteomic subtype, and further validated on independently curated dataset.

Conclusion
In conclusion, our newly built analytics pipeline facilitates mitigating technical confounders associated with integrating different datasets. We uncovered a novel BC subtype, a mix of luminal and HER2 characteristics, with associated protein signatures, proving the power of our approach for identifying novel information from systematically integrated diverse data sets. This pipeline can also be applied for dissecting heterogeneity of other diseases and identifying their pertinent new molecular features.

PP01.103: An EasyPep Magnetic Solution for Automated Proteomics Sample Preparation

Maowei Dou, United States

Unlike the genomic and transcriptomic fields, the standardization of reagents and methods for proteomic sample preparation remains a challenge. Current methods are time-consuming, laborious, prone to variability, inefficient in removing contaminants, and not suitable for processing large sample numbers. To address these challenges, we introduce a new EasyPep sample preparation workflow using magnetic beads for automated proteomics sample preparation.
Protein extraction, reduction, alkylation, and digestion are facilitated by the same ready-to-use buffers developed for the original EasyPep sample workflow. To enable the magnetic-based sample preparation clean-up, we developed a novel, magnetic version of our Ultralink beads. These magnetic beads exhibit excellent compatibility with a wide range of solvents, pH (pH 3-12) and are free from leachables. Peptide binding and elution have been optimized for different sample input amounts (10μg-2mg) and concentrations (0.1-1mg/mL). Notably, the eluted peptides can be directly injected into liquid chromatography-mass spectrometry (LC-MS), eliminating the conventional and time-consuming SpeedVac drying and reconstitution steps.
We demonstrate streamlined preparation of up to 96 samples in approximately 3 hours with a minimal manual intervention using KingFisher and Hamilton automated systems. Validation studies using mammalian cells and plasma samples (10-100 μg) demonstrated exceptional digestion efficiency with a missed cleavage rate below 10% and complete cysteine reduction and alkylation. Our sample preparation process exhibits robustness and reproducibility, with peptide and protein identifications displaying less than 5% coefficient of variation (CV) and proteome quantification based on protein abundances showing less than 10% CV. To enhance the capabilities of our automation solution, we integrated tandem mass tag (TMT) multiplexing strategies, facilitating multiplexed proteome analysis. This enables the identification and quantitation of thousands of proteins in a single experiment with exceptional precision and accuracy. The comprehensive EasyPep Magnetic solution maximizes laboratory productivity while significantly improving the speed and reproducibility of high-quality proteomics sample preparation.

PP01.105: Optimizing Protein Precipitation of Human Cell Lines for Profiling Proteomics via LC-MS

Jaehee Ha, Republic of Korea

Introduction
Protein precipitation (PPT) plays a critical role in proteomics research as it facilitates the separation of proteins from cell lysates for further analysis. However, the absence of universally accepted standard method for PPT poses challenges, particularly in effectively precipitating low-mass proteins. This study aimed to evaluate ten established PPT methods and assess their efficiency in recovering both whole proteins and low-mass proteins.

Methods
Ten PPT methods were selected, incorporating three different salts (ammonium sulfate, sodium chloride, and zinc sulfate) and eight solvents (water, acetone, ethanol, methanol, chloroform, perchloric acid, and trichloroacetic acid). HeLa cells were used for the experiments, and the protein concentration in all cell lysates was carefully adjusted. Protein quantification was performed using the Bicinchoninic Acid Protein Assay (BCA), and subsequent analysis was conducted using liquid chromatography-mass spectrometry (LC-MS) to assess protein recovery.

Results
Based on the BCA and LC-MS analysis, the recovery rates of the top 300 highly abundant proteins in HeLa cells were determined for each PPT method. Overall, the inclusion of a salt in the solvent demonstrated superior efficiency in terms of protein recovery compared to solvents without salt. Moreover, the specific type of salt utilized had a notable impact on protein recovery. However, it is important to consider that for LC-MS analysis, the inclusion of salt necessitated an additional desalting step.

Conclusion
This study evaluated ten established PPT methods and proposed an optimized approach for comprehensive proteomic profiling. The focus was on improving the precipitation of low-mass proteins in cell lysates, presenting a powerful strategy to enhance their recovery and detection. The findings contribute to the advancement of proteomics research and pave the way for more accurate and comprehensive analysis of proteomic samples.

PP01.107: High-throughput Proteomics on a Novel High-resolution Accurate Mass (HRAM) Platform

Daniel Hermanson, United States

Single-shot LC-MS based proteomics is an essential tool to help researchers unravel the proteomes of complex biological samples but there is a growing necessity to identify more proteins in less analysis time. We report on the use of a novel HRAM mass spectrometer in combination with a very short pillar array-based separation column to achieve comprehensive high throughput proteome analysis with a single LC-MS set-up.

2 ul of 100 ng lyophilized mammalian cell digest resuspended in 200 ul 0.1% FA was injected on column. The separation was performed on a Vanquish Neo UHPLC system with cycle times of 8, 14.4, and 24 minutes using a 5.5 cm long µPAC Neo High Throughput column in a trap-and-elute workflow. The LC was coupled to a novel HRAM mass spectrometer operated in DIA mode. Data files were processed with Proteome Discoverer 3.1 software.

A set of robust high-to-medium throughput LC-MS methods with variable flow rates was developed for use in large cohort study settings. A trap-and-elute configuration and performing column re-equilibration in parallel to sample loading achieves a significant increase in instrument productivity. Utilizing variable flow rates ranging from 2.5 to 0.3 µL/min LC-MS instrument productivity of 68, 79, and 87% are achieved for cycle times of 8, 14.4, and 24 min.

The performance of these different methods was evaluated by injecting 200 ng of HeLa digest sample. Because of the high transmission efficiency and scan rate of the novel HRAM-platform, DIA experiments were performed with an isolation width of 2 Th. At a sample throughput of 180 samples per day (8 min injection-to-injection) 7900 protein groups were identified on average from 5 technical replicates. Within 14.4 and 24 min of total LC-MS time an average of 8579 and 9167 protein groups were identified, respectively. These results demonstrate unprecedented performance for high-throughput proteomics.

PP01.109: Protein Array Analyses Identify STAT5-GLDC as Potential Mediators of Lactation Stimulation of Breast Tumorigenesis via Metabolic Remodeling

Shixia Huang, United States

Epidemiological studies have demonstrated that a first birth before age 25 lowers breast cancer risk, while a first birth after age 35 increases breast cancer risk. Studies of the underlying mechanisms have generally focused on pregnancy but not the ensuing lactation. To address the contribution of lactation in older women to breast cancer risk, we compared the tumor latencies of nulliparous, pregnancy alone, brief lactation, and full-term lactation mice in two different somatic models of breast cancer. These models harbor small numbers of precancerous lesions before mating. We did not detect a significant impact of pregnancy itself or a brief lactation. Rather, the ensuing full-term lactation increased the risk of mammary tumors significantly. Reverse phase protein array (RPPA) profiling the eventual tumors showed a very distinct protein profile in the full-term lactation group, particularly the prolactin signaling. Prolactin signaling activates signal transducer and activator of transcription 5 (STAT5). We next showed that forced STAT5 activation in these somatic cancer models also promoted tumor formation and further RPPA profiling in these models identified glycine decarboxylase (GLDC) as a potential novel mediator of STAT5 in driving breast cancer risk, which was confirmed by biochemical and genetic experiments. GLDC knockdown and subsequent analyses suggest that GLDC impacted cancer transformation by regulating nucleotide metabolism. Together, these data suggest that a full-term lactation in women with precancerous lesions increases breast cancer risk through STAT5-GLDC-mediated metabolic remodeling. Overall, our studies uncover potential breast cancer risk from lactation in high-risk women including those who chose a late-age pregnancy-lactation when precancerous lesions are more likely to have developed than in those with an early age pregnancy, and suggest a need for close monitoring and preventive treatment in this group of women.

PP01.111: Highly Multiplexed APEX2 Proximity Labeling for Spatiotemporal-Resolved Profiling of Submitochondrial Proteome

Donggi Jang, Republic of Korea

APEX2 is an engineered peroxidase that is commonly used in proximity labeling techniques, to study protein-protein interactions and subcellular localization. By coupling APEX2 labeling with mass spectrometry-based proteomics, we can identify and quantify interacting proteins of interest. Here, we synthesized isotopically coded desthiobiotin-phenol (DBP) probes to enable multiplexed quantification in the context of APEX2 labeling. By employing the Spot-ID protocol, DBP labeled peptides were directly analyzed and distinguished in mass spectrometry, allowing for their accurate quantification using conventional database search engines. Applying this approach, we successfully mapped the sub-organellar proteomic architecture of the mitochondria. Additionally, we demonstrated that the multiplexity of APEX2 based proximity labeling can be enhanced to 10-plex when combined with SILAC approach. By leveraging SILAC-based quantification, we were able to quantify 10-plexed proximity labeled samples prepared under different experimental conditions in a single experiment. These providing an unbiased and comprehensive view of the mitochondrial matrix proteome dynamics in response to mtDNA damage response. Our approach significantly reduced the variability associated with sample preparation and improved throughput, highlighting the advantages of MS1 quantification techniques.

![HUPO Busan 2023]

See you in Busan!
BEXCO, Busan, Korea
🌐 2023.hupo.org
🐦 f 📷 in #HUPO2023

Together Through Proteomics

PROGRAM

PP01.113: Evaluation of the Relative Quantitative Performance Using Tandem Mass Tags on a New High-resolution Accurate Mass Platform

Julia Kraegenbring, Germany

The new high-resolution accurate mass platform has a top scan speed of up to 200 Hz in MS2 mode, limited by the maximum injection time.

Shorter maximum injection times results in higher protein identification (ID) numbers for individual samples but can limit the S/N of isobaric tag reporter ions resulting in fewer quantifiable proteins for multiplexed samples. This limitation can be overcome by increasing maximum injection times to provide sufficient reporter ion S/N for precise quantitation.

Increasing the maximum injection times for TMT-labeled samples results in speeds between 20 and 50 Hz for MS2 scans in DDA mode. Despite using slower scan speeds compared to label-free samples, the improved instrument speed and sensitivity for this new HRAM platform results in >3,000 quantifiable protein groups and >20,000 quantifiable peptides using a 70-minute gradient method for the TMT11plex yeast digest standard. This is a 1.8x increase in quantifiable protein IDs and 2x increase in quantifiable peptide IDs compared to results generated previously on Orbitrap hybrid instruments.

The optimized parameters and acquisition scheme from TMT11plex yeast digest standards were applied to the TMT-labelled plasma sample and used as starting point for assessing a 3 proteome mixture labeled with TMTpro 16plex reagents.

Preliminary data from the TMT16plex-labeled plasma sample mixture shows > 97% quantifiable protein groups in a 60 minute gradient. Preliminary data from the 3 proteome mixture shows >6,000 quantifiable protein groups in a 90 minute gradient.

All samples showed good quantitation results. The preliminary data of 3 proteome mixture revealed 90% of peptides had a CV of less than 20% and the missing value for this sample was less than 20%.

PP01.115: High Throughput and High Coverage Workflow for Plasma Proteome Analysis with Automation and Multi-proteases Strategy

Qingrun Li, China

Introduction
With the stability of plasma proteome expression profiles and its strong correlation with clinical tests, plasma proteomics has greater clinical translational value in precision medicine for individuals. However, few novel markers have been truly applied in the clinic. With the development of 4D proteomics technology, the scanning speed and detection sensitivity of mass spectrometry have been greatly improved.

Methods
We developed a workflow combining multiple enzymatic digestion technique to thoroughly analyze the plasma proteome semi-automatically. Specifically, using our customized and automated sample processing platform, samples were underwent digestion with 4 enzymes (trypsin, LysC, AspN, and GluC) and prepared with high reproducibility. The platform allowed us to perform preparation of 500 samples in 3 hours (including digestion).

Results
The results showed that our workflow identified a total of 7104 peptides, with an average of 4144 peptides per sample and a qualitative CV of 6.9%. A total of 862 proteins were identified, with an average of 600 proteins per sample and a CV of 5.6%. Further analysis of 20 QC samples verified the accuracy of this strategy for quantitative protein identification. Briefly, proteins quantification variation with CV <30%, 20%, and 10% accounted for 84%, 70% and 39% of the total identified proteins, respectively.

Conclusions
The automated sampling workflow improves the speed of sample preparation and reduces operational errors. Multiple enzymes strategy increases the depth of protein identification and sequence coverage, thereby establishing a high-depth spectral library. Combined with data-independent acquisition (DIA) and a "multi-in-one" strategy, high coverage and rapid plasma proteomics can be achieved.

PROGRAM

PP01.119: New Set of Isobaric Labeling Reagents for Quantitative 16Plex Proteomics

Xiaolian Ning, China

Isobaric tag is a powerful approach in quantitative proteomics. However, there is still a large room to improve the reagent quality such as increasing throughput and reducing ratio suppression. Herein, we redesigned the original chemical structure of the IBT-10plex developed in our laboratory, termed ass IBT-16plex, and systematically assessed the reaction conditions for IBT-16plex labeling peptides. The original chemical structure of IBT-10plex was resigned and optimized to create a new set of 16-plex isobaric tags. BSA and HeLa cell as test objects, a series of optimizations were conducted including labeling efficiency, quantitative consistency, collision energy, matrix effect, and concentration dependence of labeling. Comparison of peptide labeling results derived from TMTpro-16plex and IBT-16plex was focused on the identified peptides and ratio suppression. Application of IBT-16plex to the biological samples was carried out in MCF-7 breast cancer cell line with glucose tolerance. We successfully synthesized the novel reagents, IBT-16plex. The evaluation of quantification performance demonstrated that intensities of 16 reporter ions were basically comparable, a linear correlation between the abundance ratios of theoretical and measured values was well accepted when concentration differences were set within 10 times, and no matrix effect of the synthesized reagents. Compared to TMTpro-16plex, IBT-16plex was 14.8% higher in peptide and 8.6 % in protein identification. In the abundance ratio ranges of 1:200 among the samples, IBT-16plex exhibited smaller compression effect than that labeled by TMTpro-16plex. After quantitative examination of MCF7 cell proteins, IBT-16plex was confirmed as feasible and useful for evaluating protein responses of glucose-starved MCF7 cells to glucose-rich medium. The IBT-16plex reagent is a potentially useful isobaric tag reagent in quantitative proteomics.

PP01.121: A Benchmarking Workflow for High-Throughput DIA Label-Free Quantification Using a Novel High Resolution Accurate Mass Platform

Anna Pashkova, Germany

Background
Bottom-up proteomics has proven to be the most suitable technology for high-throughput analysis of very complex biological samples, such as cell lysates or blood. As the obtained data become more and more employed in biomedical research, the challenge of analyzing the smallest amounts of samples in the shortest time remains. To meet these challenges, an integrated workflow for label-free quantitative proteomic studies has been developed. Two samples containing three-proteome digests mixed in known ratios, were analyzed in DIA mode using a novel HRAM platform.
Methods
Mixtures of three proteomes (Yeast, Ecoli, Human) with total protein loads from 50 to 500 ng were separated using either a µPAC Neo 50 cm column in a Direct Injection mode or a PepMap 15 cm column in Trap-and-Elute mode, using a Vanquish™ Neo UHPLC system under sub-microflow conditions. Different gradient lengths were employed, ensuring throughput from 24 to 180 samples per day. MS data were acquired in DIA mode with 2Th window, and analyzed using Biognosys™ Spectronaut™ 17.
Results
The new benchmarking workflow enabled the quantification of over 13,000 protein groups (7,600 Human, 4,100 Yeast and 1,400 E.coli) and 160,000+ unique peptides, based on 2 sample mixtures analyzed in triplicates. 87% of identified proteins had CVs less than 20% and 72% of identified proteins had CVs less than 10%, with a median CV 4.7%. Experimental vs. theoretical average ratios were: 1.016 vs. 1 for Human, 0.49 vs. 0.5 for Yeast, and 1.993 vs. 2 for E.coli. The dynamic range of mass-spectrometric signal intensities spanned at least 5 orders of magnitude. With such performance, yeast proteins with expression levels from 100s to 100,000s copies per cell could be reliably quantified.
Conclusions:
The novel HRAM platform provides unsurpassed throughput while ensuring deep proteome coverage, thus opening new frontiers for life sciences applications.

PROGRAM

PP01.125: Chimerys Server: Deploying the Power of the Cloud in your Basement

Tobias Schmidt, Germany

Background:
Chimerys is a cloud-based search engine on Amazon Web Services (AWS) with a client interface for Proteome Discoverer software (PD). While AWS offers scalability and accessibility, transferring large data or user preference for in-house data storage may limit cloud service practicality. To address this, we adapted Chimerys as an on-premises solution for single servers and HPC environments, combining the best of both worlds.

Methods:
The current service utilizes containerized, distributed applications orchestrated by Kubernetes (K8s) and communicates through high-performance protocols. To simplify deployment on single servers, we replaced cloud-specific services with compatible interfaces and transitioned to K3s, a lightweight Kubernetes distribution. We also developed a CLI for direct job submission to Chimerys for streamlined integration into custom workflows.

Results:
The goal was to port Chimerys to a local server featuring off-the-shelf hardware, capable of parallel processing of 16 raw files in parallel. To simplify server deployment, we utilized pre-built virtual components and automated hardware setup with PXE-boot-based auto-deployment. The server ran a minimal Debian with a GUI for KVM hypervisor or Ubuntu on bare metal. GPU was directly passed through to an Ubuntu virtual machine in a virtualized setup. Chimerys' microservice architecture was modified to fit this deployment, trimming unnecessary cloud functionalities, and using compatible interfaces like Argo workflows for processing queues. RAM limitations were addressed by reducing Mokapot's RAM footprint and streaming data from fast NVMe drives. We developed a CLI and browser-based GUI for easy integration of the local Chimerys service into customizable workflows. Benchmarking showed the server can analyze 16 HeLa 1h Q-Exactive HFX raw files in 30 minutes, making large-scale experiments feasible on locally deployed infrastructure.

Conclusions:
Porting the cloud-native CHIMERYS software to a local server allows the performant processing of data in high-privacy scenarios or for capex-bound customers.

PP01.127: Automated Sample Preparation for Human Plasma Proteomics by SP3

Malte Sielaff, Germany

Proteomics analysis of human plasma holds great promise for biomarker discovery. However, large-scale experiments pose significant challenges in sample preparation, as any induced variability will influence subsequent analyses. Automation offers the potential for high-throughput and highly standardized sample preparation, leading to improved data quality and comparability across experiments. Here, we present an automated sample preparation method using a Single-Pot, Solid-Phase-enhanced Sample Preparation (SP3) protocol for plasma proteomics implemented on a Beckman Biomek i7 liquid handling platform.
A standard sample of human blood plasma was diluted under denaturing conditions and transferred to a 96-well microtiter plate. Semiautomated sample preparation was performed using a Beckman Biomek i7 liquid handling robot and an adapted SP3 protocol, including reduction, alkylation, magnetic bead-based protein clean-up, tryptic digestion and peptide recovery. Peptides were loaded on Evotips and analyzed by LC-MS using an Evosep One (60 samples/day) coupled to a Bruker timsTOF Pro 2 mass spectrometer (diaPASEF, 0.9 s cycle time). Peptide identification and label-free protein quantification (LFQ) were performed in DIA-NN using a library-free approach based on a Homo sapiens UniProtKB/Swiss-Prot FASTA database.
Results from our automated SP3-based sample preparation method revealed excellent reproducibility in terms of peptide (>4,600) and protein identifications (330), as well as LFQ as measured by pairwise correlations of protein intensities (r>0.97) across multiple replicates. Moreover, the integration of Evotip-based peptide purification allowed seamless transfer of samples, further streamlining the workflow and reducing potential sources of variability.
In conclusion, our investigation demonstrates the potential of an automated sample preparation approach utilizing an SP3 protocol for high-throughput plasma proteomics. Due to the flexibility of the SP3 method in terms of the bead types and solvent composition used for protein capture, we expect that further optimization of this approach is possible, ultimately contributing to the discovery of disease biomarkers and advancements in precision medicine.

PP01.129: An Automated High-throughput Sample Preparation platform utilizing S-Trap Digestion

Haneul Song, Republic of Korea

In large-scale proteomic analysis, accurate and precise sample preparation is essentially required on quantitative analyses to obtain high-level reproducibility. To this end, various proteolysis methods, such as in-solution digestion, filter-aided sample preparation (FASP), and Barocycler digestion have been automated with many liquid-handling robotics. In this presentation, we have realized an automated Suspension Trapping (S-Trap) digestion procedure, in which efficient protein purification was achieved by forming aggregate through 90% methanol. The procedure was implemented using liquid robotics (Tecan A200TM, Männedorf, Switzerland), which offers advantages such as the simultaneous treatment of 96 samples and rapid processing times of less than 4 hours. To demonstrate the effectiveness of the automated S-Trap digestion, 24 Clinical Human Plasma (CHP) of 8 Ovarian cancer and 16 Oral cavity cancer samples were processed, using a 96-well S-Trap plate for high-throughput analysis. The samples were analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) including label-free quantification (LFQ). The reproducibility of the procedure was assessed through the identification of peptides and proteins, as well as the analysis of missed cleavages and the number of tryptic termini (NTT). This study demonstrates the feasibility and reliability of the automated S-Trap digestion procedure for efficient and high-throughput proteomics analysis of clinical samples.

PP01.131: GeLC-FAIMS-MS: Multidimensional Sample Prefractionation for In-Depth Middle-Down Proteomics

Nobuaki Takemori, Japan

In top-down proteomics, sample fractionation prior to mass spectrometry is effective in detecting trace proteoform components. We previously developed GeLC-FAIMS-MS, a three-dimensional protein fractionation method combining SDS-polyacrylamide gel electrophoresis (SDS-PAGE), reversed-phase liquid chromatography (LC), and high-field asymmetric ion mobility spectrometry (FAIMS), and demonstrated its effectiveness in deep top-down proteomics (DOI:10.1021/acs.analchem.2c02777). Although the establishment of GeLC-FAIMS-MS has greatly improved the number of detectable intact proteoforms, obtaining more complete information requires new analytical strategies for proteoforms larger than 50 kDa, which are difficult to detect by current top-down analysis. In this study, we developed an innovative in-depth middle-down proteomics workflow combining Glu-C digestion and GeLC-FAIMS-MS to obtain proteoform information from high-molecular-weight regions. In the workflow, middle-down peptides generated by optimized limited Glu-C digestion conditions are first separated by SDS-PAGE. Size-based fractionated peptides are recovered in solution from the gel by PEPPI-MS, a highly efficient passive extraction method, and finally provided to the FAIMS-Orbitrap MS system after additional separation by C4 reversed-phase LC. Orthogonal fractionation in the development workflow effectively reduces the complexity of the digestion mixture, resulting in improved middle-down peptide identification by mass spectrometry and increased detectable peptide length. This promising approach has the potential to increase the possibility of obtaining proteoform information by middle-down analysis.

PP01.133: Ultrahigh-throughput Quantification of Acute Phase Plasma Proteins Using Acoustic Ejection Mass Spectrometry (AEMS) and Peptide Enrichment
Jennifer Van Eyk, United States

For epidemiological/population studies that require the study of large cohorts (tens to hundreds of thousands of samples), strategies for higher throughput analysis while maintaining quantitative accuracy are needed. We have shown previously that Acoustic Ejection Mass Spectrometry, or AEMS, using the Echo MS system can provide reproducible peptide quantitation at 1-2 seconds per sample on simplified peptide samples. Ten acute phase plasma protein biomarkers representing different pathological pathways were incorporated into an immuno-enrichment assay from SISCAPA Assay Technologies, where target peptides representing each protein are enriched from plasma in a 96-well format. Here, a pilot cohort consisting of healthy, unhealthy and COVID-infected samples was analyzed using the combined peptide enrichment/AEMS workflow to test this ultra-high-throughput approach.

Protein enrichment using the SISCAPA workflow was automated using the Biomek i7 workstation (Beckman Coulter). 272 plasma samples were prepared (3x 96-well plates) then samples were transferred to a 384-well plate for the Echo MS system, where each sample plate was analyzed 3 times for each peptide. AEMS methods for quantification of the 10 light/heavy peptides for the acute phase protein multiplex were optimized (Alb, A1AG, C3, CRP, Hx, IgM, LPSBP, MBL, MPO and SAA). Two MRM transitions were monitored per peptide, for both light and heavy.

To assess AEMS data reproducibility, the heavy peptide peak areas were evaluated, and the %CV was 6-8% (n=3). A subset of the enriched samples were analyzed on a 2.5-min microflow LC-MRM workflow on the SCIEX 6500 system, and the peak area ratio correlation (light/heavy) was very good (r2 >0.98). These results confirm that AEMS can provide reproducible quantitative results, similar results to traditional LC-MS, but with significantly higher throughput (272 samples in triplicate in 3.5 hours for 10 proteins).

PP01.135: Evaluation of Two Sample Preparation Kits for Analyzing Chick Choroid Proteome Using Microflow LC Zeno-SWATH Mass Spectrometry
FENGJUAN JESSICA YU, Hong Kong

Background
Although choroid has been proposed as a predictor of myopia progression, the technical difficulties involved in tissue collection and limited material have resulted in a lack of information on choroidal proteome analysis. This study aims to compare two commercially available kits to effectively study the choroidal proteome of chick, as well as provide a comprehensive library using Zeno-SWATH 7600 MS.
Methods
The choroid tissues of normal chickens (PN17) were divided into S-Trap™ (n = 4) and EasyPep™ groups (n = 4). Proteins were extracted using 50 μl 1x SDS buffer or EasyPep™ buffer (Thermo Fisher Scientific™) respectively. One microgram of digested peptides from each sample was analyzed using ZenoTOF™ 7600 (SCIEX). The data were individually searched by DIA-NN against the UniProt chicken protein database (51486 proteins). Gene Ontology (GO) analysis was performed using PANTHER gene classification system.
Results
Despite that S-Trap™ workflow offered a higher protein yield (318 ± 8 μg) than EasyPep™ workflow (232 ± 28 μg), EasyPep™ group resulted in a higher number of protein identification (3567 ± 137 proteins with 27547 ± 1384 peptides) than S-Trap™ group (2941 ± 67 proteins with 22751 ± 502 peptides) at 1% FDR. A library of 5095 choroidal proteins after combining all searches was created for GO analysis. According to the findings, binding (41.7%) was the primary category in molecular function. Cellular process represented the largest proportion (33.2%) in biological process. The majority (75.1%) of cellular component was attributed to cellular anatomical entity.
Conclusion
This study generated a comprehensive protein profile of chick choroid with two workable protocols. EasyPep™ protocol exhibited greater efficacy, resulting in higher protein and peptide IDs. Overall, this study provided feasible workflows for exploring the potential of choroid proteomics in the context of myopia or other ocular diseases using ZenoTOF™ MS.

PROGRAM

See you in Busan!
BEXCO, Busan, Korea
2023.hupo.org
#HUPO2023

Together Through Proteomics

PP01.137: Target Identification of Natural Products and Chinese Medicine Using Mass Spectrometry

Yuling Chen, China

Introduction and Objectives
Natural products are the sources of new drugs over the nearly four decades. Although many natural products have health-promoting effects on the human body, most of their targets remain unclear. This study is aimed to investigate the targets of two flavonoid compounds with well-known anti-aging effects and one kind of traditional Chinese medicine used to stop bleeding and promote wound healing.

Methods
Thermal proteome profiling (TPP) was used to identify the targets of flavonoid compounds including 4,4'-dimethoxychalcone (DMC) and quercetin, and Chinese medicine Yunnan Baiyao. Cellular Thermal Shift Assay, isothermal titration calorimetry, hydrogen–deuterium exchange mass spectrometry and molecular docking were used to confirm the binding of compound to target proteins and explore the active sites of targets. According to the properties of the targets of each compound, enzyme activity test, quantitative PCR, western blotting and many other methods were chosen to be used to illustrate the function and mechanism of compound in cell.

Results
17 proteins and 21 proteins were identified as targets of DMC and quercetin respectively. Our results showed DMC promoted ferroptosis by increasing the content of Fe through the inactivation of FECH. FECH is the enzyme catalyzing heme synthesis by inserting Fe^{2+} into the porphyrin, and DMC could insert into PPIX-binding pocket as well as bind to dimer interface of FECH protein, leading to the decreased hem synthesis. Another flavonoid compound quercetin can also bind to FECH, suggesting flavonoids might play anti-aging role by inducing ferroptosis and ferritinophagy in aging cells. ALDH family proteins were the other main targets of these two flavonoids. Our results also showed TULA-2 was the target of Yunnan Baiyao, which negatively regulates platelet signaling through dephosphorylating SYK.

Conclusions
We identified the targets of bioactive compounds by using simple system or complex mixture, providing new insight into their mechanism study.

PP01.139: Drug Target Identification of a Multi-Pass Transmembrane G Protein Coupled Receptor Antagonist Using Limited-Proteolysis Coupled Mass Spectrometry (LiP-MS)

Yuehan Feng, Switzerland

Introduction
In drug discovery, target identification (ID) after phenotypic screens is a resource-intensive endeavor aimed to understand compound's mechanism of action. Target ID for membrane proteins is particularly challenging due to hurdles such as poor protein solubility, instability and low expression levels. Addressing these hurdles, recent proteomics-based strategies allow to analyze proteins in their native environment, and do not require compound modification or genetic manipulation of target cell lines.

Methods
Limited proteolysis coupled to mass spectrometry (LiP-MS) is a peptide-centric strategy that exploits structural protein alterations and steric hindrances induced by drug to detect drug-protein interactions, estimate potency (EC50) and predict binding sites across the proteome. Our previous reports (Piazza & Beaton et al. 2020, Hendricks & Beaton et al. 2022) showed the applicability of LiP-MS on cytosolic proteins such as kinases and phosphatases.

Results
Here, to monitor proteins in plasma membrane, live cells were treated with the compound in a DR curve for a short period of time before lysis and subsequent LiP-MS analysis.We evaluated the performance of this workflow using a tool compound targeting specific G-protein coupled receptors. Our unbiased LiP scoring identified atypical chemokine receptor 3 (ACKR3), the primary known compound target, among the top 3 hits. Additional 82 proteins were perturbed in drug-treated samples based on the LiP scores. Mapping the altered peptides on the GPCR signaling network showed enrichment of perturbations in ACKR3 downstream pathways, providing additional evidence of ACKR3 binding.

Conclusion
Taken together, we demonstrate that the live cell LiP-MS is applicable for target ID of multi-pass membrane proteins. The approach also provides a system-level view of protein and pathway perturbations downstream of a signaling protein, revealing possible mechanisms of its endogenous action. Further optimization of LiP-MS protocol is sought to accommodate different classes of receptors.

PP01.141: Identification of Target Proteins of Natural Compound in NAFLD Using DARTS-LC-MS/MS Proteomic Analysis and Mode of Action Study

Yunyeong Jang, Republic of Korea

Autophagy has been highlighted as a critical regulator of cellular homeostasis, the dysregulation of which is associated with several diseases. In particular, the autophagy of cytoplasmic lipid droplets is known as lipophagy. A link between non-alcoholic fatty liver disease(NAFLD) and lipophagy related mechanisms remains to be elucidated. To explore the role of autophagy in lipid regulation, we identified a natural compound (ACA) as a novel anti-NAFLD agent. Notably, ACA suppressed lipid accumulation and attenuated the expression of adipogenesis related factors without exhibiting cell toxicity. Furthermore, ACA activates lipid reduction via autophagy in vitro and exhibits anti-NAFLD effects in vivo. To investigate the mode of action of ACA, we applied a label free small molecule target identification method called DARTS with LC-MS/MS proteomic analysis. The target protein of ACA (TPA) was identified and validated using DARTS and CETSA methods. Collectively, these results demonstrated that ACA binds to TPA and exhibits anti-NAFLD activity through inducing of dissociation of the mTORC1 complex to alleviate steatosis, and that TPA acts as a core factor in the treatment of NAFLD disease through autophagy-mediated regulation.

See you in Busan!
BEXCO, Busan, Korea
2023.hupo.org
#HUPO2023

Together Through Proteomics

PROGRAM

PP01.143: Harnessing the Power of Limited Proteolysis and Ultra-Deep Mass Spectrometry Proteomics for Small Molecule Drug Target Identification and MOA Deconvolution

amaury lachaud, Switzerland

The identification of protein targets and elucidation of the mechanism of action (MoA) for small molecule drugs is critical for understanding their pharmacological activity and optimizing therapeutic potential. Limited proteolysis coupled to mass spectrometry (LiP-MS) has recently emerged as a versatile technique and has gained prominence for target deconvolution studies, by enabling the identification of proteins with altered conformation or stability upon small molecule binding. Here, we present a case-study of how LiP-MS target deconvolution methodology has enabled the identification of the targets of SAM-002, a small-molecule autophagy stimulator obtained from a phenotypic screen.

Further, we describe how ultra-deep phosphoproteomics has enabled the MoA studies of SAM-002, including the elucidation of drug-induced changes in cellular signalling networks that function to upregulate autophagy, as well as its specialized sub-process known as reticulophagy, or ERphagy.

Our work underscores the utility of integrating LiP-enabled target deconvolution with phosphoproteomic analyses to comprehensively investigate the protein targets and MoA of small molecule drugs. These powerful, complementary techniques can expedite the drug discovery process, inform drug development strategies, and even contribute to the development of biomarkers that help guide clinical trials.

PP01.145: Discovery of Pan-IAP Degraders via a CRBN Recruiting Mechanism

Gaseul Lee, Republic of Korea

Inhibitors of apoptosis proteins (IAPs), defined by the presence of baculovirus IAP repeat (BIR) protein domain, are critical regulators of cell survival and cell death processes. Cellular IAP 1/2 (cIAP1/2) and X-linked IAPs (XIAPs) regulate the innate immune signaling pathway through their E3 ubiquitin ligase activity. Peptidomimetics or small-molecule IAP antagonists have been developed to treat various diseases, such as cancer, infection, and inflammation. In this study, we synthesized and characterized IAP–cereblon (CRBN) heterodimerizing proteolysis-targeting chimera (PROTAC), which induces the degradation of cIAP1/2 and XIAP but not CRBN. We demonstrated that this PROTAC inhibits tumor necrosis factor alpha (TNFα)-induced innate immune response and cancer cell migration and invasion, leading to apoptotic cell death. Our study is the first to demonstrate that both cIAPs and XIAP are degradable when applied to the PROTAC strategy.

PP01.147: Revealing Protein-Protein Association Networks Through Proteome Thermal Stability Changes

Daniel Martinez Molina, Sweden

With the Cellular Thermal Shift Assay celebrating its first decade since the PoC publication, the method has gained much interest in the basic science field as well as become an industry standard in applied drug discovery setting. In addition to information about drug-protein interaction, thermal profiling of cells can help describe different aspects of cell biology as it gives insight into the activating and rewiring of protein-protein interaction networks inside the cell upon stimuli.
In the work presented here, we have used several hundred well described molecular probes and marketed drugs to measure cellular responses by Proteome Integral Solubility Alteration protocol of CETSA (cCETSA/PISA) to study drug-protein and protein-protein association networks.

Monitoring changes in thermal stability among more than 8,000 proteins reveal specific compound "fingerprints". Despite the short incubation time, proteins with compound-induced thermal stability shifts are not only targets/off-targets (direct binders), but also downstream, and sometimes upstream, pathway members and general cellular responses.
All data combined makes the basis of the Target Engagement Atlas. Here we can see clusters of compounds, sharing similar protein binding profiles. Among the most prominent ones are mTOR inhibitors, NSAIDs, as well as tubulin binders. Alternatively, one can use this resource to reveal clusters of "pharmacologically" associated protein networks. We show that pharmacological perturbation allows identifying clusters representing tight protein complexes (ribosome), know metabolic pathways (folate biosynthesis), molecular functions (kinases), as well as networks of proteins linked only by ligand binding specificity. With that, the Target Engagement Atlas provides a near orthogonal source of information in addition to transcriptomics and proteomics atlases presented before.

PP01.149: Integrated Glycomics and Proteomics in Cell Therapy to Uncover Cell-Specific Glycan Signatures and Protein Networks

Myung Jin Oh, Republic of Korea

Cell therapy is gaining attention as a promising biopharmaceutical approach for treating incurable diseases like cancer and autoimmune diseases. Understanding the biomolecules present on the cell surface is crucial in cell therapy, as the therapeutic effects are intricately tied to the physiological activities of living cells, influenced by external factors such as cell origin and culture conditions. Protein glycosylation is known as a regulator of cell function and development but has not been fully investigated. In this study, an integrated approach combining glycomics and proteomics was employed to explore the role of glycosylation and evaluate the quality of cell therapy products. To investigate specific glycome signatures for each cell line used in cell therapy, glycan phenotypes of various cell-based therapeutics, including fibroblasts, NK cells, induced pluripotent stem cells (iPSCs), and iPSC-derived cardiomyocytes (CM) was comprehensively characterized. Cursory examination of the glycan class profiles revealed a significantly higher abundance of pauci mannose-type glycans in fibroblasts compared to other cell types. NK cells exhibited distinct features such as bisecting glycans and highly antennary-neutral glycans. Remarkably, as iPSCs were differentiated into CM, the glycan was changed dramatically, revealing a completely different glycan phenotype. iPSC displayed relatively elevated expression of highly fucosylated and high mannose-type glycans, whereas CM showed an abundance of bisecting glycans and highly antennary-acidic glycans. Additionally, proteomics analysis was conducted to comprehensively identify and quantify the relevant proteome in various types of cell sources. Cell-specific protein landscapes were obtained from different cell types, encompassing pathways, and compartments such as cell signaling, differentiation, immune responses, DNA replication, cytoskeleton, cell adhesion, and ribosomes. This study underscores the importance of deeper characterization of membrane proteins and glycoforms in expanding our understanding of cellular mechanisms of action. It also highlights the potential to define specific molecular markers for product quality attributes of cell therapies.

PP01.151: Identification of Proteomic Landscape of Drug-binding Proteins in Living Cells by Proximity-dependent Target Identification

Cheolhun Park, Republic of Korea

Introduction: Understanding the interactions between drugs and their target proteins is crucial for unraveling drug mechanisms and optimizing therapeutic interventions. Proximity-dependent target identification (PROCID) has emerged as a powerful technique for systematically mapping the proteomic landscape of drug-binding proteins in living cells. This study aims to demonstrate the efficacy of PROCID in identifying drug-target interactions and expanding our knowledge of drug-protein interactomes.
Methods: The PROCID methodology utilized fusion proteins, including HaloTag-TurboID, and covalently bound drug derivatives to the HaloTag protein. By employing proximity labeling techniques, drug-HaloTag-TurboID complexes were formed and brought into proximity with their target proteins within the cellular context. The labeled protein complexes were then isolated and subjected to mass spectrometry analysis.
Results: Through PROCID, we successfully identified the prominent target proteins of two drug molecules, dasatinib and SAHA. The mass spectrometry analysis revealed a comprehensive interactome network, shedding light on the functional associations and potential downstream effects of these drugs. Notably, SMARCA2 was identified as a novel target protein for dasatinib, highlighting the power of PROCID in uncovering previously unknown drug-protein interactions.
Conclusions: PROCID represents a robust and reliable approach for identifying drug-binding proteins and elucidating their interactomes in living cells. The methodology provides a systematic and comprehensive view of drug-target interactions, enabling the discovery of novel target proteins and facilitating a deeper understanding of drug mechanisms. By expanding the application of PROCID to other small molecules and exploring its integration with E3 ligase ligand moieties in PROTACs, we aim to further expand the scope and utility of this technique in drug discovery and chemical biology.

PP01.153: Anti-cancer Compound Target Identification via Chemical Proteomics

Sein Park, Republic of Korea

Many studies have been attempted to develop new agents that target EGFR mutants or regulate downstream players in various cancers. A new small molecule, YCGE, was discovered through cell-based screening to inhibit EGFR-mutation cancer cells. Previous studies have shown that YCGE effectively inhibits anchorage-independent 3D growth of sphere-forming cells transfected with EGFR mutant cDNA. However, the underlying mechanism remains to be elucidated. The determination and validation of the target protein of a bioactive compound will help to answer this question and provide new insights into the understanding of the EGFR mutation mediated signaling pathway in cancer cells. In this study, we investigated the target protein of YCGE by combining DARTS with LC-MS/MS using label-free YCGE as a bait and HepG2 cell lysates as proteome pool. As a result, YCGEP was identified as one of the binding proteins of YCGE that is responsible for the biological activity of the compound. The interaction between YCGEP and YCGE was validated by DARTS and CETSA methods. In addition, genetic knockdown of the identified target YCGEP was validated in respect to its association with cell proliferation. Taken together, YCGEP is identified as a biologically relevant target of YCGE to address an anti-cancer activity of the compound and these results provide insights into a role of YCGE as a downstream player of EGFR mutant.

PP01.155: Comparative Proteomic Analysis of Drug Shikonin Addition to Liver Cancer

Zening Wang, China

Traditional Chinese Medicine   TCMs) represent one of the most ancient therapeutic systems and have been widely accepted as a complementary medicine approach in both Asian and Western countries. TCMs play an important role in anti-cancer treatments due to their low toxicity, high efficacy, and safety, and can even reverse MDR (Multi Drug Resistance).

Shikonin, a naphthoquinone compound extracted and purified from the roots of Lithospermum Erythrorhizon (also known as "Zicao" in TCM), has recently been found to be effective against a variety of types cancer. Several in vitro and in vivo studies have shown the strong anti-tumor effect of Shikonin via different molecular pathways. Despite of Shikonin's excellent anti-cancer activity, there are limited researches that have systematically investigated Shikonin-mediated signal pathways in different HCC cell lines.

Herein, we focused on Shikonin and used proteomics technology to reveal its molecular mechanism. To investigate the anti-cancer effect of Shikonin on cell growth and global proteomics alterations in the HCC cell line, different concentrations of Shikonin were tried till the optimal one was chosen to run the proteomic analysis. To reveal the mechanism of inhibitory effect of Shikonin on the three cell lines   we performed a comprehensive proteome analysis of three HCC cell lines under Shikonin treatment using DIA-MS based label free quantification. The differential expressed proteins were identified by mass spectroscopy. Our work established a connection between the Shikonin and HCC cell lines, which provided assistance in clinical application of Shikonin and the potential disease marker can be found to guide the treatment of liver cancer.

PP01.157: Formalin-Fixed Paraffin-Embedded Thyroid Nodule Proteomics and Machine Learning Analysis to Distinguish Carcinoma and Benign

Hee-Sung Ahn, Republic of Korea

Preoperative diagnosis of follicular thyroid carcinoma (FTC) is challenging as it cannot be distinguished from follicular adenoma (FA) or benign nodules (BN) by sonographic or cytologic features. Here, we analyzed FFPE tissue proteomes of FTC (N=68), FA (N=72) and BN (N=62) by LC-MS/MS. The total number of proteins is 8,341. Based on the protein abundance, the three groups were moderately separated by PLS-DA and functionally showed differences in glycolysis, inflammatory response, PI3K/AKT/mTOR, and Myc signaling. By unsupervised learning, FTC was divided into two groups, one of the which had strong Myc and mTORC1 signals with many RAS mutations and poor prognosis. In addition, a machine learning predictive model was built with a panel of 9 proteins that differentiated FTC from the benign subtypes and showed an AUROC of 0.828 (95% CI: 0.819-0.837). Whereas thyroglobulin is the most abundant protein in thyroid and its iodination is important in making thyroid hormones. From clinical samples, 27 iodination were found in 66 tyrosines and 16 iodination in 39 histidines of thyroglobulin. Moreover, 47 iodinated proteins were found, and these proteins were typically involved in thyroid hormone metabolism and showed antioxidant activity, which seems to be due to hypoxic conditions in thyroid collide. Although further clinical validation of our findings is necessary, it is expected that needle biopsies of tissue samples enable the discrimination of FTC through the protein biomarkers.

PP01.159: Novel EGFR-Specific Peptides Identified Using Ribosome Display

Kiattawee Choowongkomon, Thailand

Particulate matter 2.5 (PM2.5) is a major environmental concern known to increase the risk of lung cancer. PM2.5 particles directly penetrate the cell membrane and activate various pathways, including the Epidermal Growth Factor Receptor (EGFR) pathway. EGFR is a transmembrane protein family that plays a critical role in cell survival, proliferation, and invasion. Overexpression of EGFR has been observed in several cancers, such as lung cancer, breast cancer, and gastric cancer. Many research studies have focused on identifying EGFR-specific molecules for the diagnosis and treatment of EGFR-expressing cancers. In this study, we used a ribosome display peptide library to select a peptide (11-mer) that specifically targets the extracellular domain of EGFR (EGFR ECD). Our results revealed that a linear peptide, named EGFRp4, exhibited a tighter binding affinity within the nanomolar range, as determined by fluorescence polarization and ligand tracer assays. Surprisingly, EGFRp4 demonstrated positive cooperativity with EGFR ECD, as confirmed by fluorescence polarization, Surface plasmon resonance (SPR), and ligand tracer experiments. Furthermore, competitive tests indicated that EGFRp4 bound to the same site as epidermal growth factor (EGF). To determine the binding site of EGFRp4 on EGFR ECD, docking studies were performed using the GOLD program. The results indicated that EGFRp4 interacted with EGFR ECD through hydrogen bonding, aromatic -sulfur interactions, hydrophobic interactions, and non-covalent interactions.

![HUPO BUSAN 2023 SEP 17-21, 22nd Human Proteome Organization World Congress](logo)

See you in Busan!
BEXCO, Busan, Korea
🌐 2023.hupo.org
🐦 f 📷 in #HUPO2023

Together Through Proteomics

PROGRAM

PP01:161: Craspase Specificity and Substrate Repertoire in Native and Model Proteomes

Konstantinos Kalogeropoulos, Denmark

In the past decade, CRISPR-Cas systems have revolutionized biological sciences. Type III-E loci were recently described as a novel CRISPR-Cas subtype, and the first instance of Cas effectors that induce downstream effects by proteolytic cleavage. This pivotal discovery indicates a system with RNA-guided endopeptidase activity, which was named Craspase. Importantly, Craspase links genotype with protein function in a direct manner, and paves the way for a potential proteome editing tool. Characterizing this novel system in regards to its natural degradome, substrate specificity, and off-target effects, will not only provide insights into the role of Craspase in adaptive immunity, but also prove to be crucial in harnessing and engineering the system for tailored applications.

With this study, we describe the Craspase degradome in the proteomes of Candidatus "Scalindua Brodae", E. Coli and HeLa cells, utilizing advanced terminomics techniques and mass spectrometry instrumentation. We demonstrate that Craspase cleaves distinct substrates associated with immunity response in E. Coli, which provide clues for its physiological mechanism of action and serve as a starting point for functional studies. Furthermore, we show that Craspase has more than a single substrate in its native organism, and that human proteins are also cut by Craspase. We also argue that structural arrangement is required for cleavage, allowing for speculation on its specificity and substrate recognition mechanisms. Finally, we validate the prototypical Craspase substrate and its cleavage site and demonstrate that the system is amenable to interrogation with degradomic workflows, suggesting a promising future for Craspase research and engineering.

PP01.163: Deep Phenotyping of Serum Proteome in Search for Early-stage Biomarker of Diabetes Neuropathy Using Micro-flow LC–MS/MS

Gurjeet Kaur, Denmark

Background: Distal symmetrical polyneuropathy (DSPN) an untreatable complication correlated with loss of sensation, chronic pain and lower life expectancy in type 1 diabetes (T1D). Mass spectrometry (MS)-based proteomics is a powerful technology which is now reaching the clinical application of early prediction and prognosis of diseases. Here, we profile in depth blood proteome in search for new DSPN treatment targets utilizing the micro-flow LC–MS/MS.

Method: We employed a single pot sample preparation in combination with data-independent acquisition (DIA) approach spanning from 20 to 120 minutes gradients using 150 serum samples with neuropathy and controls. We have investigated the replication of proteome originating from cerebrospinal fluid (CSF) and blood using micro-flow LC–MS/MS. Moreover, fractionation and depletion methods for the identification of in-depth serum proteome was applied.

Results: We conducted an evaluation of the undepleted single-pot serum, plasma, and CSF sample preparation protocol in a clinical setting within a minimal time frame. To enhance the robustness and efficiency of the system, a micro-flow setup was implemented, enabling an active gradient of 12 minutes, allowed us to process a substantial number of samples, with a sample per day (SPD) rate of 120. We identified approximately 500 proteins in undepleted serum samples 60 mins gradient. We applied our optimized 40-minute active gradient and achieved the identification of 900 proteins in CSF. In our fractionation and depletion protocol, we can identify 700-800 serum proteins which holds great promise for DSPN biomarker discovery offers a valuable contribution to clinical sample analysis.

Conclusion: The outcome of this study provides the robust proteomics workflow in the utilization of micro-flow LC–MS/MS holds significant potential to revolutionize the proteomics in clinical settings. We present a comprehensive assessment of the advantages of utilizing the micro-flow LC–MS/MS for identification of early biomarkers of DSPN in blood.

PROGRAM

PP01.165: nanoCSC Reveals Novel Insight into the Surfaceome of Primary Human Cardiomyocytes in Heart Failure.

Mane Polite Roneldine Mesidor, United States

Advanced heart failure is associated with structural remodeling of cardiomyocytes and cardiac fibroblasts, leading to impaired cardiac contractility and fibrosis. Cell surface glycoproteins play crucial roles in maintenance of cardiac cell structure and function, but we do not yet fully understand how cell surface glycoproteins in human heart cells change in response to or contribute to advanced heart failure. Due to limited availability of human heart tissue, cell surface proteomic analysis of human heart cells is challenging. Here, we developed nanoscale Cell Surface Capture (nanoCSC) for surfaceome mapping of sample limited cell types. nanoCSC is a miniaturized, automated workflow that combines centrifugation-free preparation and biotinylation of cell surface glycans, automated protein digestion optimized for membrane proteins, and our novel LEVITATE system for automated magnetic bead-based surface peptide enrichment and cleanup. nanoCSC was applied to human B cells for performance testing and subsequently to primary cardiomyocytes isolated from non-failing and failing human hearts. Peptides were analyzed with an Exploris 480 MS, and data were analyzed using Proteome Discoverer, Spectronaut, R, and annotated with Veneer. From just 1000-4000 ng total peptide (0.5-5 million cells), nanoCSC quantified 400-600 cell surface N-glycoproteins / experiment with CV <20%. Applying nanoCSC to primary cardiomyocytes isolated from failing and non-failing human hearts revealed proteins associated with ischemia-reperfusion injury and cardiovascular diseases. Hierarchical clustering and principal component analysis groups samples by condition and demonstrates that nanoCSC is sensitive enough to distinguish protein differences among cells isolated from failing and non-failing hearts. We also identified previously unreported changes in the surfaceome of cardiomyocytes between failing and non-failing hearts and identified >20 proteins associated with cell proliferation and GPCR cell signaling. In summary, nanoCSC enables surface proteomic studies of small sample sizes and reveals cell-type specific targets for elucidating pathophysiological mechanisms and future drug development for heart failure.

PP01.167: Neoantigen Identification in Renal Cell Carcinoma Tissue by Mass Spectrometry

Poorichaya Somparn, Thailand

Renal cell carcinoma (RCC) is a heterogeneous malignancy characterized by genetic alterations, including somatic mutations that generate tumor-specific neoantigens. These neoantigens have the potential to elicit a robust immune response and serve as valuable targets for immunotherapy. Mass spectrometry (MS) has emerged as a powerful tool for identifying and characterizing neoantigens due to its high sensitivity, resolution, and ability to detect low abundance peptides. Here, we identify neoantigen-specific T cell responses in a tumor of an individual with renal cell carcinoma. We employed a comprehensive approach, which involved integrating whole exome sequencing (WES) to establish individualized databases and immunopeptidome to identify neoantigens in a cohort of 13 renal cell carcinoma (RCC) patients. We found that the number of HLA-I presented peptides ranged from 133 to 17,233 depending on the tissue amount. We also found a significant correlation between tissue amount and the number of identified peptides (R=0.87, P=0.002). Among these 17,233 unique immunopeptides, one neoantigen, JADE2-Thr391Ser was identified with a variant allele frequency (VAF) of 0.46 and 0.53 from the PBMC and tumor, respectively This neoantigen was confirmed by synthetic peptides using mass spectrometry. We also confirmed the immunogenicity of RCC5 PBMC with mutant and wild-type peptides and found that the spots of IFN-γ-producing T cells were significantly greater with the mutant peptide than the wild-type peptide. These results indicate that the identification of immunopeptides and potential neoantigens can be established directly from clinical tissues.

See you in Busan!
BEXCO, Busan, Korea
2023.hupo.org
#HUPO2023

Together Through Proteomics

PROGRAM

PP01.169: Unleashing Rapid Kinase Activity Analysis Using FRET-based Biosensor for Drug Screening Platform

Lueacha Tabtimmai, Thailand

Introduction: One of the challenges in developing kinase inhibitors lies in the limitations of screening platforms. These limitations have raised doubts about the precision and accuracy of current methods used to identify and develop effective kinase inhibitors. Overcoming these limitations and improving screening platforms is crucial for the development of targeted therapies that specifically modulate protein kinase activity and contribute to the treatment of various diseases and pathogenic conditions.
Methods: Phospho-binding domain linked to EGFR-specific peptide flanking with yPET/CFP FRET pair in both N-and C-terminus was genetically introduced into multi-copy number plasmid and produced in a bacterial expression system. The purified FRET-based biosensor was further characterized in terms of optimal reaction, enzyme selectivity, substrate specificity, and kinetic parameter using fluorescence spectroscopy. Inhibitor screening was done with natural compounds and known kinase inhibitors.
Results: The FRET-based biosensor exhibited rapid detection of kinase activity through conformational changes in the presence of ATP. A minimum of 200 ng of recombinant TK-EGFR enzyme was required to observe a detectable FRET signal using the biosensor. Interestingly, among the tested kinases, the biosensor showed selective phosphorylation by TK-EGFR while JAK-2 did not exhibit this activity. Further investigation revealed that the double mutant T790M/L858R TK-EGFR had a greater Km value compared to TK-EGFR. The kinetic parameters indicated that TK-EGFR preferably transferred the phosphate group from ATP to the biosensor, highlighting its preference for ATP as a phosphate donor. Moreover, using both kinetic and endpoint modes, the FRET-based biosensor was employed to screen the inhibitory effects of both natural compounds and known kinase inhibitors.
Conclusion: Overall, these experiments and screenings provided a comprehensive characterization of the FRET-based biosensor, shedding light on its optimal conditions, substrate preferences, and potential inhibitors, thereby enhancing our understanding of its utility as a tool for kinase activity analysis.

PP01.171: Accounting for Common Genetic Variation in Proteomics

Marc Vaudel, Norway

In genomic studies, common variation is essential to the study of population diversity and of complex traits. In proteomics however, all data are projected onto sequences derived from an arbitrary reference genome. Hence, a bias towards populations most similar to the reference is introduced, and the missing sequences might be incorrectly matched by search engines to other proteins.

Here, we show how to build sequence databases of common human protein haplotypes derived from publicly available genome sequences, and highlight challenges arising from the combination of multiple variants in linkage disequilibrium. We chart the prevalence of single and multi-variant peptides encoded by these haplotypes compared to the reference proteome. We evaluate the search space enlargement induced by these additional sequences, and its influence on the performance of proteomic searches. We demonstrate how these variant sequences might be mistaken for other peptides, possibly modified, and show how the inclusion of retention time and fragmentation predictors can alleviate these problems. Finally, we discuss challenges posed by the inclusion of population diversity in proteomic workflows.

Altogether, our results demonstrate that accounting for common genetic variation poses major challenges to current proteomic bioinformatic workflows. Alleviating these will require the development of novel data interpretation strategies, but more importantly, common sequence variation needs to be included in our collective representation of the human proteome. Capturing this fundamental information is key to accounting for human diversity, which will be essential to realize the promise of precision medicine.

PROGRAM

PP01.173: Improved Prediction of Response to Immune Checkpoint Blockade Therapy Across Multiple Cancer Types

Seonjeong Woo, Republic of Korea

Immune checkpoint blockade (ICB) such as programmed cell death protein 1 (PD-1) inhibitor provides remarkable clinical benefits for patients of many cancer types, but predictors of response to ICB are incompletely characterized. In this study, we collected and analyzed public RNA sequencing data from seven different studies involving 198 patients with gastric cancer, melanoma, non-small cell lung cancer (NSCLC), and head and neck squamous cell carcinoma (HNSCC) prior to receiving anti PD-1. Based on reference studies on immune-related factors, including CD8+ T effector and interferon-γ gene signature, we identified key genes that have the potential to serve as predictive factors for ICB therapy. Lastly, we constructed random forest models based on these key genes in collected RNA sequencing data, and furthermore performed model validation in targeted transcriptome data from 40 patients with cervical cancer, uterine cancer, and ovarian cancer. These key genes are expected to be clinically valuable in predicting ICB response, serving as useful tools for guiding therapeutic decisions in patients with cancer.

PP01.175: Investigating the Molecular Neighbourhood of a Key Target in Alzheimer's Disease

Weronika Buczek, United Kingdom

In this study, we investigate the molecular neighbourhood of a critical target in Alzheimer's disease (AD). AD is the most common form of dementia, imposing a significant burden on economies and societies globally. Since the number of people with AD is growing rapidly, new therapeutics are urgently needed. One of the early events observed in AD is a decrease of acetylcholine (ACh) at neuronal synapses in the brain. As a result, ACh binding to α7 nicotinic ACh receptors (nAChR) is disrupted, which perturbs signalling between neurons and leads to memory loss observed in AD. In addition, multiple accessory proteins tightly regulate α7 nAChR assembly and function, yet our knowledge about synaptic scaffolding and stability of α7 nAChR at cholinergic synapses is scarce.

In this study, we used a proximity labelling approach to characterise molecular partners of α7 nAChR. We generated a stable human neuroblastoma cell line expressing the receptor fused with a TurboID enzyme that biotinylates neighbouring proteins. These potential interactors were purified and analysed by liquid chromatography-mass spectrometry (LC-MS/MS).

Out of 449 proteins significantly enriched compared to control samples, seven were known α7 nAChR interactors. Gene ontology analysis revealed 42 proteins associated with autophagy, which indicates a role for α7 nAChRs in this process. In addition, the most abundant proteins seem to be involved in α7 nAChR biogenesis and transport to the cell membrane.

We will next use this and other cellular models to test how toxic peptides present in AD perturb the α7 nAChR interactome. This novel cellular model is a useful tool for studying α7 nAChR function. It can allow us to investigate receptor stability and scaffolding at nicotinic synapses and potentially discover critical determinants for nicotinic cholinergic transmission affected in Alzheimer's disease.

![HUPO BUSAN 2023 SEP 17-21, 22nd Human Proteome Organization World Congress]

See you in Busan!
BEXCO, Busan, Korea
🌐 2023.hupo.org
🐦 f 📷 in #HUPO2023

Together Through Proteomics

PROGRAM

PP01.177: Investigation of Proteomics Changes in Schizophrenic hiPSC-derived Brain Organoids Using Mass Spectrometry and Imaging

Lucrezia Criscuolo, Denmark

Schizophrenia (SCZ) is a complex and severe neurodevelopmental disorder with unknown etiology. Patients are characterized by alterations in multiple cerebral functions, leading to a combination of positive, negative, and cognitive symptoms. Post-mortem studies have shown evident brain changes, including reduced brain volume, decreased spine densities and abnormal neuron distribution, in addition to synaptic dysregulations and neurotransmission deficit.

SCZ is a challenging disease, and it is not easily modelled in vitro, due to the inaccessibility of proper models mimicking the high heterogeneity observed in brain tissue. However, new opportunities have emerged with three-dimensional brain organoids (BOs) derived from human induced pluripotent stem cells (hiPSCs). BOs resemble the complexity of the early developing brain, recapitulating the 3D self-organization and self-maturation.

In the present project, we have investigated the SCZ pathomechanisms using dorsal forebrain organoids (DFBOs) derived from hiPSCs from schizophrenic and matched healthy individuals (GM23760, GM23762, UKIBIO04-A, WTSLI008-A). The DFBOs were grown for 100 days, and the development was traced over 4 time points (day 30, 60, 80, and 100) with particular focus on synapse specific proteins. Liquid chromatography tandem mass spectrometry (LC-MS/MS)-based proteomics and immunohistochemistry-based imaging were used to characterize the overall alterations of SCZ DFBOs over the 4 time points. In parallel, at the latest timepoint, several post-translational modifications were studied and crude synaptosomes were enriched using a differential centrifugation protocol and the protein content from the enrichment was analyzed by LC-MS/MS. Finally, to describe the modulation of protein expression, a combination of bioinformatics tools was used to establish the significantly regulated gene ontology terms and the involved pathways.

Although the study is still ongoing, our project offers valuable insights for a better understanding of SCZ molecular mechanisms which can be used in the future for developing new treatment strategies for the disease.

PP01.179: Thermal Proteome Profiling of Astrocytes with Variants in Alzheimer's Disease Risk Gene APOE

Jungsu Kim, United States

Apolipoprotein E (APOE) is the strongest genetic risk factor for sporadic Alzheimer's diseases (AD). Variants in APOE gene is associated with a differential risk of developing AD; APOE4 is associated with the highest risk of developing AD whereas APOE2 is associated with a lower risk. The molecular mechanism by which variants in APOE affect the proteome is relatively unknown. To this end, we use thermal proteome profiling to gain molecular insight into the structural and functional effects of APOE variants on the proteome. Proteins exist in many states during physiological processes that correspond with changes in post-translational modifications, protein interactions, unfolding, or protein degradation. Thermal proteome profiling (TPP) detects changes in the temperature at which the protein would melt relative to other protein states. Because astrocytes produce the majority of APOE protein in the brain, we conducted TPP on primary astrocyte cultures derived from the forebrains of mice. Lysates of cells of each APOE genotype were aliquoted for eight temperatures and subjected to heat treatment. After centrifugation, the proteins in the supernatant were subjected to protein precipitation, processed, and analyzed using TMT-based LC-MS/MS. Protein melt curves and melt temperatures were determined using R-based statistical analysis. To determine the differences in the stability of protein complexes, Thermal proximity coaggregation analysis was performed on the normalized thermal melt curves with validated protein-complex and protein-protein databases. This work will give insight into the functional proteome-level changes in astrocytes due to variants in APOE and highlight the potential of thermal profiling proteomics as a powerful tool for investigating protein interactions and protein stability in AD and other neurodegenerative disease research.

PROGRAM

PP01.181: Weighted Protein Co-expression Network Analysis of Plasma Proteomes in Affective Disorders Using Multiple Reaction Monitoring-Mass Spectrometry

Yeongshin Kim, Republic of Korea

Introduction: The conventional differentiation of affective disorders into major depressive disorder(MDD) and bipolar disorder(BD) is based on the history of (hypo)manic symptoms. As treatment regimens and outcomes differ between these disorders, there has been considerable effort to differentiate these disorders, including the use of biological correlates. Top-down biological approaches have expanded our knowledge to facilitate differentiation of these disorders. However, there are limitations regarding inconsistency and modest accuracy. Understanding affective disorders based on biological correlates with a transdiagnostic bottom-up approach may explain these limitations and deepen our knowledge of the pathophysiology of these disorders. In this study, Weighted protein co-expression network analysis(WPCNA) was performed to identify biologically meaningful modules of interconnected proteins in plasma samples from individuals with affective disorders(including both MDD and BD). Furthermore, meaningful traits and hub proteins associated with these modules were determined.
Methods: The plasma proteomes of 299 patients with MDD or BD(aged 19-65 years old) were quantified using Multiple Reaction Monitoring-Mass Spectrometry(MRM-MS) in targeted proteomics approach. WPCNA was performed based on 420 protein expression levels. Significant clinical traits with protein modules were determined using correlation analysis. In addition, top hub proteins were determined using intermodular connectivity, and significant functional pathways were identified.
Results: WPCNA revealed six protein modules. The eigenprotein of a protein module with 68 proteins including complement components as hub proteins was associated with the total Childhood-Trauma-Questionnaire-score(r=-0.15, p=0.009). Another eigenprotein of a protein module of 100 proteins including apolipoproteins as hub proteins was associated with the overeating item of the Symptom-Checklist-90-Revised(r=0.16, p=0.006). Functional analysis revealed immune responses and lipid metabolism as significant pathways for each module. No significant protein module was associated with the differentiation between MDD and BD.
Conclusions: Childhood trauma and overeating symptoms were significantly associated with plasma protein networks, and should be considered as important endophenotypes in affective disorders.

PP01.183: Multi-proteomic Analysis of 5xFAD Mice Reveals New Molecular Signatures for Early-stage Alzheimer's Disease

Seulah Lee, Republic of Korea

Early diagnosis of Alzheimer's disease (AD) is important because treatments are effective only at an early stage. However, the current diagnosis has limitations in that clinical examinations and amyloid plaque imaging are detectable in the mid-term or later of AD. The situation originated from insufficient biological understanding, which cannot provide notable molecular mechanisms and biomarkers to explain AD progression. To find new molecular signatures that can explain the molecular pathology of AD, the AD lesions such as hippocampus and cortex and blood plasma extracellular vesicles (EVs) from 3- and 6-month-old 5xFAD mice were analyzed by a reliable quantitative proteomics approach. The 3- and 6-month-old hippocampus and cortex proteome in both age groups showed similar features in functional annotation and canonical pathway analysis, but the significantly changed proteins rarely overlapped. Furthermore, the plasma EVs proteome showed significantly different informatic features compared with other proteomes. Depending on the AD stage, new molecular signature candidates of AD progression were found. According to subsequent multi-dimensional experiments, including a machine-learning approach, we finally found new molecular signatures and diagnostic biomarkers for the early AD stage. In conclusion, the present study provides insights into AD pathogenesis and suggests novel early-stage AD biomarkers.

PP01.185: Regulation of Neuroinflammation in Alzheimer's Disease via the Immune Checkpoint Molecule Discovered by Proteomic Data Science Approach

Hagyeong Lee, Republic of Korea

Alzheimer's disease (AD) is one of the neurodegenerative diseases. The main cause of AD revealed to date is an accumulation of abnormally folded β-amyloid. According to previous studies, there was less improvement in cognitive ability when it targets β-amyloid inhibition. Indeed, inflammatory molecules such as IL-1β, IL-6, TNF-α were shown to be elevated in the post-mortem brain of AD patients. Instead of focusing on β-amyloid, many studies have argued that chronic neuroinflammation, which is one of the pathophysiology of AD, might play a role as the novel and critical target nowadays. On this wise, chronic neuroinflammation is believed as an essential factor in the progression and pathogenesis of AD and is on the rise as a new target of AD therapy. Immune checkpoint molecules could be a new regulator of neuroinflammation. Treatment using immune checkpoint molecules was already noticeable method in cancer therapy. However, there has been less research on immune checkpoint molecules in AD.

In this study, we aimed to discover the role of immune checkpoint molecules as potential therapeutics in AD. To discover molecular signatures related to the neuroinflammation of AD, we used 5xFAD mice model. Using the reliable quantitative proteomics and subsequent data science study, we found changes in signal modules related to inflammation pathway. Furthermore, we found that particular immune checkpoint molecules show abnormal expression in the 5xFAD cortex and hippocampus through biochemical experiments, including Western blotting and IHC. Then we checked the regulatory effect of the discovered immune checkpoint molecules in the AD model. The treatment using immune checkpoint molecules may serve a novel view of therapeutic methods in AD.

PP01.187: Galectin 3 Binding Protein as Potential Biomarker for Early Detection of Glioma

Rashmi Rana, India

Background: Gliomas, or malignant brain tumors that grow from glial cells, are the most prevalent form. They account for roughly 40% of all primary brain tumors and 70% of all primary malignant brain tumors. The only procedures available for diagnosing different stages of glioma are surgical incision and biopsy; however, our work focuses on finding a minimally invasive way for early glioma diagnosis. Identification of proteins released by cancer cells is of particular interest in this area, as it could lead to a better understanding of tumor growth.
Methods: Extracellular vesicles (EVs) were extracted from pooled plasma of healthy individuals (n=03) and glioma grades patients (Grade I, II, or III). The size and concentration of Plasma derived-EVs marker were determined using NTA, western blot, and FACS. In furthermore, an iTRAQ-based LC-MS/MS analysis of EVs protein was performed. The candidate protein galectin-3 binding protein was validated using ELISA and other methods (LGALS3BP)
Results: Total 123 proteins were identified from plasma derived pooled EVs and 34, 12 and 14 proteins were found to be differentially abundant by more than 1.3 in the different grades of glioma grade I, grade II, grade III, respectively, in comparison with the control samples. A total of seven proteins-namely, CRP, SAA2, SERPINA3, SAA1, C4A, LV211, and LGALS3BP-showed differential abundance in all the three grades. LGALS3BP is the only protein which found to be strikingly high in all three grades in a progressive manner.
Conclusion: LGALS3BP was shown to be elevated across all grades, and ELISA analysis of individual blood plasma and plasma-derived extracellular vesicles validated the enhanced expression of LGALS3BP in glioma patients. That shows the possible biomarker for early diagnosis of glioma and improved patient survival. This study would further provides the information of progression and monitoring the tumor grades (grade 1, grade II, grade III).

PP01.189: Gender- and Brain Region-Specific Alterations in Protein N-glycosylation in an Animal Model of Depression Induced by Chronic Adolescent Stress

InWoong Song, Republic of Korea

Adolescence, the transitional phase between puberty and adulthood, plays a crucial role in acquiring the necessary knowledge and skills for adult life. Major depressive disorder (MDD) is highly prevalent during adolescence, making it one of the most common psychiatric disorders, with a prevalence of 4-5% in mid-late adolescence. Moreover, depression during this critical developmental stage acts as a significant risk factor for suicide and substance abuse. Additionally, it imposes a substantial burden on society due to its propensity for relapse and persistence into adulthood. However, the underlying causes of depression in adolescence and effective treatment approaches have not been adequately elucidated. In recent years, there has been a surge in multiomics studies dedicated to unraveling the biological and molecular alterations associated with major depressive disorder (MDD). Specifically, investigations into blood N-glycan alterations in MDD patients have revealed correlations with disease severity and the expression of inflammatory markers, shedding light on the significance of N-glycan modifications in MDD pathophysiology. As a result, glycomics has gained attention as a potential avenue for MDD treatment. The objective of our study was to explore the pathological role of brain N-glycans in depression linked to adolescent stress. We conducted N-glycomic analysis on male and female mice exposed to chronic stress during adolescence, focusing on nine distinct brain regions. The depression models were validated using the novelty suppression feeding test. Our findings unveil gender- and brain-region-specific alterations in N-glycans, underscoring their involvement in adult depression stemming from stress experienced during adolescence. This study provides novel insights into the influence of adolescent stress on N-glycan modifications in different brain regions and sexes. Furthermore, these abnormal N-glycosylation patterns present potential therapeutic targets for addressing adult depression arising from stress encountered during adolescence.

PP01.191: Proteomic Analysis of Tau-BiFC Mouse Model Exposed to Particulate Matter

Ji Hyun Back, Republic of Korea

While it is known that particulate matter (PM) can increase the risk for neurodegenerative disorders such as Alzheimer's disease (AD), there have been few studies to investigate proteomic changes of mouse model subsceptible to AD under the exposure of PM. Here, we directly exposed PM particles with size ranges of 200-700 nm to a Tau-BiFC mouse model, which is able to visualize neuronal degeneration in the brain. Abnormal tau aggregation and accumuluation is a pathological mark of multiple neurodegenerative diseases collectively called tauopathies inlcuing AD. In the brain of tau-BiFC mice, tau protein is conjugated with each of the N- and C-terminal fragments of yellow fluorescent protein. Under pathological stimulation, tau proteins become aggregated and the N- and C-terminal fragments become closely located resulting in turning-on yellow fluorescence. At 6 month-old, Tau-BiFC transgenic mice were exposed to PM (175 mg/m^3) for 18 days. Tau-BiFC analysis indicated that Tau-BiFC intensity increased 2-fold in the cortex and 1.5 fold in the hippocampus compared with non-PM-exposed control group. This result indicates that PM exposure could induce tau aggregation, which will lead to neuronal degeneration liked to multiple neuro-degnerative disorders. To sucutinize PM-induced changes in the proteomic levels, comprehensive proteomic analyses of samples including plasma and tissues derived from the Tau-BiFC transgenic mouse model with and without exposure of PM are being pursued by using liquid chromatography-tandem mass spectrometry (LC-MS/MS) in combination with tandem mass tag (TMT) labeling, which enabled simultaneous comparisons of relative protein abundances of multiple samples, in order to find proteins that are specifically changed in the Tau-BiFC transgenic mouse model with exposure of PM. Then, we will investigate how the proteomic changes are releated to the neuronal degeneration patterns obtained from the brain imaging under the effect of PM exposure.

PP01.193: Integrating MALDI-MSI with LCM-MS: Advancing Spatial Multi-omics Analysis in Brain Tissue

Byoung-Kyu Cho, United States

Background
Spatial omics has revolutionized biomolecular analysis by preserving molecular spatial context in tissues, cells, and diseases. This advancement has found applications in neuroscience, cancer biology, and drug discovery. To enhance current analytical techniques, we introduce MALDI-MSI-guided LCM-MS, combining mass spectrometry imaging and laser capture microdissection. Our approach enables comprehensive profiling and quantitative analysis of proteome, metabolome, and lipidome, providing new insights into complex biological systems and molecular distribution.

Methods
Fresh frozen mouse brain tissues (n=3) were used for method development. MALDI-MSI was performed using timsTOF fleX MALDI-2 and processed with SCiLS, METASPACE, and SwissLipids. LCM-proteomics employed timsTOF Pro2, processed with Mascot and Scaffold Quant. LCM-metabolomics and lipidomics used Orbitrap IDX Tribrid Mass Spectrometer, processed with Compound Discoverer. For the biological system, six mouse brain tissues were used, comprising old and young groups (n=3 each). These samples underwent MALDI-MSI and LCM-proteomics.

Results
MALDI-MSI identified over 300 putative metabolite and lipid identifications in mouse brain, with high abundance of [PC (38:2) +K]+ and [PC (32:0) +Na]+ in the hippocampus and cortex, respectively. LCM-omics analyses of MALDI-guided focused regions (1 million cells) identified over 300 small molecules and 2800 proteins. To validate our approach, we applied MALDI-MSI guided LCM-proteomics to a biological system involving different age groups of mice. By examining the distinct spatial distribution of molecules in the hippocampus and cortex using MALDI-MSI, we identified over 3900 proteins using LCM-proteomics. Among these, 778 and 161 proteins showed differential expression in the cortex and hippocampus, respectively, between the two age groups. Biological network analysis highlighted the involvement of differentially expressed proteins in mitochondrial dysfunction, glutamatergic synapse, and AMPK signaling pathways associated with aging.

Conclusions
Our integrated approach of MALDI-MSI and LCM-MS in spatial omics provides a comprehensive understanding of complex biological systems and holds potential for future neuroscience research.

PP01.195: Lipidomics Strategies via MALDI-MSI Based on HR-MS for Molecular Signature in Tissues from Pancreatic β-cells BAG3 KO Mouse Models

Federica Di Marco, Italy

Background: Given that an important source of information that is often lacking when using cell models or biofluids is the preservation of the spatial distribution of molecules, the Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry Imaging (MALDI-MSI) can guide a more comprehensive knowledge in terms of identification, abundance, and distribution of lipids, metabolites, and proteins, within tissues. The hyphenation of such ionization technique with the MS systems can unveil putative biomarkers to be accurately identified together with their spatial distribution dimension, thus supporting the unbiased discovery phase. Furthermore, the non-destructiveness of MALDI source enables the recycling of intact tissue slices for staining techniques to drive a comparison of structural features with the MS data. The development of MALDI-MSI methods is strongly recommended to drive research in cancer. In this context, we developed a strategy for spatial lipidomics on tissues being associated with the pancreatic β-cells knockout of BAG3 (BAG3βKO), that is involved into several tumors, but mostly pancreatic ductal adenocarcinoma (PDAC).
Methods: The MassTech™ Atmospheric-Pressure Matrix-Assisted Laser Desorption/Ionization (AP/MALDI) source with ultra-high spatial resolution (UHR) has been coupled with an Orbitrap Fusion™ Tribrid mass spectrometer for high resolution in situ analysis on pancreas and liver slices collected from BAG3βKO and wild-type (WT) mice. The analysis was conducted on positive and negative ionization mode.
Results: An evaluation of the optimal sample preparation of mouse pancreas and liver tissues for MALDI-MSI -based lipidome analysis was conducted, in terms of embedding polymer, size of the raster, matrix and impact of aqueous wash before matrix deposition. Then, we applied the optimized method to compare the lipid signature of pancreas and liver between BAG3βKO and WT mice. The analysis revealed some lipids suggesting pancreatic amyloidosis and hepatic steatosis in β-cells KO models. These preliminary data pave the way for a better comprehension of the physiopathological role of BAG3.

PP01.197: Spatial Proteome Mapping of Annotated Tumour Regions in Pancreatic Ductal Adenocarcinoma Tissues Reveals Eight Distinct Tumour Subpopulations

Lay Cheng Lim, Malaysia

Introduction: Spatial proteomic heterogeneity in pancreatic ductal adenocarcinoma (PDAC) is poorly understood. MALDI imaging mass spectrometry (MALDI-IMS) is a powerful tool for mapping spatial distribution of peptides/proteins across tissues. Here, we aim to identify and map tumour subpopulations in annotated tumour regions of PDAC tissues using intact proteins analysis of MALDI-IMS.
Methods: We used MALDI-IMS intact proteins analysis to map the spatial proteomics profiles in histopathologically-annotated frozen sections of three PDAC tumour and one paired non-tumour adjacent tissue. Tumour subpopulations were delineated via hierarchical clustering of MALDI-IMS spectra, and representative m/z peaks were determined using linear discriminant analysis (LDA) and colocalization analysis.
Results: We have delineated eight tumour subpopulations in the annotated PDAC tumour regions, each with distinct m/z spectrum. Six of such subpopulations were specific to their respective PDAC tumour samples, while two were common across different PDAC tumour samples. Colocalization analysis of each identified tumour subpopulations have unearthed their associated dominant m/z peaks. Moreover, LDA analysis revealed distinctive m/z peaks when compared between paired non-tumour adjacent tissue and PDAC tumour tissues, as well as in between the three tumour tissues.
Conclusions: Our results demonstrated that PDAC tumours exhibit high intra- and inter-tumour proteomic heterogeneity. Further proteogenomic characterization of these tumour subpopulations will be performed to elucidate their phenotypic properties. This spatial proteomics data is necessary to eventually uncover the underlying molecular mechanisms and to identify the potential therapeutic targets in PDAC.

PP01.199: Exploring the Potential of Transplanted Colon Organoids to Mimic Human Physiology in Mice Using Deep Visual Proteomics

Frederik Post, Denmark

Background: Organoids are of great potential to study human biology and explore new therapeutic applications. With their 3D structure and ability to recapitulate physiological differentiation patterns, organoids provide valuable insights beyond traditional immortalized cell lines. Moreover, colon organoids can be successfully transplanted into mice (Watanabe et al., 2022), which enables the examination of epithelial cells in their in vivo context.

Methods: Stem cell-derived colon organoids in culture, in vivo, and healthy human colon samples were analyzed using the Deep Visual Proteomics (DVP) workflow (Mund et al., 2022), incl. markers for stem cells, epithelial cells, goblet cells, immune cells, and fibroblasts. Proteomes of the cell populations were acquired using the timsTOF SCP in py_diAID acquisition mode coupled to an Evosep running Whisper 40 gradient on a 15 cm Ionopticks Aurora ELITE column.

Results: By combining high-resolution imaging, single-cell image analysis, laser micro dissection, and mass spectrometry, we got unprecedented insights into organoid biology. More than 4000 proteins were identified across cell populations. We captured proteome dynamics of organoids from growing in culture, to transplantation and establishment within the microenvironment of the murine colon. The comparison of cell populations in healthy human colon crypts and transplanted organoids enabled us to assess to which extent organoids in vivo can recapitulate human colon tissue.

Conclusion: To further explore the potential of in vivo organoid studies, we adapted the DVP workflow for the analysis of experiments using organoids. By utilizing the capabilities of spatial and single-cell analysis of DVP, researchers can enhance organoid research, such as by uncovering the mode of action of therapeutics in organoid drug screens.

References:
Mund et al., 2022. Deep Visual Proteomics defines single-cell identity and heterogeneity. Nat. Biotechnol. 1–10. https://doi.org/10.1038/s41587-022-01302-5
Watanabe et al., 2022. Transplantation of intestinal organoids into a mouse model of colitis. Nat. Protoc. 17, 649–671. https://doi.org/10.1038/s41596-021-00658-3

PP01.201: Mapping the Spatial Proteome: Exploring Tissue Heterogeneity Through TMT Labeling and Multiplexed Mass Spectrometry Analysis

Yuanwei Xu, United States

Spatial proteomics is an emerging field that seeks to understand the spatial organization and subcellular localization of proteins in their native environment. Proteins perform their functions in an intricately compartmentalized fashion and the alterations in their dynamics and locations can have notable effects on cellular homeostasis and function. Advances in mass spectrometry, machine learning, imaging technologies as well as single-cell omics have brought spatial proteomics into the near horizon for a deepened understanding of cellular functions and disease mechanisms. Here we present in situ proteome labeling by tandem mass tag (TMT) on fresh frozen prostate cancer tissue slides. The results revealed molecular differences among different Gleason score sections at a spatial resolution.

PROGRAM

PP05.79: Estrogen Signaling as a Putative Target for Never-Smoker Lung Adenocarcinoma Patients Without EGFR Mutation and ALK Fusion from Proteogenomic Characterization
Seung-jin Park, Republic of Korea

Never-smoker lung adenocarcinoma (NSLA) is prevalent in Asian populations and even more in women. Since epidermal growth factor receptor (EGFR) mutations and anaplastic lymphoma kinase (ALK) fusions are major alterations found in NSLA, studies have focused on NSLA with EGFR and ALK alteration (EA), but not for NSLA without EGFR and ALK alteration (NENA). To reveal the proteogenomic landscape of NENA, we selected 101 NSLA tissues without EGFR and ALK by targeted sequencing of 1597 FFPE samples, and performed multiomics analyses including whole genome, transcriptome, methylation EPIC array, total proteome, and phosphoproteome. Genome analysis revealed that TP53 (25%), KRAS (22%), ROS1 fusion (13%), SETD2 (11%), and ERRB2 (9%) were the most frequently mutated genes in NENA. Proteogenomic impact analysis found that STK11 and ERBB2 somatic mutations had more profound effects on cancer-associated genes in NENA. From DNA copy number alteration analysis, we identified 22 prognostic proteins whose expression was controlled through transcriptome from copy number alterations Intriguingly, from gene set enrichment analysis, estrogen signaling emerged as the key pathway activated in NENA compared with EA. Evidence from multiomics analysis including copy number gains in chromosomes 14 and 21, STK11 mutation, and DNA hypomethylation of LLGL2 and ST14, also supported the increased estrogen signaling. Finally, the saracatinib, an Src inhibitor, was suggested as a potential drug for targeting activated estrogen signaling in NENA. Taken together, the proteogenomic landscape for NENA from this study will enhance our understanding of the etiology of NSLA.

Session Date/Time: Monday, September 18, 2023 - 01:15 PM - 02:15 PM

ISS04: Deep Exploration into Proteome Universe Combining Proteograph XT Workflow with Orbitrap Astral MS

13:15   **Speaker**
               Daniel Hornburg, United States

Session Date/Time: Monday, September 18, 2023 - 01:15 PM - 02:15 PM

ISS05: Seminar Supported by Bruker

13:15   **Sub-minute Gradient Capillary LC/MS/MS for High-speed, High-sensitivity and In-depth Proteomics**
               Yasushi Ishihama, Japan

13:40   **High-sensitive Spatial and Cell-type Proteomics**
               Ruijun Tian, China

Session Date/Time: Monday, September 18, 2023 - 01:15 PM - 02:15 PM

ISS06: Complementing Mass Spectrometry with Immunoaffinity Techniques

13:15   **Going Deep – Complementing Mass Spectrometry-based Methods with Affinity Probing to Enrich Plasma Proteome Coverage**
               Stefanie Hauck, Germany

See you in Busan!
BEXCO, Busan, Korea
🌐 2023.hupo.org
🐦 f 📷 in #HUPO2023

Together Through Proteomics

PROGRAM

13:45 **Multiplex Techniques in Clinical Proteomics: A Complementary Tool for Biomarker Discovery**
Frank Schmidt, Qatar

Session Date/Time: Monday, September 18, 2023 - 02:30 PM - 03:35 PM

CS05: New Technologies for Large Scale Proteomics

Chair
Sheri Wilcox, United States

Chair
Alexey Chernobrovkin, Sweden

14:31 **CS05.01: Keynote Speaker - Deep Plasma Proteomics at Scale: 8,000+ Protein Groups Across a 3,000 Subject Biomarker Study**
Bruce Wilcox, United States

14:56 **CS05.02: An In-Depth Investigation Into the Performance of Affinity and Mass Spectrometry Platforms on Large-Scale Plasma Proteomics Studies**
Sara Ahadi, United States

As the interest in non-invasive biomarker discovery and therapeutic target identification grows, plasma protein profiling platforms are becoming increasingly more comprehensive, precise, and specific. There are several proteomics platforms that can be used for human plasma analysis; however, the choice of platform is not that clear and requires better understanding of each.
We conducted an analysis of 80 plasma samples from young and old subjects (with gender ratio of 1:1) using three proteomics platforms: SomaLogic, Olink, and data-independent mass spectrometry with the aim of better understanding the scope of each platform in profiling plasma proteins. In our study we have analyzed the deepest proteomics datasets of human plasma, utilizing three primary proteomics platforms, resulting in highest overlap of proteins between all three methods. We measured 7596, 2943, and 3576 assays for SomaLogic, Olink, and mass spec platforms, respectively and there are 1051 proteins that are overlapped between all three platforms.
Our results indicate that all three platforms measured 30-40% of the biological variability in our study, however the technical CVs measured in the three assays are vastly different. The median CV measured in SomaLogic assays was 7%, 11% in Olink, and 24% for mass spec. Our data suggests that missing values are the least in SomaLogic assays, with 96% of assays measured in all samples, whereas only 60% of all measured proteins are present in all Olink or mass spec samples. The correlation between these overlapped proteins, was highest between SomaLogic and Olink platforms (Spearman Rho=0.61) and lowest between SomaLogic and mass spec platforms (Spearman Rho=0.41). In conclusion this study provides a deep comparison between the three common proteomics platforms and highlights the synergies, differences and similarities between affinity and mass spec-based proteomics platforms. Understanding the scope and limitations of each method helps with better interpretation of proteomics results.

See you in Busan!
BEXCO, Busan, Korea
2023.hupo.org
#HUPO2023

Together Through Proteomics

PROGRAM

15:09   **CS05.03: Broad Target Scanning (BTS) - A Pragmatic LC-MS Acquisition Method for Collecting Population-Scale Real-World Proteomics Data**
Dmitry Avtonomov, United States

Introduction
Despite advancements over the past two decades, challenges remain in population-scale proteome profiling with liquid chromatography-tandem mass spectrometry (LC-MS/MS), mainly due to prohibitive cost of biological sample collection and limited throughput of traditional LC-MS discovery workflows. We address this challenge with Broad Target Scanning (BTS), which integrates proteome profiling into established clinical LC-MS assays. BTS maintains the same level of rigor as selected reaction monitoring (SRM) while enabling accumulation of real-world proteomics data concurrently for population-scale discovery studies.

Methods
BTS uses a Thermo Exploris 480 coupled with a proprietary Dual-LC system. Dual-LC allows robust quantification and uninterrupted processing of hundreds, potentially thousands, of samples using fast gradient and optimal exploitation of MS acquisition time. MS acquisition consists of three experiments: Gas-Phase Fractionation (GPF) MS1, Targeted MS2 with multiplexing (MSX), and GPF data-independent acquisition (DIA). Dual-LC fully exploits 15-minute LC-MS/MS experiment time by eliminating column equilibration-related "dead time" using two mutually independent chromatographic columns. Thermo iAPI was employed for control over MS scan scheduling. MSX for targeted MS2 was used to minimize confirmation MS/MS scan times. Quantification is performed using MS1 data, GPF was used to reduce ion suppression effects.

Results
We applied BTS to the analysis of over 500 pancreatic ductal adenocarcinoma samples. Samples were spiked with a mix of 77 stable isotope labeled (SIL) peptides, selected for quantification of 77 proteins. BTS analysis runtime matched that of SRM and parallel reaction monitoring (PRM), achieving equivalent targeted quantification quality to SRM and PRM. Additionally, we identified over 3000 protein groups per run from the DIA component of the method.

Conclusion
BTS offers a viable alternative to SRM and PRM for economical, large-scale, real-world proteomic data collection. It harmonizes smart targeted monitoring, simultaneous DIA discovery, and robust liquid chromatography, proving to be a transformative tool in large-scale proteomic research.

15:22   **CS05.04: Multiplexed Deep Plasma Profiling Using a Novel High-Resolution Accurate Mass Spectrometer**
Vincent Albrecht, Germany

Background
Proteomic technology has progressed tremendously in the past years fuelling the revival of mass spectrometry (MS)-based plasma proteomics. Interestingly, a workflow that allows to accurately quantify proteins in great depth and high-throughput well suited for the analysis of large cohorts has been yet out of reach.

Methods and Results
To tackle this challenge, we describe a plasma proteome profiling workflow that comprises three key elements: a robust and economical enrichment protocol, highly efficient sample multiplexing using multiplexed data-independent acquisition (mDIA), and a novel high-resolution accurate mass spectrometer (Thermo Scientific). Specifically, we have optimized and streamlined a cost-effective and highly automatable protein enrichment method to improve the detection of low-abundance proteins. It builds on a classic and recently revived protein precipitation by perchloric acid (PCA). Abundance of many of the top plasma proteins is decreased, significantly improving the depth of proteome coverage. For multiplexing, we leveraged our recently published mDIA workflow. Three channels are isotopically encoded with non-isobaric methylation-based reagents, tripling our throughput. Identifications and quantitative accuracy are significantly improved by decoupling identification from quantification using our in-house developed software AlphaDIA. Conversely, multiplexing comes at the cost of further increasing spectral complexity of DIA MSMS spectra, potentially decreasing identification rates per channel. To overcome this problem, we utilize ultra-fast MSMS scanning speeds of up to 200 scans per second with MSMS resolution of 100,000 on the next generation Thermo Scientific MS instrument to implement DIA methods with narrow DDA-like MSMS isolation windows. Protein and peptide identifications have substantially improved compared to previous instruments in a three-plex sample on a 60 samples per day (SPD) Evosep gradient, corresponding to a throughput of 180 SPD.

Conclusion
In conclusion, we developed a deep plasma profiling workflow that allows fast, economical and deep plasma profiling of large cohorts.

Session Date/Time: Monday, September 18, 2023 - 02:30 PM - 03:35 PM

CS06: 3 Minute Thesis Competition

14:30   **3MT01.01: 3 Minute Thesis Finalist**
Mane Polite Roneldine Mesidor, United States

14:35   **3MT01.02: 3 Minute Thesis Finalist**
Alireza Nameni, Belgium

14:40   **3MT01.03: 3 Minute Thesis Finalist**
Dina Schuster, Switzerland

14:45   **3MT01.04: 3 Minute Thesis Finalist**
Janaina Silva, Brazil

14:50   **3MT01.05: 3 Minute Thesis Finalist**
Justin Sing, Canada

14:55   **3MT01.06: 3 Minute Thesis Finalist**
Dafni Skiadopoulou, Norway

15:00   **3MT01.07: 3 Minute Thesis Finalist**
Xue Sun, China

15:05   **3MT01.08: 3 Minute Thesis Finalist**
Di Tang, Sweden

15:10   **3MT01.09: 3 Minute Thesis Finalist**
Marvin Thielert, Germany

15:15   **3MT01.10: 3 Minute Thesis Finalist**
Takehiro Tozuka, Japan

15:20   **3MT01.11: 3 Minute Thesis Finalist**
Yun-Jung Yang, Taiwan

15:25   **3MT01.12: 3 Minute Thesis Finalist**
Yu Zong, China

Session Date/Time: Monday, September 18, 2023 - 02:30 PM - 03:35 PM

CS07: Extracellular Vesicles

Chair
Birgit Schilling, United States

Chair
Yong Tae Kwon, Republic of Korea

14:31   **CS07.01: Keynote Speaker - Proteomics in Mammalian and Bacterial Extracellular Vesicles and Integrated Database EVpedia**
Yong Song Gho, Republic of Korea

![HUPO BUSAN 2023 SEP 17-21 22nd Human Proteome Organization World Congress]

See you in Busan!
BEXCO, Busan, Korea
🌐 2023.hupo.org
🐦 f 📷 in #HUPO2023

Together Through Proteomics

PROGRAM

14:56   **CS07.02: Prostate Cancer Reshapes the Secreted and Extracellular Vesicle Urinary Proteomes**

Thomas Kislinger, Canada

In males, the urinary tract runs through the prostate, a secretory gland that produces prostatic fluid. The urine is a remarkably complex biofluid. Because it is effectively filtered blood, it serves as a non-invasive snapshot of organismal state, but because it spends significant time within the genitourinary tract, it can also be reflective of those tissues. The composition of urine can vary dramatically across individuals, and over time. Urine has been widely proposed as an ideal non-invasive longitudinal biomarker matrix. Several studies have identified tumour DNA, RNA or proteins within the urine. Urinary proteins can originate in different ways. From active secretion of soluble proteins with a signal peptide, through indirect release due to cellular turnover or from secreted extracellular vesicles (EVs). EVs are nanosized particles with a lipid bilayer that are actively released by cells into the extracellular milieu. They vary in size, ranging from 30 to 2,000 nm in diameter, and are heterogeneous in their mechanism of biogenesis, molecular composition, and function. EVs play a crucial role in cellular physiology and contribute to cancer pathogenesis. EVs and secreted proteomes are hypothesized to be context-driven, and tissue-specific, but their presence, population variability and disease-relevance in urine remain largely unknown. We generated the most comprehensive urinary proteomic landscape from 190 treatment-naïve men with a range of benign and malignant conditions, covering the entire disease spectrum of prostate cancer, resulting in the quantitation of over 6,000 proteins. We evaluate the tissue and subcellular origins of urinary proteins and EVs and quantify how the urine proteome changes over time in individuals using longitudinal sampling. We demonstrate that cell lines are a poor proxy for cancer tissue EV secretions, but that prostate tumor-specific proteins can be used to distinguish men with and without prostate cancer and identify context-dependent urine EV cargo.

15:09   **CS07.03: Proteogenomic Identification of Circulating Mutated Proteins in Extracellular Vesicles**

Koji Ueda, Japan

Introduction
Cancer cell-derived EVs 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 EVs.
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. We also constructed the multiple reaction monitoring (MRM)-based absolute quantification method for detection of the ccRCC mutated protein panel and analyzed EVs isolated from plasma samples collected from each patient before and 2-4 months after surgery.
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. Subsequent mutated proteome analysis identified 11,417 proteins (FDR < 0.01), in which, importantly, 3 mutated proteins were included. Further absolute quantification measurement of the mutated protein panel for pre/post operative plasma EV samples showed that drastic reduction or complete disappearance of EV mutated proteins were observed in all post-operative cases.
Conclusions
Cancer-specific mutated proteins were detectable in plasma or urine EVs, suggesting that our circulating mutated protein-based liquid biopsy could serve as an effective tool for diagnosis of kidney cancer detection or monitoring.

PROGRAM

15:22   **CS07.04: Mass Spectrometry Based Proteomic Profiling of Exosomal Proteins in Diabetic and Non-diabetic Ischemic Stroke Patients: A Case-Control Study**
Muhammad Umar Sohail, Qatar

Background: Acute ischemic stroke is the most common cause of neurologic dysfunction caused by focal brain ischemia and tissue injury. Diabetes is a major stroke risk factor, exacerbating disease management and prognosis. Therefore, discovering new diagnostic markers and therapeutic targets is critical for stroke prevention and treatment. Due to their distinct properties, exosomes have emerged as novel molecules for biomarker discovery and therapeutic application.
Methods: The present case-control study compared mass spectrometry-based high throughput proteomics of exosomes collected from non-diabetic stroke (nDS = 14), diabetic stroke (DS = 13), and healthy control (HC = 12) subjects. Physical and chemical characterization of exosomes was performed using DLS, TEM, and CD63 Western blot analysis. Mass spectrometry identified 1288 proteins. Three statistical comparisons using a general linear model (log2fc ± 0.58 and p ≤ 0.05) were performed for nDS vs HC, DS vs HC, and DS vs nDS. DS vs HC and DS vs nDS comparisons produced 155 and 159 differentially expressed proteins, respectively.
Results and Conclusion: PCA reveals a distinct clustering pattern, wherein the DS group clustered distinctly from the HC and nDS groups at PC1. FIBG, FIBB, TTC16, CHD7, IKKE, BD1L1, and PR14L were the most differentially expressed proteins in the DS group compared to the HC and nDS groups. In the DS group, acute response signaling, blood coagulation, and complement system pathways were activated (z-score ≥ 2; p ≤ 0.05). Diabetes in stroke patients was associated with significant changes in protein levels, which overactivated these systemic pathways. These findings emphasize the importance of exosome proteomics in discovering potential biomarkers for stroke management and prevention. Further studies are warranted to investigate the clinical application of these markers.

Session Date/Time: Monday, September 18, 2023 - 02:30 PM - 03:35 PM

CS08: Immunity and Immunopeptidomics

Chair
Wei Wu, Singapore

Chair
Aleksandra Nita-Lazar, United States

14:31   **CS08.01: Keynote Speaker - Great Expectations: Mass Spectrometry-Assisted Definition of the First Physiological Interactomes of TCR-like Antibodies**
Nicola Ternette, United Kingdom

14:56   **CS08.02: Awesome Activities out there: Proteomics Discovery of Intercellular Signaling Circuits Regulating Inflammation**
Felix Meissner, Germany

Inflammation is a natural mechanism to restore tissue homeostasis, and its deregulation causes disease. Intercellular signaling proteins orchestrate the crosstalk between cells; however, sterile pathologies independent of known cytokines, have alluded to unexplored signals regulating inflammatory reactions. I will present in vitro and in vivo cell-type selective, biochemical and mass spectrometry-based proteomics approaches for the systematic investigations of cellular communication in primary cell populations. Our studies highlight concepts to dissect complex secretory programs with distinct physiological functions and reveal proteins and proteoforms with previously unexplored intercellular activities. By establishing biochemical and computational social cellular networks, we unveil the architecture of communicating cells and novel signaling circuits between cell types. Together our studies will contribute to elucidating the fundamental principles of cellular crosstalk in sterile inflammatory conditions relevant for understanding the pathogenesis of chronic metabolic and age-related disorders.

PROGRAM

15:09 **CS08.03: Differential Enhancement of Antigen Presentation by Interferons in Lung Epithelia**

Anthony Purcell, Australia

Interferons drive inflammatory responses during infection with respiratory viruses like influenza and SARS-CoV-2. The interferon 'system' comprises three classes of molecules, each with distinct signalling kinetics, potency and receptor distribution. Types I and II interferon receptors are expressed by most cells whereas type III interferon receptors are restricted to epithelial and some immune cells. Interferons are key mediators of antigen presentation, a process whereby viral proteins are processed into short peptides and displayed at the cell surface in complex with human leucocyte antigen (HLA) molecules for immunosurveillance. Type II interferons upregulate antigen processing enzymes and HLA molecules leading to a diversification of the repertoire of peptide ligands; known as the 'immunopeptidome'. Modulation of the immunopeptidome by type I and III interferons are less well explored. To study the role of each interferon type in antigen presentation, we initiated a study to perform paired proteomic and immunopeptidomic analyses of lung epithelial cells treated with interferon-α (type I), -γ (type II) and -λ (type III), to assess changes in the antigen processing and presentation machinery and how this impacts on the display of peptides by HLA molecules at the cell surface. Interferon-α and –γ significantly increase expression of the cellular factors involved in antigen processing and presentation (e.g. TAP1/2, tapasin and proteasomal subunits), whereas interferon-λ had a less pronounced impact on these factors. IFN-alpha demonstrated more pronounced upregulation of classically defined interferon-inducible anti-viral proteins compared to the other IFNs. We present the the shared and distinct interferon-induced proteomic changes in lung epithelial cells, and describe how interferon-induced changes to antigen processing machinery alter the immunopeptidome. Such information will be key to the design of next generation vaccines to a range of respiratory viruses and more broadly in infectious diseases and cancers.

15:22 **CS08.04: The Impact of Varying Toll-like Receptor Activation on Intrinsic and Cross-presented MHC Peptidomes**

Jessica Hung, United States

Activating toll-like receptors (TLRs) with corresponding ligands has been demonstrated to promote dendritic cell (DC) maturation and enhance transient exogenous antigen uptake in vitro. However, how the activation of each distinct TLR alters the presentation of unique intrinsic and extrinsic antigens presented by DCs has not been thoroughly investigated. Here, we performed a proteomic antigen discovery strategy to measure how the activation of three different TLRs, TLR2 (Pam3CSK4), TLR4 (LPS), and TLR9 (CpG), modulate the major histocompatibility complex (MHC) class I and II presented peptidomes. We used mouse bone marrow-derived DCs (BMDCs) as a simple and efficient system to define the immunopeptidome under each condition. After being stimulated with TLR agonists for 16h, 1~2.25×10⁷ BMDCs were harvested for whole-cell proteomics and MHC immunoprecipitation followed by mass spectrometric analysis. Of over 5,200 quantified proteins, TLR activation resulted in 234 (4.0% of all quantified proteins), 300 (5.1%), and 264 (4.5%) differentially expressed proteins in response to TLR2, TLR4, and TLR9 stimulation, respectively. Consistent with previous findings, TLR agonists upregulated proteins involved in pathogen recognition and inflammatory cytokines and chemokines signaling pathways, indicating a more immune-active DC phenotype. Furthermore, TLR4 and TLR9 upregulated MHC-I molecules, but TLR2 uniquely upregulated proteins involved in phagocytosis, suggesting distinct mechanistic roles in antigen presentation. Peptide repertoire analysis revealed increased exogenous antigen presentation on MHC-II following TLR2 stimulation. A moderate decrease in the number of unique antigens presented by MHC-I was observed with all three TLR stimulations compared to the unstimulated control, suggesting TLR activation actually restricted the presentation of endogenous antigens. In summary, TLR2 activation reshapes antigen repertoires in vitro, highlighting this strategy's unique potential in antigen discovery in multiple disease models.

Session Date/Time: Monday, September 18, 2023 - 03:35 PM - 04:30 PM

PP01: Even Numbered Posters - Poster Viewing with Coffee Break

PP01.02: Extracellular Vesicles from β-thalassemia/HbE Reduced Endothelial Nitric Oxide Production

Pornthip Chaichompoo, Thailand

Thrombosis is a significant complication leading to morbidity and mortality in the β-thalassemia/HbE patients. The incident is increased in splenectomized patients and correlated with the increased levels of circulating medium extracellular vesicles (mEVs). Nitric oxide (NO) is an important protective molecule in the vascular system. It plays important role in thrombosis by regulates blood vessel tone and suppresses platelet aggregation, leukocyte migration, and cellular adhesion to the endothelium. Herein, effect of mEVs from splenectomized β-thalassemia/HbE patients on NO production by endothelial cells was examined. Decreased NO production was observed in human pulmonary artery endothelial cells (HPAECs) treated with splenectomized mEVs. Endothelial nitric oxide synthase (eNOS) is regulated by phosphorylation at multiple sites including at Ser1177 and Thr495, which activate and inhibit enzyme activity, respectively. However, splenectomized mEVs have no effect on eNOS phosphorylation at neither Ser1177 nor Thr495. Interestingly, splenectomized mEVs have increased hemoglobin content. Since hemoglobin can react rapidly with NO to form biologically inactive nitrate, NO scavenging was determined and revealed higher NO scavenging by splenectomized mEVs compared with mEVs from normal subjects. These findings suggested that medium EVs from splenectomized β-thalassemia/HbE patients modulate endothelial cell production of NO by directly scavenging NO, which may contribute to vascular dysfunction and thrombosis in β-thalassemia/HbE patients.

PP01.04: Leveraging Deep Proteome Profiling of Plasma- and Serum-derived Extracellular Vesicles for Melanoma Biomarker Discovery and Disease Dissection

Yuehan Feng, Switzerland

Introduction
Extracellular vesicles (EV) play an important role in melanoma progression but their potential as clinical biomarkers has yet to be realized. EVs can be found in most liquid biopsies and exosomes are the most prominent subcategory of EVs. Blood and its liquid components plasma/serum are the most frequently used matrix for biomarker discovery due to the ease of collection. However, most proteomic platforms for plasma/serum profiling are unable to profile EV proteins due to the high dynamic range of protein concentrations in EV preparations. This is due to 1) EV isolation methods that vary in their capabilities to separate EVs from free proteins, and 2) the presence of a natural corona of high-abundant blood proteins attached to the EV surface.

Methods
To tackle this challenge, we developed SEC-DIA-MS, an integrated workflow combining size-exclusion chromatography, EV concentration, and optimized LC-DIA-MS to enable deep profiling of the proteome of the enriched vesicles. (Lattmann et al. under revision)

Results
From 200 µl of plasma/serum from a test melanoma case control cohort (n=9), we quantified 2,242 exosome-associated proteins, achieving a 2.5-fold increase in depth compared to previous melanoma studies. To gain a better understanding of the exosome enrichment efficiency, we extensively characterized the plasma/serum proteome by analyzing native, depleted, and EV-enriched blood from the same donors. We successfully validated well-known exosome markers such as CD9, CD63, CD81 and TSG101, and found that EV samples are significantly enriched in intact membrane proteins and those related to T cell biology, further underlining the uniqueness of the EV proteome composition. Furthermore, known melanoma markers (e.g. MCAM, TNC, TGFBI) were upregulated in melanoma plasma-derived EV but not depleted plasma samples, highlighting the specific information contained in EVs.

Conclusion
The ease of automating and scaling the presented workflow enables a broader application to other indications and biological matrices.

PP01.06: Proteomics Profiling of Tumor-derived Extracellular Vesicles for Triple-negative Breast Cancer Diagnosis

Juyong Hyon, Republic of Korea

Tumor-derived extracellular vesicles (tdEVs) have been emerging as potential biomarkers for cancer diagnosis because the tdEVs precisely reflect tumor cell alterations with significantly increased production. The proteomic profiling study of tdEVs represents a promising approach in a non-invasive manner to novel biomarker discovery for early detection and targeted therapy of cancer. Previously, we have developed a novel microfluidic chip for rapid and selective isolation of tdEVs. This microfluidic chip enables the selection of two types of EVs by using breast tumor-derived proteins (EpiCAM & CD49f) within two minutes. In this study, we compared and analyzed proteomics of EVs isolated from several breast cancer cell lines by using a commercialized EV isolation kit based on the CD63 marker and our microfluidic chip which can selectively isolate tdEVs with the cancer cell-specific markers EpiCAM & CD49f. A total of 3,700 proteins were identified, and as a result of GO analysis of 474 unique proteins from EVs isolated by CD63, the proteins were related to EV production and transport. On the other hand, GO analysis showed that 389 proteins expressed only in EpiCAM & CD49f, which are cancer cell-specific markers, are related to signal transduction and RNA metabolism. As a result of GO analysis of different proteins, mRNA processing body, autophagosome-related and RNA degradation pathway proteins characteristically appearing in TNBC patients could be identified. In particular, four potential EV markers contributing to the progression and relapse of breast cancer were relatively high in EVs isolated from TNBC patients compared to the normal control group. The best EV protein marker showed excellent performance for discriminating patients with early- and advanced-stage breast cancer from normal control with 83% sensitivity, 80% specificity, and over 0.8 area under the receiver operating characteristic curve; Moreover, several other EV protein markers had similar diagnostic utility.

PP01.08: Small Extracellular Vesicle Protein Biomarker Profiles for Breast Cancer Diagnosis and Monitoring Progress

Yujin Lee, Australia

Introduction: Breast cancer (BC) is the most common cancer in women worldwide. Current methods for detecting BC are not accurate and reliable and cannot provide real-time information for monitoring cancer progression. Small extracellular vesicles (sEVs) are secreted by all cell types, containing various biological cargoes that reflect their cellular origin. sEVs are an important intercellular communicator and mediator in cancer metastasis, immunity, and therapeutic resistance. Therefore, disease specific proteins within sEVs are considered as a superior choice for non-invasive liquid biopsy biomarker source in BC.
Aims: The objective of our study was to optimise the most effective sEV isolation approach for proteomic analysis and identify potential sEV protein biomarkers from BC cell lines and human BC plasma samples for accurate diagnosis and progression monitoring.
Methods: sEVs derived from three BC cell lines, one normal breast cell line, BC patients, and non-cancer control plasma samples were isolated using ultracentrifugation (UC), Total Exosome Isolation kits (TEI), and a combined approach of UC and TEI (UCT). The isolated sEVs were characterised to compare the isolation capacity of the three methods and profiled using LC-MS/MS proteomics to identify potential sEV protein biomarkers.
Results: The UC isolates showed significantly higher number of sEV proteins and identified 10 potential sEV protein biomarkers in BC cell lines. The UCT isolates showed the highest proportion of sEV related proteins and lowest percentage of lipoprotein-related proteins. UCT isolates demonstrated 9 potential sEV protein biomarkers in BC plasma samples.
Conclusion: The combined approach, UCT enhances the concentration of sEV and facilitates the identification of a greater number of potential sEV protein biomarkers in BC plasma samples. Our findings demonstrate that the UCT isolation technique is a promising method for identifying potential sEV protein biomarkers for BC diagnosis and monitoring progression.

PP01.10: Size-exclusion Purification of Extracellular Vesicles and Sample Characterization by Multiplex Fluorescent Western Blottinng.

Kenneth Oh, United States

Background:

Recently, size exclusion chromatography (SEC) has seen greater adoption in EV purification schemes due to the gentile and reproducible nature of the technique, capable of fractioning EVs with high purity and integrity for further evaluation. We present the characterization of SEC resin Bio-Gel A-1.5m for the enrichment of EVs obtained from serum-free conditioned cell culture media. The quality of the corresponding SEC separations via Bio-Gel A columns were assessed by nano-particle tracking analysis (NTA) and Multiplex Fluorescent Western Blotting (MFWB).

Methods:

Serum-free conditioned cell culture medium obtained from MCF-7 cells was 100x concentrated and enriched for EVs with a self-packed with 15 ml of resin Bio-Gel A 1.5m . The 1.5 cm diameter column was operated with the NGC medium pressure chromatography system and 250 µl fractions were collected with the Bio-Frac fraction collector equipped with a nano-drop head. Particle concentration and size distribution of individual SEC fractions were measured with NTA, followed by MFWB for verifying the presence of EVs and possible co-contaminants in pooled SEC fractions.

Results:

EVs were successfully and reproducibly enriched and purified by SEC with the Bio-Gel A 1.5m resin. Chromatograms were recorded at 220, 260, 280 nm wavelengths and revealed a bulk-like elution of EVs at high (~80%) particle yield. EV-containing elutions were pooled and further analyzed for EV-specific proteins by MFWB, evaluating up to 6 proteins per lane. Typical EV-specific proteins (CD9, CD81 and ALIX) are highly enriched after SEC while contaminating proteins were significantly reduced.

Conclusions:

SEC is a gentle and scalable method and resins with diverse pore characteristics are available to enrich and purity EVs from various sources. Data from nano-particle tracking analysis (NTA) and multiplex fluorescent Western blotting (MFWB) indicate that Bio-Gel A 1.5m can be an effective resin for enriching EVs at a high yield and purity.

PP01.12: The Involvement of Exosomes in Intercellular Communication During Chemotherapy-Induced Chemoresistance in Ovarian Cancer Cells

Polina Shnaider, Russian Federation

It was recently shown that intercellular communication among cancer cells during chemotherapy can impact the development of chemoresistance. To delve deeper into this phenomenon, we focused our research on a cell model of ovarian cancer, one of the most deadly gynecological diseases. Our previous research has proven that dying cancer cells emit molecules (including spliceosomal proteins) that lead to chemoresistance in recipient cells, but the mechanism behind this process remains unknown.

In this work, therapy-induced (TIS) or control secretomes were collected from donor cancer cells treated or untreated with cisplatin, respectively. Recipient cells were incubated with obtained TIS or control secretomes for 3 days. Our proteomic analysis revealed that TIS increased the abundance of proteins involved in RNA splicing and DNA repair in recipient cancer cell. Furthermore, we demonstrated that spliceosomal proteins are encapsulated in TIS vesicles.

Next, we established that TIS rescues recipient cells from different DNA-damaging agents but not other drugs. Using fluorescent microscopy/cytometry, comet assay, and cell cycle analysis, we found that pre-incubation of recipient cells with TIS leads to acquired chemoresistance by extending S-phase of the cell cycle, reducing DNA fragmentation and cisplatin-DNA adducts, as well as decreasing the phosphorylation of RPA2 which indicate that recipient cells have improved adduct resolution and reduced replicative stress.

These collective findings show that therapy with DNA-damaging agents triggers dying cancer cells to secrete signaling molecules, including spliceosomal proteins. These molecules effectively rescue residual cancer cells from DNA damage by regulating the cell cycle and promoting DNA repair, ultimately resulting in chemoresistance.

In conclusion, this study reveals the mechanism that lies behind chemoresistance and provides significant insights into targeting the intracellular communication of cancer cells during treatment.

PP01.14: Functional Characterization of EV Cargo and Surface Protein Complexes Towards Immunological and Organotropic Targeting in Vivo

Wei Wu, Singapore

Extracellular vesicles (EVs) are nano-sized, membrane-encapsulated compartments that enable distant relay of signaling function within an organism. The specificity of such crosstalk is determined by membrane interactions that enable organotropic EV docking, transfer of activated receptors by membrane fusion, and thereby, effectively bypassing ligand-activation in the recipient cell. Understanding these processes of targeting and fusion require conformational studies, yet structural characterization within natural membranes has been immensely difficult to achieve. We have previously shown that the external plasma membrane surface can be crosslinked by MS-cleavable disuccinimidyl sulfoxide (DSSO), where we observed higher-order HLA class I and HLA class II hetero-complexes that are apparently locked in T-cell inaccessible conformations. Here, we developed an intact EV crosslinking mass spectrometry approach (iEVXL) using partially membrane permeable crosslinker disuccinimidyl suberate (DSS). We show that structural mapping and modeling of protein complexes within intact EV membranes is feasible, and structures obtained are congruent with membrane topology, solvent accessible distances and Alpha-fold predictions of previously uncharacterized regions. We envision this technique as an important strategy in understanding structural determinants of EV docking and specificity, to unlock the utility of EVs for in vivo targeted delivery.

PP01.16: pXg: Comprehensive Identification of Noncanonical MHC-I-associated Peptides from de Novo Peptide Sequencing using RNA-Seq Reads

Seunghyuk Choi, Republic of Korea

Introduction
Proteogenomics has been widely applied to discover noncanonical peptides. Recent research indicates that various noncanonical peptides that bind to major histocompatibility complex I, termed ncMAPs, may be desirable immunotherapeutic targets. De novo peptide sequencing can identify peptide sequences from their mass spectra without any reference to a sequence database, making it an ideal method for ncMAP discovery. However, due to the lack of a suitable method for estimating false-positive rates, this approach has not been widely used for ncMAP identification.

Methods
We present a novel pipeline, called pXg, to comprehensively and reliably identify immunopeptides from de novo peptide sequencing. pXg directly matches de novo peptides to six-frame translation of reads and those of reverse sequences to generate target and decoy PSMs, respectively. In contrast to exist pipelines, it utilizes not only proteomic features but also genomic features such as RNA-Seq abundance and sequencing quality to improve both sensitivity and specificity of identification using Percolator.

Results
We identified 24,449 cMAPs and 956 ncMAPs from ten samples. Among them, 1,611 cMAPs and 387 ncMAPs were novel identifications not reported elsewhere. Due to the unrestrictive search space, we identified 11 immunopeptides derived from squirrel monkey retrovirus in Human leukemia monocytic cell line. We further suggested that 70 putative tumor-specific antigens by strictly selecting higher immunogenicity values predicted by three prediction tools, demonstrating the usefulness of pXg for novel target discovery.

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.

PP01.18: Unveiling the Hidden Potential of HLA-E in Cancer Immunity Through a Combinatorial Approach to Studying Non-Classical HLA-Peptide Repertoires

Joshua Fehring,

HLA-E is a non-classical molecule that has long been recognized as a pivotal component in immune evasion and self-recognition. While extensive research has focused on its role in natural killer cell recognition and "loss of self", evidence in viral and bacterial infection suggests a broader role of HLA-E restricted presentation to CD8+ T cells, though little is known in the context of cancer. To gain deeper insights into this relationship, we have investigated the nature of peptides presented on HLA-E following pro inflammatory cytokine treatment. With the limited availability of robust and specific HLA-E- antibodies, we employed a series of complementary molecular techniques to devise alternative strategies that facilitate a more comprehensive and confident analysis of the HLA-E ligand repertoire. Specifically, we employed separate approaches utilising a single-chain HLA-E dimer, HLA-C knockout to mitigate antibody cross reactivity, and a secreted HLA-E system.

Our findings demonstrate that HLA-E exhibits the ability to present tumor-antigen derived peptides across multiple tumour-derived cell lines. This discovery carries significant implications for the fields of immunotherapy and immunopeptidomics, as the monomorphic nature of HLA-E opens up avenues for potential pan-cancer therapeutic interventions. Furthermore, the observation that these molecules can present peptides beyond self-recognition sequences in perturbed states fundamentally reshapes our understanding of tumour evasion and escape mechanisms. Ultimately, these findings hold promise for revolutionising our approach to cancer treatment and advancing the field of immunotherapy.

PP01.20: Through a Direct Contact Mechanism, CD4 T Cell Can Generate FLT3LG, which Enables Them to Neutralize Cancer Effectively.

Yeo Jin Im, Republic of Korea

Breast cancer is the most common cancer among women worldwide, with particularly high incidence in South Korea and other Asian countries. Immunotherapy has emerged as a critical approach in breast cancer treatment. The fms-like tyrosine kinase 3 ligand(FLT3LG) is known as a growth factor for hematopoietic stem cells and dendritic cells. Previous studies have demonstrated reduced FLT3LG mRNA expression in CD4 T cells derived from peripheral blood mononuclear cells (PBMCs) of dogs with mammary gland tumors. However, there are currently no identified immunotherapies specifically targeting CD4 T cells from PBMCs and involving FLT3LG in the context of cancer. Therefore, we focused on the interaction between breast cancer and CD4 T cells from PBMCs, and firstly aimed to investigate its impact on T cells. To understand the molecular mechanism, we applied in-vitro co-culture systems that can mimic the immune environment and initially confirmed FLT3LG mRNA expression in human primary T cell using RT-qPCR. The results did not show significant findings when treating primary T cells solely with SKBR3 breast cancer cell-conditioned media. However, in the direct co-culture with T47D breast cancer cells, FLT3LG mRNA expression was observed to decrease in primary T cells. These findings suggest a direct interaction between cancer and T cells, rather than the presence of a specific substance secreted by cancer, leading to the reduction of FLT3LG in T cells. Further study is necessary to elucidate the specific mechanism underlying the decrease of FLT3LG in T cells within the cancer-environment. it is anticipated that elevated levels of FLT3LG could play a role in neutralizing cancer, and by promoting significant secretion of FLT3LG from CD4 T cells, a therapeutic effect in suppressing cancer may be expected. To further explore the findings, an in-vivo system capable of mimicking the human immune system and targeting CD4 T cells is required.

PP01.22: Efficient Identification of Immunopeptidomics and Clinically Relevant Neoantigens Presented on Lung Cancer by Mass Spectrometry

Ziyi Li, China

Neoantigens, which are mutated peptides specifically presented by MHC-I molecules on the surface of tumor cells, are attractive targets for immunotherapies. However, discovery of neoantigen solely based on genomic information have a validation rate of only 0.5~2%. In contrast, neoantigen accurately detected by mass spectrometry from a primary tissue will be the actual in vivo naturally. Here, we present a robust method for the efficient identification of MHC-bound peptides from clinical tissue, by capturing of naturally processed MHC-peptide complexes with the method of immunoprecipitation. Our method identified a range of 1,082 to 11,412 MHC-I peptides of 82 tissues from 41 patients with lung cancer, with an average of 5,778 peptides per sample and a modal length of 9 (61% of peptides). These results show that our method for the direct identification of immunopeptidomics is robust and high specificity. Moreover, we established two strategies for screening neoantigens. The first strategy involved generating a personalized reference database by stringent somatic single nucleotide variant (SNV) calling from exome and RNA sequencing data, and searching MS data against this database to identify the classical neoantigen. For the second strategy, a de novo platform trained with large amounts of immunopeptidomic data was used to directly search for both the classical and nonclassical neoantigens. These strategies led to identification of more than two hundred candidate neoantigens. To investigate their immunogenicity, we synthesized the mutant peptides for testing against pre-existing antitumor T cell responses. The results demonstrated that direct identification of mutated peptides by MS improved the ability to develop neoantigen-targeted immunotherapies. These findings could lead to more effective treatment options for cancer patients.

PROGRAM

PP01.24: Modulation of the Immunopeptidome by Serine Protease HtrA1

Elizabeta Madzharova, Denmark

Despite significant advances in analysis of tumor antigen presentation and recognition of cancer cells by the immune system, mechanisms of cancer immunity are far from being understood, hampering progress of promising immunotherapies. We have identified a new link between abundance levels of serine protease high temperature requirement A1 (HtrA1) in cancer cells and major histocompatibility class I (MHC-I) tumor antigen presentation with a potential dual mode of action. First, HtrA1 appears to regulate protein abundances of major components of the MHC-I complex at increasing cell density and second, through its protease function, HtrA1 seems to alter the immunopeptidome by proteolytic cleavage and functional modification of the PI31 proteasome inhibitor. Since low expression of HtrA1 is associated with cancer aggressiveness, its downregulation might lower susceptibility of cancer cells to immune surveillance contributing to diminished responses to immunotherapies. In this project, we will use engineered cell models, immunopeptidomics, and degradomics to investigate how HtrA1 influences antigen presentation and T cell recognition of cancer cells. With these studies, we will relate HtrA1-dependent activities to cancer cell phenotypes and provide novel mechanistic insight into HtrA1 functions as tumor suppressor. Moreover, we will contribute to a better understanding of cancer immune surveillance and establish HtrA1 as a potential novel biomarker for response to immunotherapy in personalized cancer treatment.

PP01.26: The SysteMHC Atlas 2.0

Wenguang Shao, China

Introduction: Comprehensive characterization of major histocompatibility complex (MHC)-bound peptides promises a better understanding of the basic mechanism of our immune system. Mass spectrometry (MS) has emerged as the method of choice to identify MHC-bound peptides. Post-translational modifications (PTMs), such as phosphorylation, cysteinylation or glycosylation, may occur on presented peptides and have been suggested to be a more routine addition to immunopeptidomics analysis for their broad biological and clinical relevance. Here, we describe the SysteMHC Atlas 2.0, an extensive collection of publicly available immunopeptidomics datasets analyzed by an optimized computational pipeline.
Methods: The pipeline utilizes multiple search engines including MSFragger, allowing a direct and effective identification of unexpected PTMs. The binding affinity is predicted by netMHCpan and spectral libraries are built by SpectraST.
Results: This release of the atlas collects 66 published datasets with 6,591 MS raw files. By analyzing 160 million MS/MS spectra with a strict peptide FDR of 1%, it covers 194 HLA-I and 142 HLA-II allotypes presenting 423,318 HLA-I and 183,449 HLA-II peptides, which greatly expands the previous SysteMHC Atlas 1.0 by 6.1 times on average. In total, by the open search strategy we identified over 50 various modification types, which are distributed to 58,078 and 48,921 modified peptides for class I and class II, respectively. The atlas also provides all the MS raw files associated with their search results, a catalog of context-specific datasets of HLA-I and HLA-II peptides, and various spectral libraries consisting of consensus spectra that represent the best quality among the replicative spectra. These libraries include allele-specific, PTM-specific and sample-specific ones, which aim to facilitate downstream analysis, such as DIA analysis and benchmarking new computational tools.
Conclusions: The SysteMHC atlas 2.0 serves as an important resource for the immunopeptidomics community, which provides insights into immune-associated questions in the context of cancer immunotherapy.

PP01.28: Enhanced Quantitative Analysis of Novel MHC-Peptides from Fusion Genes using Synthetic Polypeptide Sequences and High-Field Asymmetric Waveform Ion Mobility Spectrometry

Zhaoguan Wu, Canada

The identification and quantitative analysis of MHC-associated fusion neoantigens encoded by fusion oncogenes have significant clinical implications for the development of innovative immunotherapeutic approaches against both pediatric and adult cancers. However, challenges remain in the accurate and sensitive detection of those unique tumor-specific peptide antigens. In this study, we propose a novel optimization workflow combining 1) proteasomal digestion of synthetic fusion polypeptides, 2) high-field asymmetric waveform ion mobility spectrometry (FAIMS) for background interference removal and 3) high-sensitivity targeted parallel reaction monitoring (PRM) for improved limits of detection (LOD) and quantitation (LOQ). Following method optimization, we aim to apply our advanced targeted immunopeptidomics method to validate presentation of MHC-associated fusion peptides encoded by the ETV6-RUNX1 and CBFA2T3-GLIS2 fusion genes expressed in the REH and ME0 cell lines, respectively. Finally, we plan to quantify the absolute abundance of cell surface MHC-associated fusion peptides in patient-derived xenograft (PDX) models. If validated and further developed, this method will enable robust and accurate detection and quantification of MHC-associated fusion peptides from clinical biospecimens for the development of targeted immunotherapeutic approaches including antibody drug conjugates (ADC), bispecific T-cell engagers (BiTEs) and cancer vaccines.

PP01.30: Discovery of Immunogenic Antigen Candidates Against Infectious Diseases for Vaccine Development and Diagnosis Platform Using Immunoproteomics Approach

Jiyoung Yu, Republic of Korea

Immunoproteomics with high resolution LC-MS platform have been one of effective 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 diseases, 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.

PP01.32: A Complete, Deep Learning-driven and Quality-controlled Workflow for Tumor Antigen (TA) Discovery

Qing Zhang, Canada

Introduction

Tumor Antigen (TA) is prevalent targets for tumor immunotherapy. To find potential antigens, an accurate and sensitive workflow is needed. It should handle both data dependent (DDA) and data independent (DIA) data and label free quantification (LFQ) is also needed.

Besides, transcriptome data is usually needed for generating personalized protein database. De novo sequencing is promising alternative since it could be performed without database,

Here we propose a complete workflow include deep learning-based de novo sequencing, DDA and DIA data identification and quantification, all results contain quality control (QC) steps to ensure an accurate and sensitive output.

Methods
Workflow steps
1.   If DDA data is available, then database search, homolog search and de novo sequencing were performed. The results were merged and re-searched to generate a FDR-controlled spectral library.
2.   The DIA dataset was searched against a spectral library (if available) and then DIA-DeepNovo sequencing. Later, based on deep learning-based prediction of detectability, mass spectra, retention time and ion mobility, the library-free search was performed.
3.   High confident peptides in previous steps were collected, re-scored and reported with the estimation of FDR.
Results
Two datasets from published paper were test.

Dataset 1(cell line)

we reported close unique peptide number (36039) to the recently published paper (36947), sequence overlap is around 70%. Further analysis of peptide length distribution and motif also show similarity between two analyses. The number we reported is also much higher than original paper (14784). All these show sensitivity and accuracy of our workflow.

Dataset 2 (tumor sample)

Next, we test our workflow on tumor sample and quantified over 7000 unique peptides (almost double than paper). More interesting, we found 5 more validated tumor antigen include 2 from DeepNovo result, showing the prospect of using DeepNovo to find potential tumor antigen without time-consuming transcriptome data.

PROGRAM

PP01.34: Comprehensive Multi-omics Study Reveals Novel Colistin Resistance Profiles of Acinetobacter Nosocomialis Clinical Isolate, KAN02

Hayoung Lee, Republic of Korea

•Background
Acinetobacter nosocomialis, an opportunistic pathogen responsible for pneumonia or bacteremia, poses a particular threat in intensive care units. The rising prevalence of colistin-resistant strains in hospital settings has raised alarming concerns regarding patient safety and healthcare outcomes. Consequently, there is an increasing need to elucidate the mechanisms driving colistin resistance, effectively track the spread of resistant strains, and develop strategies to overcome the resistance.

•Methods
We conducted a comparative genomic analysis to uncover the antimicrobial resistance genes or underlying mechanisms by comparing susceptible and resistant bacterial isolates. Additionally, we examined the molecular characteristics of A. nosocomialis strains under colistin-exposed conditions (4 mg/L for 4 hours) using quantitative transcriptomics and proteomics. Label-free proteomics was performed through in-gel digestion, and the eluents were analyzed using LC-MS/MS with Orbitrap Q Exactive Plus.

•Results
Through comparative genomic analysis, we discovered a distinct subtype of the capsular polysaccharide (K) locus in A. nosocomialis KAN02, a colistin-resistance strain. This K locus is closely associated with capsular polysaccharide (CPS) production, which contributes to antimicrobial resistance and promotes survival through surface modification. Furthermore, we identified differentially expressed genes (DEGs) and differentially expressed proteins (DEPs). Our comprehensive transcriptomic and proteomic analysis revealed a total of 2,289 DEGs and 660 DEPs. Notably, 14 out of the 22 genes within the K locus showed significant upregulation in both omics datasets, indicating increased synthesis of the capsule in the KAN02 isolate.

•Conclusion
Notably, this study represents the first comprehensive multi-omics investigation aiming to enhance our understanding of the mechanisms driving colistin resistance in A. nosocomialis. Through multi-omics analysis, we identified molecular characteristics associated with the mechanisms of colistin resistance in A. nosocomialis KAN02. Our findings revealed that the upregulation of the K locus, as assessed through multi-omics analysis, plays a significant role in conferring colistin resistance in A. nosocomialis KAN02.

PP01.36: Accelerating the Development of Vaccines Against Nosocomial Infections: Proteomic Techniques Applied to AcinetoVax

Gustavo Adolfo Sánchez-Corrales, Spain

The challenge of multidrug-resistant bacteria requires the improvement and updating of classical methods. Vaccines against some of the most dangerous pathogens such as Acinetobacter baumannii are becoming increasingly urgent. The difficulties in dealing with these pathogens start at the product development stages, in this study we work with a potent vaccine candidate against infections caused by A. baumannii, AcinetoVax, vaccine technology developed by Vaxdy, S.L. AcinetoVax is able to express antigens of other highly dangerous bacteria such as Pseudomonas aeruginosa. However, the development of highly complex products is an analytical challenge for the selection and characterization of the best candidates.
Here we propose the use of proteomic methods to accelerate vaccine development, employing LC- MS/MS for this purpose. The studies are based on the characterization of the proteome of different vaccine candidates by FASP preparation of lysates. Membrane protein enrichment procedures were applied by biotinylation of exposed regions and affinity purification with streptavidin.
The proteomic approach is a fast and efficient tool to characterize not only vaccines, but also a great variety of biological products. It allows us to know the abundant proteins and potential antigens, to evaluate the expression of heterologous antigens of interest and to compare expression levels with homologous proteins. Some membrane proteins were clearly dominant and could be used to monitor product stability and consistency.

PP01.38: Multiproteomics Data Reveal Specific Associations Between Plasma Proteomes and Auto/pathogen Immunoproteomes in a COVID-19 Timeline Study of ICU Patients

Frank Schmidt, Qatar

Background: Intelligently linking different layers of proteomes is one of the main tasks of modern proteomics in the post-genomic era and can ideally lead to new insights into disease-specific questions. In the wake of the global COVID-19 outbreak, it has become very important to use blood to accurately characterize the status of infection, particularly proteomes and immunoproteomes that may be relevant to virus infection. In the present study, data were collected from different layers of blood proteomes and their correlation in networks and association with disease parameters were analyzed.

Methods: From a COVID-19 ARDS time-line cohort, we acquired data using multiple technologies including DIA mass spectrometry, selected panels from targeted Olink-PEA, NULISAseq, KREX human autoantibody and microbial pathogen antibody arrays from plasma collected from healthy controls (HC = 44) and 4 time points from COVID-19 ARDS ICU patients (C = 83). All data were further normalized and statistically analyzed using linear models, and their intercorrelations and clinical parameter associations were calculated. In addition, different visualization methods and prediction models were applied, complemented by network calculations.

Results and conclusions: PCA shows a clear pattern with the HC group clustering clearly from the C group in PC1 in both techniques with time trends in PC2. We also observed a large number of highly significant regulated features between the controls and the different time points for all techniques applied. Proteomic changes of the T- and B-cell pathway were significantly altered in the early phase of ICU admission, with corresponding alterations in the autoimmune profile mainly observed in the later phase of hospitalization. Furthermore, pathogen antibodies were detected in the early phase of admission, confirming secondary and tertiary infections with known pulmonary pathogens. Taking all data together, specific network analysis revealed a strong interaction between the different proteome layers, especially for TNF-related receptor types.

PP01.42: A Proteomics Insight into Streptolysin O's Multifunctionality: Unmasking Its Plasminogen Binding Role

Di Tang, Sweden

Background

Streptolysin O (SLO), a major virulence factor of Group A Streptococcus (GAS), is known for its pore-forming activity on the eukaryote membrane. In this study, we employed proteomic approaches to explore the host-pathogen protein-protein network centered on SLO, and identified Plasminogen (PLG) as a direct SLO-binding plasma protein. Given that GAS's propensity to exploit the PLG system to promote its pathogenesis and survival, we further validated this novel interaction and assessed its biological significance using integrated proteomic strategies coupled with a panel of immuno/bio-chemical assays.

Methods

Affinity purification-mass spectrometry (AP-MS) was used to identify potential SLO-binder among plasma proteins. Indirect enzyme-linked immunosorbent assay (ELISA) was applied to validate the direct binding of SLO to PLG. Plasminogen activation assays were conducted to assess the biological relevance of this interaction. Crosslinking mass spectrometry (XL-MS) was utilized to map the SLO-PLG interface, generating distance restraints for pairwise protein complex modeling.

Results

Our proteomics-driven approach identified PLG as a direct interactor with SLO. We demonstrated that SLO binding to PLG enhances tissue plasminogen activator (tPA) catalyzing plasmin generation, which may contribute to bacterial dissemination and tissue invasion. The molecular basis of the binding was unveiled through XL-MS data-derived interaction analysis, identifying key residues involved in the SLO-PLG interface and shedding light on a unique GAS pathogenic mechanism.

Conclusions

This study reveals a novel SLO-PLG interaction, highlighting the complex interplay between host hemostasis and bacterial pathogenicity. Our findings not only uncover a previously unrecognized moonlighting function of SLO but also suggest potential therapeutic value in PLG-targeted thrombolysis. Furthermore, this work underscores the power of integrated MS- based approaches for unraveling uncharacterized associations between proteins, contributing to a deeper understanding of host-pathogen interactions, paving the way for future research on bacterial virulence mechanisms and their potential therapeutic targets.

PROGRAM

PP01.44: Novel Machine Learning Algorithm for Real-time Optimization of Mass Spectrometry-based Proteomics Data Acquisition to Improve Protein-protein Interaction Detection Sensitivity.

Iryna Abramchuk, Canada

Optimizing mass spectrometry-based proteomics data acquisition remains a challenge in maximizing biological information extracted from samples. Most data-dependent acquisition techniques redundantly collect tandem mass spectra from abundant proteins at the expense of less abundant ones. Techniques employing real-time peptide and protein identification during experiments can help prevent such redundant data acquisition and favour more efficient mass spectrometry resource distribution. Despite these efforts to avoid redundant data collection, affinity purification experiments coupled to mass spectrometry acquire large amounts of tandem mass spectra from contaminating proteins. The resources used to identify such biologically irrelevant interactions can be better redistributed to detect bona fide protein-protein interactions.

Herein, we present a novel machine learning algorithm for the real-time detection of contaminating proteins in affinity purification experiments coupled to mass spectrometry. Our approach improves and supplements MealTime-MS (Pelletier et al., 2020) - a supervised learning tool that assesses the confidence of protein identifications in real-time and prevents further data acquisition from proteins upon their confident identification. We extended MealTime-MS by building a logistic regression classifier that uses ion mobility information to improve data acquisition control on Bruker timsTOF Pro instruments. We show using simulations that our new model identifies 98% of the proteins identified in standard human cell lysate mass spectrometry analyses using only 72% of the mass spectra.

We then supplemented MealTime-MS with a Bayesian inference model to detect contamination events in real-time during affinity purification experiments coupled to mass spectrometry. When tested on a subset of the BioPlex (Huttlin et al., 2021) dataset, the model identified 3258 protein-protein interactions (false discovery rate=15.1%), comparing favourably to the popular tool SAINT (Choi et al., 2011), which identified 3326 interactions (false discovery rate=24.5%) for the same data.

Overall, our novel method uses real-time mass spectrometry data analysis to efficiently distribute resources and improve protein-protein interaction characterization.

PP01.46: Multi-layered Proteomics Approach to Elucidate Molecular Mechanisms of EGFR Signaling Diversity

Akihiro Eguchi, Denmark

EGFR induces diverse cellular signaling and function depending on ligand and biological context. This phenomenon is known as functional selectivity, but the molecular mechanisms responsible for this diversity in signaling remain unclear. When EGFR is activated, phosphorylated tyrosine sites in its C-terminal tail serve as docking sites for adaptor proteins that function as signal transducers. This EGFR-centric interaction network and the resulting phosphorylation cascades triggered by the recruited proteins are determinants of receptor fate and downstream signaling. Thus, elucidating the molecular mechanisms underlying the functional selectivity requires a comprehensive understanding of the molecular events happening on a global scale.

To investigate this, we applied a multi-layered high-throughput proteomics approach that combines time-resolved proteome, phosphoproteome, and proximity ligation-based interaction network analysis. We characterized the effects of natural and engineered EGFR ligands as well as the importance of individual phosphotyrosine sites on EGFR and linked this to signaling kinetics and endocytic fate of the receptor. With this comprehensive set of data, we show how signaling proteins are differently regulated and dissect the temporal changes of phosphorylation and interactions to explore the mechanisms underlying functional selectivity. Clustering of 1,218 regulated interactors in response to the six highest-affinity ligands showed nine distinct temporal profiles providing information on timing of receptor recruitment, e.g., early, intermediate, or late, and whether the activated signaling response is transient or sustained. Moreover, to pinpoint where the interaction network changes, we precisely evaluated the selectivity of phosphorylated tyrosine residues in the EGFR C-terminal tail for recruited proteins using tyrosine mutated EGFR constructs and found both known and new EGFR interactors that are recruited in a site-specific manner. The integration of these data provides a huge resource of the molecular network of EGFR signaling, supporting a deeper understanding of biased-ligand receptor signaling mechanisms and the discovery of novel drug targets.

PP01.48: ChIP-MS Reveals the Local Chromatin Composition by Label-free Quantitative Proteomics

Dennis Kappei, Singapore

Chromatin Immunoprecipitation (ChIP) has been a cornerstone for epigenetic research over the last decades, but even coupled to sequencing approaches (ChIP-seq) it is ultimately limited to one protein at a time. In a complementary effort, we here combined ChIP with label-free quantitative (LFQ) mass spectrometry (ChIP-MS) to interrogate local chromatin compositions. We reasoned that as a gold standard, ChIP-MS should be able to enrich multiple proteins that independently bind to the same chromatin fragment. To this end, we first demonstrate our workflow at telomeres as a particularly well-characterised genomic locus and establish that our gold standard expectation requires double cross-linking for stabilisation of chromatin-bound complexes. In return, cross-linking is a source of potential false-positives, and we exemplify how to mitigate this challenge in ChIP-MS reactions via loss-of-function controls such as CRISPR knock-outs or degron knock-ins. Our approach is highly versatile as demonstrated across telomere-binding proteins and general transcription factors using both cell line and tissue samples. Finally, to address locus-specificity, we adopted the use of endonuclease-deficient Cas9 (dCas9) as an anchor protein and demonstrate specific enrichment of telomeric chromatin. In sum, we have established a simple, robust ChIP-MS workflow based on comparably low input quantities that can be applied to any DNA-binding protein and in a locus-specific manner using dCas9.

PP01.50: Global Interactome Mapping Reveals Pro-tumorigenic Interactions of NF-κB in Breast Cancer

Petr Lapcik, Czech Republic

Introduction: NF-κB pathway plays a key role in immune response and inflammation, however, our previous results supported by data from other studies show its role also in cancer development and progression, including lymph node metastasis of luminal A breast cancer [1]. Here we used size exclusion chromatography (SEC) fractionation and protein correlation profiling (PCP) [2] to study the impact of NF-κB modulation on global protein interactome dynamics in luminal A breast cancer model.

Methods: Liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis of 160 SEC fractions of native MCF-7 lysates with inhibited or innate NF-κB activity was performed and the PrInCE algorithm [3] was applied for protein interaction mapping. AlphaPulldown methodology was employed for protein complex prediction. Immunoprecipitation with LC-MS/MS detection was used for characterization of NF-κB factor RELA interactome.

Results: The co-fractionation experiment led to identification of 5460 protein groups in total (FDR = 0.01) and to detection of 7568 interactions among 1520 protein groups. Of these, 2564 interactions have been validated in independent datasets. NF-κB modulation was associated with rearrangement of protein complexes involved in NF-κB signaling and immune response, cell cycle regulation and DNA replication. Central NF-κB transcription factor RELA co-eluted with interactors of NF-κB activator PRMT5, both established and new complexes were confirmed by AlphaPulldown prediction. A complementary immunoprecipitation experiment recapitulated RELA interactions with other NF-κB factors, and associated NF-κB inhibition with decreased binding of NF-κB activators to RELA.

Conclusions: This study describes an extensive network of pro-tumorigenic NF-κB interactions and its rearrangement in breast cancer that may have a therapeutic implications in tumors with high NF-κB activity.

References:
1.   Bouchal P. et al.: Mol Cell Proteomics, 14(7):1814-30. (2015)
2.   Kristensen A.R. et al.: Nat Methods, 9(9):907-9. (2012)
3.   Stacey R.G. et al.: BMC Bioinformatics, 23;18(1):457 (2017)

PROGRAM

PP01.52: Mass Spectrometry Analysis of the ADORA2A Interactome in a PDRN Environment Using LC-MS

Wonseok Lee, Republic of Korea

Introduction
PDRN(Poly DeoxyRiboNucleotide) is a tissue regeneration substance that closely resembles human DNA and is present in human cells, mullets, salmons, and flatfishes. It stimulates physiological regeneration and metabolic activity. The regenerative and metabolic effects of PDRN are attributed to the activation of ADORA2A (Adenosine A2A Receiver), which increases the production of angiogenesis factors. Activation of ADORA2A also leads to an increase in the expression of interactome proteins with similar function. Therefore, our objective was to investigate the differential expression of ADORA2A and interactome proteins in the presence of PDRN. To ensure reliable analysis, proteins were enzymatically digested, and peptide-level analysis was conducted using LC-MS.

Methods
The control group consisted of mock samples, while the experimental group comprised HUVEC cells treated with salmon sperm, flatfish sperm, and flatfish testist for 6 hours. Cell disruption was performed using a bioruptor to extract proteins, which were then digested using trypsin. Finally, the peptide-level analysis using LC-MS confirmed the abundance of ADORA2A and interactome proteins.

Results
We quantified the differential expression of known ADORA2A-interacting sequences in humans. The analysis results were normalized using a 6×5 reference mix, and the fold change (FC) ratio was calculated. Statistical analysis was conducted to determine the significance of differences between the groups.

Conclusion
By comparing the groups, we observed differential expression of peptide sequences in HUVEC cells treated with flatfish sperm and salmon sperm. This finding indicates an increased abundance of ADORA2A-interactome when PDRN is present, suggesting individual variations in PDRN levels

PP01.54: Interactome Profiling of UGP2 Splice-forms Differently Prevailing in Normal and Cancer Tissue

Ekaterina Poverennaya, Russian Federation

Background. Multiple studies examined the phenomenon of isoform switching in human cancers and discovered that isoform switching is widespread, with hundreds to thousands of cases observed within cancer types. Although all of these studies used slightly different definitions of isoform switching, which in part led to a rather poor overlap between their results. Despite this, the presence of this phenomenon can be explained by the different functions of these isoforms (splice-forms), i.e. participation in diverse biological processes, and therefore interaction with distinct proteins.
Methods. For target gene selection the reanalysis of RNA-seq data available in TCGA was performed. Additional data about the possibility of obtaining modified Ha-tag splice-form sequences using genetic editing based on CRISPR/Cas9 was considered. CRISPR-Cas9 was used to obtain the label HA-tagged splice-forms for AP-MS.
Results. The UGP2 gene was chosen for functional annotation. This gene encodes two splice-forms, where the canonical form (Q16851-1) prevails in normal samples, and alternatively spliced form (Q16851-2) dominates in intestinal adenocarcinoma samples. HaCaT cells with equivalent expression of both splice-forms, were chosen based on analysis of public RNA-seq data. As a result of AP-MS analysis seven unique partner proteins for splice form 1 and four partner proteins for splice form 2 were found.
Conclusion. Comparative interactome profiling of UGP2 splice-forms in different cell lines allows us to fully reveal their cellular role.

PP01.56: Flashlight into the Function of Unannotated C11orf52 Using Affinity Purification Mass Spectrometry

Yeji Yang, Republic of Korea

For an enhanced understanding of the biological mechanisms of human disease, it is essential to investigate protein functions. In a previous study, we developed a prediction method of gene ontology (GO) terms by the I-TASSER/COFACTOR result, and we applied this to uPE1 in chromosome 11. Here, to validate the bioinformatics prediction of C11orf52, we utilized affinity purification and mass spectrometry to identify interacting partners of C11orf52. Using immunoprecipitation methods with three different peptide tags (Myc, Flag, and 2B8) in HEK 293T cell lines, we identified 79 candidate proteins that are expected to interact with C11orf52. The results of a pathway analysis of the GO and STRING database with candidate proteins showed that C11orf52 could be related to signaling receptor binding, cell−cell adhesion, and ribosome biogenesis. Then, we selected three partner candidates of DSG1, JUP, and PTPN11 for verification of the interaction with C11orf52 and confirmed them by colocalization at the cell−cell junctions by coimmunofluorescence experiments. On the basis of this study, we expect that C11orf52 is related to the Wnt signaling pathway via DSG1 from the protein−protein interactions, given the results of a comprehensive analysis of the bioinformatic predictions.

PP01.58: Unraveling Molecular Pathways and Implications of Triclosan Exposure on Adipocyte Dysfunction: Insights from Proteome Integral Solubility Alteration.

Susana Cristobal, Sweden

Adipocyte differentiation, a fundamental process in energy homeostasis and obesity development, is increasingly recognized as being influenced by environmental chemicals. Triclosan, a widely used antimicrobial agent found in numerous consumer products, has raised concerns regarding its potential adverse effects on human health. Perturbations in endocrine function are among the recognized health concerns associated with triclosan exposure. Identifying the specific targets of triclosan in adipocytes is essential for predicting the potential impact on adipose tissue dysfunction and the subsequent development of metabolic disorders, including obesity. In this study, we applied the Proteome Integral Solubility alteration (PISA) assay to identify protein targets of triclosan within the soluble proteome of 3T3-L1 preadipocytes across a wide range of concentrations. Subsequently, we characterized the altered molecular pathways associated with triclosan exposure. Our findings highlight impairment in clathrin-mediated endocytosis, which disrupts receptor internalization, including those involved in glucagon signaling. Additionally, compromised PKA activation hampers glucagon signaling, impeding the activation of adenylate cyclase and various metabolic processes, including CREB1 phosphorylation. Furthermore, the metabolism of cofactors, crucial for adipocyte metabolic activities, may also be affected. These interconnected pathways are pivotal in regulating adipocyte metabolism and function, underscoring the potential of this strategy to predict the health impact of exposure to metabolism-disrupting chemicals.

PP01.60: Transcriptome-wide Association Study Reveals Cholesterol Metabolism Gene Lpl is a Key Regulator of Cognitive Dysfunction

Wei Hu, China

Cholesterol metabolism in the brain plays a crucial role in normal physiological function and its aberrations are associated with cognitive dysfunction. The present study aimed to determine which cholesterol-related genes play a vital role in cognitive dysfunction and to dissect its underlying molecular mechanisms using a systems genetics approach in the BXD mice family. We first systematically analyzed the association of expression of 280 hippocampal genes related to cholesterol metabolism with cognition-related traits and identified Lpl as a critical regulator. This was further confirmed by phenome-wide association studies that indicate Lpl associated with hippocampus volume residuals and anxiety-related traits. By performing expression quantitative trait locus mapping, we demonstrate that Lpl is strongly cis-regulated in the BXD hippocampus. We also identified ~3300 genes significantly ($p < 0.05$) correlated with the Lpl expression. Those genes mainly involve in the regulation of neuron-related traits through MAPK signaling pathway, axon guidance, synaptic vesicle cycle, and NF-kappa B signaling pathway. Further, Protein-protein interaction network analysis identified several direct interactors of Lpl, including Rab3a, Akt1, Igf1, Crp, and Lrp1, which indicates that Lpl involves in the regulation of cognitive dysfunction through Rab3a-mediated synaptic vesicle cycle and Akt1/Igf1/Crp/Lrp1 mediated MAPK signaling pathway. Our findings demonstrate the importance of the Lpl, among the cholesterol-related genes, in regulating cognitive dysfunction, and highlight the potential signaling pathways, which may serve as novel therapeutic targets for the treatment of cognitive dysfunction.

PP01.62: Advances in Metabolomics Using Untargeted Ion Chromatography Coupled with an Orbitrap Mass Spectrometer for Profiling.

Wai-Chi Man, United Kingdom

Background:
Untargeted metabolomics profiling attempts to identify, quantify, and pathway map the metabolites present in a biological system. Because metabolites are the end products of processes occurring in cells, tissues, and organs, metabolomics provides a snapshot of a biological system not possible with proteomics or genomics.
Method:
Coupling an Ion Chromatograph such as Thermo Scientific™ Dionex™ ICS-4000 Capillary High-Pressure™ Ion Chromatography System (HPIC™) with the Thermo Scientific™ Q Exactive™ Hybrid Quadrupole-Orbitrap Mass Spectrometer (IC-MS), utilizing anion exchange to separate the polar and very hydrophilic metabolites. The mobile phase is a potassium hydroxide KOH) eluent that is electrolytically generated to ensure purity and constant reproducibility. Once, that is through the anion exchange separator column, the KOH is desalted by a suppressor before entering the mass spectrometer. This can also be achieved using a HPIC with microbore setup.
Results:
During the preliminary evaluations of the 42 metabolite standards, exceptional results for the tricarboxylic acid cycle (TCA) and glycolysis cycle metabolites were achieved using IC with High-resolution accurate-mass (HRAM) Orbitrap mass spectrometer-based detection. As these metabolites are traditionally difficult to analyze, the remainder of the experiments were focused on the 21 polar metabolites. The separation and responses were obtained by IC-HRAM analysis of the 21 polar metabolites at 600 ppb and 60 ppt, and by hydrophilic interaction liquid chromatography (HILIC)-HRAM analysis at 600 ppb.
Conclusion:
The outstanding resolution of IC enabled separations of isomeric polar metabolites and isobaric metabolites with identical MS/MS spectra and identification based on RT matches with standard compounds. When applied to metabolic profiling, IC-MS detected significantly more peaks than HILIC-MS and RP-UHPLC-MS. In particular, IC-MS detected a larger number of sugar-phosphate variants. Enhanced separation and detection of polar anionic metabolites establish IC-HRAM analysis as a technique that complements HILIC-HRAM and Revere-Phase (RP)-UHPLC-HRAM analyses for metabolomics applications.

PP01.64: C18ORF25 is a Novel Exercise-Regulated AMPK Substrate Regulating Skeletal Muscle Function

Yaan Kit Ng, Australia

Introduction: Exercise regulates a diverse array of phosphorylation networks thought to promote numerous health benefits. These networks hold great promise as novel therapeutic targets. We have recently shown the uncharacterised protein, C18ORF25, is significantly phosphorylated at Ser-67 in human skeletal muscle subject to sprint, endurance, and resistance exercise. A machine-learning approach also revealed Ser-67 as a probable AMPK substrate. Given the well described role of AMPK in metabolic adaptations during exercise, we hypothesise that C18ORF25 is a novel regulator of exercise adaptations.

Methods: We generated a phospho-specific antibody for Ser-67 on C18ORF25 to probe for upstream signalling. To functionally characterise C18ORF25, we generated a whole-body knockout (KO) mouse model which we subjected to a phenotyping protocol. Investigation of downstream signalling involved performing proteomics and contraction-induced phosphoproteomics on skeletal muscle of wild type (WT) vs KO mice.

Results: Here, we validate phosphorylation of Ser-67 on C18ORF25 as a novel exercise-regulated AMPK substrate. Our data reveal significantly increased adiposity and decreased lean mass in KO mice. Interestingly, KO mice displayed no major differences in whole body glucose tolerance or skeletal muscle insulin sensitivity as assessed by ex vivo insulin-stimulated glucose uptake. Furthermore, forced treadmill exercise and ex vivo contractile function testing revealed KO mice fatigue quicker and have significant reductions in soleus force production compared to WT siblings. This was associated with a drastic reduction in fibre cross sectional area.

Moreover, proteomic analysis of tibialis anterior muscles coupled with contraction-induced phosphoproteomic analysis of soleus muscles from KO mice revealed impaired PKA-dependent signalling, and attenuated contraction-induced phosphorylation of several contractile and calcium handling proteins.

Conclusion: Taken together, our data suggest C18ORF25 plays a vital role in AMPK-mediated skeletal muscle adaptations to exercise and that loss of C18ORF25 attenuates several known exercise-induced signalling pathways and kinases including PKA that mediate skeletal muscle contractile function.

PP01.66: Analysis of m6A Modifications in HepG2 Cells

Viktoriia Arzumanian, Russian Federation

Introduction: A weak correlation between human transcriptome and proteome can be explained by different post-transcriptional processes including RNA modifications. Among these modifications, the methylation of adenosine in the N6 position (m6A) is the most prevalent and affects both RNA splicing and translation. Recently direct RNAseq by Oxford Nanopore technologies (ONT) emerged as a tool facilitating the detection of m6A modification. This study aimed to investigate the impact of m6a modifications on the translatome and proteome.
Methods: We analyzed transcriptome (direct RNAseq) and translatome (cDNA) data obtained by ONT for a single sample of the HepG2 cell line using Guppy (version 6.2.1), minimap2 (version 2.24-r1122) and salmon (version 1.6.0) to count gene expression. To identify m6A modifications from direct RNAseq we used nanom6A. The transcriptome was sequenced in five repeats, while the translatome was sequenced once. Additionally, the proteomics profiling was performed for the same samples.
Results: We detected 481 genes containing m6A modifications for HepG2 cells. More than half of these modified genes show a low correlation with protein expression. In contrast, only 10% of these genes did not correlate with the results obtained from translatome sequencing. These findings highlight a stronger relationship between the transcriptome and translatome, emphasizing the critical role of the translation step in accurately deciphering the effects of m6A modifications on protein expression.
Conclusion: Our study underscores the importance of RNA modifications at the epitranscriptome level in regulating gene expression and protein formation. The observed discrepancies between m6A-modified genes and protein expression further emphasize the intricate regulatory mechanisms underlying post-transcriptional processes.

PP01.68: ExpressVis: a Biologist-oriented Interactive Web Server for Exploring Multi-omics Data

Cheng Chang, China

In the era of multi-omics in life sciences, biologists are benefited from the big data of multi-omics, resulting the new data-driven research paradigm. However, conveniently obtaining biological insights from multi-omics data is an evitable challenge for biologists with few coding skills. To solve this issue, web server is an ideal choice which usually does not need many computational resources. Unfortunately, the existing web servers hardly make a good balance between professionality and usability.

Here, we propose an easy-to-use and interactive web server named ExpressVis, which contains six modules covering the complete procedures from data preprocessing to biological enrichment analysis and finally to phenotype-associated analysis. The first module "ImportData" directly reads the files in public format in transcriptomics (RNA-seq and microarray) or the result files of the commonly-used tools in proteomics. Users can import multiple datasets and then analyze simultaneously. The second module "DiffExp" performs differential analysis using two popular algorithms (DESeq2 and Limma) and provides an interactive table and a volcano plot, which is updated from PANDA-view we published before (Bioinformatics 2018, 34, 3594-3596). Users can perform functional analysis for the differentially expressed genes. The third module "ClusterExp" provides clickable heatmaps for hierarchical clustering or k-means clustering, where users can easily select genes/proteins of interest to perform functional enrichment analysis. The fourth and fifth modules "KeggExp" and "PPIExp" are the updated version of our previous works (Bioinformatics 2019, 35, 1430-1432 and J Proteome Res 2019, 18, 633-641). In this updated version, users can view genes expression profiles and highlight differentially expressed genes in multiple datasets. The sixth module "ClinicalExp" provides interactive Kaplan-Meier curves and forest plots for hazard ratio when users upload prognosis information. Based on these biologist-friendly new features, ExpressVis (https://omicsmining.ncpsb.org.cn/ExpressVis) provides an easy-to-use solution for exploring multi-omics data.

PP01.70: Therapeutic Target Discovery Using Proteome-Wide Analyses in Large Population Health Studies Like the UK Biobank

Ray Chen, United States

Understanding the dynamics of the human proteome is crucial for identifying biomarkers to be used as measurable indicators for disease severity and progression, patient stratification, and drug development. The Proximity Extension Assay (PEA) is a technology that translates protein information into actionable insights across large samples sizes in both healthy and disease samples. The high-throughput nature of the assay is enabled by linking protein-specific antibodies to DNA-encoded tags that can be read out on a next generation sequencer. Here we have combined the unique PEA technology with automated sample preparation and high-throughput sequencing readout for parallel measurement of ~3,000 proteins for up to 384 samples at a time, generating over 1 million data points per run. Characterizing the proteome alongside genetic and clinical data enables a pQTL framework to not only validate known clinical targets and identify new clinical targets but to also suggest repurposing opportunities of clinical candidates for new indications. Join us to hear how proteomics is impacting large population health studies like the UK Biobank, SCALLOP and TOPMed to advance precision and personalized medicine.

PP01.72: Multiome Reveals the Molecular Regulation in Post-TKI Lung Adenocarcinoma

YiJing Hsiao, Taiwan

Background:
EGFR mutation is the leading driver oncogene in NSCLC, especially in East Asia. Several EGFR-Tyrosine Kinase Inhibitors (TKIs) are available, but most NSCLC patients develop EGFR TKI resistance within one year. Although several TKI-resistant mechanisms are elicited by DNA aberrations, how to treat the remaining patients is a clinical unmet need.
Methods:
Using isobaric labeling with TMT-16plex, the peptides from the tumor and adjacent normal tissues were labeled in each channel. More than 10,000 unique proteins and >40,000 phosphopeptides were quantified from 49 patients. Moreover, the transcriptome was also analyzed by RNAseq. Unsupervised consensus clustering was further used to classify the TKI-treated LUAD patients into molecular subtypes.
Results:
The proteome and phosphoproteome profiles categorize into 3 clusters for proteome and 4 clusters for phosphoproteome. We identified 3040 differentially regulated proteins between the three subtypes (log2 T/N values, ANOVA, $p < 0.05$). Proteome subtype 1 (P1) showed the enrichment for KEGG pathways in transcription/translation and repair, having significantly overexpressing proteins in spliceosome, RNA processing, and DNA repairs. Interestingly, proteome subtype 2 (P2) showed key pathways in infectious disease and immune related signaling pathways. Proteome subtype 3 (P3) showed an overall higher protein expression level in metabolism. We identified 246 differentially regulated phosphosites through the kinase-substrate regulation between the phosphoproteomic subtypes (log2 T/N values, ANOVA, $p < 0.05$). About 100 upregulated phosphosites enriched in phosphoproteomic subtype 2 (PP2) displayed pathways in spliceosome, proteoglycans in cancer, and homologous recombination (Benj. Hoch. FDR < 0.1). Interestingly, phosphosites from key pathways, including MAPK signaling pathway, showed an overall higher phosphorylation in subtype 3. The RNAseq results reveal that the cell cycle is significantly associated with relapse.
Conclusions:
The significant pathways of proteome/phosphoproteome reveal the missing puzzle of EGFR-TKI resistant mechanisms. Proteome/phosphoproteome provides another drug target option for the TKI treatment of failed patients.

PP01.74: Building of a Massive Spectral Library Based on Experimental Data Applying Real Analysis Conditions

Dooun Jang, Republic of Korea

Liquid chromatography-mass spectrometry (LC-MS) is a powerful technique for quantifying disease-specific target proteins. However, the complexity of the obtained results can pose challenges in interpretation. To address this issue, spectral libraries have emerged as valuable resources for improving identification accuracy and facilitating the interpretation of complex LC-MS results. These libraries are generated from either experimental data or in silico-generated spectra based on protein sequence databases. While experimental data can be accessed as an open resource, building and integrating it into a large-scale library poses resource-intensive challenges. The aim of our research is to create a new and comprehensive spectral library by building and integrating experimental data.

We utilized raw data obtained from ProteomeXchange, the National Cancer Institute Proteomic Data Commons, and iProX to construct a substantial spectral library. The analysis conditions for each spectral library were collected from published white papers, the library was constructed using MSAmanda as the searching algorithm. By applying this library, we identified proteins and peptides that overlap with reviewed human proteins as well as those from various organisms. Furthermore, we compared the results obtained using our library with those derived from the existing spectral library, identifying significant differences.

Through LC-MS analysis using the newly constructed spectral libraries, we confirmed the presence of overlapping proteins and peptides between human and other organism models. Moreover, while the MassIVE-KB library applies the same analysis conditions to all experimental data sets, our approach considers specific analysis conditions for each experimental set, resulting in expected variations inaccuracy.

This study employed a large-scale spectral library constructed with consideration of actural experimental conditions to validate LC-MS analysis results. The utilization of such a library is expected to bridge the gap between the application results obtained from the MassIVE-KB library, which was built under a single set of conditions.

PROGRAM

PP01.76: Proteogenomic Analysis of Longitudinal Trajectory of Glioblastoma Evolution

Kyung-Hee Kim, Republic of Korea

The evolutionary trajectory of glioblastoma after therapy is a multifaceted biological process that extends beyond discrete genetic alterations alone. Here, we performed a proteogenomic analysis of 123 longitudinal glioblastoma pairs, temporally separated by standard-of-care treatment. Integrative analyses identified a prevalence of a highly proliferative cellular state at diagnosis and its replacement by recurrent tumors sharing activation of neuronal differentiation and synaptogenic pathways. Proteomic and phosphoproteomic analyses revealed that the molecular transition from the proliferative to the neuronal state at recurrence is marked by coherent post-translational activation of the WNT/PCP signaling pathway and the BRAF protein kinase. Multi-omic analysis of the Patient-Derived Xenograft model demonstrated similar patterns of evolutionary trajectory, marked by activation of neuronal signaling programs that were consistently observed in longitudinal patients. Inhibition of the BRAF kinase with small molecule inhibitors impaired both neuronal differentiation and migration capability of recurrent glioblastoma cells, which are the phenotypic hallmarks of glioblastoma progression after therapy. This work provides comprehensive insights into the biological mechanisms of glioblastoma evolution and treatment resistance and highlights new therapeutic opportunities to effectively counter them in the clinic.

PP01.78: Deep Unbiased Interrogation of the Human Plasma Proteome in a Cancer Cohort of 3000 Subjects

Joon-yong Lee, United States

Untargeted plasma proteomics using mass spectrometry (MS) has emerged as a powerful tool for biomarker discovery in human diseases. However, the technology has achieved limited success in translation to actionable clinical tests due to the challenges of achieving reproducible detection and quantification with the desired statistical power, mainly stemming from the complexity of the human plasma proteome and existing technological limitations.

To overcome these challenges, PrognomiQ has developed a high-throughput proteomics platform, integrating the Seer Proteograph™ Product Suite with Bruker TIMSTOF HT™ mass spectrometers. This platform enables robust quantification of thousands of proteins per sample across thousands of subjects.

Here we report results from a 3044-subject study with K2-EDTA plasma samples collected across 58 clinical sites including 1287 pathology-confirmed, untreated cancer cases and 1757 non-cancer controls. The samples were processed using Seer's 5-nanoparticle protocol and run across four Bruker TIMSTOF HT mass spectrometers with a throughput of 168 subject samples per week. Intra- and inter-instrument median coefficient of variation for control samples were 24.3% and 34.3%, respectively. In an interim analysis on data from 1400 subjects (7000 sample injections across five nanoparticles) we uniquely identified 46155 peptides and 5390 protein groups, present in at least 25% of the subjects. The identified proteins covered 55.9% of the 3486 proteins in the Human Plasma Proteome Project (HPPP) database. We detected high abundance proteins like albumin and ceruloplasmin with estimated concentrations of 0.78 and 0.83 mg/ml, respectively, and low abundance proteins like guanylate cyclase and phospholipase A-2 (~5 pg/ml), demonstrating the quantitative ability of the PrognomiQ platform across the dynamic range of the human plasma proteome.

In conclusion, we demonstrated the ability to successfully identify a large number of unique peptides and protein groups across large cohorts with precise quantitation. These findings hold promise for the development of highly sensitive diagnostic tests.

![HUPO BUSAN 2023 SEP 17-21 22nd Human Proteome Organization World Congress]

See you in Busan!
BEXCO, Busan, Korea
🌐 2023.hupo.org
🐦 f 📷 in #HUPO2023

Together Through Proteomics

PROGRAM

PP01.80: Proteogenomic Landscape of East-Asian Breast Cancer Reveals Pathogenesis and Subtypes for Prognostic and Therapeutic Opportunities

Kuen-Tyng Lin, Taiwan

Breast cancer (BC) in East Asia is characterized by remarkably higher and increasing incidence of 10-15 years younger generations, high prevalence in luminal type compared to Western women. However, the etiology and molecular mechanisms underlying the disease remain poorly understood. To study the early-stage, younger and luminal subtype-predominant Asian BC, we presented a deep proteogenomics landscape of a prospective cohort of 140 treatment-naive patients consisting of majority at early stage (78.6%) and luminal type (79.3%) disease. We performed a comprehensive proteogenomics analysis of patient-matched BC tumors and normal adjacent tissues (NAT), which were enriched in younger premenopausal patients with a high prevalence of luminal subtypes from Taiwanese patients. The integrated multi-omics dataset and clinical feature were used to identify subtypes and their molecular characteristics and druggable pathways. The multilayer proteogenomic architecture revealed distinct endogenous and environmental carcinogen-associated mutagenesis, including unique etiology in younger patients potentially benefiting from standard adjuvant chemotherapy. A proteomics-informed classification resolves luminal heterogeneity, defines high-risk recurrent luminal disease and nominates biomarkers and actionable druggable pathways for potential targets. In summary, this study provides a proteogenomics-transformative early-management guide to guide prognosis, patient stratification and new molecular insight to precision therapeutics beyond standard of care.

PP01.82: Drug Identification Using Genomic Feature, Kinase Activity Profiles, and Drug Screening with Patient-derived Models in Giant Cell Tumor of Bone

Rei Noguchi, Japan

[Background]Giant cell tumor of bone (GCTB), a rare intermediate malignant bone tumor, exhibits both local recurrence and pulmonary metastasis after curative surgery. The rarity of disease causes difficulty to promote preclinical research and novel therapy. We aimed drug repositioning, a process of finding new therapeutic indications for existing drugs, as a strategy to identify druggable candidates in GCTB, and focused on tyrosine kinase inhibitors which were approved by FDA in oncology.
[Materials and Methods] Multi-omics analysis of patient-derived GCTB cell lines was performed using genomics features, kinase activity profiles, and drug screening data. The patient-derived cell lines were established from tumor tissues. H3-3A mutations known as GCTB hotspot mutations were examined. Copy number alterations and somatic mutations were examined using SNParray and NCC OncoPanel, respectively. The comprehensive kinase activity profiles were investigated by three-dimensional peptide array for 100 tyrosine kinases. Using the kinase activity profiles, single-sample gene set enrichment analysis (ssGSEA) was performed. Tumor suppressive effects of 60 kinase inhibitors were screened.
[Results] Eight patient-derived GCTB cell lines were established. H3-3A hotspot mutations were identified in all cell lines. Recurrent copy number variants and somatic mutations were not found. Comprehensive kinase activity analysis revealed that PDGFRB, Lck, ZAP70, and Syk were highly activated, and ssGSEA demonstrated that EGFR pathway was significantly enriched in the cell lines. Drug screening elucidated nine kinase inhibitors targeting ALK, c-MET, EGFR, PDGFRB, or VEGFR. Integrated with kinase activity profiles and drug screening data, sunitinib and sunitinib malate targeting PDGFRB were identified as kinase inhibitors in all cell lines.
[Conclusions] Genomic features showed no genomic alterations in targetable kinases. Kinase activity profiles detected highly aberrant kinases, and drug screening data identified two kinase inhibitors consistent with detected aberrant kinase. Consequently, druggable candidates were elucidated by proteomic approach rather than comprehensive genomic profiles.

PP01.84: Omics Approaches for the Understanding of Herbs Used in Kampo Topical Medications

Manon Paul-Traversaz, France

The Kampo is the traditional Japanese herbal medicine, which offers treatments for a several therapeutic indications including dermatological ones. Among Kampo formulas, three topical preparations are used for skin wound healing: Shiunko, Chuoko and Shinsen taitsuko. All have in common sesame oil and beeswax in which crude drugs are extracted. Among the herbs used are representatives of the botanical genera Angelica, Lithospermum, Curcuma, Phellodendron, Paeonia, Rheum, Rehmannia, Scrophularia and Cinnamomum.

While the Kampo drinkable formulas are widely standardized, it is not the case yet for topical formulas. Therefore, for these preparations a great chemical and therapeutic variability can be observed depending on the origins and species of the plants used, and on the treatments and extraction methods applied to the crude drugs. Better understanding of this diversity and the possible rationalization of these topical treatments is consequently of great interest.

The study of topical Kampo ointments is complex because of their lipophilic aspect which makes both metabolomic and lipidomics studies challenging. However, suitable experimental protocols allow the assessment of the metabolites' diversity and of the biological action of Kampo topicals. Studies on oily plant extracts involved in Kampo ointments are carried out by LC/MS and LC-MS/MS. The raw data are then analyzed bioinformatically with tools such as W4M or MetGem. The realization of multivariate studies but also the one of molecular networks allow to better understand the influence of biotic and abiotic factors, the impact of traditional preparation and extraction processes, on natural compounds diversity. These influent parameters, correlated to a variety of biological effects on wound healing, need to be studied further in order to identify compounds favorably involved in wound healing.

PP01.86: The Effect of Exercise on the Serum Proteomes and Metabolomes of Elite-level Athletes in Different Sports Disciplines

Yoondam Seo, Republic of Korea

Proteomics and metabolomics are closely interconnected fields that provide valuable insights into the complex mechanisms underlying biological systems. Proteins play a pivotal role in metabolic processes by influencing the production and utilization of metabolites through their expression levels and activities. The integration of proteomic and metabolomic data facilitates a deeper understanding of biological phenomena, enabling a holistic exploration of molecular interactions and dynamics within organisms. In this study, we focused on investigating proteomics and metabolomics profiles in the serum of elite-level athletes representing diverse sports disciplines. To conduct proteomic analysis of serum samples, we employed a series of experimental techniques. Initially, serum pools were subjected to pre-treatment to construct a data-dependent acquisition library. Subsequently, gas-phase fractionation was utilized for data-independent acquisition analysis, allowing for comprehensive coverage of the proteome. Concurrently, for metabolomic analysis, an in-house library containing approximately 1000 standard substances was established to facilitate metabolite identification and quantification in serum samples. In this study, we conducted protein profiling and metabolite profiling on serum samples obtained from 300 elite-level athletes. The athletes were divided into two groups: the high-power group and the high-endurance group. Additionally, we compared these groups to a sedentary control group and an elite-level athlete group. Exercise induces systematic changes in the average concentrations of proteins and metabolites related to energy metabolism and various branches of metabolism. These results suggest that elite athletes participating in high-power and high-endurance activities exhibit a distinct metabolic profile.

PROGRAM

PP01.88: Integrating DIA-NN Software Analysis of Data-independent Acquisition Data into a Cloud Processing Pipeline

Katherine Tran, Canada

Combined omics disciplines have proven to be more powerful than individual disciplines. However, multi-omics data analysis workflows can be time-consuming, as consolidating and interpreting various results outputs especially when using different scoring schemas and criteria is often challenging. A cloud-based suite of software tools has been demonstrated to provide researchers with tools for multi-omics data management, compound identification and quantification, statistical analysis and pathway analysis to streamline biomarker discovery studies. To further facilitate data processing for high-throughput proteomics workflows, this cloud-based software suite extends its SWATH DIA (data independent acquisition) data processing by supporting visualization and statistical interpretation of DIA-NN software results. DIA-NN software is a widely used proteomics processing platform that leverages neural networks and powerful quantification and inference algorithms to achieve confident protein and peptide identification. Here, a processing workflow for proteomics analysis is presented using data-dependent acquisition (DDA) and DIA data. In this workflow, a spectral library was generated from DDA data using the Ion Library app in the cloud processing suite. DIA data were subsequently processed using this ion library in DIA-NN software and evaluated in the DIA Results app. Results generated using this workflow can be rapidly and securely shared with collaborators.

PP01.90: Understanding all of Biology: Simultaneous, High-throughput Si-Trap Multiomics Sample Preparation

John Wilson, United States

Life is influenced by multiple classes of biomolecules, but sample preparation often focuses on removing or destroying all classes except the one of interest. However, this limitation hampers our ability to explore diagnostic, prognostic, therapeutic, or predictive biomolecules. Integrating omics fields is particularly challenging during sample preparation. We present advancements in Si-Trap technology that enable reproducible and rapid sample preparation of multiple biomolecule classes in an affordable and high-throughput manner.

Healthy and cancerous tissue samples, along with adjacent normal pairs, were processed using Si-Trap. Samples were dissolved in a detergent-free Si-Trap lysis buffer and applied to the Si-Trap column, which separated small and large molecules. Large molecules were then processed in-situ using chemical and enzymatic methods. Proteomics and metabolomics analysis were performed on various recovered molecular classes. Proteomics results were compared to S-Trap sample processing.

Si-Trap demonstrated its ability to provide a simultaneous multiomics analysis platform in a study of clear cell renal carcinoma and adjacent noncancerous tissue sections. Si-Trap's detergent-free sample dissolution allowed the recovery of minimum metabolomic and proteomic fractions, while lipidomics, glycomics, and transcriptomics fractions could also be generated. Si-Trap sample processing was rapid, taking only minutes per sample, and could be performed in loose spin columns or automated 96-well format. Proteomics and metabolomics analysis revealed concordant changes in enzymes and substrates, highlighting the role of the carnitine system in cancer metabolic plasticity. Si-Trap showed comparable performance to S-Trap sample processing.

Si-Trap sample processing can be automated using an affordable Tecan A200 positive pressure workstation, making it accessible to research and clinical labs. It offers a high-throughput solution for multiomics analyses and holds promise for future clinical implementations. Si-Trap is expected to become an essential tool in laboratory and clinical settings, enabling novel discoveries and ushering in a new era of clinical proteomics.

PP01.92: Transcriptional Landscape and Novel Candidate Genes in Heart Failure

Fuyi Xu, China

Heart failure (HF) is a significant cause of morbidity and mortality worldwide. However, its underlying genetic regulating mechanisms have not been fully elucidated. Here, we assayed electrocardiogram and echocardiogram traits, genome-wide genotype, and cardiac transcriptomes in ~40 BXD recombinant inbred strains, a large murine genetic reference population derived from crosses between C57BL/6J and DBA/2J mice over 20 generations. Our results demonstrated the cardiac traits showed strong heritability and a total of 38 genetic regulating loci were identified with quantitative trait locus (QTL) mapping. Genes such as Ptp4a1, Epc2, and Dap3 were nominated as novel QTL candidates. We further constructed the cardiac transcriptional landscape with expression QTL (eQTL) analysis and found ~2000 genes were cis-regulated and ~5500 were trans-regulated, respectively. Moreover, those eGenes were largely regulated with 10 genome-wide eQTL hotspots, with Nr2f1 and Pkdcc being defined as the master transcriptional regulators. In addition, by applying gene co-expression network analysis, pathway enrichment analysis, and Fisher's test, three gene co-expression modules and their hub genes (Mia3, Cep68, and Lig3) were found significantly enriched in the HF positive signals from human genome-wide association studies. Our findings provide novel insights into the genetic basis for HF that may ultimately contribute to new biomarkers, better diagnostics, and improved approaches to prevent or treat in HF patients.

PP01.94: Targeted Detection of Protein Complexes by Mini-Complexome Profiling (mCP)

Hugo Alejandro Amedei, Germany

Living organisms are sophisticated machinery that carries out intricate procedures to alter their surroundings, translate, and maintain homeostasis. On the molecular level, the majority of cellular functions, including energy generation, cell division, and replication, rely on the actions of protein complexes (PPCs). During the last decade, the co-fractionation mass spectrometry experiment was an emerging method to detect PPCs from a system biology perspective. Much effort has been made to detect PPCs in a system biology approach1, which usually includes big datasets from a thousand fractions2 to 81 fractions up to 546 PPCs detected in model cells like Hek2933.

Here we present a novel and systematic workflow, called mCP, for wide targeted detection of PPCs. It includes mild extraction of PPCs, fractionation by mini-Blue Native PAGE, in-gel digestion, and Mass spectrometry Data Independent Acquisition detection. PPCs detection was done using bioinformatics targeted analysis by our mCP custom-developed R package with a controlled False Discovery Rate (FDR) approach based on Monte-Carlo simulation.

We applied our protocol as a proof of principle on an established cell type for PPCs detection, Hek293 cells. About 362 annotated PPCs were detected. Then, we challenged mCP by investigating changes in the complexome of different heart compartments in isolated mouse cardiomyocytes. A total of about 5900 protein groups and around 30 annotated PPCs per compartment were detected. The resulting data provides a comparison of PPCs coming from heart compartments of a single experimental unit for the first time. Taken together, a considerable reduction of measurement time was achieved in comparison to traditional approaches3, i.e. 1day per cell-type vs to 2 months from previous studies3. A successful mini complexome profiling, with only 35 fractions and controlled FDR detection, was achieved. The reduced material requirements open the possibility of applying this workflow to sparse/rare samples, i.e. patient biopsies.

PP01.96: Standardized, High-throughPut Platform for Automated, Rapid, and Extensive Plasma Proteome Characterization

Manuel Bauer, Switzerland

Background
Blood plasma represents a rich source of disease biomarkers. However, discovering protein biomarker candidates in human plasma is exceptionally challenging. The high complexity and wide dynamic range of proteins complicate the in-depth profiling of the plasma proteome at the throughput required for the study of large cohorts in a reasonable time We developed a pipeline of automated sample preparation on the Fluent® liquid handling system using a novel plasma workflow that also facilitates the identification and quantification of low abundant proteins. Combined with the next-generation dia-PASEF® MS acquisition, it is designed for high-throughput yet profound characterization of the plasma proteome of large cohorts.
Methods
The automation platform consists of the Fluent® liquid-handling system with the positive pressure module Resolvex® A200 (Tecan). The automated sample processing covers every step from enrichment of low abundant proteins to LC-MS ready peptides employing the robust iST technology (PreOmics). A total of 96 samples/run can be prepared within one working day processing 20 μL of blood plasma/sample. Peptides were separated with a 20-min gradient on the nanoElute 2 system and analyzed using dia-PASEF® acquisition on the timsTOF HT (Bruker) Spectronaut 17 was used for direct DIA analysis (Biognosys).
Results
The high-throughput plasma pipeline was evaluated by processing 96 samples in parallel using human plasma from 3 healthy donors. From collected plasma samples to peptides, the automated sample preparation takes ~5 hours and requires minimal user intervention. Data obtained from analyzing 300 ng of peptides with the plasma enrichment step demonstrated a more than 2-fold increase in protein identifications compared to the raw plasma analysis. low technical variability within replicates was obtained confirming the robustness and reliability of the presented automated plasma pipeline.
Conclusions
This automated plasma proteome pipeline combines throughput with profound proteomic characterization, bringing simplicity into the challenging field of plasma proteomic.

PP01.98: Solution-stabilized TMT & TMTpro Reagents in 96 Well Plates for High-throughPut Sample Processing

Ryan Bomgarden, United States

TMT and TMTpro isobaric tags are the gold standard reagents for highly multiplexed by mass spectrometry (MS)-based proteomics, enabling relative quantification of up to 18 samples in a single LC-MS/MS acquisition. Due to their high reactivity, TMT reagents are typically stored as dry powder with desiccant to prevent tag hydrolysis with stability limited to just a few weeks after reconstitution in anhydrous, organic solvents such as acetonitrile (ACN). In addition, ACN solutions are difficult to handle using automated liquid handling platforms due to high volatility, low viscosity, and risk of chemical incompatibility with commonly used plastic consumables. To enhance reagent integrity during storage after reconstitution and improve automation compatibility, we have developed a dimethyl sulfoxide (DMSO)-based stabilization solution for TMT reagent stock solutions. Accelerated stability studies of TMT and TMTpro reagents stored in the stabilized DMSO solution as bulk stocks indicate >80% reactivity at -20°C for several years. Small amounts (<50 μg) of solution-stabilized reagents in foil-sealed, 96-well PCR microplates show >80% reactivity for 18 months at -20 °C and at a minimum of 5 weeks at room temperature. Preliminary experiments comparing reagents in stabilized DMSO versus ACN for HeLa digest labeling using standard protocols indicate equivalent or better performance in terms of labeling efficiency (≥99%) and numbers of identified and quantified peptides. In contrast to standard workflows which require a dry-down step to remove ACN prior to solid phase extraction (SPE) clean up, samples containing DMSO diluted to <10% showed equivalent labeled peptide recovery from C18 or mixed-mode SPE resins without need for a dry-down step. The ready-to-use 96-well PCR microplate format of solution-stabilized TMT & TMTpro reagents are ideal for sample labeling on automated liquid handling platforms used for high-throughput proteomics applications.

PP01.100: Optimizing Experimental Design for Multi-kit TMT Labeling of Mixed Proteomes

Jenna Cleyle, Canada

Introduction: Isobaric labels are well-known in proteomics as they allow the analysis of up to 18 samples in a single mass spectrometry run, while reducing technical variability and improving identification. As tandem mass tags (TMT) become more widely used, it is increasingly common to use multiple TMT kits in a single study. Many groups have accommodated the use of multiple kits by including a pooled sample in each kit to normalize between runs - however, this approach has primarily been used when working with a single cell type. We hypothesize that a single (or repeated) pooled sample does not make up a significant enough proportion of the mixed proteome sample. Thus, this does not trigger peptide identification in all kits, leading to an increase in missing values. We propose that TMT experiment design can be optimized to mitigate this by ensuring that the protein mixture is the same in each kit.

Methods: To evaluate this, we worked with three human cell lines derived from different tissues with substantially different proteomes and combined them using three TMT kits in various iterations: 1) one cell type per kit with a pooled sample; 2) one sample of each cell type per kit with a pooled sample; or 3) one sample of each cell type per kit with no pool. We investigated the ways in which these combinations affected sample clustering, peptide identification, and quantification.

Results and Conclusion: We found that having the same background proteome in each kit minimizes missing values and simplifies normalization. Despite the small reduction in identification that comes as a consequence of more complex mixtures being analyzed, combining samples in this way makes quantification more robust overall, minimizes missing values and should be adopted in multi-kit, multi-proteome studies to provide more reliable and reproducible results.

PP01.102: BeatBox and iST for Standardized FFPE Tissue Processing: A robust, High-throughput, Xylene-free Sample Preparation for Proteomic Analysis

Zuzana Demianova, Germany

Background
Formalin-fixed paraffin-embedded (FFPE) tissues are an invaluable resource for retrospective clinical studies due to their clinical metadata and huge biobanks. However, they require harsh conditions to remove cross-links and paraffin to effectively extract proteins. As a result, most protocols include a xylene-based deparaffinization step that is time-consuming, toxic and may result in sample loss. Here we present a xylene-free, high-throughput workflow combining the BeatBox tissue homogenizer and robust iST sample preparation allowing the parallel processing of up to 96 samples in one day.

Methods
This innovative BeatBox-iST sample preparation workflow begins with homogenizing the 10μm FFPE curls in the BeatBox (10 min, high settings), followed by a one-hour incubation at 80-95°C to de-crosslink, extract, reduce and alkylate the proteins. After spinning and cooling, the samples are transferred to clean plasticware with the solidified paraffin remaining in the primary tube. Finally, the samples were digested using trypsin/LysC, followed by optimized peptide clean-up to ensure complete removal of paraffin. Peptides were analyzed on a nano-LC coupled to a timsTOF mass spectrometer.

Results and conclusions
A step-by-step benchmark of this new workflow against a traditional xylene-based deparaffinization/sonication workflow was performed using freshly frozen or FFPE-stored mouse heart, kidney, and liver tissue. For FFPE tissues, BeatBox outperformed sonication, revealing a >10% increase in protein IDs, with a 43% increase for mouse heart tissue. Comparing FFPE tissues with the corresponding fresh tissues revealed up to 87% of shared proteins and similar dynamic ranges. BeatBox-iST protocol achieved excellent repeatability with median CVs <10% intraday and between 2 days (n=4).
In conclusion, the BeatBox-iST workflow for FFPE tissues enables safe, high-throughput and efficient sample preparation. It provides an answer to large-scale retrospective studies of FFPE tissue through the simultaneous processing of up to 96 samples in less than 6 hours.

PP01.104: Ultra-fast Narrow Window DIA Approach for Quantitative Analysis of Comprehensive Proteomes with High Sequencing Depth and Quantitative Accuracy

Ulises H Guzman, Denmark

The synergy of faster and more sensitive MS instruments with Data-Independent Acquisition (DIA) and advanced software solutions maximizes proteome coverage in a rapid and robust manner. In this study, we assessed the effectiveness of combining a new ultra-fast scanning narrow-window DIA strategy with single shot and multi-shot strategies for comprehensive proteome profiling. This new acquisition strategy is possible by coupling quadrupole and OrbitrapTM mass analyzers with a novel HRAM analyzer. The combination of these three analyzers enables DIA analyses with 2-Th narrow precursor isolation windows across the entire mass range dissolving the main differences between Data-Dependent Acquisition (DDA) and DIA. Briefly, the new mass spectrometer combines a high-resolution Orbitrap MS1 full scan with the ultra-fast MS2 scan speed of the new analyzer (~200Hz), covering the entire mass range with narrow windows and short cycle time. The ultrafast scanning speed of the new HRAM platform (> 10,000 MS/MS per minute) coupled with fast chromatographic separation (5-minutes LC gradient (180 SPD)) provides decreased peptide peak widths and increased MS signals while maintaining a high identification rate (1350 proteins/min) when compared to longer gradients. We used this set-up to acquire comprehensive proteomes by offline fractionating a HEK293 tryptic digest by high-pH reversed-phase (HpH) chromatography and injected 200 ng per fraction. We identified 12,135 protein groups (PG) and 223,596 peptides in only 6.1 hours. Further optimization of HpH scheme to 34 and 23 resulted in a similar proteome coverage (99.4% and 96.7% of the total, in 4.5h and 3.1h respectively). In contrast to multi-shot approaches, single-shot analysis allowed to confidently quantify >9,000 protein-coding genes in 30 min LC gradient. Altogether, we provide a solution for full proteome profiling by using HpH fractionation to achieve fast acquisition with a comparable depth to next-generation RNA sequencing. In addition, we provide guidelines for obtaining comprehensive single-shot proteomes.

PP01.106: Proximity Extension Assay in Combination with Next Generation Sequencing Continues to Increase Throughput in Proteomics

Sara Henriksson, Sweden

Background

Proteins are the building blocks of life and the key to understanding real time biology. The Olink assays enable the analysis of the low abundant dynamic plasma proteome and can be used to better understand the difference between health and disease, get insights into population health, and accelerate drug development.

The Proximity Extension Assay (PEA) technology used in Olink assays is based on the dual antibody recognition of targeted proteins that is translated into DNA barcodes that can then be read out using qPCR or Next Generation Sequencing (NGS).

Method

In PEA, two oligonucleotide-coupled antibodies bind to their target proteins. Upon binding, the oligos are brought into close proximity and hybridize to form a unique DNA barcode that can be read out using either a qPCR system such as a Olink® Signature Q100 or with Next Generation Sequencing (NGS) on the Illumina® platform.

Results

Moving from a qPCR to NGS readout has enabled a higher degree of multiplexing while retaining exceptional specificity, overcoming the challenges typically associated with multiplexed affinity-based assays. It has also enabled a higher sample and data throughput by utilizing a miniaturized and automated library preparation protocol, resulting in millions of data points from a single run.

Conclusions

PEA in combination with NGS readout has enabled increased throughput while retaining the same level of specificity when studying the dynamic low abundant plasma proteome. When using NGS there is no limit on the number of assays that can be multiplexed, and that number can continue to increase as the output from sequencing increases.

PP01.108: A New High-Throughput Platform for Proteomics: Orbitrap unites with a Novel High-resolution Accurate Mass Analyzer

Christian Hock, Germany

Introduction

Speed and sensitivity in combination with high-resolution and accurate mass (HRAM) remain key to further propel large cohort studies and advance single-cell proteomics to the next level. This need is addressed in this work by a novel instrument platform that combines Orbitrap™ analyzer with the novel Astral™ analyzer. The combination of these two analyzers allows for unprecedented performance that raises mass spectrometry to the next level.

Methods

A new platform has been developed that unites a novel HRAM analyzer with the state-of-the-art Orbitrap analyzer. The new platform allows for acquisition of HRAM MS1 spectra with up to 480K resolution in Orbitrap analyzer. In parallel, HRAM MS2 spectra are recorded at up to 200Hz in Astral analyzer. In total, 5 ion packages are processed in parallel to maximize usage of ions and allow for unprecedented sensitivity and speed so that an entire MS/MS cycle can be executed in under 5ms.

The new platform and its key components that enable speed, sensitivity, mass accuracy and resolving power of up to 100K in the Astral analyzer will be presented. Special emphasis will be given to the ion handling, ion processor, Astral analyzer, and high-dynamic range detector which allows for single-ion detection efficiency.

Results

For performance characterization, the new platform has been characterized with Pierce™ Flexmix™ calibration solution, HeLa digest and proteins. For HeLa digest, this resulted in >8,000 protein groups identified at 180 samples-per-day (SPD), >10,000 at 60 SPD, and >12,000 at 24 SPD. For low sample load (250pg HeLa), > 5000 protein groups can be detected. In addition, significant improvements for quantitation based on TMT™ labeling and suitability of the novel analyzer for proteins will be demonstrated.

Conclusion

A novel instrument with unique new technologies represents a significant improvement that will help to bring proteomics to the next level.

PP01.110: Development of High Through-put Proteomics Using an LC-Quadrupole-Orbitrap Mass Spectrometer with Data-Independent Acquisition

Masaki Ishikawa, Japan

In recent years, mass spectrometry (MS)-based proteomics have been applied in both clinical and medicinal fields. An improvement in the analytical throughput is requisite for these applications, because of the requirement of analyzing a large number of samples. Quadrupole-Orbitrap mass spectrometer (Q-Orbitrap MS) has been widely used in proteomics, along with Q-TOF MS. Despite this, Q-TOF MS has been mainly applied for the development of high through-put (HT) proteomic methods. So far Q-Orbitrap MS is considered unsuitable for HT proteome analysis because of its relatively slow scan speed arising from ion accumulation. However, it has not been fully investigated whether Q-Orbitrap MS is unsuitable for HT proteomics.

In the study, we aimed to establish a HT proteomic method based on a 5-min gradient LC and Q-Orbitrap MS. We systematically evaluated data independent acquisition (DIA) parameters for 5-min gradient LC and reached a depth of >5,000 and 4,200 proteins from 1,000 and 31.25 ng HEK293T cell digest in a single shot run, respectively. The throughput of our method enabled the measurement of approximately 80 samples/day, including sample loading, column equilibration, and wash running time. We demonstrated that our method is applicable for the screening of chemical responsivity via a cell stimulation assay. These data show that our method enables the capture of biological alterations in proteomic profiles with high sensitivity, suggesting the possibility of large scale screening of chemical responsivity.

PP01.112: Human Biofluids Analysis Using a Scalable, Deep, Unbiased, Automated, Nanoparticle-based Proteomics Platform

Wei Jiang, United States

Introduction
Biospecimen proteomics including characterization of non-blood biofluids like cerebral spinal fluid (CSF), and cell line conditioned media (CM) have the potential to reveal insights to human disease. While recent advances in sample collection and mass spectrometry have deepened our understanding of biofluid proteomes, the field is still plagued by non-standardized and complex workflows to characterize proteomes at acceptable depth. In this work, we evaluated the Proteograph™ workflow, a standardized, automated nanoparticle-based deep plasma proteomics workflow, to interrogate a variety of disease conditions spanning CSF and CM sample types.
Methods
To evaluate the performance of the Proteograph platform, biospecimens were sourced from commercial biobanks. For human CSF, we profiled samples from normal donors as well as donors with Alzheimer's disease, Amyotropic Lateral Sclerosis, and Parkinson disease. To assess in vitro secretome models, CM samples from breast, colon, cervical, prostate cancer cell lines, and induced pluripotent stem cells (iPSCs) were analyzed. Samples were processed directly using the Proteograph Product Suite, and in parallel using conventional sample prep techniques as a control. Tryptic peptides were analyzed by 60-min DIA LC-MS analysis using an Orbitrap Exploris 480, and data processing performed using Proteograph Analysis Suite.
Preliminary Results
When comparing Proteograph workflow against traditional sample preparations, we observed a >3-fold increase in protein group IDs (3204 vs. 1030, n = 12 for cancer cell lines) and a >10-fold increase for iPSCs (3230 vs. 269, n = 3), while observing ~1.6-fold increase for CSF samples (2239 vs. 1406, n = 15). We demonstrate feasibility of CSF and CM analysis on the Proteograph platform in rapid, deep, and unbiased fashion with improved coverage compared to conventional sample preparation.
Conclusions
The new Proteograph XT workflow simplifies analysis of biofluids without the need for complex sample manipulation and fractionation, offering a robust solution for proteomic and biological insights.

PP01.114: Spectra-Sum Method for Protein Quantification Using LC-MS/MS and TMT Labeling Data

Hahyun Lee, Republic of Korea

There are two methods used to calculate data for protein quantification using LC-MS/MS with TMT labeling. Spectra-sum method is calculated as a sum of the intensities of TMT report ions in spectra level. In other hand, Spectra-ratio method is calculated as a median of the ratio intensities with the global reference ion. In order to find a best quantification method, we prepared Hela standard peptides with TMT-6-plex labeling of difference protein content ratio as 0.5:0.5:1.0:1.0:2.0:2.0. First, we identified 3,684 proteins with FDR < 1% as spectra and global protein level using Integrated Proteomic Pipeline and ProteinInferencer. Of 30 replicated experiment of day by day, 16,384 is median number of identified spectra. We calculated the ratio value between the different protein content samples using the TMT reporter ions. As a result of spectra-sum method, the mean value of the ratio of number of proteins with CV < 30% was 0.865, and its standard deviation (SD) was low (0.07). The other hand of spectra-ratio method, the mean value was 0.45 and SD was high (0.11). It is indicated that spectra-sum method is more accurate quantification, where 87% of proteins could be quantifiable.

PP01.116: Comparison of Guinea Pig Retinal Proteomes Prepared Using S-TrapTM and EasyPepTM Protocols for Label-Free Proteomics

Daqian Lu, Hong Kong

The guinea pig retina is an emerging tissue for studying eye growth in myopia research. However, given the potential variability of diverse sample preparation protocols on retinal proteome, we aim to investigate the performance of two available sample preparation protocols, S-TrapTM and EasyPepTM in label-free proteomics.

Six retinae were collected from 41-day-old pigmented guinea pigs, and were allocated equally into two groups: EasyPepTM (n=3) and S-TrapTM (n=3). All retinal proteins were first extracted using the Precellys EvolutionTM homogenizer, then underwent alkylation, reduction, and digestion using either EasyPepTM or S-TrapTM according to the manufacturers' instructions. With 500ng per injection, each sample was loaded with technical replicate on a microflow-LC (15-min gradient) coupled with ZenoTOFTM 7600 running on a ZenoSWATH (64 variable windows) protocol. Protein identification was conducted using DIA-NN and Uniprot guinea pig proteome library.

There were 4127 proteins (25565peptides) commonly identified in all six injections in EasyPepTM group, compared to 3368 proteins (18617peptides) in S-TrapTM group. Comparing the two methods, 3138 common proteins were found overlapped, accounting for 76% of EasyPepTM group and 93% of S-TrapTM group. Gene ontology (GO) analysis on the two proteomes revealed similar top enrichment in biological processes (cytoplasmic translation, synapse translation, mRNA processing), and molecular functions (ribosome constituent, translation activity, bindings). Non-overlapping proteins between the EasyPepTM (989proteins) and S-TrapTM (500proteins) groups also shared top identical GO ontology terms, including mRNA and RNA stabilization, regulation of mRNA catabolism.

The EasyPepTM protocol yielded significantly more proteins and peptides, which was a preferred approach for studying guinea pig retinal proteome. However, both protocols provided a substantial number of proteins and showed similar enrichment in GO terms, suggesting the preservation of core biological functions and protein compositions using different sample preparation methods. This study compared and demonstrated sample preparation protocols for retinal proteomics for studying myopia and other retinal diseases.

PP01.118: Innovative High-throughput ENRICH-iST Workflow Facilitates Fast and Robust Plasma and Serum Proteome Profiling.
Chloé Moritz, Germany

Introduction
Blood plasma/serum is one of the least invasive biopsies and a valuable sample for clinical research and patient health monitoring. However, the high dynamic range as well as sample heterogeneity and complexity pose significant challenges for LC-MS-based proteomics, limiting thereby the access to the full proteome information and making in-depth profiling difficult at the rate required to study large cohorts. The ENRICH-iST workflow provides a robust and easy-to-use solution to the dynamic range challenge in plasma/serum and enables fully automatable high-throughput sample preparation for LC-MS-based plasma proteomics.

Methods
Starting with 20 µL plasma/serum, the novel ENRICH technology allows dynamic range compression by enriching low-abundance proteins onto non-functionalized paramagnetic microbeads. For subsequent LC-MS sample preparation, on-bead denaturation, reduction and alkylation, digestion and peptide clean-up were performed according to the iST-BCT protocol. Peptides were analyzed by nanoLC coupled to a timsTOF instrument (Bruker) using diaPASEF® acquisition mode.

Results and discussion
Parallel processing of up to 96 samples using the ENRICH-iST workflow can be completed within 5 hours. Human plasma and serum samples processed with ENRICH-iST were compared to neat samples (iST-BCT) showing an increase in protein identifications by ≥2-fold for plasma and 1.5-fold for serum while demonstrating excellent repeatability with median CVs <10%. Analysis of the protein intensity ranking reveals that reduction of high-abundance plasma/serum proteins allows the detection of lower abundant proteins.
In addition, the effect of donor and anticoagulant diversity was evaluated indicating a strong effect of the pre-analytical parameters and the importance of standardized plasma collection. For example, plasma collected with citrate (~1000 protein IDs) resulted in lower protein identifications compared to EDTA plasma (~1400 protein IDs) from the same donor.
Furthermore, the ENRICH technology is not specific to human samples and was successfully applied to mouse and rat plasma making it particularly interesting for preclinical research studies.

PP01.120: Automated Data Analysis and Tools for Mass-Spectrometry-Based Omics: Introducing SpAC9 Data Foundry
Gun Wook Park, Republic of Korea

Mass-spectrometry-based omics analysis offers the ability to quantitatively analyze thousands of proteins and glycoproteins. However, the field faces challenges stemming from experimental and computational limitations. In this study, we introduce the SpAC9 Data Foundry as a solution that provides tailored automation workflows, optimized for omics search. These workflows encompass LC-MS/MS analysis, omics data analytics, statistics analysis, bio-network analysis, and scientific visualizations. Additionally, we offer visualization tools that facilitate the effective interpretation of omics data analysis results and provide customized options to address specific customer requirements.
We have successfully established and validated the efficacy of the SpAC9 Data Foundry in analyzing proteins and glycoproteins with various types of post-translational modifications (PTMs). By fully automating omics analysis workflows with the SpAC9 Data Foundry, scientists can significantly reduce search time and costs, maximizing R&D efficiency, without deep domain knowledge of each of the fields.
The SpAC9 Data Foundry holds promise as a universal solution for the quantitative mapping of PTMs and enabling the discovery of drug and biomarker candidates. As this innovative platform can be readily adopted by many laboratories worldwide with LC-MS capabilities, its widespread implementation could revolutionize the field.

PP01.122: Label-Free Quantitation of Protein Mixtures Using Data-Independent Acquisition (DIA)

Patrick Pribil, Canada

Data-Independent Acquisition (DIA) has become a preferred workflow for the detection and quantitation of peptides and proteins using mass spectrometry, as it offers distinct advantages over data-dependent acquisition (DDA) methods. In addition, the ability to detect and quantify peptides and proteins without the need for expensive labeling strategies makes DIA methods attractive to researchers. Quadrupole time-of-flight (QTOF) hybrid systems are ideal for high-throughput characterization of complex protein samples because of their high acquisition speed along with enhanced sensitivity in the MS/MS space. DIA therefore is the ideal method for quantitative label-free proteomics. Tryptic digests of yeast, human and E. coli lysates were mixed at 2 different ratios and analyzed by LC-MS using nanoflow reverse-phase separation (at a 300 nL/min flow rate) and DIA on a QTOF system. Triplicate injections of 500 ng on-column loadings were done using a DIA method consisting of 100 variable-width windows. The resulting data were processed with DIA-NN software using a library-free approach, with searches performed against the combined UniProt database FASTA sequences for the 3 organisms. Using DIA, over 10,000 protein groups (>110,000 precursors) could be detected and over 9,500 protein groups (>95,000 precursors) could be quantified with a CV of <20% in both sets of lysate mixture samples. Enhanced MS/MS sensitivity for DIA provides a significant boost in the total number of protein groups (> 1,500) and precursors (> 27,000) quantified. The ratios of the lysate mixtures in each sample matched closely to the expected ratios with excellent precision and accuracy, demonstrating the fidelity of quantitation using DIA.

PP01.124: Single-shot LC-MS Workflow for Comprehensive Proteome Identification on a Novel High-Resolution Accurate Mass Platform

Julian Saba, Canada

Introduction
Recent advances in mass spectrometry technologies have led to higher proteomic depths mostly with pre-fractionated samples. Such approaches add considerable labor, requires high sample amounts and may not be feasible for processing tens or hundreds of samples. Also, these methods are not suitable for analysis of medium to large sample cohorts considerably adding sample to sample variation. Here, we present a single-shot LC-MS/MS workflow with identification of >10,000 protein groups from human cell lines on a novel HRAM platform.
Methods
HCT116, A549, K562, MCF7, MCF10A, MDA-MB-231, HEK293T, OVCAR8, OV90, PEO1, Jurkat and CHO cell line lysates were processed using AccelerOme automated sample preparation platform for reduction, alkylation, in-solution trypsin digestion and peptide clean-up. The digested peptides were vacuum dried and reconstituted in 0.1% TFA. The reconstituted peptides were separated using a µPAC™ Neo 50 cm column on a new high-resolution accurate mass platform interfaced with Thermo Scientific™ Vanquish Neo™ UHPLC system. The raw files were analyzed using CHIMERYS in Proteome Discoverer 3.1 beta version.
Results
Using the end-to-end workflow from sample prep with AccelerOme automated platform, peptide separation using Vanquish Neo UHPLC system with µPAC™ Neo column, data-independent acquisition (DIA) on a novel high-resolution platform and data analysis using Proteome Discoverer, we identified ~10,000 proteins from the various cancer cell lines with >80% of identified proteins <20% CV. Our single-shot LC-MS workflow at different sample loads from 5 ng to 500 ng of HeLa digest resulted in identification of 5,000 -10,000 protein groups. The unique peptides identifications at these loads were from 50,000 to 120,000. This clearly demonstrates the utility of our single-shot LC-MS workflow for in-depth proteome analysis. The higher-throughput method with 180 samples per day resulted in identification of >8,000 proteins.
Conclusions
Single-shot LC-MS workflow for high-throughput in-depth proteome analysis on a novel high-resolution accurate mass platform.

PROGRAM

PP01.126: Data - Information - Knowledge Effortlessly: Combining timsTOF data with PaSER information and Mass Dynamics Knowledge to Accelerate Proteomic Discoveries

Nobuyuki Shimura, Japan

Mass spectrometers are generating data at unprecedented rates, such that the bioinformatics of 'data to knowledge' is the primary bottleneck for large studies. PaSER bridges the 'data to information' gap by including on-the-fly database searching and decision-making based on the data generated. Here, we complete the 'information to knowledge' gap by leveraging Mass Dynamics - a seamless and modular web-based software that simplifies and templates complex proteomics analysis.

A typical proteomics pipeline is a time-consuming process and consists of steps including sample preparation, data acquisition, processing, data analysis using statistical analyses and various solutions to generate key insights. We showcase how the integrated PaSER and Mass Dynamics pipeline alleviates the time-consuming elements to facilitate researchers to generate key insights from their proteomics analyses.

We re-analyzed a previously published dataset (doi.org/10.1007/s13365-022-01077-0) using PaSER with a high pH library and a predicted spectral library. The combined experiments were uploaded to Mass Dynamics for statistical analysis, normalization and visualization.

Re-analysis using the PaSER and Mass Dynamics integrated pipeline allowed faster comparisons of identified proteins.

As demonstrated in Ahmed et al. 2022 and broadly more common to proteomics pipelines, at least 8 independent processes, utilizing at least 4 piecemeal post-processing solutions are required to derive results. This time-consuming process requires multiple points of human intervention, resulting in delays to generate insights, report findings, and publish results. Conversely, using this new pipeline results in the ability to produce comparable results with functionality for sharing, collaboration, and figure generation. This allows the capacity to directly link the experiment results with external knowledge bases and generate figures in near-real time.

Taken together, streaming data from a timsTOF mass spectrometer to PaSER, where on-the-fly information generation is performed, followed by a cloud-based platform accepting pre-processed data, results in high-confidence biological knowledge in a seamless and expedited fashion.

PP01.128: Next-Generation Protein Sequencing on Quantum-Si Platinum™: Advances in Protein Identification

Kenneth Skinner, United States

Title: Next-Generation Protein Sequencing on Quantum-Si Platinum™: Advances in Protein and Proteoform Identification

Abstract: Studies of the proteome would greatly benefit from methods that enable direct sequencing and digital quantification of proteins, as well as the detection of post-translational modifications with single-molecule sensitivity. Here, we showcase single-molecule protein sequencing using a dynamic approach with the Platinum™ instrument, where individual peptides are probed in real-time by dye-labeled N-terminal amino acid recognizers and simultaneously cleaved by aminopeptidases. Through integrated semiconductor chip analysis, we annotate amino acids and identify the peptide sequence via fluorescence intensity, fluorescence lifetime, and binding kinetics. This information-rich capability enables recognizers to discern single amino acid substitutions and post-translational modifications.

To demonstrate the versatility of this core methodology and its kinetic principles across proteins, we sequenced a mixture of five proteins: cerebral dopamine neurotrophic factor (CDNF), fibroblast growth factor 2 (FGF2), interleukin-4 (IL4), glia maturation factor beta (GMFB), and protein disulfide isomerase (PDIA1). The resulting peptides generated distinct kinetic signatures aligned with their respective sequences, highlighting the Quantum-Si sequencing platform's ability to effectively analyze multi-protein mixtures at reduced input concentrations. Additionally, we successfully sequenced human serum albumin (HSA) enriched from a urine sample, identifying eight unique peptides using the Platinum™ instrument. These results demonstrate how Quantum-Si's protein sequencing technology enables in-depth analysis of biomarkers extracted from biofluids.

PP01.130: Nanoparticle Enrichment Mass-Spectrometry Proteomics Identifies Protein Altering Variants for Precise pQTL Mapping

Karsten Suhre, Qatar

Genome-wide association studies (GWAS) with proteomics generate hypotheses on protein function and offer genetic evidence for drug target prioritization. Although most protein quantitative loci (pQTLs) have so far been identified by high-throughput affinity proteomics platforms, these methods also have some limitations, such as uncertainty about target identity, non-specific binding of aptamers, and inability to handle epitope-modifying variants that affect affinity binding. Mass spectrometry (MS) proteomics has the potential to overcome these challenges and broaden the scope of pQTL studies. Here, we employ the recently developed MS-based Proteograph™ workflow (Seer, Inc.) to quantify over 18,000 unique peptides from almost 3,000 proteins in more than 320 blood samples from a multi-ethnic cohort. We implement a bottom-up MS-proteomics approach for the detection and quantification of blood-circulating proteins in the presence of protein altering variants (PAVs). We identify 184 PAVs located in 137 genes that are significantly associated with their corresponding variant peptides in MS data (MS-PAVs). Half of these MS-PAVs (94) overlap with cis-pQTLs previously identified by affinity proteomics pQTL studies, thus confirming the target specificity of the affinity binders. An additional 54 MS-PAVs overlap with trans-pQTLs (and not cis-pQTLs) in affinity proteomics studies, thus identifying the putatively causal cis-encoded protein and providing experimental evidence for its presence in blood. The remaining 36 MS-PAVs have not been previously reported and include proteins that may be inaccessible to affinity proteomics, such as a variant in the incretin pro-peptide (GIP) that associates with type 2 diabetes and cardiovascular disease. Overall, our study introduces a novel approach for analyzing MS-based proteomics data within the GWAS context, provides new insights relevant to genetics-based drug discovery, and highlights the potential of MS-proteomics technologies when applied at population scale.

PP01.132: High Throughput Plasma Proteome Profiling of Inflammatory Bowel Diseases Using a Novel High-resolution Accurate Mass Platform

Jennifer Van Eyk, United States

The Inflammatory bowel diseases (IBD) are a spectrum of common chronic hyper-inflammatory diseases associated with poor quality of life, impaired ability to work, and the need for recurrent surgeries. Anti-tumor necrosis factor (TNF) strategies remain the most effective therapies for IBD. Our IBD cohort includes subjects on anti-TNF therapy and other advanced therapies with longitudinal follow-up to assess proteomic variation with disease activity and drug responses. Age, sex and ancestry matched healthy controls were also included. The proteomics experiments were carried out with a novel high-resolution accurate mass (HRAM) mass spectrometer (MS) for deep, reproducible, quantitative and robust plasma proteome profiling of subjects. Plasma protein denaturation, reduction, alkylation, trypsin digestion and desalting were performed on a Beckman i7 workstation. Digested plasma peptides were analyzed using a Vanquish Neo UHPLC system coupled with the new HRAM platform. MS1 scans (240,000 FWHM) were collected in parallel with ultra-high speed (up to 200 Hz) DIA scans (80,000 FWHM). Data analysis was done with a beta version of Proteome Discoverer Software using CHIMERYS™. The healthy plasma from 100 females and 100 males was employed to evaluate the workflow at three throughputs 180, 100, 60 samples per day (SPD), respectively. The sensitivity, reproducibility, and data completeness were evaluated using loading curves ranged from 12.5 ng to 500 ng with 5 repeated injections at each concentration. With 300 ng loading, the new HRAM-MS identified >1300

proteins, >6000 unique peptides with 60SPD. Importantly, CRP protein as circulating plasma protein associated with IBD disease activity was quantified and detected 5 out of 5 injections. LC MSMS analysis has been carried out for the IBD cohort (289 controls and 205 IBD subjects) with 60SPD workflow. The novel HRAM platform-based workflow demonstrates quantitative precision and proteome coverage of naive IBD plasma proteome at high-throughput.

PROGRAM

PP01.134: Unlocking the Potential of Large-cohort Proteomics Studies with a Novel High-resolution Accurate Mass Platform

Yue Xuan, Germany

Large-cohort proteomics analysis using mass spectrometry is a powerful approach to discover and validate new biomarkers. Yet, to achieve meaningful biological insights in large-cohort studies, robust, reproducible, and comprehensive proteome profiling in a high-throughput manner remains challenging.
Here, we use a novel high-resolution accurate mass (HRAM) platform to enable high-quality and robust protein quantification across thousands of LC-MS/MS analyses.
To evaluate proteome profiling performance, reproducibility across instruments and time, and robustness over thousands of injections, we designed our study to simulate a large-cohort study. Multiple LC-MS/MS systems were operated in DIA mode either with or without FAIMS Pro device in a 24/7 operation mode at a throughput of 100samples/day. Undepleted plasma digest was analyzed with >1000 injections on each LC-MS/MS setup. HeLa digest as QC were inserted every 12hours in triplicates. To effectively manage and analyze these thousands of data files generated, the resulting DIA raw data files were immediately and automatically transferred to a server, then processed by Chimerys in a beta version of Proteomics Discovery software. Benefiting from the ultra-high scan speed (200Hz), a narrower isolation window width of 2Th was applied in the DIA method, identifying ~9000 proteins from HeLa digest and ~700 proteins from undepleted plasma digest at a throughput of 100sample/day. More than 80% of the proteins were reproducibly identified and quantified from all the runs on each LC-MS/MS setup, indicating a great reproducibility longitudinally. Stable and robust peptide quantitation was observed by extracting peptides with high, medium, and low abundant across the runs. Importantly, HeLa digest as QC showed no performance degradation throughout the entire study, indicating high robustness of the entire LC-MS/MS setup.
These results demonstrate this novel HRAM MS platform can comprehensively analyze the proteome of >1000s of sample robustly and reproducibly in a high-throughput manner, addressing the needs in large-cohort studies.

PP01.136: Development of LC-MS/MS Analysis Method for High Throughput Screening of Protein Covalent Compounds and Verification Through Inter-laboratory Study

Sanghyun Bae, Republic of Korea

This study aims to develop and validate an LC-MS/MS method for efficient screening of covalent binding interactions between electrophilic compounds and nucleophilic residues within proteins, specifically focusing on cysteine residues. To evaluate the formation of covalent bonds, we selected 30 electrophilic inhibitors from a cysteine-focused compounds library and divided them into three groups of 10 compounds each. Cysteine was introduced into the druggable pocket of the MDM2 protein, serving as the target protein for our experiments. Each group of 10 compounds was incubated with MDM2 (M62C), followed by trypsin digestion and subsequent LC-MS/MS analysis. We calculated the relative intensity ratios of the inhibitor-bound target peptide to their corresponding internal standard peptides for ranking the relative binding of the compounds to the protein. To ensure the reproducibility of our method, we conducted an inter-laboratory study involving seven laboratories. Each laboratory performed LC-MS/MS analysis using identical procedures. By comparing the results obtained from each laboratory, we evaluated the universal applicability of our LC-MS/MS method for high-throughput screening of covalent binding between electrophilic compounds and nucleophilic residues in proteins. Our findings demonstrate that this method can serve as a valuable tool for conducting such screenings in diverse laboratory settings.

PP01.138: Understanding the Molecular Effects of Trilaciclib, a CDK4/6 Inhibitor, in the Treatment of FLT3-mutated Acute Leukaemia: Insights from Proteomic Analysis

Amy George, United Kingdom

Background: Although advances in acute myeloid leukaemia (AML) treatment improve survival in younger patients, elderly patient outcomes remain dismal. The poor prognosis associated with FLT3-mutated AML is partly due to the rapid emergence of resistance to various FLT3 tyrosine kinase inhibitors. Recent studies have suggested a significant role of cyclin-dependent kinase 6 (CDK6) in the transcriptional regulation of FLT3, in addition to influencing cell cycle progression and promoting leukemic cell growth. Consequently, targeting CDK6 may serve as a potential therapeutic strategy for FLT3-mutated AML.
Methods: Multiple leukaemia cell lines, representing various subtypes, with differing FLT3-ITD+ mutation status treated with trilaciclib or vehicle for 24 hours. Whole cell lysates were then digested using S-trap Micro-columns, and a single-shot analysis performed using data-independent acquisition on a Q-Exactive HF. Data was analysed by DIA-NN for relative quantification, and differential analysis between treatment and vehicle controls performed.
Results: Trilaciclib treatment resulted in the downregulation of proteins associated with S-Phase transition and DNA synthesis in all tested leukaemia cell lines, reflecting inhibited CDK4/6 activity and consequential cell cycle arrest in G1 phase. Notably, cell-lines possessing FLT3 mutations were 10-times more sensitive to CDK4/6 inhibition than wild-type cells. Significantly perturbed proteins exclusive to FLT3-mutated cell lines were subject to Gene Ontology enrichment analysis, highlighting signalling pathways implicated in both CDK4/6 and FLT3 activity.
Conclusion: Understanding the intricate relationship between CDK6 and FLT3 signalling pathways may offer new avenues for developing combination therapies that synergistically target these key components of AML pathogenesis. By harnessing the potential of Trilaciclib as a CDK4/6 inhibitor, we aim to contribute to the development of more effective and personalized treatment strategies for elderly AML patients.

PP01.140: Identifying the Anticancer Target of Ruthenium (III) Pyrazole Compounds in Colon Cancer by Chemoproteomics

Jilin He, Hong Kong

Ruthenium(III) pyrazole complex RuP-01 is a potent cytotoxic compound towards human colon cancer cell lines compared to NAMI-A, which is the prototypical ruthenium based anticancer compound entering clinical trial. RuP-01 was proved to show its potential as prodrug that could be activated by reduction preferentially under hypoxic tumor microenvironment, while sparing normal tissues from toxic effects. By using both thermal proteome profiling and reactive-cysteine profiling, we revealed that RuP-01 engaged with multiple molecular targets including aldehyde dehydrogenase 18 family member A1 (ALDH18A1), citrate synthase (CS) and heat shock protein 90 (HSP90). Treatment of cancer cells with RuP-01 resulted in inhibition of both the glycolysis and mitochondrial respiration and the downregulation of proto-oncogene, c-Myc. Our findings show the promise for developing new ruthenium based metallodrugs by multiple targeting in colon cancer.

PP01.142: Unveiling the New Player in ER-Mitochondria Interactions by DARTS-LC-MS/MS Proteome Analysis

Minjeong Ko, Republic of Korea

Mitochondria Associated ER Membrane (MAM) is involved in several physiological activities, and increased MAM formation promotes excessive calcium transport to mitochondria, leading to several diseases. In this study, we observed increased ER-mitochondria interaction and impaired autophagy in vascular endothelial cells stimulated with oxidized low-density lipoprotein (oxLDL) and in the apoE-/- mouse model atherosclerosis. We found that CTS, the active compound found in Danshen, exhibited anti-atherosclerotic effects both in vitro and in vivo. In addition, CTS induced autophagy and reduced the association between ER and mitochondria. To elucidate the underlying mechanism of CTS, DARTS-LC-MS/MS analysis was performed using averaged quantitative SWATH analysis. A list of candidate targets that showed more than 10% resistance to proteolysis was generated, and we found that they were mainly located in the ER or mitochondria among different organelles. Considering the high sequence coverage, we identified the ER-resident chaperone CTS-BP as the binding partner of CTS. Validation of the interaction between CTS and the target protein was further confirmed by silencing the target gene, resulting in MAM disruption and increased autophagy. Taken together, our study provides novel insights into the mechanism by which an anti-atherosclerotic natural compound targets the ER-mitochondria contact complex to induce autophagy.

PP01.144: High-Depth Multiplexed Drug Profiling with the Orbitrap Ascend

Amanda Lee, United States

Chemoproteomics is a powerful approach to drug discovery. However, sensitivity and throughput remain limiting factors to interrogating full-proteome drug effects. We evaluated the new Orbitrap Ascend mass spectrometer combined with TMTpro16 to assess whole-proteome drug effects. The Ascend offers new hardware and software features that improve throughput, sensitivity, and quantitative accuracy. We assessed the effects of novel inhibitors of peptidyl-prolyl isomerase PIN1 on proteins across the whole proteome, revealing changes at superior depth. A biological quadruplicate of four human cell lines was digested, labeled using TMTpro16 reagents, pooled, and fractionated. HCT116 cells were treated with PIN1 inhibitors in triplicate at 10 µM for 24 h, then processed in the same manner. A two-proteome mixture containing yeast peptides at defined ratios and human peptides at all 1:1 ratios was prepared similarly without fractionation. Samples were analyzed on the Orbitrap Ascend and the Orbitrap Eclipse using FTMS2 and SPS-MS3 with real-time search. Analysis of the two-proteome mixture revealed that quantitative precision was improved with the Orbitrap Ascend. The Ascend quantified 29% more peptides and 13% more proteins. In the four-cell-line study and the PIN1 inhibitor study, the Ascend quantified 21–22% more peptides and 6.0–8.1% more proteins (a gain of ~500–700 proteins) than the Eclipse and improved quantitation. The Ascend required 30% fewer fractions to achieve these gains. Proteins quantified only on the Ascend have lower iBAQ values, suggesting that the Ascend is revealing low-abundant proteins. In the PIN1 inhibitor study, the Ascend identified 175 more statistically significant hits than the Eclipse. One protein not identified by the Eclipse, FGFR1, is involved in some of the same oncogenic pathways as PIN1, revealing potentially relevant compound-target interactions. The improvements within the Orbitrap Ascend enable enhancement with multiplexed quantitative proteomics and deeper characterization of whole-proteome compound effects.

PP01.146: A Proteomic Study on Traditional Chinese Medicine: Bavachinin Regulates Bone Homeostasis in T2DM via Inhibition of Ferroptosis Pathway.

Jingwen Liu, China

Introduction:
As peroxisome proliferator-activated receptor γ (PPAR γ) full agonist, Thiazolidinediones (TZDs) have been proven to effectively prevent the process of Type 2 Diabetes Mellitus (T2DM); however, the side effects have curtailed their use in the clinic, including weight gain and bone loss. Here we present a selective PPAR γ modulator, Bavachinin (BVC), isolated from seeds of traditional Chinese medicine Psoralea Corylifolia L., which increases insulin sensitivity and potently reduces bone loss.
Methods:
Two classic T2DM animal models, leptin receptor-deficient mice, and diet-induced obesity mice, were applied to evaluate the intervention of BVC in bone homeostasis in vivo. MC3T3-E1 pre-osteoblast cells were assessed for osteogenic differentiation activities and proteomic analysis. The proteins and pathways regulated by BVC in high glucose-treated MC3T3-E1 were identified and quantified with the Data-independent acquisition (DIA) method. The candidates of proteins and pathways involved in BVC regulation were further confirmed by Western Blot in vitro and in vivo.
Results:
Compared to full PPAR γ agonist, BVC was found to prevent weight gain, ameliorate lipid metabolism disorders, improve insulin sensitivity, and maintain bone mass and bone biomechanical properties in two classical models of T2DM mice. In vitro, BVC significantly increased osteogenesis differentiation activities of MC3T3-E1 pre-osteoblast cells under high glucose conditions. DIA analysis showed that the ferroptosis signaling pathway was considerably enriched under BVC treatment. In particular, GPX4 was identified as a potential target to reverse high glucose-induced ferroptosis under BVC treatment.
Conclusion:
BVC exhibits the function of insulin sensitizer and beneficially minimizes the side effects of TZDs, including bone loss and undesirable weight gain.

See you in Busan!
BEXCO, Busan, Korea
🌐 2023.hupo.org
🐦 f 📷 in #HUPO2023

Together Through Proteomics

PROGRAM

PP01.148: Genetically Encodable Click Reactions(GEN-Click) for Spatially Restricted Protein and Metabolite Labeling

Pratyush Mishra, Republic of Korea

Chemical reactions for in situ modification of biomolecules within living cells continue to be developed. Among the re-actions available, bio-orthogonal reactions such as click chemistry using copper(I) and Staudinger ligation are now widely used for specific biomolecule tracking in live systems. However, currently available live cell copper(I) catalyzed azide/alkyne cycloaddition (CuAAC) reactions are not designed for spatially-resolved manner. Therefore, we developed a "GEN-Click" system that can target the CuAAC reaction catalysts proximal to the protein of interest (POI) that could be genetically expressed in a live cell. The genetically controlled, spatially restricted metal-catalyzed biorthogonal reaction can be used for proximity biotin-labeling of various azido-bearing biomolecules (e.g. protein, phospholipid, oligosaccharides) in living cell systems. Using GEN-Click, we could successfully detect the metabolite-transferring events at the cell-cell contact sites.

PP01.150: Proteomic Characterization of Triple-negative Breast Cancer Treated with Nucleolin-targeting Aptamer AS1411

Hyoung Min Park, Republic of Korea

Breast cancer is one of the most common malignant diseases worldwide. Triple-negative breast cancer (TNBC) is infamous for being the most aggressive and most associated breast cancer with poor prognosis. Unlike other breast cancers, TNBC lacks effective molecular targets, limiting therapeutic options such as hormone or tyrosine kinase inhibitor therapy. In our previous studies, we found that nucleolin (NCL)/astrocyte elevated gene-1 (AEG-1) protein complex promotes migration and invasion of TNBC. Here, we show that an NCL-targeting aptamer AS1411 reduced the viability as well as the migration of TNBC cells, while other breast cancer cell lines and normal breast epithelial cells were unaffected. This suggests aptamer-based NCL targeting could be an effective therapeutic strategy against TNBC. To further investigate the mechanism for the suppression of TNBC progression, the global proteome of TNBC cells exposed to AS1411 was analyzed. For comparison, cells exposed to AS1411 for 8, 16, and 24 h were processed by the filter-aided sample preparation and fractionated into 7 portions for extensive protein identification. LC-MS analyses were performed using an EASY-nLC 1000 HPLC coupled to Orbitrap Eclipse™ Tribrid™ Mass Spectrometer equipped with a custom electrospray ionization source. A total of 8,659 proteins were identified. Bioinformatic analysis of differentially expressed proteins (87 up-regulated and 153 down-regulated proteins in 8 h; 111 up-regulated and 492 down-regulated proteins in 24 h) revealed that AS1411 affects translational machinery in TNBC. Moreover, we identified 4 down-regulated proteins (HPRT1, CHEK1, SRP9, ATP6AP1), whose over-expression was previously associated with poor prognosis of breast cancer. Further functional validation of these differentially expressed proteins would provide useful insights into the therapeutic potential of AS1411 in treating TNBC.

PP01.152: Proteomics Application in Discovering Therapeutic Effect of Dental Mesenchymal Stem Cell Secretome on Stroke.

Sehoon Park, Republic of Korea

Background
Stroke is one of the global health problems that lead to deaths and severe disabilities. Although there are effective treatments for acute stroke, their narrow time window and high risk of complications require alternative therapeutic strategies. Recently, the secretome of mesenchymal stem cells(MSCs) has received attention because of its potential for regenerative medicine. Particularly, since dental MSCs are from the neural crest, they have higher potential for neurogenesis compared to other MSCs.

Method
LC-MS which is a highly sensitive bioanalytical technique was applied to this study. To confirm the therapeutic effect of secretome on stroke, in-vivo study was conducted. Hippocampus from mice was used for the in-vivo study. Proteins were quantified by label-free quantification with data-dependent acquisition mode. Differentially expressed proteins were identified, and the biological pathways were enriched from those proteins that may elucidate the effect of secretome on stroke.

Result
Differentially expressed proteins were filtered by fold change>1.5, p-value <0.05, and they were used for GO enrichment analysis. Comparing the stroke group with secretome against the group without it, 'positive regulation of immune response' was enriched by up-regulated proteins, while 'negative regulation of nervous system development' was enriched by down-regulated proteins. In addition, most of the differentially expressed proteins that were associated with therapeutic effect of secretome were confirmed to be up-regulated in the group with secretome. Further possibilities of therapeutic mechanism of secretome on stroke were suggested by assessment of protein interaction in MAPK signaling pathway which is considered to be associated with stroke recovery.

Conclusion
Secretome has effects on immune response and nervous system development, and DEPs that are involved in positive therapeutic effects on stroke were increased by secretome. Furthermore, there is a possibility of MAPK signaling being involved in the pathogenesis of stroke.

PP01.154: Comprehensive Characterization of the Mechanism of Action of GSK3 Inhibitors in Stem Cells Using Functional Proteomics

Pierre Sabatier, Denmark

Small molecule inhibitors of Glycogen Synthase Kinase 3 (GSK3) are extensively used in biology to mimic the activation of the Wnt/β-catenin pathway. In stem cell research and regenerative medicine, these inhibitors help guide cell differentiation toward specific cell lineages for organ modeling or transplantation. Although a clinical trial involving cells developed using GSK3 inhibitors is currently underway in Sweden for treatment of Parkinson's disease, most differentiation protocols lack reproducibility, and producing clinical-grade cells remains challenging. The supposed reasons are that the dosage of these inhibitors is crucial and that they may exhibit off-target effects. While recent protocols try to address the former and some inhibitors were tested for their specificity against panels of recombinant kinases to address the latter, their mechanism of action is understudied at the molecular level in relevant cell models. In this project, we systematically profiled eleven commonly-used GSK3 inhibitors in human induced pluripotent stem cells (hiPSC) using phosphoproteomics and proteome integral solubility alteration (PISA) analysis in intact cells, at multiple drug concentrations. We also tested a subset of the drugs in another hiPSC line, in the embryonic stem cell line H9, and in the colon cancer line HCT116. Surprisingly, some inhibitors were highly specific to GSK3A/B in PSCs in contrast to previous work with recombinant kinases. Interestingly, CHIR99021 showed high specificity towards GSK3A/B in PSCs but little target engagement in HCT116. This highlights the importance of using relevant cell models in drug discovery. Lastly, we provide a comprehensive proteomics analysis of each drug and ranking according to target specificity. Collectively, this study represents the most detailed functional proteomics analysis of a single type of inhibitor, it also demonstrates that GSK3 inhibitors are more specific than previously assumed and provide guidance to researchers interested in employing these compounds in stem cell research, medicine, and biology in general.

PP01.156: Time-resolved Phosphoproteomics of Colorectal Cancer Liver Metastases Resistant to Adjuvant Chemotherapy Reveals PI3K-PAK1 Axis as a Potential Therapeutic Target

Jun Adachi, Japan

Colorectal liver metastases (CRLMs) are the leading cause of cancer-related death in colorectal cancer; after curative resection of CRLMs, adjuvant chemotherapy is used to prevent recurrence. However, tumor recurrence after chemotherapy remains a significant therapeutic challenge.

To investigate novel therapeutic targets for chemotherapy-resistant CRLM, we compared tissues from the same patients before and after chemotherapy using a phosphoproteomic approach.

Eighteen patients were treated with chemotherapy after resection of liver metastases and developed recurrent tumors during or after chemotherapy. TMT-based proteomic and phosphoproteomic analysis was performed on tumors and NATs obtained from patients and on 35 colorectal cancer cell lines (CRC35). Kinase activity profiles were obtained using PTM-SEA. A drug sensitivity-kinase activity correlation map was created using kinase activity data for CRC35 and drug sensitivity data from the publicly available small molecule sensitivity data set (CTD2).

Patients were divided into two groups: recurrence during adjuvant chemotherapy (During group: n=8) and recurrence after chemotherapy (After group: n=10). Overall survival was significantly shorter in the During group than in the After group. PAK1 activity was significantly upregulated in patients in During group. We then systematically selected PI3K inhibitors as drug candidates using Drug sensitivity-Kinase activity correlation map and confirmed anti-cancer effect in vitro and in vivo.

Our integrated analysis of pharmaco-phosphoproteomic data revealed PAK1 kinase activation associated with poor prognosis cases and PI3K kinase as a potential therapeutic target for chemotherapy resistance CRLM.

PP01.158: Using Proteomics to Improve Risk Prediction Models for Common Diseases in Participants from the UK Biobank Pharma Proteomics Project

Shing Wan Choi, United States

Background
Traditional risk factors form the cornerstone for disease prediction, prevention, and management approaches used in clinical practice. Recent availability of large-scale proteomic datasets offers the possibility of improving disease risk prediction by incorporating proteomic data into clinical risk scores.
Methods
We combined clinical factors, genomic scores, and large-scale proteomics data to improve disease risk prediction for several common diseases using data from 54,306 participants from the UK Biobank Pharma Proteomics Project. A total of 2,939 plasma proteins were measured using the Olink Explore 1536 and 3072 platforms. Participants were randomly divided into 70% training and 30% validation cohorts. We employed forward stepwise Cox regressions to identify proteins and aggregate them into proteomic scores. Risk prediction models for chronic obstructive pulmonary disease (COPD), cardiovascular disease (CVD), osteoarthritis and osteoporosis were developed by integrating clinical and genomic scores with proteomic scores in the training cohort. Performance of these models was then evaluated in the validation cohort.
Results
Incorporating proteomic scores significantly improved disease risk predictions beyond those from genomic scores. Adding proteomic scores to risk prediction models enhanced the identification high-risk patients (top decile of risk scores) for 10-year incidence by 8.29% for CVD, 31.7% for osteoporosis, 18.2% for COPD, and 19.5% for osteoarthritis. These composite risk scores were also significantly associated with secondary clinical outcomes, including COPD related hospitalization or death with an odds ratio per standard deviation (OR) of 1.87 ($p=9.85 \times 10^{-12}$), knee and hip joint replacement in osteoarthritis patients (OR of 1.66; $p=1.65 \times 10^{-23}$), recurrent CVD events in CVD patient (OR 2.43; $p=8.06 \times 10^{-57}$), and bone fractures (OR of 1.72; $p=5.77 \times 10^{-47}$).
Conclusion
Addition of proteomics scores to current risk prediction models may lead to earlier diagnosis and improved management of several chronic conditions.

PP01.160: Facilitating Precision Medicine through Targeted Proteomics Analysis of Dried Plasma from Fingerpricks

Andreas Hober, Sweden

With the boundaries of proteomics being pushed further, in terms of quantitative precision, sensitivity and multiplexing, the prospect of proteomics as a tool for precision medicine for the general population is ever-increasing. Through longitudinal and cost-efficient monitoring of biomarkers, truly preventative medicine can become a reality. A crucial requirement for longitudinal monitoring of the general population is a reproducible, reliable and non-invasive sampling that preferably can be performed at home. In this study we have evaluated a novel microsampling device for at-home sampling that readily prepares dried plasma spots (DPS) from a single fingerprick. By collecting paired plasma, DPSs and fingerprick samples, the device could be benchmarked against the more traditional specimen for plasma profiling. An extraction protocol was optimized for targeted LC-MS/MS analysis using stable isotope labelled recombinant protein fragments (qRePS) and selected reaction monitoring, which allows for reproducible and precise quantification of a selection of plasma proteins spanning a diverse concentration range. A 1-hour long extraction in 1% SDC, 10 mM TCEP and 50 mM ammonium bicarbonate followed by alkylation and tryptic digestion achieved a reproducible sample preparation resulting in robust quantification of 60 peptides from 18 different proteins were the vast majority have a coefficient of variance (CV) below 10%. The median CV of the quantified peptides was 2.3% (mean CV = 3.9%). The quantified peptides showed good concordance with neat plasma, and the difference between samples obtained using the microsampling device and neat plasma showed a mean difference of 12% and a median difference of 6.4% at a protein level. The results showcase the potential of microsampling devices for longitudinal monitoring of individuals for precision medicine purposes. This novel sampling strategy has the potential to transform how clinical trials and health screenings are conducted as it retains precision and robustness while improving patient convenience.

PP01.162: Metabolomic Profiling Identified Serum Metabolite Biomarkers and Muscle Pathophysiology of Idiopathic Inflammatory Myopathy

Jihyun Kang, Republic of Korea

Idiopathic inflammatory myopathy (IIM) is a diverse set of autoimmune diseases with a range of clinical symptoms, responses to treatment, and prognoses. Since IIM is a rare disease, diagnosis for IIM can be challenging without muscle biopsy. This study aimed to identify metabolic biomarker panel for IIM detection by using metabolomics approach in serum samples from patients and healthy control and explore metabolomic signature in tissue samples from mouse model. We obtained serum samples from 10 healthy volunteers, 30 ankylosing spondylitis (AS) patients, and 50 IIM patients. More, we obtained serum and muscle tissues from C-protein-induced myositis (CIM) which is a murine model of IIM. All serum and tissue samples were processed using the AbsoluteIDQ p180 kit with liquid chromatography mass spectrometry. Significant metabolites were analyzed using ANOVA with false discovery rate adjusted p-value less than 0.05 in human serum. Following ANOVA test, a multiple comparison was performed to identify a total of 37 IIM specific metabolites. A combination of metabolites model to detect IIM calculated from logistic regression analysis with backward elimination. In addition, two machine learning algorithms, support vector machine and random forest, were used to validate the model. To distinguish IIM from healthy control and AS group, metabolite panel including lysophosphatidylcholine and phosphatidylcholines showed the highest AUC score of 95.5% after 5-fold cross validation. A total of 68 metabolites were significantly changed in CIM-vehicle. Important metabolic pathways in mouse tissue sample such as beta-alanine pathway and polyamine pathway are revealed as evaluative biological pathways for muscle disease. Our metabolomic approach offers the possibility of potential biomarker to classify IIM and provided metabolic pathways associated with IIM.

PP01.164: Proteomic Representation of the Genetic Architecture of Monogenic Diabetes

Ksenia Kuznetsova, Norway

Monogenic diabetes is a Mendelian disorder following the autosomal-dominant pattern of inheritance. Despite the relative simplicity of the genetic architecture of Mendelian diseases, monogenic diabetes poses challenges for precise diagnostics due to its symptomatic similarity with the other diabetes types. The aim of our work is to move away from the standard diabetes classification toward a precision approach in diagnostics and treatment. The analysis of proteome, as the actual functioning part of the biological system, may contribute to the personalized profiling of patients and add to the fundamental understanding of disease pathogenesis.

We have collected all the available information on the rare genetic variants associated with monogenic diabetes and translated them into protein sequences mapped to all the isoforms from Ensemble. This is implemented in our developed and publicly available Python-based pipeline. These variant sequences have been added to a human protein database containing common protein haplotypes enabling the account of the common genetic variation and avoiding search space inflation. This database is ready to be used for the sequence-level identification of the protein product of the monogenic diabetes-causing genetic variants.

As a model experiment, we analyze mass spectrometry-based proteomics data of cell cultures and mouse model samples with introduced genetic variants associated with monogenic diabetes. The resulting spectra are searched against our databases accounting for the products of the variants of interest. In this way, we are able to trace the variant amino acid sequences on the level of the proteome.

We are developing the instruments for the thorough investigation of the proteomic representation of the genetic architecture present in monogenic diabetes. Such a complex and personalized approach will move the diagnostics and treatment of diabetes mellitus further toward precision medicine and make a difference for patients.

PP01.166: Next Generation Proteomics of New Liquid Biopsy in Early Lung Cancer

Yoonha Park, Republic of Korea

Background
Lung cancer is one of the most common cancers in Korea. Over the past 20 years, the early detection rate of gastric cancer and colorectal cancer has increased in Korea due to the development of endoscopy and its active application to health examinations. However, there is no such endoscope for health checkup for lung cancer. In the case of small lung cancer lesions or ground-glass nodules (GGN), it is very difficult to apply tissue biopsy. Therefore, as an alternative, we propose liquid biopsy to identify tumor and classify tumor-proteotypes through analyzing the EV proteome of BAL fluids in lung cancer patients.

Methods
Using the data-independent acquisition (DIA)-based proteomics, we explored the proteome of extracellular vesicles (EVs) isolated from bronchoalveolar lavage (BAL) fluid. With pooled patient's BALF EV proteome, we identified proteins and established the spectral library for DIA analysis. The protein sample has also used as quality check between multiple runs to monitor nano-LC-MS/MS system. We performed DIA analysis for quantification of large proteomes from patient specimens (WT, EGFR 19-Del, and EGFR L858R in early and late lung cancer).

Results
While our previous DIA-based proteomics has been effective for late-stage lung adenocarcinoma patients, its potential for early detection has not been established. Here, we used the DIA-based proteomics to examine a small set of EV proteome samples from early and late-stage lung cancer patients with EGFR mutations (L858R or E19Del). Our study identified molecular differences according to EGFR mutation type and stage of cancer in early lung cancer.

Conclusions
These findings suggest the Data-Independent Acquisition-based Deep Proteomics for BALF-EV may be a promising approach for early detection of lung cancer and selection of EGFR mutant drug-responsive patients.

![HUPO BUSAN 2023 SEP 17-21 22nd Human Proteome Organization World Congress]

See you in Busan!
BEXCO, Busan, Korea
🌐 2023.hupo.org
🐦 f 📷 in #HUPO2023

Together Through Proteomics

PROGRAM

PP01.168: A Protein-based Prognostic Prediction Model to Stratify Pediatric Patients with Papillary Thyroid Carcinoma

Yaoting Sun, China

Papillary thyroid carcinoma (PTC) is among the most common endocrine malignant tumors in pediatric patients (≤ 18 years). Compared to adult PTC, pediatric PTC (PPTC) has more aggressive clinical features. However, unlike adults, there are currently no widely adopted risk stratification criteria for PPTC patients who, thus, do not receive individualized treatments or prognosis assessments. Therefore, we aimed to find an efficient way to stratify pediatric patients into high- or low- recurrence risk groups. In this retrospective study, we evaluated patients (≤18 years) with thyroid nodules, including 85 pediatric malignant (PM) and 83 pediatric benign (PB) thyroid nodules, who underwent surgery with a median follow-up time of 71 months between November 2007 and April 2021. And we also included 66 adult malignant (AM) patients with PTC as controls. We quantified 10,426 proteins from the 234 tissues by tandem mass tag-labeled quantitative proteomics. From them, we further identified 243 significantly dysregulated proteins between the two pediatric groups (PM vs. PB) and 122 between the pediatric and adult malignant groups (PM vs. AM). Functional annotations showed enhanced activation of the inflammatory and immune system in PPTC patients compared with adult PTC patients. The infiltration of CD8+ T cells were significantly altered in PPTCs, which was analyzed by CIBERSORTx and verified by immunofluorescence staining. Further 19 proteins were selected from the dysregulated proteins to generate a machine learning model for predicting the recurrence risk of PPTC patients. Our optimized model achieved an accuracy of 88.24%. In summary, these results suggest the potential of the classifier to provide a reference for clinical decision-making and individualized treatment for pediatric patients with PTC.

PP01.170: Mass Spectrometric Blood Metabogram: Characterization and Application to Disease Diagnostics

Oxana Trifonova, Russian Federation

Recently, the mass spectrometric metabogram was developed as a simplified and clinically applicable way of measuring the blood plasma metabolome. For this, direct infusion mass spectrometry (DIMS) of the low molecular fraction of blood plasma, principal component analysis (PCA), and metabolite set enrichment analysis (MSEA) were used for metabolome analysis of blood plasma samples from healthy male volunteers (n = 48) of approximately the same age. The seven components of the metabogram, which cover ~70% of blood plasma metabolome variance, were characterized as related groups of the blood metabolites associated with humoral regulation, lipid, carbohydrate, and amine metabolism, lipids intake into the organism, and liver function, thereby providing clinically relevant information. To confirm the expected clinical significance of the metabogram it was applied to analyze patients with various degrees of metabolic dysfunction associated with obesity. The study involved 20 healthy, 20 overweight and 60 with class 1, 2, or 3 obesity individuals. The results showed that the metabogram reveals the metabolic features of obesity, including changes in the blood level of steroids, amino acids and phospholipids, which is consistent with the accumulated scientific data to date. In addition, the metabogram brings clarity to the analysis of metabolically unhealthy overweight/obese patients, providing both a general overview of their metabolic abnormalities and revealing their individual nature. Thus, the metabogram as a rapid clinical test, together with its demonstrated clinical relevance, justifies further efforts to introduce it into clinical practice.

PROGRAM

PP01.172: Kitted Universal MAM: Automatable Sample Processing for all Stages of Biological Drugs

John Wilson, United States

Twenty-six of the top 50 highest-grossing therapeutics are biological drugs, including antibodies. Multiple Attribute Monitoring (MAM) is an LCMS technique used to monitor critical quality attributes (CQAs) in biologics. It has gained popularity for biotherapeutic characterization and is replacing single-attribute assays in QC and release testing. To address the challenges of diverse biotherapeutic manufacturing, we present a universal, automatable MAM kit based on the S-Trap 96-well plate. This kit effectively removes contaminants from salts to surfactants to excipients to dyes, streamlining the workflow from bioreactor to final product.

The modified MAM-specific kit was rigorously tested using the NISTmAb RM 8671 monoclonal antibody in challenging samples, including viscous cough syrup and solutions with PEG or surfactants. MAM sample preparation was performed manually, semi-automated on the Tecan A200 workstation, or fully automated on the Tecan EVO fluid handler with an A200. The kit demonstrated excellent reproducibility for unmodified and PTM-modified peptides, accurately detecting glycosylations. Residual host cell proteins (HCPs) were also detected.

The MAM kit removes contaminants such as sweeteners, salts, surfactants, antioxidants, emulsifiers, binders, bulking agents, lubricants, coatings, dyes, and small molecules. It provides a standardized protocol applicable at all stages of biopharmaceutical manufacturing. The kit's performance matches previous studies on surfactant-containing bioreactor supernatant. Variations in peptide and HCP detection were observed among instruments, but the variability resulting from sample preparation, especially automated preparation, was similar to technical replicate injections.

With the ability to apply the same protocol without modification, the MAM kit has the potential to facilitate broader adoption across therapeutics manufacturing. It is suitable for R&D, characterization, process control, formulations, QC, and release testing.

PP01.174: Deciphering Deregulated Mechanisms Associated with Huntington's Disease and X-linked Dystonia Parkinsonism Pathogenesis in Human Medium Spiny Neuron Models

Joanna Bons, United States

Huntington's disease (HD) and X-linked dystonia parkinsonism (XDP) are rare neurodegenerative diseases characterized by motor disorder and loss of medium spiny neurons (MSNs) in the striatum. HD is caused by a CAG expansion in the huntingtin HTT gene, resulting in altered HTT protein, and XDP is associated with mutated TAF1 gene coding for TATA-binding protein-associated factor 1, including SINE-VNTR-Alu (SVA) element retrotransposon insertions. To date, no cure is available for these diseases, and HD and XDP pathogenesis is not fully characterized. We investigated the proteomes of MSNs generated from human HD and XDP patient induced pluripotent stem cells (iPSCs) to gain insights into disease mechanisms.

Here, HD patient iPSCs and isogenic corrected iPSCs were used to generate MSNs. Regarding XDP, cell lines were obtained from two XDP patients, two related controls (patients' sons) and control SVA deletion. Samples were analyzed using FAIMS-DDA and DIA on Orbitrap Lumos and Eclipse platforms. Data were processed using Proteome Discoverer (Thermo) and Spectronaut (Biognosys).

To explore disease pathogenesis, HD and XDP disease models were developed using iPSC technology to mimic brain development and generate MSNs. Comprehensive proteomic analysis yielded over 4,000 quantified protein groups (2+ unique peptides), with ~10% of the MSN proteome being altered. More particularly, we observed a common deregulation of DNA helicase MCM subunits with HD and XDP. HD-MSNs were additionally characterized by dysregulated septins, involved in synaptic plasticity, and lipid metabolism typified by lipid droplet accumulation and lipophagy deficit. Weighted co-expression network analysis on XDP-MSN proteomics revealed altered RNA splicing, including for TAF1 mRNAs, and altered mitochondrial metabolism as highlighted by upregulated mitoribosome subunits.

Altogether, we present a powerful proteomic workflow to obtain deeper insights into the role of MSNs in HD and XDP and, thus, identify molecular determinants of pathogenesis and potential druggable targets.

SEP 17-21
22nd Human Proteome Organization World Congress

See you in Busan!
BEXCO, Busan, Korea
🌐 2023.hupo.org
🐦 f 📷 in #HUPO2023

Together Through Proteomics

PROGRAM

PP01.176: Integrative Proteomic Analysis with Neurotransmitters for the Brain of PS19 Alzheimer's Disease Model Mice

Eunji Cho, Republic of Korea

Alzheimer's disease (AD) is the most common cause of dementia. It is a slowly progressive neurodegenerative disease associated with cognitive, functional, and behavioral impairments characterized by the progressive accumulation of extracellular amyloid beta plaques and intracellular neurofibrillary tangles of the accumulation of hyperphosphorylated-tau protein. AD is associated with dysfunction in several important neurotransmitter systems, such as dopamine, and individuals with AD have been shown to have impaired neurotransmission. While levels of neurotransmitters in the brain have been associated with AD pathology, in-depth profiling studies on changes in neurotransmitters are still insufficient. Thus, we integrated the profiling of neurotransmitters and proteome in PS 19 mice, one of the representative animal models of AD. Considering the age at which phenotypes appear, wild/hetero-type mice of 3 months and 6 months were used in the study. Neurotransmitters in tissues of 7 brain regions were measured using high-performance liquid chromatography (HPLC-ECD) with an electrochemical detector, an analytical technique commonly used to measure neurotransmitters. Six neurotransmitters, including dopamine, of 6 months age wild/hetero-type mice showed significant changes in 6 brain regions. To discover molecular signatures for the neurotransmitter changes, the tissue proteome of 6 brain regions was analyzed by the reliable quantitative proteomics approach. According to the integrative analysis of the two types of data, we confirmed the distribution of neurotransmitter levels by phenotype and found neurotransmitter-associated molecular pathways. This study will be helpful in understanding the molecular pathology of Alzheimer's disease through new integrative proteomics analysis.

PP01.178: Newly Synthesized Proteome of Parkinson's Disease Peripheral Blood Mononuclear Cells

Dani Flinkman, Finland

Introduction: Parkinson's disease (PD) is the second most common neurodegenerative disease, causing progressive motor symptoms. This disease affected 6.1 million people globally in 2016 and is currently the fastest growing neurodegenerative disease. In our earlier work we have observed decreased protein synthesis in PD patient skin samples, which is in line with earlier work from other groups in post-mortem brain. Here we utilize non-canonical amino acid labelling, to study newly synthesized proteome from peripheral blood mononuclear cells (PBMC) to find biomarkers of PD from blood.
Methods: PBMC cells from 14 healthy, 12 PD individuals, and 12 individuals with other motor disease (non-PD) were labelled with Azidohomoalanine (AHA), which is incorporated in to protein at methionine sites during synthesis. Samples were trypsinized with S-Trap midi columns, and AHA containing peptides were enriched with modified DiDBiT protocol. Samples were analysed in data independent mode in in Q-Exactive HF, and resulting raw files with Spectronaut 14. Downstream data analysis was done with in house developed R package PhosPiR.
Results: We find that peptides belonging to 126 and 203 genes are significantly changing between PD vs healthy, and PD vs non-PD respectively. Strikingly there is a large overlap with enriched pathways and GO terms between these two comparisons. Among the most enriched KEGG pathways Salmonella infection and spliceosome are overlapping, and GO Biological process terms related to mRNA splicing and processing overlap.
Conclusions: Bacterial infections and environmental factors have been linked earlier to PD. Our data highlights proteins that might predispose certain individuals to develop PD in response to exposure to infectious environment. Ongoing work in the lab is to find out, which of the significantly changing peptides are changing at the total proteome level by targeted proteomics technique SureQuant™.

PROGRAM

PP01.180: Rnf146 Disrupts Wnt/β-catenin Signaling Pathway in a VPA-induced Mouse Model of Autism Apectrum Disorder

Seoyeon Kim, Republic of Korea

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by impaired social behavior, communication difficulties, repetitive behaviors, and restricted interests. Various factors contribute to ASD development, including both genetic and environmental influences. One well-known environmental factor is the exposure to valproic acid (VPA) during pregnancy, which has been linked to ASD. However, the mechanisms behind how in utero VPA exposure leads to behavioral deficits have remained unclear.
In this study, we performed a quantitative proteomic analysis on the prefrontal cortex (PFC) of mice exposed to VPA in utero using high-resolution mass spectrometry. We found a significant overlap between differentially expressed proteins (DEPs) in the PFC of VPA-exposed mice and ASD risk genes, including differentially expressed genes (DEGs) from postmortem cortex samples of ASD patients. Among these DEPs, RNF146 showed notable upregulation, which causes dysregulation of Wnt/β-catenin signaling pathway.
Consistent to VPA exposure, Rnf146 overexpression in rodent PFC impaired social behaviors with the dysregulation of the Wnt/β-catenin signaling pathway at adult stage of mice. Furthermore, Rnf146-overexpressing PFC neurons showed increased excitatory synaptic transmission, which may explain the behavioral deficits in mice. Overall, these results demonstrate that Rnf146 is critical for ASD development and contributes to social deficits in VPA-exposed mice, suggesting Rnf146 as a potential therapeutic target for intervention.

PP01.182: Multiplexed Quantitative Proteomics Revealed Proteome Alterations in Two Types of Rodent Traumatic Brain Injury Models

Young Sik Kim, Republic of Korea

In many cases of Traumatic brain injury (TBI), conspicuous abnormalities such as scalp wounds or intracranial hemorrhages abate over time. However, a number of unnoticeable symptoms such as cognitive, emotional, and behavioral dysfunctions often last from weeks to years after the trauma even in the mild injury. Moreover, the reason for the persistence of symptoms has not been fully explored. Recent studies suggested that the dysregulation of the molecular system in the injured brain could be the major players, necessitating in-depth analysis investigating the relevant proteins and signaling pathways involved in regulating the consequences of TBI. Therefore, in this study, the brain proteomes of two types of TBI models were explored by quantitative proteomics over the recovery period to elucidate the molecular mechanism of TBI. The result showed distinct alterations in proteome expression in both TBI models. Further bioinformatics analysis showed strongly activated/inhibited signaling pathways as well as the core proteins that play an important role in the biological processes after the brain injury. These findings would help uncover the molecular mechanisms underlying the persisting consequences of TBI and discover novel targets of drug intervention.

PP01.184: Plasma Proteomic Signature of Mild Cognitive Impairment Using Proteograph Workflow

Seung Joon Lee, Republic of Korea

Mild Cognitive Impairment (MCI) is a critical prodromal stage preceding Alzheimer's disease (AD), necessitating the need for early diagnosis. This study addresses the need for reliable biomarkers by employing LC-MS/MS proteomics with the Proteograph. The Proteograph leverages nanoparticles with diverse surface functionalities to enable precise and unbiased proteomic analysis across the dynamic range of the blood proteome. Plasma samples from 44 MCI patients and 13 cognitively normal individuals were analyzed using the Proteograph and a conventional depletion method for comparison. Data Independent Acquisition (DIA) mode and a 1-hour nanoLC method were utilized. The Proteograph identified 1,864 proteins, including 298 differentially expressed proteins (DEPs), while the depletion method only detected 832 proteins and 160 DEPs. Importantly, the Proteograph identified 172 low-abundance proteins (LAPs) with concentrations below 100 pg/mL, compared to only 5 detected by the depletion. Functional enrichment analysis of the DEPs revealed their association with cognitive decline progression and AD. Additionally, the study demonstrated the potential of deep proteome profiling to discriminate between MCI subtypes. Overall, the comprehensive analysis of the plasma proteome using the Proteograph provides insights into molecular signatures for predicting MCI risk and enables accurate diagnosis and classification. These findings advance early detection and personalized management of MCI, benefiting patient outcomes.

PP01.186: Deep Plasma Proteomic Landscape of Alzheimer's Disease: An 1800-Sample Cohort Study

Khatereh Motamedchaboki, United States

Introduction

 The mechanisms and biomarkers that underline the development and progression of Alzheimer's Disease (AD) are still not fully understood and work continues to identify biomarkers that can be used to diagnose the disease earlier and develop more effective treatments. Here, we leveraged recent advances in plasma proteomics to conduct an unprecedentedly deep and unbiased study of the plasma proteome in AD, tracking proteome changes over time.

Methods

Our cohort included approximately 1,800 samples from individuals with three different conditions: control, AD and dementia affected groups. Some of the samples were from the same individuals over time. The plasma samples were randomized based on conditions, but all samples from the same individual were kept on the same plate. The samples were processed with the ProteographTM Product Suite and then analyzed using a high-throughput Liquid Chromatography (LC) coupled to a Data Independent Acquisition (DIA) on a Thermo Fisher Scientific OrbitrapTM ExplorisTM 480 MS.

Results

LC-MS data was acquired on 1,802 samples, of which 12 failed the mass spectrometry quality control protocol. We investigated several DIA spectral library strategies, including library-free analysis with match between runs and a project-specific gas-phase fractionation (GPF) library. The experimental work was completed in 11 weeks on a single MS instrument. We identified 5,253 protein groups using the library-free search and 4,007 using the GPF library, both at a false discovery rate of 1%. Our preliminary analysis showed a strong biological signal, demonstrating the statistical power of the data. Using 10-fold cross validation and a customized AutoML framework, we were able to separate controls from dementia-and AD affected individuals.

Conclusions

Our preliminary analysis is promising, and we plan to extend this work to better understand the trajectory of the changes over time and the biological pathways that underlie the observed differences.

PP01.188: Uncovering Brain Region-specific O-glycophenotypes of Mice with Depressive-like Behavior

Youngsuk Seo, Republic of Korea

Depression is a common mental disorder worldwide, with an estimated 5% of the adult affected. Symptoms of depression can vary from sadness to fatigue, which is caused by a complex interplay of social, psychological, and biological factors. Therefore, investigating molecular mechanisms in the brain is necessary to understand the biological basis of the disease and develop effective treatments. Recently, differential glycosylation characteristics have been revealed in patients with depression. In this study, we explored brain O-glycans of mice with depressive-like behavior. Initially, we induced depressive-like symptoms in mice by exposing stressful conditions such as tail suspension, electrical foot shock, and restraint in tube. Depressive state of the mice was evaluated by novelty suppressed feeding test, exhibiting high level of innate fear in a novel space. Prefrontal cortex (PFC) and hippocampus (HP) of mouse brain, regions most consistently impaired in depression, were selectively sectioned to determine region-specific and disease-associated O-glycosylation. We isolated O-glycans from brain tissues for in-depth profiling of heterogeneous glycans. In the following LC-MS analysis, approximately 20 native O-glycans were observed in both control and chronic variable stress (CVS)-induced mice. They were classified into core 1, 2, and 3 type O-glycans and O-mannose glycans. For O-glycosylation in PFC, an increase in fucosylation and a decrease in sialylation were observed in CVS-induced mice. Meanwhile, both fucosylation and sialylation were slightly reduced in HP. Taken together, the mice with depressive-like behavior exhibited differential brain O-glycosylation features according to regions. Stressful environments could cause an alteration in O-glycosylation of brain proteins.

PROGRAM

PP01.190: Exploring Proteomic Alterations and Co-expression Modules Associated with Tau-induced Pathologies in Human Tau-transgenic Mice

Kazuya Tsumagari, Japan

Abnormally accumulated tau protein aggregates are one of the hallmarks of neurodegenerative diseases, including Alzheimer's disease (AD). To investigate proteomic alteration driven by tau aggregates, we implemented quantitative proteomics to analyze disease model mice expressing human MAPT (P301S) transgene (hTau-Tg) and quantified more than 9,000 proteins in total. We applied the weighted gene co-expression analysis (WGCNA) algorithm to the datasets and explored protein co-expression modules that were associated with the accumulation of tau aggregates and were preserved in proteomes of AD brains. This led us to identify four modules with functions related to neuroinflammatory responses, mitochondrial energy production processes, cholesterol biosynthesis, and postsynaptic density. Furthermore, phosphoproteomics uncovered phosphorylation sites that were highly correlated with these modules. Our datasets represent resources for understanding the molecular basis of tau-induced neurodegeneration, including AD.

PP01.192: A Routine Workflow of Spatial Proteomics on the 0.002mm2 of FFPE Tissues

Hao Chen, China

In the era of single cell biology, spatial proteomics has emerged as an important frontier, whereas it is still facing several challenges in technology. Tissues with formalin-fixed paraffin-embedded (FFPE) are an important material in spatial pro-teomics, in which the fixed tissues are excised by laser capture microdissection (LCM) followed by protein identification with mass spectrometry. How to efficiently extract and identify proteins from micro-FFPE tissues prompts our effort to systematically assess the key steps in such analysis like necessity of de-staining, complete de-crosslinking, and size-limitation of FFPE proteomics. Although de-staining is generally accepted in the proteomics on the FFPE tissues stained by hematoxylin-eosin (HE), a micro-FFPE tissue excised with LCM was found no need of destaining to prevent peptide loss. After comparing how to place a container that held the micro-FFPE slice during de-crosslinking, the hang-down posi-tion was attained to achieve a satisfied identification of proteins. To make a better peptide recovery from micro-FFPE tissues, a simplified process was established. A routine workflow of proteomic analysis on micro-FFPE tissues, therefore, was proposed by integration of all the optimized technologies. Based on this method, excision size at 0.002mm2 was workable to detect over 800 proteins, while a region of mouse brain tissues fixed by FFPE was continuously LCM-cut at 0.05mm2 and was undergone for proteomic analysis. A spatially proteomic atlas was preliminary constructed according to protein abundance and functional enrichment of proteins in that region.

PP01.194: Spatial Proteomics of a Human Brain Tumour

Simon Davis, United Kingdom

Background:
The spatial protein abundance profiles within tissues are a key factor in understanding disease pathology and cellular function. These profiles are typically studied by antibody-based imaging techniques that provide high spatial resolution, but can only probe a limited number of proteins. In order to more precisely define molecular phenotypes in disease, 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 high-throughput LC-MS/MS-based proteomics is well placed to meet this need.

Methods:
Tissue voxels were isolated from 10 µm-thick sections of a human brain tumour in a gridded pattern. Captured tissue was processed using an SP3-based protocol prior to analysis by LC-MS/MS on a timsTOF Pro using 17-minute gradients. Moran's I tests were used to investigate spatial autocorrelation of quantified proteins. Spatial protein profiles were used for unbiased spatial clustering by affinity network fusion, ANOVA significance testing and pathway analysis between spatial clusters, with validation by immunohistochemistry.

Results:
Proteomic depth was comparable between the two platforms, with approximately 5,000 proteins detected across the experiment. Quantitative protein measurements can be plotted in their spatial context to generate proteomic tissue maps. Over 3,000 proteins show significant evidence of spatial autocorrelation, respectively (Moran's I test, q ≤ 0.05). Immunohistochemistry staining for three highly spatially autocorrelated proteins is consistent with the proteomic dataset. 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.

Conclusions:
Spatially-resolved proteomics methods which spatially profile thousands of proteins will push the boundaries of understanding tissue biology and pathology at the molecular level. Recent mass spectrometry technology developments were key to achieving this throughput and depth, and this approach stands to benefit from further technological developments.

PP01.196: Development of Robust Spatial N-Glycomics and Proteomics Techniques for Human Tissue Analysis

Young Ah Goo, United States

MALDI-MSI has emerged as a powerful tool for the spatial mapping of N-linked glycans and proteins in a variety of tissue types, including fresh/frozen tissues, FFPE tissue, and tissue microarrays to aid in the identification of potential biomarkers. Here we describe the development of a robust protocol for profiling the spatial distribution of N-glycomics and proteomics within the serial tissue sections.

FFPE tissue blocks were sectioned at 7 µm thickness. Slides were heated, dewaxed, and rehydrated for heat-induced antigen retrieval. PNGase F was applied to the slide for spatial N-glycan analysis using a TM-Sprayer and incubated in a humidity chamber. The slide for spatial proteomics is stained overnight with photocleavable probes targeting 6 specific proteins. CHCA matrix is used in both workflows. Bruker timsTOF Flex MALDI2 was operated in positive ion mode over an m/z range of 1000–5000. Imaging data files were imported into the SCiLS lab software. Using the NGlycDB, a novel database of N-glycans available publicly on METASPACE, the data was rapidly annotated, visualized, and interpreted.

We were able to putatively annotate >200 N-glycans from the human pancreas tissue, analyzed at a 10% FDR on METASPACE. Several of these glycans specific in the tumor region can be traced back to being fucosylated, an interesting trend in disease. To obtain additional information from the same samples, spatial proteomics was performed on serial sections using the established protocol from AmberGen and a panel of proteins, CD3Ɛ, CD68, Vimentin, Collagen 1A1, PanCK, and Ki67, was visualized.

Through these workflows, we are building robust sample preparation methods as well as instrument methods to aid in mapping of spatial distribution of N-glycomics and proteomics.

PP01.198: Phosphoproteomic Analysis to Identify the Mechanism Underlying the Development of Childhood Autism on Prenatal VPA Exposure

Hazara Begum Mohammad, Republic of Korea

Phosphoproteomic analysis to identify the mechanism underlying the development of childhood autism on prenatal VPA exposure

Hazara Begum Mohammad, Hung M.Vu, Thy N.C.Nguyen, Jung-Hyun Lee, Yeji Do, Min-Sik Kim*

Department of New Biology, DGIST, Republic of Korea

Abstract

Autism spectrum disorder (ASD) is a heritable and heterogeneous neurodevelopment disorder characterized by impairments in social communication and interaction, sensory anomalies, repetitive behaviors, and varying levels of intellectual disability in children. ASD development has been linked to numerous genetic variations and environmental triggers. Recently, we have shown that an in vivo genetic alteration such as Cntnap2 knock-out can lead to proteome-wide change related to multiple biological processes including metabolic processes, synaptic activity, and neurogenesis, demonstrating how quantitative proteomics can help understand the underlying pathophysiology of ASD. Valproic acid (VPA) is an anti-epileptic drug to use as monotherapy and adjunctive therapy for complex partial seizures. However, it has been reported that prenatal exposure to VPA to can also lead to ASD-like phenotype. In this study, we carried out TMT-based quantitative proteomic, phosphoproteomic and CETSA analyses from neuroblastoma cells treated with VPA to discover underlying signaling mechanisms for ASD development. Further bioinformatic analysis such as GSEA and PTM-SEA using differentially expressed proteins and phosphorylations showed that various signaling pathways including Wnt signaling are altered with VPA treatment. We also found that these enriched signaling pathways play a significant role in the development of autism in children. Then, we performed TMT-based quantitative CETSA experiment to find direct targets of VPA. Several molecules including known ones have been recapitulated, and were found to be involved in signaling pathways identified by proteomics and phosphoproteomics. In conclusion, we successfully discovered the pivotal signaling pathways that lead to VPA-associated ASD development through a series of TMT-based quantitative mass spectrometry analyses.Can be utilized to advance an effective treatment for ASD.

PP01.198: A Spatiotemporal Single-cell Type Map of Human Tissues Based on High-resolution Antibody-based Imaging

Cecilia Lindskog, Sweden

For a fundamental understanding of human health, molecular medicine and targeted treatment, it is necessary to map processes unique to each tissue and cell type. We here aimed to set up a stringent workflow for mapping human tissues at the single-cell type level, and utilize this workflow to create a high-resolution spatiotemporal map of adult human testis. Testis is a complex organ where thousands of genes and proteins are activated or repressed through multiple cell states, from spermatogonial stem cells to mature sperms. Understanding the intricate functions and mechanisms at each step of this process requires a multi-dimensional approach that integrates several quantitative methods.

Based on reanalysis of publicly available single-cell RNA sequencing data, we identified 12 distinct differentiation states of testicular germ cells linked to unique biological processes and molecular functions, such as cell division. By utilizing a large-scale multiplex immunofluorescence pipeline and automated image analysis using deep learning, we then performed an in-depth characterization of ~600 proteins expressed in germ cells, to map their exact localization to specific cell states. The integrated data allowed us to study temporal mRNA and protein expression gradients along with maturation processes and identify which mRNAs that are directly synthesized into proteins from those that are translationally sequestered from a spatiotemporal aspect. We were also able to assign functions to numerous uncharacterized proteins not previously described in the context of male reproduction.

In summary, we present a spatiotemporal single-cell type reference map of adult human testis that decodes the complexity of human germ cells, cell heterogeneity, and homeostasis and links quantitative data with tissue morphology. The data is freely available on www.proteinatlas.org and the validated workflow will be further utilized to generate a single-cell type map of other major normal tissues, contributing to valuable insights into molecular function and disease-related processes.

PROGRAM

PP01.200: MitoAtlas, a Mitochondrial Proteome Map Created with Super-Resolution-Proximity-Labeling

Nirmali Sharma, Republic of Korea

Introduction: Mapping the sub-mitochondrial proteome and understanding its spatial organization are vital for elucidating mitochondrial functions. In this study, we employed super-resolution proximity labeling (SR-PL) with APEX2 and BioID baits to map the sub-mitochondrial proteome of HEK293T-REx cells. This allowed us to make a mitochondrial proteome database and study the effects of environmental contexts on the labeling reactions.

Methods: APEX2 and BioID baits with various sub-mitochondrial localizations were stably expressed in HEK293T-REx cells, and SR-PL was used for analysis of labeled products. Logistic regression algorithms were employed to classify the sites and proteins into sub-mitochondrial spaces, and protein topology were assigned. Further computational investigations were done on the dataset to find the factors that affect the reactivity of sites. AlphaFold-Multimer was used to predict protein interactions of specific bait-prey pairs. PEX3 and PEX16 KO HEK293T-REx cells were also studied using mito-APEX2 and TOM20-TurboID.

Results: Total of 13,348 biotin or DBP-modified sites were identified, and 11,660 of them were classified into distinct sub-mitochondrial spaces. 858 mitochondrial proteins were identified with detailed sub-mitochondrial localization and topological information. 120 mitochondrial orphan proteins were newly discovered. Surface exposure of specific probe radii and certain motifs showed relationships with site reactivity, depending on the labeling chemistry. PEX3 and PEX16 knockout HEK293T-REx cells displayed increased ROS as well as reduced near-MTS labeling in the matrix but the opposite at the outer membrane.

Conclusion: We mapped the sub-mitochondrial proteome of HEK293T-REx cells using SR-PL, resulting in MitoAtlas—a mitochondrial proteome map of 858 proteins with sub-mitochondrial localization and topological information. Furthermore, our study revealed mitochondrial orphan proteins, relationship of environmental context with the labeling reactions, and the impact of PEX3 and PEX16 knockout on the mitochondrial proteome and import. These findings contribute to our understanding of mitochondrial biology and are accessible through the website mitoatlas.org.

Session Date/Time: Monday, September 18, 2023 - 04:30 PM - 05:35 PM

CS09: Pharmaco/Chemical Proteomics

Chair
Tadashi Kondo, Japan

Chair
Garry Corthals, Netherlands

16:31   ### CS09.01: Keynote Speaker - Unraveling Drug-Target-Phenotype Interaction Using Label-Free Small Molecules Based Chemical Proteomics and Its Translational Implications
Ho Jeong Kwon, Republic of Korea

See you in Busan!
BEXCO, Busan, Korea
2023.hupo.org
#HUPO2023

Together Through Proteomics

PROGRAM

16:56   **CS09.02: High-Throughput (Phospho)Proteomics Drug Screening in Triple-Negative Breast Cancer Cell Lines Uncovers Targetable Nodes for Rational Drug Combination Therapies**

Kristina Bennet Emdal, Denmark

Triple-negative breast cancers (TNBCs) comprise the most challenging breast cancer subtypes due to a high degree of tumor heterogeneity and lack of clinically targetable nodes. Consequently, patient survival is poor, which underscores the need for improved therapies. We previously demonstrated that monitoring immediate phospho-signaling changes in response to single-drug treatment in leukemic cells revealed signaling rewiring and actionable targets with potential for effective co-targeting by rational drug combination therapies (Emdal et al., Cell Reports, 2022). Therefore, we hypothesized that a similar approach would be valuable to identify drug combinations with clinical benefits for TNBC patients.
Here, we characterized the immediate cellular responses at the phospho- and proteome level in seven TNBC cell lines using a panel of clinically relevant drugs: eight FDA-approved chemotherapeutic agents, six CDK4/6 inhibitors, and an immune checkpoint inhibitor targeting PD-L1. To establish a drug response discovery platform, we streamlined our sample processing workflows for phospho- and proteome analysis in a high-throughput 96-well format making use of automated Opentrons and KingFisher systems. TNBC cells were treated with 2 doses of each drug individually for 1 hour (phosphoproteome) or 24 hours (proteome) in 5-6 replicates per condition, and samples were analyzed by mass spectrometry with short liquid chromatography gradients using Evosep One system coupled to Orbitrap Exploris 480 in DIA (1,344 single-shot proteomes) and hybrid-DIA mode for simultaneous targeted and discovery phosphoproteome analysis (selected cell lines). We identified >6,000 proteins per cell line and >11,000 phosphorylation sites including sensitive quantification of 120 targeted phospho-peptides covering seven major signaling pathways. These data show drug dose-dependent protein abundance changes and phosphorylation site regulation. We are now focusing on validating key findings using thermal proteome profiling and drug synergy screens with clinically relevant kinase inhibitors and chemotherapeutics to identify novel and rational drug combinations with clinical potential in the TNBC setting.

17:09   **CS09.03: Proteome-Wide Pharmacophore Selectivity Profiling by Chemical Proteomics**

Polina Prokofeva, Germany

Most of the human proteome remains therapeutically unaddressed, and we lack the chemical tools to validate all potential pharmacologically targetable proteins. As a result, the majority of drug discovery campaigns revolve around a small number of already validated protein targets. We present a novel molecule-centric strategy that capitalizes on affinity-based proteome profiling to systematically interrogate the full bioactivity potential of a chosen pharmacophore in a target-agnostic fashion.

To exemplify this concept, we delineated the interactomes of a focused library of 35 structurally related molecules designed around a lophine kinase inhibitor scaffold. We deployed the set of linkable lophines and their respective tailored affinity matrices in a dose-resolved competitive chemoproteomic target deconvolution assay, which established the selectivity profiles of all molecules against all bead-bound proteins. The results showed that 11 compounds interacted with 44 different protein targets with submicromolar affinities. Comparison of the selectivity profiles revealed proteome-wide Structure-Affinity Relationships (SAR) between compound features that bury deeply inside protein pockets. Some of the new molecules were rather unselective, but three were exquisitely selective for either the kinases ALK5 and RIPK2 or, unexpectedly, Sepiapterin reductase (SPR). We confirmed that compound binding translates into enzyme activity inhibition by orthogonal assays, demonstrating the power of the method.

The examples aside, the perspective of this novel target-agnostic approach is that any chemotype of medicinal chemistry interest may be tested against a complete proteome. The data illuminate common or distinct chemical features responsible for binding to an individual protein, and the overall profile constitutes the pharmacophore's target space. The potency of binding to any protein and the target selectivity of each newly synthesized molecule are simultaneously measured, allowing to drive synthesis efforts towards chemical probes and expansion of the druggable proteome.

PROGRAM

17:22   **CS09.04: Perturbation Proteomics: Insights from 16,000 Perturbed Triple-negative Breast Cancer Proteomes**
Rui Sun, China

Background Triple-negative breast cancer (TNBC) is associated with poor clinical outcome. Treatment options for TNBC remain limited. Perturbation proteomics offers unique insights in drug discovery research.

Methods We employed a 15 min-gradient data-independent acquisition (DIA) strategy to perform perturbation proteomic analysis of 15 TNBC cell lines and two non-TNBC cell lines treated 63 FDA-approved drugs for 0, 6, 24, 48 h at 10 µM. Triple biological replicates were obtained for each treatment. The efficacy of each drug (IC50) was evaluated on at least two cells every two months. In the DIA-MS analysis, the nine TNBC cell line pool sample and randomly selected technical replicates in each batch were used as quality control samples. We built a spectral library containing 130130 peptides and 9355 proteins. The DIA data were searched with DIA-NN using this spectral library. Subsequently the reproducibility of quality control samples was evaluated by Pearson correlation and CV.

Results We acquired 20,000 perturbed proteome dataset, characterizing 5432 proteins. The median correlation index of biological replicates, technical replicates, and pool samples were 0.79, 0.94, 0.96, respectively. With a model composed of 50 selected proteins, we could predict the sensitivity of an unknown chemical with an AUC of 0.83 in TNBC. The data exposed multiple signaling pathways which could be potentially targeted to treat refractory TNBC patients. We compared the most perturbed proteins and pathways with the most frequent mutant genes in published genomic and transcriptomic dataset of TNBC patients. Our data uncovered proteins and pathways sentinels for phenotypic changes in cancer cells. Lastly, we built a model to predict mode of actions of a compound based the perturbed proteome.

Conclusion Our study presents a rich perturbation proteomics dataset in prediction of drug responsiveness and mode of actions of a compound and uncovering protein and pathway sentinels for cell phenotypic changes.

Session Date/Time: Monday, September 18, 2023 - 04:30 PM - 05:35 PM

CS10: Proteomics and Biology

Chair
Peter Hoffmann, Australia

Chair
Sergio Encarnación-Guevara, Mexico

See you in Busan!
BEXCO, Busan, Korea
2023.hupo.org
#HUPO2023

Together Through Proteomics

PROGRAM

16:31   **CS10.01: A Protein-Based Differential Diagnosis Classifier for Follicular Thyroid Neoplasms**

Yaoting Sun, China

Differential diagnosis of follicular thyroid adenoma (benign, FTA) and carcinoma (malignant, FTC) before and after surgery is an excellent challenge that may lead to unnecessary thyroidectomy. To precisely differentiate follicular thyroid tumors, we constructed a deeply covered proteomic resource of 645 tumors from 13 clinical centers. We used pressure cycling technology and tandem mass tags labeling proteomic strategy combined with data-dependent mass spectrometry acquisition to quantitatively measure proteome expression in retrospective formalin-fixed paraffin-embedded slide samples. The acquired mass spectrum raw files were processed by Proteome Discoverer and FragPipe, and the matrix comprising 11,837 proteins was divided into a discovery set (271 FTA and 238 FTC) and an independent test set (70 FTA and 65 FTC). We found 178 differentially expressed proteins (DEPs) for distinguishing FTA from FTC in the discovery set, which mainly participate in pathways of LXR/RXR activation, coagulation system, and protein kinase A signaling. We also proposed a novel signaling pathway of IGF2R-MVP-HDAC-H1.5 which was validated by co-immunoprecipitation (IP) assays through western blot and IP-MS. To differentially diagnose follicular tumors, we built a classifier using machine learning and DEPs. The extreme gradient boosting (XgBoost) algorithm performed optimally regardless of the number of features chosen and achieved the highest area under the curve (AUC) with 25 proteins. After optimizing the hyperparameters, the model attained an AUC of 0.90 with 0.88 sensitivity, 0.84 specificity, 0.84 positive predictive value, and 0.88 negative predictive value in the independent test set. In summary, we constructed a 25-protein classification model through deep proteomic identification combined with the XgBoost algorithm, which has great potential to distinguish FTA from FTC and improve clinical decision-making.

16:44   **CS10.02: Measurement of Proteins at Scale Using Protein Identification by Short-epitope Mapping (PrISM)**

Parag Mallick, United States

Introduction: The combination of single-molecule resolution and comprehensive proteome coverage has the potential to improve sensitivity and reproducibility of protein analysis. Here, we demonstrate Protein Identification by Short-epitope Mapping (PrISM), which aims to provide analysis of >95% of the proteome with broad dynamic range at single-molecule sensitivity by interrogating immobilized, intact proteins in parallel using multi-affinity probes.

Methods: PrISM uses non-traditional affinity reagents, termed multi-affinity probes, which are designed with high affinity and low specificity in order to bind short epitopes present in multiple proteins across the proteome. Proteins were conjugated to DNA scaffolds and deposited onto a high-density patterned flow cell at optically resolvable locations. Multi-affinity probes were applied to proteins over multiple cycles to generate binding patterns for each single-molecule protein, which are translated to protein identifications and quantities using machine learning. We acquired data on complex protein mixtures and control samples using over 100 multi-affinity probes targeting trimer or tetramer sequences, validating PriSM as a viable approach for proteome analysis.
Results: We report single-molecule deposition of billions of DNA nanoparticle complexes on a flow cell. We demonstrate measurement of individual protein molecules from complex mixtures through iterative probing with multi-affinity probes using the PrISM methodology. Further, we demonstrate the ability to accurately estimate the false identification rate of these proteins using a target-decoy based statistical approach.

Conclusions: Combining single-molecule analysis, intact (non-digested) proteins, and iterative probing, PrISM provides a new tool for quantitative proteomics. The ability to make comprehensive measurements of intact proteins at single-molecule resolution will accelerate proteomic research.

16:57　**CS10.03: Enhancing Consistent Quantification and Site-Localization of PTMs in Large-Scale DIA-MS Experiments using Dynamic Alignment and Ion Mobility**
Justin Sing, Canada

Introduction:
Post-translational modifications (PTMs) play a crucial role in cellular processes, diversifying the proteome. However, achieving consistent quantification across data-independent acquisition mass spectrometry (DIA-MS) runs and accurately site-localizing partial/co-eluting peptidoforms remains challenging. To address these issues, we developed a dynamic programming alignment method for aligning extracted ion chromatogram (XIC) features and leveraged ion mobility separation in diaPASEF data.
Methods:
To improve peptidoform quantification in DIA-MS runs, we utilize a dynamic programming alignment method for XICs. Alignment paths within peptidoform sets were considered monotonic, facilitating confident site-determining ion propagation and peptidoform inference. Benchmarking included synthetic phospho-peptides, complex phospho-enriched data, and N-Glycosylated enriched blood plasma from five solid carcinomas. For site-localized peptidoform quantification in dia-PASEF, we implemented a U-Net architecture for 2D feature peak-picking, training it with watershed feature boundaries generated from binary masks and peak maxima. Incorporating robust 2D quantification and ion mobility into a hierarchical Bayesian framework (IPF) will improve site-localization performance.
Results:
Our method outperformed the standard workflow, achieving a 24% reduction in false site-localization and recovering 17% of missed signals at 5% FDR in a synthetic phospho-peptide dataset. True identifications increased by 9%, reaching a true positive rate of 94%. Notably, the U2OS dataset exhibited remarkable improvement, with a 21% point gain in quantified phosphopeptides and 97% consistent identification post-alignment. Ongoing evaluation of our 2D feature quantification using a challenging phospho-enriched EGF dataset demonstrates promising separation of partially co-eluting peptidoforms in retention time and ion mobility.
Conclusions:
We address the challenge of inconsistent quantification and potential loss of identifications in DIA-MS caused by liquid chromatography variation and low signals of site-determining ions. Our method enables consistent quantification of site-localized PTMs across runs, improving downstream statistical analyses and reducing the coefficient of variation (CV). Additionally, the integration of ion mobility via diaPASEF shows promising potential for resolving site-localized PTMs.

17:10　**CS10.04: Different Impact of Oscillating, Transient, and Sustained Activations on the Same Kinase: An Optogenetic-Phosphoproteomic Study**
Yansheng Liu, United States

Both the abundance and dynamic pattern of protein phosphorylation dictate the output of cell signaling networks. However, how cells decode different activation patterns from the same kinase into distinctive downstream signaling and phenotypic outcomes remains largely enigmatic. To address this interesting question, we focused herein on the example of AKT, a central kinase node in cell signaling. We performed a systems-level analysis that integrates methodological advances in optogenetics, mass spectrometry-based phosphoproteomics, and bioinformatics to elucidate how Akt1 intensity, duration, and stimulation pattern lead to distinct temporal phosphorylation profiles in vascular endothelial cells. Firstly, we developed an Optogenetics-phosphoproteomics DIA-MS system, namely Optop-DIA, in which blue light was used to stimulate Akt1 phosphorylation with great specificity, high precision and minimal invasiveness. Optop-DIA enabled us to measure and compare 27 conditions of Akt1 activation (3 light intensities× 3 patterns× 3 time points). Through the analysis of 35,000 Class-I phosphorylation sites across these conditions, we identified a series of signaling circuits downstream of Akt1, categorizing kinase substrates that are preferentially activated by oscillating, transient, and sustained Akt1 signals. We found that transient phosphorylation of Akt1 substrates appeared sufficient to induce sustained phosphorylation of unique sets of phosphorylation sites (P-sites), which could be potential substrates for MAPKs, CK2, and CDKs. Moreover, the upregulation of MYLK3 and ROCK1 substrates strikingly favored oscillatory pulses over constant stimulation. Furthermore, we applied Optop-DIA to dissect the pathway crosstalk between Akt1 and growth factor signaling, identifying kinases and phosphosites preferably regulated by vascular growth factors rather than by Akt1 alone. Finally, using a novel mTRAQ-phosphoproteomics-based plex-DIA MS method and phosphosite-specific antibodies, we validated a list of 112 phosphorylation sites that covaried with Akt1 phosphorylation across all experimental conditions as potential Akt1 substrates. Our OptoCore dataset demonstrated even higher specificity than PhosphoSitePlus and improved our current understanding of AKT motifs.

PROGRAM

See you in Busan!
BEXCO, Busan, Korea
2023.hupo.org
#HUPO2023

Together Through Proteomics

Session Date/Time: Monday, September 18, 2023 - 04:30 PM - 05:35 PM

CS11: Metabolism and Regulation

Chair

Fernando Corrales, Spain

Chair

Min-sik Kim, Republic of Korea

16:31   **CS11.01: Keynote Speaker: Proteome-Wide Systems Genetics Identifies UFMylation as a Regulator of Muscle Function**
Benjamin Parker, Australia

16:56   **CS11.02: Integrated Multi-Omics Analysis Reveals Enhanced Branched-Chain Amino Acids Metabolism Alleviates Non-alcoholic Steatohepatitis**
Hyeong Min Lee, Republic of Korea

Nonalcoholic steatohepatitis (NASH) is a chronic liver disease characterized by hepatic steatosis and hepatic lipotoxicity, which can lead to cirrhosis and hepatocellular carcinoma (HCC). Recently, it has been reported that Vutiglabridin, shown to be a paraoxonase 2 modulator, may enhance mitochondrial function. However, the underlying mechanisms that lead to the alleviating NASH remain obscure. In this study, we used an Amylin-Liver NASH (AMLN) diet-induced obese (DIO) mouse model and performed the transcriptomic, proteomic, phospho-proteomic, and metabolomic analyses to investigate the mechanism of action of Vutiglabridin. Vutiglabridin enhanced mitochondrial function, which activated BCAA catabolism, leading to reduced hepatic BCAA levels, inhibition of mTOR signaling, and activation of AMPK signaling. Unexpectedly, we observed that cholesterol synthesis was activated despite a decrease in hepatic free cholesterol and cholesteryl esters. Enhanced mitochondrial function decreased cholesterol produced from acetyl-CoA. To maintain cholesterol homeostasis, leucine served as a primary precursor for cholesterol synthesis, and cholesterol is used for cholesterol metabolism, including bile synthesis. Enhancing BCAA metabolism may have the potential to treat NASH by reducing hepatic BCAAs and modulating cholesterol homeostasis.

17:09 **CS11.03: Integrated Multiomics Examinations in the Study of Obesity and Type 2 Diabetes**

Eva Csosz, Hungary

Background
Obesity and type 2 diabetes (T2D) can be considered as major health-impairing conditions affecting millions of people worldwide. It would be of high importance to acquire information that could make possible patient stratification or can predict the appearance of disease-related complications with an emphasis on cardiovascular complications.
Methods
Serum and plasma samples collected from patients with obesity, and/or T2D were examined using liquid chromatography-mass spectrometry (LC-MS) and the concentration of 23 amino acids and 10 biogenic amines were studied. In parallel to this we examined with proximity extension assay (PEA) the relative changes of 366 proteins related to cardiovascular diseases. Along with sample collection a thorough clinical examination was carried out, including classical laboratory tests (lipid profile, liver, and kidney metabolism, etc.), some gene polymorphism analyses, and imaging. Classical statistical analysis along with correlation and network analysis was performed to map biological processes relevant to obesity and T2D.
Results
Using our data, we were able to construct a combined big data-based repository integrating clinical and laboratory analysis and imaging data, demographics, and other medical records. This database allows us to do a complex analysis of clinical information, metabolomics, and proteomics data as well as patient data.
Conclusion
Using the complex examination of proteomics and metabolomics data along with network analysis we could identify pathways that are characteristic to either or common to both conditions.

17:22 **CS11.04: Identification of Secreted Unannotated Small ORF Microproteins from Adipocytes and in Plasma to Elucidate Function of Novel Gene Product Proteins**

Christopher Barnes, United States

Unannotated small ORF (smORF) microproteins (MPs) are a potentially rich source of uncharacterized metabolic regulators. Applying ribosome profiling (Ribo-seq) with RNA-Seq enables the exploration of novel protein translation within non-coding regions of the transcriptome. However, validation of stable protein production is fundamentally important to begin to understand the function of these new genes. Numerous known metabolic hormones are circulating small peptides such as glucagon-like peptide 1 (GLP-1) suggesting that MP discovery could be fruitful in discovering new secreted, small metabolic effectors. To find metabolism-related MPs, we used Ribo-seq to curate 3,877 unannotated MP-encoding smORFs in primary brown, white, and beige mouse adipocytes. Using mass spectrometry (both DDA and DIA) along with peptide fractionation, we validated 85 MPs including 33 circulating MPs in mouse plasma. We show that numerous MP-encoding mRNAs are regulated in adipose tissue in vivo and are often co-expressed with known metabolic genes. Our Ribo-seq efforts provide evidence for the translation of the long non-coding RNA, predicted gene Gm8773 in mouse ("mGm8773") whose expression is high in the arcuate nucleus of the hypothalamus. This led us to make a recombinant protein form of mGm8773-MP to test using intracerebroventricular administration where we subsequently saw orexigenic activity in obese mice, which confirmed that mGm8773 is indeed the homolog of human and chicken FAM237B. With the aim of identifying circulating MPs with novel hormone function, we've extended our DDA- and DIA-based fractionation methods to human plasma. We've now identified a small catalog of novel MPs from human smORFs using combinations of small protein enrichment, high pH reversed phase fractionation DDA measurements, and gas phase fractionation DIA methods. Together, these data underscore the value of combining Ribo-seq, RNA-seq, complex fractionation-based MS that uses combinations of DDA with DIA, and recombinant protein-based functional studies to elucidate functions for these new gene products.

Session Date/Time: Monday, September 18, 2023 - 04:30 PM - 05:35 PM

CS12: Infectious Diseases

Chair

Bernd Wollscheid, Switzerland

PROGRAM

Chair

Junho Park, Republic of Korea

16:31 **CS12.01: Keynote Speaker - Defeating Human Host Cell Defences by Stealth SARS-CoV-2 Infection Executed by NSP5/3CLpro Main Protease.**
Christopher Overall, Canada

16:56 **CS12.02: Lipopolysaccharide-induced Changes in the Macrophage RNA-binding Subproteome.**
Aleksandra Nita-Lazar, United States

The interactions between RNA and protein within cellular signaling pathways, known as the interactome, have significant modulatory effects on RNA binding proteins' (RBP's) effector functions, which contribute to post-transcriptional control of inflammatory cytokine release in the innate immune response. Post-translational modifications (PTMs) are known to have a regulatory effect on a protein's effector function at nearly every step of a cellular signaling network. Thus, investigating the effect of RNA-protein interactions, specifically long noncoding RNAs (lncRNA) as a type of PTM, can answer questions central to our understanding of cellular signaling.

We investigated changes in the RNA bound proteome of immortalized mouse macrophages following treatment with lipopolysaccharide (LPS). Isobaric labeling (SILAC) of cells in culture followed by purification of RNP complexes at three time points after LPS exposure and bottom-up proteomic analysis by LC-MS/MS provided a list of candidate RBPs. Global RNA sequencing was used to characterize the correlation of transcript abundance to RBP candidate protein abundance, as well as the differential gene expression in response to LPS at different time points. Notably, Il1α, MARCKSl1, and ACOD1 (RBP candidates involved in innate immune signaling) were significantly differentially expressed in the RNA- seq dataset and showed concordance with proteomics dataset. One functionally relevant candidate, WDR26, was not differentially expressed in the RNA-seq dataset but was changing in response to LPS in the proteomics dataset showing discordance between mRNA and RNA-binding protein product. The binding sites of the RBP and RNA conjugates at amino acid resolution have been investigated by enriching the peptides containing phosphate group (coming from the oligonucleotide backbone) after the trypsin digestion of the RBPs. Additionally, microRNAs implicated in the innate immune pathways were observed upon analysis of RBPs and their LPS-dependent changes were investigated using nanoString.

This research was supported by the Intramural Research Program of NIAID, NIH.

17:09 **CS12.03: Uncovering Novel Anti-virulence Strategies to Combat Fungal Infections**
Brianna Ball, Canada

Combatting the increasing rate of fungal infections is a top priority to protect global health. Cryptococcus neoformans is an opportunistic fungal pathogen found ubiquitously within the environment and is equipped with sophisticated virulence factors (e.g., polysaccharide capsule, melanin, extracellular enzymes) that modulate the host immune system and promote fungal survival in immunocompromised individuals. The lack of adequate antifungal treatments and the presence of drug-resistant strains often results in fatal cases of cryptococcal meningitis. Recently, anti-virulence strategies designed to block virulence factors characteristic of fungal pathogens have been explored as an alternative therapy to reduce fungal burden within the host and reduce the selective pressure towards resistance. This study aims to identify and characterize novel anti-virulence strategies by profiling infection dynamics between host immune cells and C. neoformans using high-resolution mass spectrometry (MS)-based proteomics. We performed quantitative proteomics of primary murine macrophages infected with C. neoformans in single runs on a Quadrupole Orbitrap mass spectrometer and identified novel candidate infection-associated fungal proteins with the potential for host interaction. Following construction and characterization of a gene deletion library in C. neoformans, we defined fungal phenotypic differences and critical roles in host infection using in vitro and in vivo models. A lead candidate, CipC, is attenuated for virulence in a murine model and demonstrates packaging into bioactive fungal extracellular vesicles with roles in host immune modulation and virulence transfer between fungal cell populations. Overall, this research will provide a new pipeline for alternative drug target discovery against fungal pathogens and a robust tool to control fungal diseases.

PROGRAM

17:22 **CS12.04: Time Dependent Characterization and SRM Based Validation of Protein Profiles Induced by Chronic Infection With Lymphocytic Choriomeningitis Virus**
Asif Manzoor Khan, Denmark

We present a comprehensive discovery-based proteomic investigation of mice with chronic infection of lymphocytic choriomeningitis virus (LCMV). To study the cellular complex protein networks in mice, we have applied a global proteome profiling and quantitative proteomics approach to improve understanding of LCMV mediated host immune interactions. We investigatedtime dependent infection with LCMV, which is capable of inducing a chronic infection.
Using high resolution liquid chromatography - tandem mass spectrometry, we report significant protein changes in different tissues like, retina, RPE-choroid, kidney and spleen in LCMV infected mice at 1, 8 and 28 weeks of infection. Using bioinformatics tools, we observed several protein pathways to be perturbed and associated with LCMV mediated inflammation and disease progression. Validation of the results were done using skyline based targeted proteomics.

The effects of the infection on the cellular proteomes varied substantially between the four different 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 was 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. Substantial 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 and increased degeneration throughout the period.

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.

Session Date/Time: Monday, September 18, 2023 - 05:45 PM - 06:30 PM

PL03: Plenary Session: Manuscript Competition

See you in Busan!
BEXCO, Busan, Korea
🌐 2023.hupo.org
🐦 f 📷 in #HUPO2023

Together Through Proteomics

PROGRAM

17:45 **PL03.01: Immobility-associated Thromboprotection is Conserved across Mammalian Species from Bear to Human**

Johannes Bruno Müller-Reif, Germany

The manuscript titled "Immobility-associated thromboprotection is conserved across mammalian species from bear to human," published in Science in April 2023, presents the discovery of a potential therapeutic target for venous thromboembolism (VTE). Our unconventional approach started with the observation that brown bears have no enhanced risk of VTE during hibernation. Thus, we investigated the platelet proteome of hibernating and active brown bears with mass spectrometry (MS)-based proteomics. We revealed that immobilization induces a functional platelet proteome conversion, culminating in an antithrombotic state that mitigates the risk of VTE. The most prominently changed protein was HSP47 (SERPINH1) with a 55-fold downregulation during hibernation.

With a platelet HSP47 deficient mouse model as well as pharmacological inhibition of HSP47, we confirmed the thromboprotective function of HSP47 downregulation. Both strategies induced a reduction of platelet activity, immune cell activation and by that reduced the formation of neutrophil extracellular traps as facilitators of thromboinflammation. To translate these findings from bears to human patients we turned to long-term immobilized paraplegic patients and provide insights into the enigmatic observation that they have a VTE risk comparable to the general population. From bed rest study patients and acute immobilized trauma patients we conclude that platelet HSP47 downregulation happens on the megakaryocyte level and the protein abundance correlates with the platelet turnover.

Mass spectrometry-based proteomics proved indispensable for this discovery, as it facilitates analyses of non-model organisms such as brown bears, which are otherwise challenging to investigate with affinity-based molecular techniques. We demonstrate the power of cross-species discovery approaches starting from organisms, adapted to a specific biological niche or environment. In our case, the adaptive molecular mechanisms were evolutionary conserved and could be directly transferred to the human physiology. This cross-species discovery strategy may give rise to new therapeutics and prognostic markers beyond the scope of human centered studies.

18:00 **PL03.02: Global Detection of Human Variants and Isoforms by Deep Proteome Sequencing**

Pavel Sinitcyn, United States

Current shotgun proteomics methods routinely enable the detection of approximately 10,000 human proteins from a single sample. However, individual proteins are typically identified by peptide sequences representing a small fraction of their total amino acids. Hence, an average shotgun experiment fails to distinguish different single amino acids variants and protein isoforms. Deeper peptide sequencing is therefore required for the global discovery of unique protein sequence variants. To accomplish this, we utilized six human cell lines, six parallel protease digestions, extensive fractionation, three MS/MS fragmentation methods, and state-of-the-art instrumentation to generate the most comprehensive proteome to date, with a median sequence coverage of approximately 80%. Our groundbreaking approach has shed light on how genomic variation and alternative splicing impact the proteome, providing the first direct evidence that most frame-preserving isoforms are translated. This discovery has implications for understanding complex biological systems and for disease biomarker discovery. Our work also serves as a valuable resource for machine learning endeavors and as a transformative experimental method for exploring the proteome in greater depth.

I am excited to see that our study has already generated significant interest and has inspired multiple collaborations in the proteomics community. We are currently exploring ways to increase its throughput and expanding its application to various biological systems. Our work represents a major advance in the field and has the potential to transform our understanding of the proteome.

I am honored to be considered for the "Proteomics Highlight of the Year" award, and I believe that our study will have a lasting impact on the field of proteomics.

PROGRAM

18:15   **PL03.03: Dissecting the Blood Ecosystem in SARS-CoV-2 Omicron Patients**

Hong Wang, China

By utilizing the concept of blood ecosystem, which we adapted from tumor and gut microbes ecosystem, we profiled the cellular and fluidic components from the peripheral blood of a large cohort of SARS-COV2 Omicron infected patients using advanced proteomics and multiple other omics, covering clinical phenome, bulk Omics and single cell Omics. We further derived the interactions among these massive cellular and molecular components through advanced multi-omics integrative approaches to reveal novel functional properties out from these newly identified components and/or interactions. Our systematic analyses of Omicron infections provide diverse mechanistic and clinical insights into potential drug targets, neutralizing antibody development strategies, and biomarkers for disease monitoring, prediction, and treatment. Our study represents a perfect example of proteomics-centric cross-omics strategies to deal with pandemic. Furthermore, our blood ecosystem approach may inspire a paradigm shift for utilizing the powerful proteomics-centric cross-omics strategies to study other systemic diseases and future emerging public health problems.

Session Date/Time: Tuesday, September 19, 2023 - 08:00 AM - 09:00 AM

ISS07: Seminar Supported by Bruker

08:00   **An Extended View on Targetable Tumour Antigens using de Novo Sequencing**

Nicola Ternette, United Kingdom

08:25   **Optimal Metagenomic Data Annotation Pipeline Facilitates Deep Metaproteomic Coverage of Microbiota by TIMS-TOF PASEF**

Liang Qiao, China

Session Date/Time: Tuesday, September 19, 2023 - 08:00 AM - 09:00 AM

ISS08: Protein Analysis – Fast, Easy, Robust Sample Preparation Workflows for Every Protein Type. Every Matrix. Every User.

08:00   **Speaker**

Debadeep Bhattacharyya, United States

08:10   **Speaker**

Kyunggon Kim, Republic of Korea

08:30   **Speaker**

Qifeng Lin, Singapore

Session Date/Time: Tuesday, September 19, 2023 - 08:00 AM - 09:00 AM

ISS09: Evolution of Mass Spec Technologies Enables Next Generation Therapeutics

PROGRAM

08:00 **Increasing Biological Insight using Alternative Fragmentation and High Sensitivity MS/MS on the SCIEX ZenoTOF 7600 System**
Patrick Pribil, Canada

08:30 **Standardized Clinical Proteomics Enabled by a fully Automated Sample Preparation strategy and Powered by Zeno SWATH DIA**
Dorte Bekker-jensen, Denmark

Session Date/Time: Tuesday, September 19, 2023 - 09:15 AM - 10:00 AM

PL04: Plenary Session: Matthias Mann, Germany - Technological Advances in MS-based Proteomics Applied to Single Cell Type Analysis in Cancer Tissues

Chair

Je Yoel Cho, Republic of Korea

09:17 **PL04.01: Plenary Speaker - Technological Advances in MS-based Proteomics Applied to Single Cell Type Analysis in Cancer Tissues**
Matthias Mann, Germany

Recent breakthroughs in mass spectrometry-based proteomics and computational biology are transforming bioscience. In this talk, I will introduce our Python-based open-source AlphaPept software suite, designed for rapid and efficient processing of large MS datasets. For instance, DirectLFQ, a new ratio-based method for sample normalization and protein intensity calculation, allows the analysis of large-scale data and quantification of 10,000 proteomes in minutes instead of days or months. I will discuss recent efforts in our group to increase robustness, throughput and sensitivity of proteome measurements. We use these capabilities for increasingly large-scale studies, such as cellular interaction and PTM maps. When coupled with low flow chromatography on the Evosep platform they also enable single cell analysis and can be used to investigate functional cellular heterogeneity. Our new workflow termed Deep Visual Proteomics (DVP) combines high-content microscopy, AI-driven image recognition, and laser microdissection with ultrahigh sensitivity MS to connect visual, spatial, and molecular proteomics data. Applied to various diseases, such as borderline ovarian cancers, rare cutaneous drug reactions, and liver diseases, this provides a direct route to cellular function at a cell type resolved level, identifying potential therapeutic targets. DVP has great potential in aiding diagnosis and prognosis and moving us towards personalized cancer medicine, which is our ambition for the future.

Session Date/Time: Tuesday, September 19, 2023 - 10:15 AM - 11:45 AM

CS13: Technological Advancements

Chair

Khatereh Motamedchaboki, United States

Chair

Sara Ahadi, United States

PROGRAM

10:16   **CS13.01: Keynote Speaker - Finding The Connection: Multi-omics To Provide Dimensionality To The Proteome**
Melanie White, Australia

10:41   **CS13.02: Keynote Speaker - Bottom-up Proteomics Using DIA**
Lukas Reiter, Switzerland

11:06   **CS13.03: Quantitative Aspects of diaPASEF on Current and Novel Instrumentation Evaluated by Response Curve Analysis**
Ben Collins, United Kingdom

The adoption of data independent acquisition (DIA) mass spectrometry by the proteomics community and beyond continues at pace due to its robust quantitative characteristics and broad applicability. diaPASEF has emerged as a leading DIA approach that leverages trapped ion mobility spectrometry to increase ion usage efficiency and selectivity[1]. While quantitative aspects of diaPASEF performance have been evaluated to some extent using 3-species mixtures these data do not provide information on limits of detection/quantification and dynamic range of linearity. Further, new developments in instrumentation promise to extend the quantitative performance of diaPASEF.

To more fully evaluate the quantitative aspects of the diaPASEF approach on current and novel instrumentation we assessed the limits of detection/quantification and linearity using response curve analysis. We used a previously published benchmarking sample set[2] consisting of 30 stable isotope labelled peptides spiked into a complex HEK293 digest background over ~6 orders of magnitude. Ongoing experiments (to be presented at HUPO) will extend this work using a (pseudo) matched matrix design[3] where a HeLa sample is diluted into yeast background using a 14 dilution points facilitating the generation of 10,000s of response curves.

We show on current instrumentation (timsTOF Pro 2) that limits of detection and quantification are in the low amol on column range and that linearity is maintained over ~4 orders of magnitude of dynamic range. Equivalent response curve data, in addition to data from applied experiments, for prototype instrumentation will be presented.

With these data we establish the current and developing quantitative performance of diaPASEF to underpin applied experiments in quantitative proteomics.

1 Meier, F. et al. Nat. Methods 17, 1229 (2020); 2 Collins, B. C. et al. Nat. Commun. 8, 291 (2017); 3 Pino, L. K. et al. J. Proteome Res. 19, 1147 (2020)

11:19   **CS13.04: The Astral Analyzer: A Rising Star for Deep & High-Throughput Proteomics.**

Hamish Stewart, Germany

Introduction
Over the last decades, mass spectrometers have improved by orders of magnitude in sensitivity, dynamic range, and spectral acquisition rate. Among other advances, MS-driven proteomics have expanded into ever higher throughout methods necessary for large patient cohort studies, and driven down the limits of sensitivity required for deep single cell analysis.

A novel open electrostatic trap high-resolution accurate-mass analyzer has been developed that delivers a generational leap in sensitivity and speed. Fed by a unique ion processing device incorporating apertureless, low energy ion transfer across pressure regions, the "Astral" analyzer operates at up to 200Hz with destructive detection to produce hundreds of single-ion sensitive, MS/MS spectra per DIA or DDA cycle.

Methods
Electrosprayed ions are concentrated and processed in a high duty cycle dual-pressure ion processor , before being extracted into the open electrostatic trap. Here they oscillate between a pair of converging ion mirrors, which over many overlapping reflections direct and focus them to the detector surface. Instrument performance was characterised with electrosprayed Pierce™ FlexMix™ calibration solution, intact proteins and digested HeLa. Overall transmission was compared against an Orbitrap™ analyzer mounted upstream of the analyzer.

Results and Conclusion
Transmission of ions to the Astral analyzer, recorded with up to 100K resolving power, was found to exceed that of the conjoined Orbitrap analyzer, whilst secondary electron detection increased relative ion signal-to-noise by an order of magnitude. This combination, along with the full duty cycle of the ion processor, massively enhanced sensitivity. In particular, MS2 spectra with low ion number appeared far better populated when generated by the novel analyser than via FTMS. High quality intact protein spectra could be generated via 10x averaging. HeLa digest was analyzed to a depth of >8000 identified proteins at 180 samples-per-day, and to >10,000 at 60, representing a highly significant step above state-of-the-art.

11:32   **CS13.05: Robust and Scalable Single-Molecule Protein Sequencing With Fluorosequencing**

Jagannath Swaminathan, United States

The need to accurately survey proteins and their modifications with ever higher sensitivities, particularly in clinical settings with limited samples, is spurring development of new single molecule proteomics technologies. Here, we describe our optimization of one such highly parallelized single molecule peptide sequencing platform, fluorosequencing, which is based on determining the positional information of select amino acid types within peptides to enable their identification and quantification from a reference database. We made substantial improvements to the technology, including identifying fluorophores compatible with the sequencing chemistry, developing a practical solution for mitigating dye-dye interactions encountered during scaling, and developing an end-to-end workflow for sample preparation and sequencing. We demonstrate by fluorosequencing peptides in mixtures and identifying a target neoantigen from a database of more than a thousand decoy MHC peptides, highlighting the potential of the technology for clinical applications.

Session Date/Time: Tuesday, September 19, 2023 - 10:15 AM - 11:45 AM

CS14: Clinical Proteomics/Biomarkers

Chair

Ji Eun Lee, Republic of Korea

Chair

Byoung Chul Park, Republic of Korea

PROGRAM

10:16 **CS14.01: Keynote Speaker - Genetics Meet Proteomics: Challenges and Opportunities for GWAS with MS Proteomics**

Karsten Suhre, Qatar

10:41 **CS14.02: Keynote Speaker - In-depth Plasma Proteomics for Cancer Biomarker Discovery**

Ayumu Taguchi, Japan

11:06 **CS14.03: Mapping Early Serum Proteome Signatures of Liver Regeneration in Living Donor Liver Transplant Cases**

Fernando Corrales, Spain

Background & Aims. The liver is the only solid organ capable of regenerating itself to regaining 100% of its mass and function after liver injury and/or partial hepatectomy (PH). This property represents a therapeutic opportunity for severe liver disease patients. However, upon complete or partial liver resection, liver regeneration (LR) might fails due to poorly understood causes. Here we wanted to fully elucidate mechanisms of LR, identify LR drivers and eventually biomarkers for an early prognosis and intervention. Methods. We performed a comprehensive analysis of LR in a well stablished PH mouse model. We have investigated the regulation of liver proteome and phosphoproteome 9 h after PH, to depict a detailed mechanistic picture of the early LR phase. Furthermore, we analyzed the dynamic changes of the serum proteome of healthy living donor liver transplant (LDLT) donors at different time points after surgery using SureQuant strategy. The molecular profiles from both analyses were then correlated.
Results. Insulin and FXR-FGF15/19 signaling were stimulated in mouse liver after PH, leading to the activation of the main intermediary kinases (AKT and ERK). Besides, inhibition of the hippo pathway led to an increased expression of its target genes (Areg, Myc, Ctgf, Fgf1, Id1) and of one of its intermediary proteins (14-3-3 protein), contributing to cell proliferation. In parallel, metabolic reprogramming coupled to enhanced mitochondrial activity cope for the energy and biosynthetic requirements of LR. In human serum of LDLT donors, we identified 56 proteins and 13 metabolites statistically differential which recapitulate some of the main cellular processes orchestrating LR in its early phase.
Conclusions. These results provide mechanisms and protein mediators of LR that might prove useful for the follow-up of the regenerative process in the liver after PH as well as preventing the occurrence of complications associated with liver resection.

11:19 **CS14.04: Proteomics of Human Colon Adenocarcinoma Tissues and Tissue-Derived Primary Cell Lines Reveal Unique Functions of Tumours and New Potential Biomarkers**

Lifeng Peng, New Zealand

Colorectal cancer is the third most common cancer type worldwide. More than 60% of CRC cases have already developed to an advanced stage by the time of detection, resulting in a survival rate around 8-9%. The aim of this study was to find new biomarkers for diagnosis, prognosis and therapeutic drug targets for this disease.
Four low-grade and four high-grade human colon adenocarcinoma (CA) tumours with patient-matched normal colon tissues as well as the tissue-derived primary cell lines from each tumour tissue were analysed. DNA sequencing was used to examine the conserved gene mutations of the cell lines. Label-free quantitation proteomics was performed to compare the proteomes of the CA tissue samples and CA tissue-derived cell lines. Function enrichment analysis was performed using STRING. Proteomics data was validated by Western blotting.
A total of 4767 proteins were identified across all tissues, and 4711 across the tissue-derived cell lines. The cell lines shared about 70% of proteins with their parent tissue, and they retained mutations to key CA-related genes and did not diverge far genetically from their parent tissues. CA tissues displayed upregulation of RNA processing, steroid biosynthesis and detoxification, and downregulation of cytoskeletal organisation and loss of normal muscle function. The cell lines exhibited increased interferon-gamma signalling and aberrant ferroptosis. 318 proteins were significantly up-regulated and 362 proteins significantly down-regulated by comparisons of high-grade with low-grade tumours and low-grade tumour with normal colon tissues from both sample types. The differences exhibited between tissues and cell lines highlight the additional information that can be obtained from patient-derived primary cell lines. DNA sequencing and proteomics confirmed that these cell lines can be considered suitable in vitro models of the parent tumours. Various potential biomarkers for CA initiation and progression and potential drug targets were identified, including seven novel markers.

11:32 **CS14.05: Comparative Evaluation of In-Depth Mass Spectrometry and Antibody-Based Proteomic Platforms for Plasma Biomarker Discovery**
Noora Sissala, Sweden

Recent technological advancements have enabled deep profiling of the plasma proteome for biomarker discovery with both mass spectrometry (MS) and affinity-based methods. Given the diversity of plasma proteomic platforms, it is essential to understand their strengths and limitations to allow informed decisions in study design and platform selection. While MS-based proteomics allows unbiased interrogation of the plasma proteome and provides accurate quantification, affinity-based methods deliver highly sensitive detection of specific target proteins. However, few studies have directly compared platform performance using in-depth MS analysis.

In this study, we compared MS-based proteomics (HiRIEF LC-MS) with the antibody-based Olink Explore 3072 assay. We profiled the proteome of 88 plasma samples from individuals with suspected lung cancer using both methods, quantifying 2578 unique proteins with MS and 2923 with Olink Explore, with 1129 proteins overlapping between the methods. We obtained an overall correlation of 0.55 between measurements, with variable per-protein correlation (median 0.59). Furthermore, we assessed the performance of the platforms in terms of key factors influencing biomarker discovery, such as proteome coverage, precision, and statistical power, investigated factors affecting measurement accuracy, and pinpointed potential protein misidentifications or antibody cross-reactivity.

In summary, we present a comprehensive analysis of the relative strengths and limitations of MS and antibody-based platforms for biomarker discovery and provide valuable insights for study design and data quality control in plasma proteomics.

Session Date/Time: Tuesday, September 19, 2023 - 10:15 AM - 11:45 AM

CS15: Spatial and Imaging Proteomics

Chair
Je Yoel Cho, Republic of Korea

Chair
Je-Hyun Baek, Republic of Korea

10:16 **CS15.01: Keynote Speaker - Bioorthogonal Chemistry-enabled Spatial-temporal Proteomics**
Peng Chen, China

10:41 **CS15.02: Keynote Speaker - A Spatio-temporal Single-cell Type Map of Human Tissues**
Cecilia Lindskog, Sweden

![HUPO BUSAN 2023 SEP 17-21, 22nd Human Proteome Organization World Congress]

See you in Busan!
BEXCO, Busan, Korea
🌐 2023.hupo.org
🐦 f 📷 in #HUPO2023

Together Through Proteomics

PROGRAM

11:06   **CS15.03: Statistical Approach to Predict Lymph Node Metastasis in Endometrial Cancer using Mass Spectrometry Imaging**
Peter Hoffmann, Australia

Classic histopathological examination of tissues remains the mainstay for cancer diagnosis and staging. In some cases, histopathologic analysis yields ambiguous results, leading to inconclusive disease classification. Here, we set out to explore the diagnostic potential of mass spectrometry-based imaging for tumour classification based on proteomic fingerprints using Mass Spectrometry Imaging (MSI). Supervised machine learning (ML) approach was applied to large MALDI MSI datasets from endometrial cancer (EC) TMAs consisting of 302 unique patients. The pathologist labels for these patients included 43 patients of primary tumour with lymph node metastasis (LNM), 214 patients with no LNM. Combining mass spectrometry with ML, we were able to predict the presence of LNM in primary tumour of EC with an overall accuracy of 80% (90% sensitivity and 69% specificity). Using a different statistic approach with the above stated MSI data using a canonical correlation analysis (CCA) based method was applied to rank the intensities of the acquired m/z values based on their power to discriminate the primary carcinomas with metastatic potential from those without. This specific statistical method was able to correctly classify all patients with 100% accuracy. This study set to establish approaches for binary sample classification problems. This is achieved based on the MSI data alone, without any need to identify the m/z values. Overall, these results highlight the potential of this technology to determine the optimal treatment for cancer patients to reduce morbidity and improve patients' outcomes.

11:19   **CS15.04: Multiplex-DIA and Deep Visual Proteomics Enhances Spatially-Resolved Proteome Resolution to Uncover the Landscape of Pancreatic Islet Biology**
Marvin Thielert, Germany

Introduction: Diabetes is a chronic metabolic disorder with increasing prevalence, in which functional beta cell loss is a well-established factor manifesting in pancreatic islet cell remodeling. While cellular models have traditionally focused on beta cells, pancreatic islets are an intricate spatial arrangement of different cell types and responsible molecular mechanisms remain poorly understood.
Methods: Recent advances in high sensitivity mass spectrometry allow the acquisition of proteomes from very low input material, paving the way for the analysis of spatial and cell type-resolved proteome signatures of pancreatic islets. In this study, we applied Deep Visual Proteomics (DVP) to characterize the diverse cell types in pancreatic islets on single-cell level within the spatial context of human tissue samples. This consisted of high-content imaging, followed by artificial intelligence-guided cell recognition, laser-capture microdissection and ultra-sensitive mass spectrometry on clinical archival tissue.
Results: Incorporating multiplex-DIA (mDIA) enables us to reduce the required input amount using a reference channel, thereby increasing the spatial resolution and throughput. This is because such a reference channel conceptually decouples identification from quantification. We designed the reference channel as a representation of the entire pancreatic islet proteome by sectioning single islets and processing them as a bulk digest. Employing this reference channel on 2.5 μm-thin human tissue slices, we achieved a proteomic depth of about 3,000 protein groups in single-cell populations of only 25 precisely isolated shapes per sample (equivalent to five to ten cells) for the different cell types within the pancreatic islet. This approach allows us to detect the common markers of the pancreatic islet cell types as well as numerous additional ones across the whole abundant range.
Conclusion: Overall, our study demonstrates that harnessing the combined power of DVP and mDIA technology enabled us to explore the landscape of pancreatic islet biology by spatially resolving their cell types.

PROGRAM

11:32   **CS15.05: Near Single Cell Proteomics on FFPE Tissue Sections Using Hydrogel-Based Tissue Expansion and DIA-Based Mass Spectrometry**
Zhen Dong, China

Introduction: Clinical tissues are normally heterogeneous. The study of protein localization and expression at the tissue level is of great value for analyzing this heterogeneity, investigating the tissue microenvironment, and assisting the diagnosis and prognosis in precision medicine.
Methods: Here we present a spatial proteomics technology using hydrogel-based tissue expansion and data-independent acquisition (DIA) mass spectrometry for near single cell proteomics of formalin-fixed paraffin-embedded (FFPE) specimens. The FFPE tissue sections are embedded in customized hydrogel and can be expanded by a linear scale of 4-5 folds. After expansion, a tiny amount of tissue area, down to near single cell level, could be easily dissected and subject to DIA-based mass spectrometric analysis.
Results: Based on our recently published ProteomEx methodology (1), we have further optimized the method. The throughput was increased by 2-3 folds, and the sample amount was reduced by 8-10 folds to 0.042 nL (under 20 HeLa cells), leading to identification of over 6700 proteins from FFPE specimens containing different stages of malignant changes of colorectal adenomas at specific positions. We also presented a free accessible online database querying for the spatial proteome expression available at https://www.guomics.com/ProteomExAct/.
Conclusions: A new spatial proteomics technology has been further developed and applied onto clinical FFPE samples. In this presentation, we will discuss its technical details, utility, pros, and cons.
1. Li, L., et al. 2022 Nature Communications 13(1): 7242.

Session Date/Time: Tuesday, September 19, 2023 - 10:15 AM - 11:45 AM

CS16: Cell Biology

Chair
Kyunggon Kim, Republic of Korea

Chair
Bernd Wollscheid, Switzerland

10:16   **CS16.01: Keynote Speaker - Biophysical Proteomics**
Mikhail Savitski, Germany

10:41   **CS16.02: Keynote Speaker - OSBPL2 Mutations Causes Hearing Loss via Proteinopathy**
Heon Yung Gee, Republic of Korea

See you in Busan!
BEXCO, Busan, Korea
2023.hupo.org
#HUPO2023

Together Through Proteomics

PROGRAM

11:06   **CS16.03: Impact of Additional Chromosomes on Cellular Phosphoproteome**

Barbora Salovska, United States

Aneuploidy, the presence of an abnormal number of chromosomes in human cells, is a hallmark of genetic diseases and cancers. However, the underlying mechanisms of how aneuploidy disrupts cellular physiology remain disputable. No systematic study has investigated the implications of aneuploidy for cellular signaling. For example, if kinases or phosphatases are upregulated due to the gain of the corresponding aneuploid chromosomes encoding them, how could we use this information to predict the downstream cell signaling network and better understand the diseases? Here, we analyzed the proteomes and phosphoproteomes of a panel 64 human fibroblast cell lines isolated from individuals with divergent karyotypes (e.g., trisomy 8, 9, 13, 18, 21, and X; triploid and tetraploid) and variable genetic background. By employing DIA-MS, we identified 10,549 protein groups and 58,792 unique class-I phosphosites (P-sites) from 6,053 phosphoproteins (peptide - and protein- FDRs of 1%) across the aneuploid and euploid cells. We observed protein abundance including abnormal kinase expressions followed the expected trends in chromosome stoichiometry, with a consistent pattern observed in the phosphoproteome data. Clustering analyses indicated the absence of a universally dominant aneuploidy-driven clustering but revealed a common phosphoproteomic impact across aneuploidy profiles. Differential expression analysis demonstrated the off-target impact of aneuploidies (i.e., trans- effect) was variable at the level of individual proteins and P-sites and not correlating with the chromosome size, nor showing preference for specific chromosomes. As an example, we observed a specific upregulation of the IFN gamma signaling in Trisomy 21 and leveraged the data to identify other aneuploidy-specific pathways. To cross-validate our results in different cell types and species, we integrated the results obtained in human aneuploid lymphoblast cells, isogenic cancer cell lines, mouse isogenic fibroblasts, and cross-linking MS datasets. In conclusion, our study revealed both on- and off-target effects of additional chromosomes on human proteome and phosphoproteome.

11:19   **CS16.04: Unveiling IRF4-steered Regulation of Context-Dependent Effector Programs in Th17 and Treg cells**

Ute Distler, Germany

Background: The transcription factor IRF4 contributes to the delicate equilibrium between pro- and anti-inflammatory immune responses and is essential for the development of fully functional effector Th17 and Treg cells. Despite its central role in T cell lineage determination, molecular mechanisms of IRF4-mediated gene expression are still poorly understood and only few studies integrate information derived from IRF4 ChIP-Seq analysis with other 'omics' data in an unbiased manner or shed light on proteins that act in concert with IRF4. To identify novel players in IRF4-mediated gene regulation, we integrated data from IRF4 interactome and IRF4-ChIP-Seq analyses with proteome data from IRF4 knock-out animals.

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 from IRF4 knock-out mice were processed as described before [2] followed by LC-MS analysis on an Exploris 480. Immunoprecipitated DNA was sequenced on an Illumina NovaSeq 6000 instrument.

Results: Here, we characterized distinct IRF4-mediated effector functions in fully differentiated Th17 and Treg cells integrating different data sets from IRF4 interactome, ChIP-Seq and proteome analyses. We could define a "core" protein IRF4 interactome in ex vivo propagated Th17 and Treg cells, which is preserved in the two functionally opposed T cell subsets. Additionally, we detected lineage-specific interactors involved in IRF4-steered gene expression including novel, uncharted interplayers in both cell types. ChIP-Seq analysis revealed a strong enrichment of motifs targeted by identified IRF4 interactors and we could define distinct IRF4-mediated transcriptional programs contributing to cellular functionality and plasticity.

Conclusions: Affinity purification MS facilitated the identification of novel players in IRF4-mediated gene regulation in CD4+ T cells.

1. Driegen, S. et al. Transgenic Res. 14, 477–82 (2005).
2. Hughes, CS. et al. Mol. Syst. Biol. 10, 757 (2014).

11:32   CS16.05: Unlocking the Secrets: Using Secretomics for Exploring Cell-to-Cell Communications

Juan Manuel Sacnun, Austria

Peritoneal Dialysis (PD) is a life-saving renal replacement therapy. However, the use of PD-fluids lead to detrimental changes in the peritoneal membrane (PM), reducing effectiveness of the therapy. Cross-talk among different peritoneal cell types modulates PD-associated deterioration. Currently, there is no model available to study the interactions of these cells in close proximity. Here, we aimed to develop a co-culture model for investigating cell-to-cell communication by analyzing the cellular proteome and secretome.
For modelling the PM, mesothelial and endothelial cells were co-cultured in transwell plates under optimized conditions for simultaneous culturing under non-starving conditions (5%FCS). Cells were exposed to PD-fluids in either co-culture or single culture conditions. To overcome current limitation on secretomics analysis in non-starvation conditions, an equalizer approach was used to deplete high abundant proteins in combination with SILAC to identify the cell origin of secreted proteins. For quantitative analysis of cellular and secreted protein abundances, LC-MS (TMT-18plex) was performed.
Co-cultured cells yielded differently regulated pathways following PD-fluid exposure compared to individual cultures. Combined proteome and secretome analysis revealed different ligand-receptors pairs expressed uniquely in co-culture. Using protein-protein interaction analysis, the identified cell-secreted proteins (~1900) formed 11 functional clusters, interacting with different receptors presented by the cells. The resulting interactome between cells through the secreted proteins in combination with differentially expressed cellular and secreted proteins revealed novel candidates affected by PD-fluids regulating pathways related to angiogenesis, TGFb and NOTCH.
This study shows that harmful effects of PD-fluid exposure on mesothelial also affect endothelial cells. Interestingly, co-culturing both cells revealed different responses compared to individual cultures, highlighting the importance of models that allow interactions between multiple cell types. We further identified novel potential signaling axes between the cell types explaining pathophysiological changes of the PM during PD that may allow identifying therapeutic targets to reduce current limitations of PD.

Session Date/Time: Tuesday, September 19, 2023 - 11:45 AM - 01:00 PM

PP03: Odd Numbered Posters - Poster Viewing with Coffee Break

See you in Busan!
BEXCO, Busan, Korea
2023.hupo.org
#HUPO2023

Together Through Proteomics

PROGRAM

PP03.01: MaxLFQ Algorithm Enables Accurate Hybrid Precursor-fragment-based Quantification of plexDIA Data in MaxQuant

Dmitry Alexeev, Germany

DIA combined with non-isobaric labels has the promise of combining high sensitivity and reproducibility with reduced measurement times. Furthermore, low-mass spectrometric signals can be made detectable by using carrier channels with higher concentrations. Applications include single-cell proteomics and pulse-chase proteomics experiments. Here we present how labeling with any number and kind of non-isobaric labels in DIA data is supported in MaxQuant and accurate intra and inter LC-MS run quantification is achieved by generalizing the MaxLFQ algorithm to multiplexed data.

Two complementary concepts for finding multiplexes in DIA data are combined in MaxDIA: the detection of MS1 n-plex features as higher-dimensional objects and the matching of in-silico predicted or measured library spectra transformed to all labeling states to the DIA samples. For incomplete n-plexes, the quantification of the missing labeling state is performed with the 'Re-Quantify' algorithm. A generalization of the MaxLFQ algorithm treats M LC-MS runs with N labeling states as M×N samples for normalization and quantification. It can be used in MS1, MS2, or hybrid mode.

Benchmarks with human cell line data with labels mixed in defined ratios show promising quantification performance for low-concentration samples. In particular, we analyze the data, provided by Pino et al.[1], with SILAC double labeling acquired in DIA mode on a Thermo Q-Exactive HFX tandem mass spectrometer. Light and heavy channels were mixed in 30/70, 50/50, and 70/30 ratios. We show that experimental ratio values correspond to the expected ones with high consistency.

Utilizing high-confidence MS1 n-plex feature identification with a generalized MaxLFQ algorithm allows for accurate quantification of the multiplexed samples in MaxDIA. All described algorithms will become available as part of the freely available MaxQuant software upon publication of the manuscript.

1. Pino LK, Baeza J, Lauman R, Schilling B, Garcia BA. J Proteome Res. (2021)

PP03.03: Mass Spectrogram Decomposition of DIA Proteomics Datasets for Identification and Quantitation of Peptides and Proteins

Jherico Geronca, Japan

DIA (Data-Independent Acquisition) is a method used in shotgun proteomics that combines the advantages of traditional data-dependent acquisition (DDA) and targeted approaches, such as parallel reaction monitoring (PRM). DIA proteomics holds the potential for precise and reliable quantification of proteins across diverse laboratory conditions and large-scale study designs. However, DIA requires the development of robust data analysis tools to handle the complex and abundant data generated. In this study, we developed a novel approach based on non-negative tensor decomposition (NTF) for the unsupervised classification of MS2 peaks using prior information known regarding the peptide ions and their associated fragment ions. The goal is to identify and quantify proteins in DIA-MS datasets. Using HeLa samples, our study highlights a novel approach that can surpass DIA-NN, a conventional tool employed in DIA-MS analysis. Our findings show that this approach exhibits promising capabilities, identifying an increase of over 11.9% in peptide ions and 9.8% in proteins. Moreover, it demonstrates a high correlation with DIA-NN in terms of protein quantification and RT annotation with a Pearson correlation coefficient (PCC) of at least 0.86 and 0.99, respectively. Notably, our model showcases superior reproducibility in protein quantification with a relative standard deviation (RSD) mean of 11.12, compared to the RSD mean of 13.16 from DIA-NN. We envision that the implementation of this approach can help revolutionize the field, offering enhanced sensitivity and accuracy in the identification and quantification of DIA-MS data.

See you in Busan!
BEXCO, Busan, Korea
🌐 2023.hupo.org
🐦 📘 📷 in #HUPO2023

Together Through Proteomics

PROGRAM

PP03.05: A New Bioinformatic Tool to Evaluate Biosimilarity of Antibody Drugs Using Intact Glycoprotein Analysis with LC-MS and Artificial Neural Network

Heeyoun Hwang, Republic of Korea

Heterogeneity and complexity of the glycosylation on biotherapeutics greatly depends on expression system, process conditions, and environment of cell culture of products. In order to evaluate biosimilarity of antibody drug such as Trastzumab, Infliximab, Bevacizumab, and Adalimumab, we develop a new informatics tool using glycome and intact glycoprotein analysis with LC-MS, and an artificial neural network (ANN). Briefly, 2,000 more number of spectra was used for training and testing the ANN model coded by Python 3.8 and Tensorflow (2.0.0). From the deconvoluted LC-MS data, ANN calculates the p-value for similarity as intact glycoprotein level against tranined antibody drug spectra. The accuracy was 100% and 94.6% at training and test set, respectively, where the accuracy was higher using decoy database. In particular, ANN model distinguishes low quality of mass spectra from all antibody drugs by the similarity. We released the trained ANN model at http://deepy.kr as a limited service.

PP03.07: Real-Time Search Improves Sensitivity of TMTpro Complementary Ion Quantification

Amanda Lee, United States

Using the balancer-peptide conjugates (TMTproC complementary ions) in the MS2 spectra for quantification circumvents the ratio distortion problem of multiplexed proteomics. These TMTproC quantification scans require long transient and ion injection times for sufficient ion statistics and spectral resolution. Real-Time Search (RTS) algorithms have been shown to increase the sensitivity of SPS-MS3 methods. By informed selection of precursor peaks for quantification, analysis time can be prioritized to peptides useful for quantifying proteins. Nevertheless, the naive implementation of TMTproC still quantifies more proteins than SPS-MS3. Here, we combine complementary ion quantification with Real-Time Search (TMTproC-RTS) to improve sensitivity while maintaining accuracy and precision in quantitative proteomics experiments at the MS2 level. Human peptides were labeled with TMTpro at 1:1 ratios across the nine 1-Da spaced channels in the complementary ion region. Similarly, yeast peptides were labeled in ratios of 0:1:5:10:1:10:5:1:0 with TMTpro. Human and yeast peptides were mixed at a ratio of 10:1 before analysis, and the mixed proteome was analyzed on an Orbitrap Ascend Tribrid. The TMTproC-RTS method collects fast exploratory ion trap MS2 scans at the turbo scan rate with quadrupole isolation and HCD fragmentation. MS2 spectra are searched immediately against a concatenated human-yeast fasta file. Successfully identified peptides were then re-isolated, and high-resolution MS2 spectra were collected in the Orbitrap with quadrupole isolation and CID fragmentation. In addition to ppm, XCorr, and dCn filters, in separate runs, the RTS filter was set to trigger a high-res MS2 scan on either peptides originating from yeast proteins or peptides from transcription factors. We significantly improved the number of quantified proteins from the desired subsets in either experiment while retaining quantitative precision. Thus, TMTproC-RTS enables the quantification of low-abundant proteins of interest, like transcription factors and signaling molecules, in multiplexed experiments.

![HUPO BUSAN 2023 SEP 17-21, 22nd Human Proteome Organization World Congress]

See you in Busan!
BEXCO, Busan, Korea
🌐 2023.hupo.org
🐦 📘 📷 💼 #HUPO2023

Together Through Proteomics

PROGRAM

PP03.09: DeepQuant, A Neural Network for Interference Correction of Precursor Quantities

An-phi Nguyen, Switzerland

Introduction:
In recent years, deep learning has been repeatedly used successfully to extract signals even from very noisy data. This ability is particularly useful in the analysis of mass spectrometry-based proteomics data, where quantification is inevitably affected by interferences, especially for low abundant analytes. Therefore, neural networks have the potential to dramatically improve the quantitative analysis of mass spectra.

Methods:
We design a neural network model to correct for the interferences affecting peaks. More specifically, the model is trained to predict a multiplicative factor. This factor can then be applied to correct the area estimated from a peak and, ultimately, to attain a more accurate quantification.
To train our model, we built a dataset from a controlled quantitative experiment (CQE) with three organisms (A. thaliana, H. sapiens, C. elegans) across 10 different conditions. The data was acquired on a Thermo HF-X instrument and processed using Spectronaut 17 (Biognosys). After further preprocessing, our training dataset consists of about 1.7 million samples.

Results:
We test our model in Spectronaut by analyzing CQE datasets different from the one used for training. We note that these test datasets include 4 organisms (H. sapiens, C. elegans, E. coli and S. cerevisiae) and were acquired with different Thermo (HF-X, Exploris480) and Bruker (timsTOF HT) instruments.
Our results show that our model can improve the quantification of precursors across different organisms, in terms of average mean squared error. We observe substantial improvements in the quantification of precursors belonging to E. coli, which is especially low in abundance in our test datasets. We highlight that E. coli was not present in our training dataset.

Conclusion:
Neural networks can correct for interferences and improve quantification across different instruments.

PP03.11: Midia-PASEF Maximizes Information Content in Data-Independent Acquisition Proteomics and Uses Machine Learning Based-Deconvolution to Generate DDA Quality Spectra

Stefan Tenzer, Germany

Here we characterize midia-PASEF, a novel DIA scan mode using mobility-specific scanning of overlapping quadrupole windows to optimally cover the ion population in the ion mobility-mass to charge plane. midia-PASEF 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. midia-PASEF maximizes information content in DIA acquisitions which enables the determination of the precursor m/z of each fragment ion with a precision of less than 2 Th. To process the resulting high-complexity datasets, we developed the Snakemake-based midiaID pipeline. midiaID integrates algorithms for multidimensional peak detection and for machine-learning-based classification of precursor-fragment relationships, which are stored in the format of a bipartite graph. Using machine learning approaches to refine the MIDIA graph based on initial database results, we significantly improved the specificity of precursor-fragment relationships, thereby surpassing the spectral purity of DDA in our deconvoluted MIDIA-MSMS spectra as indicated by database search score distributions.
Our midiaID pipeline enables fully automated processing and multidimensional deconvolution of midia-PASEF files and exports highly specific DDA-like MSMS spectra with a fragment ion mass accuracy below 10 ppm. In contrast to DDA, midiaPASEF is non-stochastic and thus generates detailed detection profiles of each fragment ion in all dimensions, which facilitates the highly specific deconvolution and scoring of precursor-fragment relationships.
Resulting deconvoluted MIDIA-MSMS spectra are exported as .mgf files which are suitable for de novo sequencing and can be searched directly with established tools including PEAKS, FragPipe and Mascot. midiaPASEF acquisition identifies over 40 unique peptides per second and provides powerful library-free DIA analyses including phosphopeptidome and immunopeptidome samples. In addition, midia-PASEF provides all benefits of DIA acquisitions, including efficient ion sampling, high duty cycle and excellent reproducibility.

PP03.13: Real-Time Proteome Identification Using Deep Learning

qianzhou wei, China

Mass spectrometry (MS) has become the most powerful proteomics analysis technology. Identification of MS spectra into peptide sequences is the first step of most MS data processing pipelines. Although numerous identification algorithms have been developed, such spectra identification still needs high computational power and professional knowledge, taking many hours or even days. This hinders wide application of MS proteomics technology, especially in the time-sensitive clinical applications. We developed a deep learning algorithm, the DeepMS, to perform real-time, one-step identification from MS spectra to peptide sequences with high accuracy. Peptide models for three species, human, E. coli, and SARS-CoV-2, were trained by collected spectra data in ProteomeXchange. We performed validation in each of the three species using data generated by other laboratories, and the accuracy reached 86%~98% in all three species. The cross-validation among the three species showed a very low false positive rate and false negative rate of only 1/10,000 at the highest, and in most cases were 0. This accuracy is sufficient for clinical testing. DeepMS resolves MS spectra at a speed of 0.35 ms per spectra in a normal notebook computer, much faster than the widely-used database search tools, and can potentially perform real-time MS spectra identification.
In sum, we exhibited that the DeepMS provided a fast and accurate solution for MS-based proteomics.

PP03.15: CAPE: Chromatogram Aware Pointwise Embeddings for Peak Group Identification in Multi-Run Multi-Transition Chromatogram-Based Data-Independent Acquisition Mass Spectrometry Data Analysis

Leon Xu, Canada

Introduction
Data-independent acquisition (DIA) is a novel mass spectrometric method that achieves high reproducibility/quantitative accuracy; however, consistency across multiple runs remains an issue due to retention time drift.

Methods
Existing peak identification algorithms focus on single run characteristics, which can result in identifying different peaks per run. To correct this, global (e.g. LOESS/LOWESS) or local alignment (e.g. DIAlignR) can be used. Here, we introduce a novel method based on deep learning which automatically learns to generate pointwise embeddings for each time point along the retention time axis of chromatograms that are aware of wider chromatographic context, and extract corresponding peaks across multiple runs. This is accomplished through the reproducibility of DIA and simultaneously learning a unique key embedding per library precursor which the resulting pointwise embeddings are queried against.

Results
On initial experiments in an LC-MS S. Pyogenes/Human plasma dataset, we identify peaks for ~79% of library precursors at 99% precision in held out test data, vs. ~60% using PyProphet. In an IM-LC-MS whole HeLa cell injection dataset, training on only a single replicate per precursor, we identify peaks for ~69% vs. ~72% for PyProphet. We show competitive results against current state of the art in automated peak detection across different data acquisition conditions.

Conclusion
Our approach is an end-to-end neural network that performs feature extraction, scoring, and pseudo-alignment in tandem, whereas current approaches rely on multiple passes over the data. This can help alleviate error propagation downstream which can occur in multi-step processes (e.g. misidentifying a peak feature but using it for alignment after). We also propose a variation of the model architecture which can use pre-annotated chromatograms as the alignment target instead of a learned embedding in cases where there are insufficient replicates to train on, opening the path for more interpretable, scalable, and controllable peak identification.

PP03.17: AIDIA: AI-Empowered Data-Independent Acquisition Proteomic Analytics for High-Throughput Phenotyping for Unexplored and Understudied Proteins Biomarkers

Fangfei Zhang, China

Background:
The accumulation of proteomic big data is happening at an accelerating pace. To address this, we have developed a novel tensor-centric analysis strategy and introduced a new data format called DIA tensor (DIAT). DIAT allows for the analysis of complete DIA-MS maps without the need for peptide precursor identification. By representing the data in a tensor format, DIAT minimizes storage space and enables direct feeding into a deep neural network on GPUs for phenotype prediction.
Methods:
We further extended our preivously DIAT framework in generation of DIAT files for various DIA-MS schemes with phenotypic prediction frameworks validated. The artitecture of the file structures were further optimized to minimize the storage space. A workflow in converting DIAT files for a general XML-based format for identification was established. Generation, conversion of DIAT files were packaged as a standalone software with   A recommendation system algorithm was used in matching novel peptidoforms as potential biomarkers being validated with targeted proteomics as well as synthetic peptides.
Results:
The generation of DIAT files compressed raw MS files from common MS instrument types without compromising the performance of identification and quantification, after the conversion of DIAT files to the conventional XML-based formats for library search. Various neural network based prediction frameworks (ResNet, MobileNetV2, ViT) of AIDIA were validated in a variety of DIA-MS datasets(DIA/SWATH, diaPASEF, ScanningSWATH, DI-SPA). Large cohort applications on thousands sample datasets confirmed the presence of known protein biomarkers from known classifier and also identifies novel proteoforms as potentially undercharacterized biomarker. The generation, conversion of DIAT files with deep learning based prediction functionality is available as a software package TensorPlayer https://github.com/guomics-lab/TensorPlayer.
Conclusions:
AIAIDIA represents a novel analytics approach for analyzing DIA-based proteomic big data. It offers the advantages of portable storage, fast phenotype prediction, and the potential to characterize the involvement of unexplored proteins.

PP03.19: SGLT2 Inhibition Reveals Kidney Reconfiguration and Metabolic Inter-Organ Communication

Anja Billing, Denmark

Background: SGLT2 inhibitors, initially used for managing hyperglycemia, have shown protective effects against cardiovascular and renal complications, regardless of the presence of diabetes. However, the precise molecular mechanisms underlying these effects remain largely unknown and cannot be solely attributed to their primary function of inhibiting renal glucose re-absorption.
Methods: To gain insights into these mechanisms, we employed LC-MS/MS techniques to investigate multiple aspects, including the proteome, phosphoproteome, gut metaproteome, metabolome, and SGLT2 interactome. For proteomics/phosphoproteomics analysis, we utilized TMT16-plex labeling or label-free approaches with an Exploris 480 mass spectrometer connected to an UltiMate3000 RSLC system. For untargeted metabolomics, we utilized a quadrupole time-of-flight Impact II instrument connected to either a Bruker Elute HPLC or an Agilent 1290 infinity HPLC device. Targeted metabolomics was conducted using a 6495C triple-quadrupole coupled to an Agilent 1290 Infinity HPLC.
Results: We extensively analyzed the proteome, phosphoproteome, and metabolome after one week of SGLT2 inhibitor treatment in non-diabetic and early diabetic mice. We integrated data from various metabolic organs and body fluids, including the kidney, heart, liver, white adipose tissue, skeletal muscle, plasma, urine, and gut microbiota. The kidney exhibited the most significant and robust response to SGLT2 inhibitors in terms of metabolic signaling and transporter reconfiguration. Furthermore, the gut microbiome showed a decrease in bacteria taxa capable of fermenting phenylalanine and tryptophan, leading to lower plasma levels of uremic toxins. P-cresol sulfate, one of the most notably affected metabolites, was confirmed in cohort studies involving heart failure and diabetic patients.
Conclusion: The metabolic communication facilitated by SGLT2 inhibitors resulted in reduced circulating waste products like p-cresol sulfate, thereby decreasing the need for renal detoxification. Combined with decreased glucotoxicity in the proximal tubules and a broad downregulation of apical transport activity, these findings provide a metabolic explanation for the observed cardiovascular and renal protection.

See you in Busan!
BEXCO, Busan, Korea
2023.hupo.org
#HUPO2023

Together Through Proteomics

PROGRAM

PP03.21: The Role of TRPA1 Cation Channel in Progression of Cardiac Fibrosis

Jessa Flores, Republic of Korea

Background: Transient receptor potential Ankyrin 1 (TRPA1) is a non-selective cation channel which is known for its roles in nociception in neurons. However, it is also found to be expressed in cardiac tissues, facilitating ion transport. This study aimed (1) to evaluate the regulation of TRPA1; and (2) to determine the role of TRPA1 in the physiology of cardiac fibrosis in vitro.

Methods: Western blot was performed to check the TRPA1 protein levels in vitro (mouse cardiac fibroblasts) fibrotic model. RT-PCR analysis was used to measure gene expression and immunocytochemistry was used to visualize the protein expression in cells. rhTGFβ1 treatment was used to create a fibrotic model while siRNA transfection was performed to create a TRPA1
knockdown model.

Results: TRPA1 levels were found to increase under fibrotic conditions and knockdown of TRPA1 decreases the levels of fibrotic markers. Accordingly, the decreased levels of TRPA1 under fibrotic conditions attenuated the phosphorylation of SMAD2 and ERK1/2 proteins which led to decreased fibrotic markers.

Conclusions: The findings of this study showed that TRPA1 inhibition potentially exerts a protective role against TGF-β1-induced cardiac fibrosis by
downregulating the fibrotic ERK1/2 signaling pathway.

PP03.23: Proteome-wide Interrogation of Idiopathic Pulmonary Arterial Hypertension

Sungseek Kim, Republic of Korea

Background: Pulmonary arterial hypertension (PAH) is a life-threatening condition characterized by elevated pulmonary arterial pressure leading to right heart failure. However, the lack of a definite treatment strategy and challenge of early diagnosis remains due to limited understanding of its pathophysiology. Moreover, current plasma proteomics approaches have limitations in capturing the complex and dynamic proteome involved in PAH.
Methods: We conducted a comprehensive global proteomics analysis of plasma proteomes in idiopathic PAH (IPAH) patients of PAH Platform for Deep Phenotyping in Korean Subjects (PHOENIKS) cohort. We employed a 2-hour LC method coupled with the Data Dependent Acquisition (DDA) of mass spectrometry (MS) and the ProteographTM workflow.
Results: We analyzed 32 patient samples and normal controls, and classified them into mild, moderate, and severe IPAH based on mean Pulmonary Arterial Pressure (mPAP). The ProteographTM identified 2,481 proteins across the groups, including 632 differentially expressed proteins (DEPs), whereas the depletion method detected only 701 total proteins and 143 DEPs. Notably, 90 DEPs were commonly detected across IPAH patient groups. For proteins below 100 pg/mL, the depletion method detected only one protein and no DEP, while Proteograph identified 357 proteins and 19 DEPs, highlighting the remarkable capability of the Proteograph in detecting clinically valuable low abundance proteins. Further analysis of the DEPs revealed candidate proteins associated with IPAH pathophysiology. Functional enrichment using GO/KEGG analysis showed candidates exhibiting similar increasing or decreasing trends as the mPAP-based IPAH severity. These candidates were linked to immune processes, CO2 transport, hyperproliferation, and metabolism, indicating their potential involvement in IPAH pathophysiology.

Conclusions: Taken together, by using LC-MS/MS and the Proteograph platform to provide comprehensive analysis of the global proteome of IPAH patients, this study provides valuable insights into IPAH for the identification of novel candidate proteins for diagnosis and classification of IPAH according to severity.

PP03.25: KATP Channel Dependent Heart Multiome Atlas

Sungjo Park, United States

Plasmalemmal ATP sensitive potassium (KATP) channels are recognized metabolic sensors, yet their cellular reach is less well understood. Here, transgenic Kir6.2 null hearts devoid of the KATP channel pore underwent multiomics surveillance and systems interrogation versus wildtype counterparts. Despite maintained organ performance, the knockout proteome deviated beyond a discrete loss of constitutive KATP channel subunits. Multidimensional nano-flow liquid chromatography tandem mass spectrometry resolved 111 differentially expressed proteins and their expanded network neighborhood, dominated by metabolic process engagement. Independent multimodal chemometric gas and liquid chromatography mass spectrometry unveiled differential expression of over one quarter of measured metabolites discriminating the Kir6.2 deficient heart metabolome. Supervised class analogy ranking and unsupervised enrichment analysis prioritized nicotinamide adenine dinucleotide (NAD+), affirmed by extensive overrepresentation of NAD+ associated circuitry. The remodeled metabolome and proteome revealed functional convergence and an integrated signature of disease susceptibility. Deciphered cardiac patterns were traceable in the corresponding plasma metabolome, with tissue concordant plasma changes offering surrogate metabolite markers of myocardial latent vulnerability. Thus, Kir6.2 deficit precipitates multiome reorganization, mapping a comprehensive atlas of the KATP channel dependent landscape.

PP03.27: Proteotranscriptomic Analysis Identified Inducer and Driven Molecular Pathway for Calcific Aortic Valve Disease

Hung M. Vu, Republic of Korea

Calcific aortic valve disease (CAVD) is the most prevalent valvular heart disease and the third leading cause of cardiovascular disease. CAVD causes thickening and impairment of the aortic valve leaflets and progresses to aortic stenosis (AS). This results in obstruction of left ventricular outflow and subsequent heart failure. Despite a significant clinical and economic burden, there are currently no proven therapeutics to reverse or prevent the progression of CAVD. Thus, there is an urgent need to identify the key molecular drivers contributing to the development of this disease. In the present study, we analyzed transcriptome and proteome data obtained from human CAVD tissues and proteome and phosphoproteome from human valvular interstitial cells (hVIC) to identify the underlying mechanisms of this disease. We evaluated a total of 10 AR and 33 AS tissue specimens to identify differentially expressed genes and proteins associated with the development and progression of CAVD. Analysis of the DEGs and DEPs revealed VWF and other proteins as common upregulated molecules associated with CAVD progression. Gene set enrichment analysis revealed that all the enriched biological processes during AS were primarily associated with an increase in the immune system; particularly, activated macrophages in AS. One of the proteins upregulated in CAVD was treated to hVIC to functionally confirm its effect on increased expression of calcification markers (e.g. RUNX2, BMP2, and OPN) that led to an increase in calcification. Interestingly, this was significantly inhibited by an inhibitor, suggesting that the protein is associated with valvular interstitial (VIC) cell calcification. In conclusion, we demonstrated altered expression of molecules in calcified aortic valves to gain insight into CAVD pathobiology by integrating proteomics, phosphoproteomics, transcriptomics, and network analyses and believe that this result may help to develop a novel effective drug for the CAVD treatment.

PROGRAM

PP03.29: Proteome Analysis of Chrna7 Knock Out Mice Suggests an Involvement of Nicotinic Acetylcholine Receptors in the Regulation of Ovarian Functions

Karolina Caban, Germany

Neuronal acetylcholine receptor subunit alpha-7 (CHRNA7) is a member of the ligand-gated ion channel superfamily. CHRNA7 forms a homo-pentameric channel that is highly permeable to calcium ions and has been linked to a variety of cellular processes, including excitatory neurotransmission, inflammation regulation, metabolism, cell growth and cytoskeleton regulation, and cell death. Although it is primarily expressed and studied in the nervous system, it has also been reported in the ovary, where its functions are not known. To shed light on the role of CHRNA7 in the ovary, we conducted a label free quantitative proteome analysis of ovaries from Chrna7 knock out (KO) and age-matched wildtype mice (WT; both in metestrous). Our analysis identified 36,662 peptides corresponding to 3951 different proteins. We found 128 differentially abundant proteins (FDR < 0.05), with 96 proteins being more and 32 proteins less abundant in KO animals. Notably, we observed an increase in the abundance of proteins related to steroidogenesis, such as CYP11A1 and HSD3B6, suggesting that CHRNA7 deficiency could lead to changes in steroid production. Additionally, our bioinformatics analysis revealed that the proteins that were more abundant in KO samples are associated with mitochondrial and fatty acid metabolic processes, which might be related to steroidogenesis. Furthermore, our results indicate that CHRNA7 may also play a role in regulating inflammatory responses in the ovary. Specifically, DDX3X and macrophages associated proteins CTSS, CNN2, SOAT1, and LCP1 were more abundant in KO samples, while TMSB10, which is related to cytoskeleton organization, was less abundant. These findings suggest that CHRNA7 may have distinct roles in ovarian steroidogenesis, metabolism, and inflammation regulation.

PP03.31: A Novel Role for RNA in Regulating the Maturation of Trigger Factor During the Initial Stages of Protein Folding

Yura Choi, Republic of Korea

Trigger Factor (TF), a conserved molecular chaperone, plays a pivotal role in the folding and assembly of newly synthesized polypeptides in bacteria. Despite its importance, the mechanism governing the transition of TF from its dimeric to monomeric state, a critical step in protein folding, remains largely unexplored. In this study, we investigated the potential role of RNA in regulating this structural transition of TF. Our findings suggest that RNA molecules can facilitate the transition of TF from its dimeric to its monomeric and active state. We further identified specific RNA sequences that induce this transition, implying a novel regulatory role for RNA in TF activity. This study provides new insights into the complex interplay between chaperones and RNA molecules in bacterial protein folding and homeostasis, highlighting the potential of RNA as a regulatory factor in the dimerization and monomerization of TF. These findings open up new avenues for understanding the intricate mechanisms of protein folding in bacteria.

PP03.33: Time-Resolved Proteome Profiling of Glioblastoma Cell Response to Type I Interferon Stimulation Using DirectMS1 Approach

Mark Ivanov, Russian Federation

Introduction:
Knowledge of interferon-dependent antiviral mechanisms in tumor cells is important for oncolytic virus therapies. Glioblastoma DBTRG-05MG cell line acquires resistance to the vesicular stomatitis virus (VSV) after interferon treatment. We found that resistance formation time strongly depends on the concentration of interferon and varies in the range of 7-12 hours. However, existing studies usually show proteins that are differentially expressed at a single time point after the treatment (e.g. 24 hours) and there is a lack of time-resolved proteomics studies.

Methods:
Glioblastoma DBTRG-05MG samples were analyzed using the previously established DirectMS1 workflow. Samples treated using two concentrations of type I interferon were analyzed at 11 time points in a range of 0-24 hours. Analysis was done using 5-min LC-MS1 mode with Orbitrap Q Exactive HF-X which took less than 1 day of total acquisition time for 150 runs.

Results:
More than 200 differentially expressed proteins between control and interferon-treated samples were detected in at least a single time point. Full time profiles for these proteins were obtained using MS1 peptide intensities. We found that the most usually reported interferon-stimulated genes are expressed at proteome level only after resistance is fully established and only two of them correlated with VSV resistance.

Conclusions:
DirectMS1 method makes it possible to implement quantitative large-scale time-resolved proteomic analysis with reasonable total acquisition time and quantification depth.

PP03.35: RNA-binding as Chaperones of DNA Binding Proteins from Starved Cells: The Role of N-terminal Lysines in E. coli Dps Assembly

Yoontae Jin, Republic of Korea

DNA-binding proteins from starved cells (Dps) are dodecameric proteins primarily produced by bacteria under stressful conditions. These proteins bind to DNA, sequester divalent iron ions, and prevent the production of harmful reactive oxygen species. The N-terminal region of Dps also facilitates interactions with RNA, suggesting a putative chaperoning function for RNA. Furthering our understanding, recent findings revealed that the N-terminal lysine residues of Escherichia coli Dps specifically interact with RNA. Our investigation using gel filtration, electrophoresis, and circular dichroism has shown that the oligomeric state of Dps changes with the presence or absence of RNA. In addition, the RNA-dependent assembly appears to be influenced by the interaction between RNA and the N-terminal lysine residues of Dps. This novel insight into the role of N-terminal lysine residues in Dps assembly and RNA binding further enriches our understanding of the complex regulatory mechanisms governing Dps function and suggests potential avenues for targeted interventions under conditions of cellular stress.

PP03.37: The Study Based on Proteomics on the Specific Function of Transforming Growth Factor- β2 in Human Umbilical Vein Endothelial Cells

Soohyeon Kim, Republic of Korea

TGF-β is a multifunctional cytokine and plays a variety of roles during development and pathophysiology. In mammalians, TGF-β isoforms exist from 1 to 3. Although the isoforms have selectivity for several TGF-β receptors and different binding affinities for them, previous studies have reported that TGF-β isoforms have almost the same function. However, recent studies have reported the possibility that each isoform may perform a different function through the involvement of various receptors and co-receptors in TGF-β signaling. In particular, vascular endothelial cells express various TGF-β receptors and co-receptors such as endoglin, and we have shown the specific function of TGF-β2 in our previous studies. Based on this, in this study, we tried to understand more broadly the TGF-β2 specific signal transduction and protein expression through proteomic analysis and to interpret the pathogenesis of TGF-β2 selective or sensitive diseases. HUVECs samples were prepared by the S-trap digestion method and protein profiling was performed using Orbitrap LC-MS equipment. For data processing, the 'Sequest HT embedded in Proteome discoverer 2.2' program was used.

PROGRAM

PP03.39: The Effects of Conditional UHRF1 Knockout in CD4+ T Cell on Mammary Tumor

Jiyoon Kim, Republic of Korea

In our laboratory, we previously conducted methylation profiling on peripheral blood mononuclear cells (PBMCs) from dogs with normal and canine mammary tumors. We selected genes that showed hypermethylation and decreased expression in canine mammary tumors compared to normal dogs. For our next project, we will first orthotopically inject breast cancer cells into C57BL/6 mice and collect blood samples to perform immunophenotyping. We aim to determine precisely which immune cell, among the PBMCs in the blood, exhibits hypermethylation and decreased expression of this gene. Once the immune cell type is identified, we will utilize the Cre, LoxP knockout system to conditionally knock out this gene specifically in that immune cell population, elucidating the role of this gene in the function of immune cells within the context of mammary tumor.

PP03.41: Proteomic Analysis of Human Dermal Fibroblasts Induced by Dermal Matrix Alteration

Sun Young Lee, Republic of Korea

The skin is the most important barrier to protect the body from external damage, skin has complex mechanisms for self-defense and regeneration of skin integrity to maintain its integrity and homeostasis after damage. The ability of tissues to recover after damage is one of the fundamental characteristics of all organisms for maintaining homeostasis in the body. In particular, fibroblasts play a key role in maintaining skin homeostasis and adjusting physiological tissue repair. Fibroblasts generate an extracellular matrix (ECM), which allows a complex interaction between fibroblasts and their microenvironment. External mechanical stimuli also act as stressors on fibroblast phenotype expression.
Here, we investigated the influences of fibroblasts under the surrounding microenvironment, especially, the ECM component ratio as a cell culture conditions. We performed proteomic analysis using nanoLC-ESI-MS/MS on human dermal fibroblasts cultured in matrices with different compositions of collagen (C) and elastin (E). Quantitative proteomic analysis revealed that changes in the proteome were most prominent at 0 hours in fibroblasts cultured with 80 % collagen (4C1E). Proteins up-regulated at 0 hour were associated with activation of calcium ion channels, and those up-regulated at 72 hours were related with collagen fibrils and ECM organization. In conclusion, we identified a mechanism by which changes in the skin environment induce calcium ion channel activity in fibroblasts, leading to collagen fibrils and ECM organization, which in turn induces fibrosis. Our findings may be used to develop therapeutic reagents for skin diseases.

PP03.43: USP-A Regulates p53 Through the DnaJ-α with Cisplatin

Yosuk Min, Republic of Korea

Ovarian cancer is the 7th most common cancer in women. Our previous research revealed that patients of ovarian cancer with a high mRNA level of USP-A have a higher survival rate compared to those with a low level of USP-A. Therefore, in this study, we studied the mechanism of USP-A to find out how patients with upregulated USP-A have higher rate of survival. USP-A is one of the deubiquitinating enzymes which detach the ubiquitin from the substrate. Ubiquitination is one of the post-translational modifications (PTMs). It affects many cellular functions, such as DNA damage repair, apoptosis, cellular signaling, and protein degradation. On the other hand, deubiquitination removes ubiquitin chains from the substrates. Cisplatin, a widely used chemotherapy agent, is commonly employed in the treatment of ovarian cancer. When DNA damage occurs under the effect of cisplatin, USP-A increases in response. Here, we found the putative binding partner of USP-A, DnaJ-α, using protein-protein interaction analysis and confirmed the bindings between USP-A and DnaJ-α using immunoprecipitation assay in vitro. Second, we performed ubiquitination, deubiquitination, and stability assay using MG132 (proteasome inhibitor) or cycloheximide. We discovered that DnaJ-α undergoes ubiquitinatoin and deubiquitinated mediated by USP-A, and the protein stability of DnaJ-α is also regulated by USP-A. Finally, since previous study revealed that DnaJ-α was associated with the binding affinity of p53 and MDM2, which is the E3 ligase of p53. Therefore, we checked the binding affinity of p53 and MDM2, the amount of ubiquitination of p53, and stability by regulating USP-A and DnaJ-α. The stability of p53 is affected by USP-A and DnaJ-α, and the ubiquitination of p53 is also altered by USP-A and DnaJ-α. Moreover, these mechanisms affect apoptosis in cisplatin-treated ovarian cells. Collectively, we suggest that the USP-A - DnaJ-α -p53 pathway is the essential molecular pathway in ovarian cancer therapy.

PP03.45: Establishment of Novel Cancer Stem-Like Cells of Cholangiocarcinoma. And Their Characterization by Proteomics

Orasa Panawan, Thailand

Introduction: Cholangiocarcinoma (CCA) is an aggressive with poor response to chemotherapy and high recurrence rate. Recent evidence suggests the impact of cancer stem cells (CSC) on the therapeutic resistance of CCA. However, the knowledge of CSC is very limited since there are few CSC model cells and their molecular markers.
Method: We established CCA-stem-like cells from CCA parental cell lines. To identify the CCA-CSC associated pathway, we have undertaken a global proteomics and functional cluster/network analysis comparing between CSC, 10%FCS-induced differentiation CSC, and Parental cells. The proteomics of those cells were performed by the LFQ using an Easy-nanoLC-Orbitrap-Fusion-Tribrid-system equipped with Nikkyo-RP-nano-Column, the data mining were performed by Proteome Discoverer, MaxQuant and Perseus software. Gene Ontology analyses and network analyses were assisted by DAVID, KEGG, and KeyMolnet.
Results: We successfully established and characterized CCA-CSC (KKU-055-CSC) which has the potential to be CSC model. CCA-CSC highly expresses the stem cell markers such as SOX2, CD44, OCT3/4, shows the drug resistance, faster and constant expansive tumor formation in xenograft mouse models, and, possesses the multi-lineage differentiation ability. In proteomics, we identified 8900 proteins in total, followed by the cluster analysis, we extracted the significantly upregulated proteins in CSC compared with DIF and Par cells (>1.5-fold, p-value <0.05). Network analysis revealed that HMGA1 and Aurora-A signaling pathway were upregulated in CSC. The silencing of HMGA1 in KKU-055-CSC suppressed the expression of stem cell markers, induced the differentiation followed by cell proliferation, and enhanced sensitivity to chemotherapy drugs including Aurora-A inhibitors. Moreover, in silico analysis indicated that the expression of HMGA1 was correlated with Aurora A expression and poor survival of CCA.
Conclusion: Our CCA-CSC could be useful as a cancer stem cell model, and the HMGA1-Aurora-signaling pathway may be a novel candidate of therapeutic target for CSC in CCA. (Cancer Science 2023 in press)

PP03.47: Assessing Human Stem Cell Models of Human Peri-Implantation Development While Contributing Towards the Chromosome-Centric HPP

Charles Pineau, France

BACKGROUND: Understanding human peri-implantation development is essential to appreciate the onset of human life but also for multiple clinical applications such as in vitro fertilization, regenerative medicine and placenta physiopathology. To overcome the technical challenges and ethical issues that result in limited access to human embryo for research, we studied stem cells lines modeling human embryo development. Pluripotent cells, developing into the fetus, and trophoblast cells, giving rise to the placenta, emerge during the first weeks. This emergence is a focus of scientists working in the field of reproduction, placentation and regenerative medicine. Most knowledge in the field has been harnessed by transcriptomic analysis. Interestingly, some genes are uniquely expressed in those cells, giving the opportunity to uncover new proteins that might play a crucial role in setting up the molecular events underlying early embryonic development.

METHODS: A strategy combining single-cell RNA sequencing (scRNAseq), data independent acquisition mass spectrometry (DIA-MS) on a tims TOF Pro instrument (Bruker Daltonics), was used to analyze naive pluripotent (hNPSCs) and trophoblastic (hTSCs) stem cells. Interestingly, the use of the PaSER 2022 database search platform (Bruker Daltonics), in addition to a classical Mascot search, was shown to be relevant and crucial for validating several unique peptides out of our MS proteomics data.

CONCLUSIONS: In this study, we discovered several new missing proteins, thus contributing to the chromosome-centric Human Proteome Project. Beyond helping to complete the catalog of protein encoded by the human genome, our work contributes to define hallmarks for pluripotent and trophoblast stem cells that are particularly important, when reliable, to ensure proper models are used for tackling hypotheses on the mechanistic of embryo development and to further assign at least a function to several key proteins.

PP03.49: Proteomic Analysis of Primary Human Nasal Epithelium Reveals the Allergic mechanisms by House Dust Mite and Diesel Exhaust Particles

Hoseok Seo, Republic of Korea

Introduction: Allergy stimulated by various causes significantly reduces the quality of life. Specifically, House dust mite (HDM) is the antigen with which many people are allergic and has become increasingly stronger as indoor life becomes more common. On the other hand, Diesel exhaust particle (DEP), a main component of urban air pollution with particulate matter, are associated with weakening respiratory and increasing the sensitivity to allergens. While the interest of HDM and DEP are increasing, the specific mechanisms of DEP on HDM allergic patients are still poorly understanding. In this study, we investigated proteome differences related to the DEP in HDM allergic patients and non-allergic patients.

Methods: We developed primary human nasal epithelium (PHNE) cells from HDM allergic patients and non-allergic patients as an in vivo-like model. After the HDM sensitization or vehicle for 13 days, cells were treated with HDM or HDM+DEP for 48 hours. After protein was digested by S-trap procedure, peptide samples were labeled with 11-plex tandem mass tag (TMT) reagents. Labeled sample was analyzed by Orbitrap Exploris 480. MS raw files were processed by Proteome Discoverer ver 3.0. Statistical analysis was performed using Perseus software.

Results: A total of about 8000 proteins were quantified in 3 sets of TMT experiments. we identified the differentially expressed proteins in three comparison. 1) HDM allergic patients and non-allergic patients in vehicle only treatment condition, 2) protein changes during HDM allergic response in HDM allergic patients, 3) protein changes in HDM+DEP and HDM alone in HDM allergic patients. Bioinformatics analysis showed that many processes were involved in HDM allergic response with DEP.

Conclusions: In this study, in depth proteomic analysis of cellular mechanisms of HDM and DEP was firstly elucidated by PHNE from HDM allergy patients. These observations provide an understanding of response and mechanisms to DEP in HDM-allergic patients.

PP03.51: Elevated Expression of ANOS1 Induce Tamoxifen Resistance and Leads to Poor Prognosis in ER+ Breast Cancer

Hoonyoung Yoon, Republic of Korea

Breast cancer is a prevalent and significant health concern affecting women globally. Tamoxifen, a selective estrogen receptor modulator, is a cornerstone in hormone receptor-positive breast cancer treatment, representing approximately 70% of cases. However, the development of tamoxifen resistance poses a considerable clinical challenge, compromising its long-term effectiveness and leading to disease progression and adverse patient outcomes. Understanding the underlying mechanisms of tamoxifen resistance is essential to overcome this hurdle and identify potential therapeutic targets.

ANOS1, also known as KAL1, is located on the X chromosome and encodes anosmin-1, a secreted glycoprotein involved in neuronal development and axon guidance during embryogenesis. Although primary role of ANOS1 has been predominantly studied in Kallmann syndrome, its involvement in breast cancer and endocrine therapy resistance has not been established.

In this study, we investigated the expression of ANOS1 in breast cancer clinical datasets and breast cancer cell lines. We observed elevated levels of ANOS1 expression in breast cancer, and its higher expression is associated with a poor prognosis in tamoxifen-treated estrogen receptor-positive (ER+) breast cancer patients. Additionally, we confirmed increased ANOS1 expression in a tamoxifen-resistant breast cancer cell line and assessed its role in cancer progression and treatment resistance.

Overall, our findings suggest a potential association between ANOS1 and breast cancer, highlighting its relevance in tamoxifen resistance. Unraveling the molecular mechanisms underlying the contribution of ANOS1 to tamoxifen resistance in ER+ breast cancer could provide valuable insights for enhancing patient outcomes through its potential as a prognostic biomarker and therapeutic target. Further investigations into the mechanisms by which ANOS1 influences tamoxifen response and its clinical implications may pave the way for improved personalized treatment strategies and patient care.

PP03.53: Temporal Omics Profiling Using a Mouse Model of Nonalcoholic Steatohepatitis-Associated Hepatocellular Carcinoma.

Yuichi Abe, Japan

Non-alcoholic steatohepatitis (NASH) has emerged as a significant cause in the development of hepatocellular carcinoma (HCC). However, the molecular mechanisms underlying the occurrence of HCC in a subset of NASH patients, even in the absence of cirrhosis, remain unclear. Therefore, there are unmet clinical needs for early diagnosis and optimal treatment strategies in NASH-associated HCC. To address these challenges, we conducted molecular profiling of plasma samples and tumor tissues collected at defined time points from melanocortin-4 receptor–deficient (MC4R-KO) mice fed high-fat diet, which faithfully recapitulate development of human NASH-associated HCC. In-depth quantitative plasma proteome profiling revealed a distinct HCC-associated signature that discriminated mice bearing early-stage HCC from the NASH mice. Profiling of plasma immunoglobulin-bound proteins uncovered the occurrence of humoral immune response in the form of autoantibodies against a set of tumor-associated antigens that are overexpressed in early-stage HCC. Finally, integrated analysis of genome, transcriptome, proteome, and phosphoproteome of HCC tissues and adjacent liver tissues resulted in the identification of signaling pathways related to metabolic reprogramming in early-stage HCC. Our study contributes to a better understanding of molecular mechanisms in the development of NASH-associated HCC and provides a rich resource for novel diagnostic biomarkers and therapeutic targets.

PP03.55: Identification of Stratifin as a Novel Biomarker for Interstitial Lung Disease by an Affinity Proteomics

Noriaki Arakawa, Japan

Among the various histopathological patterns of drug-induced interstitial lung disease (DILD), diffuse alveolar damage (DAD) is associated with poor prognosis. However, there is no reliable biomarker for its accurate diagnosis. To search for new biomarker candidates for DAD diagnosis, we performed an affinity proteomic analysis, SOMAscan assay, using blood samples from DILD patients. The study included two independent cohorts (including totally 26 patients with DAD) and controls (total 432 samples). We found stratifin/14-3-3σ (SFN) as a biomarker candidate found in the proteomic analysis. We developed an in-house ELISA for SFN and validate the SOMAscan results. SFN was specifically elevated in DILD patients with DAD, and was superior to the known biomarkers, KL-6 and SP-D, in discrimination of DILD patients with DAD from patients with other DILD patterns or other lung diseases, including bacterial pneumonia. SFN was also increased in serum from patients with idiopathic DAD, and in lung tissues and bronchoalveolar lavage fluid of patients with DAD. In vitro analysis using cultured cells derived from human alveolar epithelium suggested that extracellular release of SFN occurred via p53-dependent apoptosis. We conclude that serum SFN is a promising biomarker for DAD diagnosis.

PP03.57: Population Serum Proteomics Uncovers Prognostic Protein Classifier and New Perspective on the Pathophysiology for Metabolic Syndrome

Xue Cai, China

Background

Metabolic syndrome (MetS) is a complex metabolic disorder with a global prevalence of 20-25%. Early identification and intervention would help minimize the global burden on healthcare systems. Plasma and serum are the predominant samples used for diagnostic analyses in clinics with low invasiveness and ease of collection and preservation. The rapid development of mass spectrometry (MS)-based proteomics provided solid technical support needed by large cohort proteomics.

Methods

We included 7,890 serum samples collected from 3840 participants from the community-based prospective cohort study. A 20-min DIA-MS method was used for MS acquisition. All data were analyzed using DIA-NN against a plasma spectral library containing 5102 peptides and 819 unique proteins. A machine learning model was built to predict the risk of developing MetS, and linear mixed models were used to explore the potential protein biomarkers of MetS.

Results

We measured over 400 proteins from ~20,000 proteomes using data-independent acquisition mass spectrometry for 7890 serum samples from a longitudinal cohort of 3840 participants with two follow-up time points over ten years. We then built a machine learning model for predicting the risk of developing MetS within ten years. Our model, composed of 11 proteins and the age of the individuals, achieved an area under the curve of 0.784 in the discovery cohort (n=855) and 0.774 in the validation cohort (n=242). Using linear mixed models, we found that apolipoproteins, immune-related proteins, and coagulation-related proteins best correlated with MetS development.

Conclusions

We generated a resource of serum proteomics using DIA-MS based on a prospective population cohort with a 10-year follow-up. Using this data, we built a model for predicting the risk of developing MetS within ten years. We also found new potential protein biomarkers of MetS that, together with their pathways, providing new perspectives on the pathophysiology of MetS.

PP03.59: Translating of Tissue Proteomics Profile Into Plasma Protein Biomarker Signature by DIA-MS -based Proteomics

Yi-ju Chen, Taiwan

Plasma-based molecular diagnosis is most attractive for early cancer detection. However, the huge dynamic range presents great challenges for mass spectrometry-based proteomics. Based on our previous proteogenomics study on lung cancer, a proteome-based classification revealed a new subtype, "late-like" in early-stage patients, characterized by their proteome profile resembling that of late-stage patients and high recurrence in follow up studies. To develop a panel of protein biomarker candidates in identification of high-risk early-stage patients for early treatment, we established a data-independent acquisition (DIA)-based platform to achieve deep plasma proteome profiling from >100 patients. Plasma samples from lung adenocarcinoma patients and healthy cohorts were collected with IRB approval. The top 12 high abundant proteins were depleted from individual plasma samples and then the samples were analyzed by DIA-MS. A spectra library was constructed by the datasets from fractionation of pooled plasma samples, followed by data-dependent acquisition (DDA). The result showed that a total of 3068 protein groups and an average of 787 proteins per patient were quantified by library-based DIA analysis from 100 healthy and 100 plasma samples of individual lung adenocarcinoma patients with stage IA to IV. Using the direct DIA analysis by Spectronaut software, 1654 protein groups, including well-known lung cancer markers EGFR, were quantified. The analysis revealed significant enrichment of 16 selected proteins with dramatic up- and down-regulation in LUAD cohort compared to the healthy cohort (p<0.05). These protein candidates will be further validated by the developed DIA-MS assay as well as ELISA assay in another independent prospective cohort. In addition, we will also design a DIA-based quantitation assay for absolute quantitation of the developed candidate panel to develop a prediction model for high-risk early recurrent lung cancer patients. In conclusion, DIA-MS-based proteomics facilitates deep plasma proteomic profiling for discovery and validation of plasma biomarker candidates.

PP03.61: Pushing DIA Proteomics Analyses of Neat Plasma to 1000 Protein Groups ID/h

Moonju Cho, Republic of Korea

Introduction
Direct proteomics analysis of neat plasma remains a challenge because of the huge dynamic range of the plasma proteins, but is also very appealing because it requires very small volumes and is unexpensive compared to new depletions technology. We focused on workflow optimization of neat plasma analysis using dia-PASEF® approach to maximize the number of proteins groups (PG) identified and quantified, while minimizing both gradient time and missing value (MV) levels.

Methods
The plasma sample cohort consisted in 15 patients affected by a rare genetic disease and 18 age-matched controls. Samples were digested with trypsin using STRAP columns, separated either by nano-HPLC (nanoElute, Bruker Daltonics) using a 25cm column on a 30min gradient (IonOpticks, Australia), or with an EvosepOne ran with the 60 Samples Per Day (60SPD) method (Endurance column, 8cm). Both LC's were connected to a timsTOF Pro™ instrument (Bruker Daltonics) via its Captive Spray source. The timsTOF Pro was operated in dia-PASEF acquisition modeDiaNN1.8.1 (Demichev group) and Spectronaut®17 (Biognosys) were used for data processing.

Results
Using the nanoElute, a single plasma analysis leads to the identification of ca.450 proteins groups, the analysis of 4 samples (enabling MBR), leads to 507+-6 proteins (2% missing values, MV hereafter). Searching 33 samples simultaneously led to 811 +-78 protein groups (33% MV), and an average of 1091 PG (+- 181) 5 runs of plasma extracellular vesicles purified from 5 healthy individuals independent form the cohort were also added to the analysis (search EV-boost). Using Spectronaut®17.6, we obtained 799 (+- 70) proteins from the 33 plasma runs and 905+-225 for 33 plasma EV boosted search.

conclusions
We will discuss the trade-off of analysis depth and quantification quality for time, for the tested softwares in both library-free and library-based mode

See you in Busan!
BEXCO, Busan, Korea
🌐 2023.hupo.org
🐦 f 📷 in #HUPO2023

Together Through Proteomics

PROGRAM

PP03.63: Performance and Validation of "Proteome Panels" as Classifications Tools of Kidney Transplants in FFPE Tissues.

Garry Corthals, Netherlands

Background
RNA expression profiling using microarrays has been a widely adopted method for identifying immunological patterns in renal transplantation biopsies, defining gene sets associated with graft rejection. NanoString Technologies (NST) have recently emerged as a unique alternative, quantifying gene expression from FFPE tissues using a panel of 770 genes as a surrogate for microarrays' extensive gene coverage.

In this study, we evaluated MS-based proteomics performance on various tissue types and biopsies, following a workflow similar to NanoString measurements in pathology clinics. We optimized parameters to capture reproducible quantitative data and aimed to identify "proteome panels" or windows of molecular information complementary to microarrays and NST, with the goal of making protein expression analysis more accessible in clinical settings.

Methods
Over several years, we analysed numerous resection and biopsy tissues, including Fresh Frozen and FFPE samples of varying thickness (4-20 um). Both Data-Dependent Acquisition (DDA) and Data-Independent Acquisition (DIA) modes were tested. The publicly available Banff Human Organ Transplant Panel (BHOT) was incorporated into our research.

Results
Several cost-effective methods are now available requiring minimal sample preparation without needing expensive automation, suitable for analysing a limited number of samples (10s). However, for large-scale analysis (100s) with high reproducibility and fast completion, automation is essential. Our findings indicate that achieving approximately 50% active proteome coverage requires less than 1 mm tissue samples.

Conclusions
With the speed and accuracy of current instrumentation, real time analysis can be achieved where one contains reproducible deep proteome coverage within minutes of MS-time, on sub mm2 tissue biopsies, offering a valuable tool for molecular profiling.

Proteome panels may play a significant role in tissue classification as they serve as valuable assays. These panels consist of hundreds of proteins that can be compared with existing methods in pathology, enabling more accurate and comprehensive diagnosis.

PP03.65: Proteomic Profiling of Cerebrospinal Fluid Reveals Protein A as a Potential Biomarker for Medulloblastoma

Ki-soon Dan, Republic of Korea

Background
The development of a sensitive diagnostic marker for medulloblastoma (MBL), a common malignant brain tumor in children, is still a challenge. Cerebral spinal fluid (CSF) contains various substances such as soluble proteins and extracellular vesicles (EVs), and may reflect pathological changes in the central nervous system.

Methods
We analyzed human CSF proteomes collected from Hydrocephalus (HC) and MBL patients using mass spectrometry-based proteomic methods. To identify proteomic signatures for the classification of two groups, we first extracted top 10 ranked features using two feature selection methods, including ANOVA and Fast Correlation-Based Filter (FCBF), from up- and down-regulated DEPs in MBL group. ELISA analysis (n =18) of soluble CSF proteins and ExoView® analysis (n=14) of isolated EVs cargo proteins for verification of four candidates were performed.

Results
A total of 35 patients' CSF were analyzed.(MBL, n = 21; HC, n = 14). 2,223 protein groups were identified. Pairwise comparison of two groups reveals that 273 differentially expressed proteins (DEPs) were found. We selected the top 4 ranked and overlapping features from each selection methods based on the upregulated proteins in the MBL group as the biomarker verification candidates. Only Protein A had a significant difference in abundance during verification process. Subsequently, we found protein A level in EVs according to tumor leptomeningeal metastasis (LM) was also significantly higher in the LM (+) group than in the non-LM (-) group. Enrichment analysis with protein A and its 11 interacting DEPs showed that the pentose phosphate pathway and glycolysis, which are tightly connected in glucose metabolism, were enriched.

Conclusion
Our study showed that proteomic analysis of whole CSF was able to distinguish between HC and MBL. Furthermore, we identified the significance of protein A in the whole CSF and its presence in circulating EVs in the CSF.

PP03.67: Proteomic Characterization of Blood Samples From Cystic Fibrosis Patients by Mass Spectrometry

Kerstin Fentker, Germany

Background
In clinical trials, the novel small molecule therapy ELX/TEZ/IVA (Elexacaftor/Tezacaftor/Ivacaftor) has demonstrated remarkable effectiveness for treating patients with cystic fibrosis (CF). Lung function (FEV1), sweat chloride levels, and respiratory symptoms such as mucus production, coughing, and breathing difficulties showed significant improvement. However, there is limited knowledge available regarding the systemic effects of ELX/TEZ/IVA and the comparison to previously approved modulator therapies, such as LUM/IVA (Lumacaftor/Ivacaftor).

Aim
This study intends to explore the pharmacodynamic changes of ELX/TEZ/IVA treatment on the plasma proteome of patients with CF. Since blood reflects the body's overall processes, systemic effects of drug treatment can be monitored. LUM/IVA was frequently employed to manage patients with a homozygous F508del mutation before ELX/TEZ/IVA approval. Our objective is to evaluate and contrast the effects of both therapies on patients.

Methods
Our study utilized label-free quantitative mass spectrometry to analyze the plasma proteome of 54 CF patients before and after three months of ELX/TEZ/IVA therapy. Additionally, we compared it to the serum proteome of 32 CF patients before and after three months of LUM/IVA therapy.

Results
We successfully quantified over 500 proteins in both cohorts. Both cohorts demonstrated a decline in inflammatory and immune markers like CRP, S100A8, and S100A9. However, the alterations were more prominent after three months of ELX/TEZ/IVA therapy overall. Furthermore, the mechanisms involved in these changes varied between the two therapies. Specifically, ELX/TEZ/IVA induced more substantial modifications in innate immune system processes such as the complement cascade, while LUM/IVA resulted in more significant changes in BCR signaling processes.

Conclusions
Our study provides a comprehensive summary of how LUM/IVA and ELX/TEZ/IVA therapies affect circulating proteins in CF patients differently. It showcases the significant improvements, particularly in reducing inflammation, but also emphasizes the ongoing requirement for further therapy developments.

PP03.69: Proteome Profiling of Clinically Relevant Pig Models for Duchenne Muscular Dystrophy: Disease Mechanisms and the Potential of Exon Skipping Therapies.

Thomas Fröhlich, Germany

Duchenne muscular dystrophy (DMD) is a severe X-linked genetic disease, caused by frameshift mutations in the dystrophin gene, leading to absence of dystrophin. DMD is characterized by progressive muscle degeneration and a dramatically reduced life span. Since a previously established tailored pig model of DMD (deficient of DMD gene exon 52) has shown to reflect important biochemical histological and functional hallmarks of the human disease, the model has become a valuable tool for preclinical testing of gene therapy approaches restoring an intact DMD reading frame. Since DMD in humans affects heart and skeletal muscle to a different extent, we generated a large set of proteomics profiles from myocardium and skeletal muscle to characterize the disease at the proteome level. To capture DMD progression, samples from animals of different ages were analyzed. As exon skipping, reframing the transcript and leading to the expression of a shortened but functional dystrophin, is a promising treatment strategy, we further analyzed cardiac as well as skeletal muscle samples from DMDΔ51-52 pigs. This animal represents the best possible therapeutic result of this treatment strategy. Strikingly, in contrast to DMDΔ52 pigs, profiles of DMDΔ51-52 pigs are highly similar to the healthy wild types. Using parallel reaction monitoring mass spectrometry, we could further demonstrate that levels of shortened dystrophin are similar to wild type levels. Taken together, we demonstrated that proteome profiling is a valuable tool to biochemically characterize DMD and enables the evaluation of new therapeutic options at the molecular level.

PP03.71: Characterizing the Effect of Adiposity on Cardiometabolic Traits and the Circulating Proteome in Qatar Biobank

Lucy Goudswaard, United Kingdom

Background
The Qatari population have a high prevalence of obesity (40%). Obesity, defined by a body mass index (BMI) >30kg/m², increases the risk of various diseases such as cardiovascular disease and several cancers. We aimed to characterise the causal effect of adiposity on cardiometabolic traits and circulating proteins in the Qatar biobank (QBB).

Methods
We used data from 2,935 QBB participants and performed (1) an observational analysis and (2) one sample Mendelian Randomization (MR) to estimate the effect of BMI on cardiometabolic traits and on 1,305 proteins measured by SomaLogic. MR analyses were performed by deriving a genetic risk score for BMI using 656 variants associated with BMI at $p<5\times10^{-8}$ and using this as an instrumental variable in a two-stage least squares analysis. Next, we compared effects with a cohort of European ancestry (N=2737) to determine evidence of ancestry-specific or shared proteomic effects.

Results
BMI was associated with >500 proteins observationally. The MR analysis gave evidence for a causal effect of BMI on 14 proteins (after Bonferroni-correction), with higher BMI corresponding to higher levels of fatty acid-binding protein 3 (FABP3), leptin and fibrinogen, and lower levels of neural cell adhesion molecule-1 (NCAM1) and brevican core protein (BCAN). Observational effect estimates across the European and Arab populations were positively correlated ($R^2=0.47$, $p=3.0\times10^{-183}$), however FABP3 was only associated with BMI in QBB. MR results were less precise but were still positively associated across ancestries.

Conclusions
We provide evidence that BMI effects proteins involved in haemostasis (fibrinogen), immune cell interaction (NCAM1) and neuronal development (NCAM1, BCAN) in an Arab population. Observational estimates suggested shared proteomic effects of BMI across European and Arab ancestries. Higher powered MR studies are required to compare causal effect estimates. Future analyses will incorporate dual-energy X-ray absorptiometry data to explore the effect of regional fat distribution on circulating proteins.

PP03.73: A Comparison of Sputum Proteome Analysis in Asthma Patients Receiving Different Biologic Treatments

Jeong-yeon Hong, Republic of Korea

Asthma is a chronic respiratory disease in which the bronchi are repeatedly narrowed by allergic inflammation. Sputum is a sticky mucous liquid produced by the bronchi and normal person secrete about 100 ml/day. It consists of 95% water and the remaining 5% protein, lipids and minerals. Sputum, a protein-rich airway biofluid, was sampled using a non-invasive method. 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, alpha-amylase being the top-ranked protein. It also the saliva-elevated protein was present at high concentration. 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.

PP03.75: Proteomics-Based Discovery of Salivary Biomarker Candidates Specific to Periodontal Disease.

Kim Inyoung, Republic of Korea

Periodontal diseases are mainly the result of infections and inflammation of the soft tissue and bone that surround and support the teeth. In its early stage, the gingiva can become swollen and red, which is called gingivitis, resulting in bleeding. In its more serious form, the periodontal tissue can be detached away from the tooth, bone, and the teeth may loosen or even fall out, which is called periodontitis. Once the teeth or bone are destructed, improper disease control can lead to a recurrence of disease. In our current study, we performed proteomic analysis using liquid chromatography-tandem mass spectrometry (LC-MS/MS) in combination with tandem mass tag (TMT) labeling which enabled simultaneous comparison of relative protein abundances of multiple saliva samples in order to discover proteins specific to periodontal diseases. Saliva samples from 10 controls, 10 periodontal positive patients(Untreated) denoted as PP, and 10 periodontal negative patients (Treated) denoted as PN were subjected to in-solution digestion using trypsin followed by labeling with two sets of 16 TMT isobaric reagents. Then, the peptides labeled with isobaric reagents were fractionated using high-pH reversed-phase chromatography and analyzed using Orbitrap Eclipse Tribrid mass spectrometer. From LC-MS/MS analyses, a total of 3,324 proteins were identified and 2,091 proteins of them had quantifiable information. When one-way analysis of variance (ANOVA) for the 2,091 proteins was applied, 582 proteins from the three groups of saliva samples showed statistical significance (P-value < 0.05). Currently, proteins showing statistically significant increases in PP group in comparison to control and PN groups, which can be simultaneously used as potential biomarkers as diagnosis and prognosis of periodontal diseases, are being investigated and some of the biomarker candidates will be pursued using enzyme-linked immunosorbent assay (ELISA).

PP03.77: Plasma Proteome Profiling for Discriminating Major Depressive Disorder and Bipolar Disorder by Data Independent Acquisition Mass Spectrometry

Eunji Jeon, Republic of Korea

Background: The diagnosis and treatment of major depressive disorder (MDD) and bipolar disorder (BD) with similar symptoms but clear diagnostic criteria are still ambiguous in the psychiatry and neuroscience fields. Because it is impossible to get direct samples of brain lesions associated with MDD and BD, many studies have recently been conducted to develop blood-based biomarkers through quantitative proteomics analysis. This study aims to develop a biomarker panel that increases the accuracy of the diagnosis MDD and BD.

Methods: We performed a comprehensive expression profiling for 149 plasma samples from 74 healthy control (HC), 30 MDD, and 45 BD participants using data-independent acquisition mass spectrometry (DIA-MS) with the Q-Exactive Plus. For pairwise comparisons of proteomes to detect differentially expressed proteins (DEPs), two-sided t-tests were used with substantial filtering criteria (p-value 0.05) by Perseus software. To avoid model overfitting, the cohort was randomly divided into training and test sets, and multiple biomarker panel was created by lasso regression and machine learning algorithms. In addition, validation was performed in independent cohorts by using multiple reaction monitoring (MRM) with corresponding heavy stable isotope labeling peptides of selected targets.

Results: Almost 400 proteins from 149 samples were quantified, and 7 DEPs for marker candidates were selected by pair-wise comparison analysis. The diagnostic model created using regression and machine learning algorithms demonstrated good discriminatory performance between MDD and BD, as well as MDD and BD compared to HC, with AUC values of 0.8 or above in all three comparison groups in the training and test sets. Collectively, the biomarker candidates in multiple biomarker panel were validated using MRM.

Conclusions: The use of our biomarker panel could be beneficial to discriminate between MDD and BD. Moreover, it is expected that our panel integrated with clinical traits may improve the diagnostic accuracy of MDD and BD.

PP03.79: Multiple Biomarkers Identification to Diagnose Metastatic Carcinoma from Thyroid Cancer Patients Plasma Using High-precision Proteomics Approach

Jinwoo Jung, Republic of Korea

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 blood biomarkers to diagnose metastatic carcinoma and distinguish a differentiation status of thyroid cancer using integrative proteomics approach.
Methods: In discovery stage, 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. Quantitative proteomics analysis based on BoxCar was used to identify differentially expressed proteins (DEPs). Validation was performed within large cohort (n= 166) using multiple reaction monitoring (MRM). Multi-biomarker panel containing the optimal combination of targets for diagnosis was constructed by LASSO regression.
Results: The 102 DEPs from discovery experiments highlighted 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, 23 proteins were selected as diagnostic biomarker candidates and those were proceeded to LASSO regression analysis. The optimal model equation includes 4 proteins and showed AUC value of 0.875 with statistical significance. Moreover, the 4-biomarkers panel produces high discriminatory power for both metastasis and non-metastasis with 91.72% and 66.70%, respectively. The overall specificity and sensitivity of panel is also beyond 80%. Collectively, our study gained confident prediction levels with the constructed panel and will be further verified with a large independent cohort.
Conclusion: Our 4-biomarkers 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.

PP03.81: False Discovery Rates: Not Just a Matter of Getting the Science Right

Daan Kenis, Belgium

Clinical peptidomics is increasingly regarded as a crucial component in implementing precision medicine for diagnostic and therapeutic purposes. However, as Maes et al. (2019) suggest in their recent review, several technical challenges must be addressed throughout the peptidomics workflow before it can be properly integrated into clinical practice. Despite the necessity of technological advancements to attain high-resolution workflows, the inherent probabilistic nature of peptidomics research ensures that uncertainty remains an irreducible aspect. For one, as Käll et al. suggest in 'Posterior Error Probabilites and False Discovery Rates' (2007), given that peptidomics experiments necessarily involve type 1 and 2 errors, "(...) biologists [would] benefit from the availability of statistical scores with well-defined semantics".
    In this paper, I argue that in addition to the technological complexities, these 'well-defined semantics' point toward deeper conceptual issues. I draw on recent insights from philosophy of science on inductive risk suggesting that appraising scientific claims (f.e. identifying peptide-spectrum matches) cannot be kept free of social and ethical values (Douglas 2009; Elliott 2017). This argument from inductive risk (AIR) holds that, for example, one's tolerance towards accepting false positives/negatives can only be set in reference to social and ethical values regarding the costs related to error.
In this presentation, I focus on inductive risk – and as such, the values involved – in setting an acceptable False Discovery Rate – a method to conceptualize the rate of false positives in peptide-spectrum matches. Although, in general, FDRs of 1% are deemed acceptable, and efforts are undertaken to increase resolution, AIR shows that threshold-setting is not merely a matter of getting the science right. In conclusion, I suggest that the specificities of one's research question not only necessitate scrutiny of experimental conditions (Maes et al. 2019) but also demand moral consideration of the inductive risks involved.

PROGRAM

PP03.83: Integrated Analysis of Proteome, Phosphoproteome, and N-glycoproteome for Immuno-oncology Biomarker Discovery

Kwang Hoe Kim, Republic of Korea

Post-translational modifications (PTMs) are catalyzed by a variety of enzymes, extensively increase protein diversity, and are involved in complex biological processes. They regulate the protein function by altering their activity state, localization, and interactions with other proteins. Thus, abnormal PTMs are associated with a variety of diseases, including cancer. In the last few decades, cancer immunotherapy has become an important part of treating cancer patients. The checkpoint blockade therapies combined with novel immune-oncology drugs become the recent focus of various clinical trials in cancer while response rates widely vary. Therefore, identification of patients who are responsive to immunotherapy strategies is important to improve the efficacy of these immune-oncology treatments. This highlights the unmet need in discovery of novel biomarkers to add predictive values for effective treatment of the cancer patients. Here, we present a liquid chromatography-tandem mass spectrometry (LC-MS/MS)-based integrated analysis of protein expression, phosphorylation, and N-glycosylation of serum samples from the cancer patients who were treated by an immune-oncology therapy. As a results, we have found potential biomarker candidates with an aberrant protein expression, phosphorylation, and N-glycosylation. Our results suggest that multi-dimensional proteomic analyses can provide complementary cancer-related information of proteins.

PP03.85: Development of a Novel Serum Biomarker Panel for Early Diagnosis of Pancreatic Ductal Adenocarcinoma

Hyeonji Kim, Republic of Korea

Introduction: Pancreatic ductal adenocarcinoma (PDAC) is a highly lethal disease for which early detection is a high-demanding unmet clinical need. While CA19-9 is available for clinical use as a PDAC biomarker, its limited diagnostic performance warrants development of novel biomarkers. Deep proteomic profiling of blood provides a great opportunity for discovering novel biomarkers. In this study, we present a multi-protein biomarker panel for highly accurate PDAC diagnosis.
Methods: Data-independent acquisition mass spectrometry (DIA-MS) was employed to analyze neat serum samples from 40 PDAC patients and 39 healthy controls (HCs). Protein identification was performed using DIA-NN, followed by bioinformatic analyses and literature mining to prioritize biomarker candidates. Multiple-reaction monitoring (MRM) was utilized for all verification and validation experiments.
Results: Our DIA-MS analysis showed a clear differentiation between PDAC patients and HCs at the serum proteome level. We prioritized PDAC biomarker candidates based on the following criteria: 1) proteins involved in pathways dysregulated in PDAC, 2) proteins previously reported as potential PDAC biomarkers, 3) significant fold-change between PDAC and HCs, and 4) known blood concentration. To validate the selected proteins, MRM was employed for a verification cohort consisting of individuals with PDAC (>150), benign cancer (~50), other cancers (~100), and HCs (>150). Based on the verification data, developed a predictive model which alone showed diagnostic performance comparable to that of CA19-9. A combined model of our biomarker panel and CA19-9 resulted in the best diagnostic performance, highlighting the additional value of our novel biomarkers. We validated our model in an independent cohort which included a multi-ethnic sample group, proving the robustness of our biomarker panel.
Conclusions: Further confirmation of our biomarker panel is underway through a confirmatory clinical trial using a large cohort. We envision our novel biomarker panel to enter clinics to improve patient survival by accurate detection of PDAC.

PP03.87: Optimized Biochemical and Analytical Workflow for High-Throughput Clinical Peptidome Profiling

Chien-Yun Lee, Germany

Background:Endogenous peptides play crucial roles in medical diagnosis and therapy. However, their untargeted identification in plasma is hindered by challenges in sample preparation, data acquisition, and analysis. Abundant plasma proteins impede the detection of low-abundant peptide biomarkers, and the low identification rate suggests the presence of non-tryptic or modified peptides overlooked by standard analysis approaches. This study aims to develop a robust workflow to efficiently identify endogenous peptides in plasma, particularly for large clinical cohorts.
Methods:We evaluated various biochemical approaches, including antibody-based depletion, solvent- or acid-based precipitation, and mixed-mode solid-phase extraction, to remove abundant proteins. Peptide separation was achieved through ultrafiltration with a molecular weight cut-off (<10 kDa). Peptides were analyzed using Orbitrap LC-MS/MS, and reproducibility was assessed using internal peptides standard across replicates. MSFragger, MaxQuant, and Prosit were employed for peptide identification and improved analysis.
Results:We established an optimized workflow for the detection of endogenous peptides in plasma. This workflow effectively depleted high molecular weight proteins and enriched low molecular weight endogenous peptides. Repetitive analysis of plasma samples from healthy subjects demonstrated high reproducibility and low variability, confirming the robustness of the workflow. Applying the workflow to patient samples, including plasma from ischemic stroke patients (n=24, 3 time points) and neurologically healthy controls (n=24) in a 96-well plate format, revealed the identification of 4,000-8,000 endogenous peptides per individual and a total of 13,000 identified peptides (10,700 quantified). These included low-abundant circulating proteins such as protein inhibitors and peptide hormones.
Conclusions:This workflow enables high-throughput clinical peptidome profiling in plasma, making it applicable to large cohorts. It addresses challenges in sample preparation, enhances the identification of endogenous peptides, and facilitates the discovery of novel biomarkers. Our results demonstrate that adding peptidome profiling has the potential to advance disease research, improve understanding of disease mechanisms, and enhance medical diagnostics and therapies.

PP03.89: Comprehensive Spectral Library Generation for Primary Human Immune Cells Using Data-Dependent Acquisition

Hyeonjeong Lee, Republic of Korea

Immune cells play crucial regulatory roles and exhibit remarkable cellular diversity in human physiology. Previous studies have extensively analyzed T-cell proteomes in order to clarify the comprehensive profiles according to the status of cells. However, generating cell-specific proteome libraries for small immune cell components like NK or B cells has not yet been undertaken due to the difficulties of sample collection. In this work, we report novel cell-specific libraries for four primary human immune cell types obtained through sorting with high-purity using fluorescence-activated cell sorting (FACS) and data-dependent spectrum acquisition. As a result, we generated 10,300 spectral libraries with 6,125 common spectra among helper T cells, cytotoxic T cells, natural killer (NK) cells, and B cells. Each primary cell library included cell type-specific protein markers and comprised 7,879, 7,347, 7,516, and 7,070 spectra for helper T cells, cytotoxic T cells, NK cells, and B cells, respectively. With these spectral libraries, we further analyzed a limited number of immune cells and identified proteins using the integrated spectral library. Obtaining sufficient protein quantities from primary immune cells, which are present in absolutely limited quantities in whole blood, poses challenges for reproducibility in sample analysis. Therefore, providing a comprehensive candidate list of proteins in small quantity of samples can enhance quantitative reproducibility among technical replicates. In this study, we observed that the identified proteins were proportionally increased with the number of cells, and functional analysis of the identified proteins revealed multiple signaling pathways characteristic of each immune cell type. In conclusion, our established immune cell-specific libraries, ensuring high cellular purity, can be readily applied to biomarker discovery, immune status assessment, and drug response monitoring, particularly in diseases associated with immune system dysfunction. Moreover, these libraries enhanced quantitative reproducibility among the technical replicates even with a limited sample amount.

PROGRAM

PP03.91: Serum Proteome Profiling for Biomarker Discovery of Current Depressive Episode

Jiyeong Lee, Republic of Korea

Depressive mood is a prevalent psychiatric symptom that causes significant impairments in daily life. However, unlike physical symptoms, psychiatric symptoms are challenging to objectively assess. Therefore, the identification of biomarkers reflecting changes in serum protein metabolism during a clinical depressive mood is crucial. In this study, we aimed to discover biomarkers using serum protein profiling in individuals who were currently experiencing a depressive episode, individuals in remission, and healthy individuals. Serum protein profiling was performed using liquid chromatography-tandem mass spectrometry. Differentially expressed proteins with a p-value < 0.05 were selected, and candidate biomarkers were verified using multiple reaction monitoring analysis for absolute quantification. The results revealed that apolipoprotein A-IV levels were lower in the current depressive episode group than those in both the remission and healthy control groups. Additionally, fibronectin levels were lower in the current depressive episode group than in the healthy control group, but not in the remission group. These findings suggest that apolipoprotein A-IV and fibronectin levels may serve as novel biomarkers of current depressive episode. The study findings have several implications. Firstly, apolipoprotein A-IV and fibronectin levels could be utilized as potential biomarkers for diagnosing current depressive episodes. Secondly, the study suggests that apolipoprotein A-IV-mediated inflammation may be involved in the development of clinical depressive moods, possibly by inducing neurological brain changes. These biomarkers could contribute to the development of improved diagnostic and treatment strategies for depression. The study's findings also suggest that apolipoprotein A-IV-mediated inflammation may play a role in the development of clinical depressive moods.

PP03.93: Proteomic Analysis of Tumor Tissues Reveals LCP1 as a Potential Regulator Involved in Oral Cancer Recurrence

Chiao-Rou Liu, Taiwan

Oral cancer is the sixth leading cause of the cancer-related mortality in Taiwan. More than 90% of oral cancers are oral cavity squamous cell carcinomas (OSCCs). The high mortality rate of OSCC is ascribed to metastasis and local regional relapse of cancer cells, suggesting that identification of proteins involved in migration and chemoresistance of OSCC cells can facilitate development of useful treatment approaches for OSCC. To identify novel targets for improvement of OSCC therapy, we have comparatively profiled the proteomes of primary and relapsed OSCC tissues with iTRAQ-based mass spectrometry. Gene expression of proteins dysregulated in OSCC tissues was surveyed with the cancer genome atlas (TCGA) database. Lymphocyte cytosolic protein 1 (LCP1) was selected as a candidate involved in development of OSCC relapse, as LCP1 is up-regulated in relapsed OSCC tissues and associated with poor survival rate of OSCC patients. Knockdown and overexpression of LCP1 resulted in reduction and increase of resistance to cisplatin in OSCC cells, respectively. We demonstrated that activation of epidermal growth factor receptor (EGFR) signaling pathway can lead to elevated levels of LCP1 and phosphorylation of LCP1 Ser5 in OSCC cells. Level of LCP1 phosphorylation can be reduced in OSCC cells upon treatment of PI3K and ERK inhibitors. Moreover, LCP1 expression and/or LCP1 phosphorylation may contribute to activation of JAK2/STAT3 signaling pathway. Collectively, we have identified an OSCC recurrence-associated molecule, LCP1, which may be upregulated by EGFR signaling pathway and promote cisplatin resistance through JAK2/STAT3 signaling pathway in OSCC cells. The results may provide important information for development of recurrent OSCC treatment.

PP03.95: An Empirical Study on Adenosine Triphosphate-binding Cassette (ABC) Transporter through RnsD Protein in Discovery of Biomarkers.

Shagufa Malik, Republic of Korea

Introduction: ATP-binding cassette (ABC) transporters constitute a superfamily of membrane transporter proteins that actively translocate a wide range of molecules, from simple molecules (fatty acids (FAs), sugars, nucleosides, and amino acids) to complex organic compounds (lipids, oligonucleotides, polysaccharides, and proteins). ABC transporters have been confirmed to be closely related to the pathogenesis of diseases such as metabolic diseases, cancer, and Alzheimer's disease based on their transport abilities. In this study we aim to find out the transportation of biomarkers. ABC transporters have been confirmed to be closely related to the pathogenesis of diseases such as metabolic diseases, cancer, and Alzheimer's disease based on their transport abilities

 Methods: Through this study, we aim to investigate and find Biomarkers through LC MS analysis of the 6 samples of non-depleted Mouse blood which was collected by Gacheon University, by extracting and pre-processing the data already present data in the Peptide Atlas library through Skyline MS software, to gain some insights about the target proteome, which can help us provide the Target list. Computational Modelling of the extracted data would be done through Whole Genome Sequencing (WGS) through R language. Metabolites sequencing would also help us in understanding set of small molecules present in a biological sample.

Results: Expected outcome of the experiments through LC/MS analysis is to identify and quantify the proteins and to understand the biological functions and insights of ATP-binding cassette (ABC) transporters in order to find biomarkers.

Conclusion: Based on the LC/MS analysis of collected samples and whole genome sequencing data, estimated results could provide potential findings of biomarkers and enhanced pathogenesis of diseases.

PP03.97: A Shotgun Proteomics Approach to Reveal New Putative Therapeutical Targets in Nephropathic Cystinosis

Jesus Mateos, España

INTRODUCTION

Nephropathic cystinosis is a rare autosomal recessive metabolic disease due to mutations in the CTNS gen codifying for cystinosin, a lysosomal symporter. It is characterized by the accumulation of cystine crystals in lysosomes causing end-stage renal damage and blindness in patients under ten years of age. Currently, there is no cure and the only palliative treatment, cysteamine, presents several limitations including the lack of cure for the disease, the adverse effects, and the complexity of the indicated treatment for life. Recent findings indicate that the intra-lysosomal accumulation of cystine alters key processes such as phagocytosis, redox balance, and autophagy, causing a molecular imbalance that, to date, has not been characterized in detail.

METHODS

We have performed a Data-Dependent Acquisition quantitative proteomic study (TMT10plex) including skin fibroblasts from patients with nephropathic cystinosis (n=3) and parental healthy controls (n=4) to obtain the proteomic signature of the patients, as well as the protein modulation by the treatment with cysteamine (48h at 200 µM). Orthogonal validation was done by Real-time-PCR and immunoblotting.

RESULTS

We have been able to observe that a group of proteins appears significantly ($p \leq 0.05$) altered in patients compared to healthy controls and that this alteration is reversed in patients when cells are incubated with cysteamine. Among those proteins modulated by cysteamine, several stand out that are implicated in renal pathology and lysosomal maturation. Targeted validation of those candidates by RT-PCR and immunoblotting confirms the shotgun results.

CONCLUSIONS

We have identified a set of new, more specific putative therapeutical targets altered in nephropathic cystinosis. Currently we are validating this set of new targets in clinical samples from a more extent cohort of patients and developing molecular tools to study the effect of their dose-specific down-regulation.

See you in Busan!
BEXCO, Busan, Korea
2023.hupo.org
#HUPO2023

Together Through Proteomics

PROGRAM

PP03.99: Investigating the Plasma Proteome of People Living with HIV (PLHIV) with ARV-associated Acute Kidney Injury in a South African Cohort.

Rethabile Mokoena, South Africa

South Africa contributes ~20% of the global burden of HIV and hosts the largest antiretroviral therapy program worldwide. ARVs used for HIV treatment can cause nephrotoxicity, which can progress to acute kidney injury (AKI) in patients. With early detection, this side-effect is reversible. However, current kidney function tests are unreliable for early detection in the South African population. Therefore, using mass spectrometry-based proteomics, differentially abundant plasma proteins that distinguish between PLHIV, with and without AKI, were identified and quantified for early detection of ARV-associated AKI in the South African population.

The AKI cohort compared 188 patient plasma samples (106 cases; 82 controls) using an in-house sample preparation protocol, with magnetic beads for automated on-bead sample clean-up and digestion. The resulting peptides were analysed with the Evosep One and Sciex TripleTOF® 5600 mass spectrometer, using a SWATH-data acquisition method. Data were processed using Spectronaut™ 16. Differential abundance testing was completed using an unpaired t-test, where identified proteins were compared between the cases (AKI) and the controls (non-AKI). A list of differentially abundant (candidate) proteins was generated; proteins with a fold-change ≥ 2 and an FDR of 1%, were considered significant. Pathways over-represented by the significant proteins were identified using the Reactome pathway database (v81).

Thirty-three candidate proteins were identified, of which 13 were considered significant; 10 proteins showed increased abundance while 3 proteins showed decreased abundance, in the cases compared to the controls. The significant proteins with increased abundances in the case group were over-represented (FDR ≤ 1%) in pathways of haemostasis, the immune system, signal transduction, cell-cell communication, the cytoskeleton, small molecule transport and metabolism, and correspond to known pathophysiological and cellular mechanisms of AKI.

The proteins identified are involved in ARV-associated AKI and require further validation for early detection of AKI within the South African population.

PP03.101: Metabolic Phenotype of Leukemic Cells : A Major Determinant of the Prognosis of AML Patients

Pascal Mossuz, France

Many studies have highlighted the oncogenic role of metabolic deregulation in acute myeloblastic leukemia (AML), suggesting that metabolic reprogramming could significantly impact patient prognosis.
In this context, we characterized the metabolome of leukemic cells by untargeted metabolomics using HRMAS-NMR on human leukemic lines and on cells from AML patients at diagnosis. We first showed that at baseline there were significant differences in metabolic profile between human leukemic lines of different molecular status, but that under metabolic stress (culture in serum-free medium, for example) , the same leukemic cells developed common mechanisms of metabolic reprogramming, in order to continue to proliferate,. In particular, we were able to show using HPTLC combined to GCMS approaches that remodeling of lipid homeostasis is a major and shared stress adaptation factor in leukemia cells. We completed this work with the HRMAS analysis of more than 50 AML patients at diagnosis, which showed that the metabolomic profiles of the patients varied between the different FAB subtypes of AML and according to the cytogenetic and molecular status. We also showed that lipidomic profiles of plasma from AML patients were significantly correlated with the prognosis of patients. In particular, we highlighted the impact of species of Phosphocholine and Phosphatidylcholine metabolism on patients' survival.
Finally, we showed that the targeting of certain metabolic pathways had a significant impact on the survival of leukemic cells, depending on their initial metabolic phenotype. Sensitivity to Etomoxir, which blocks fatty acid oxidation, was dependent on the initial lipidomic profile of the cell lines suggesting that only particular "lipid phenotypes" are sensitive to drugs targeting lipid metabolism. Likewise, sensitivity to specific drugs targeting glutamine/glutamate and alanine pathways, seems to be dependent of the baseline amino acids profile of leukemic cells.

PP03.103: Discovery of Biomarkers Related to Chronic Kidney Disease Through Comparative Plasma Exosome Proteome Analysis Via SWATH LC-MS Platform

Yumi Oh, Republic of Korea

Chronic kidney disease (CKD), one of final complication of diabetes mellitus (DM), have been major factor that aggravate the quality of life for DM patients. Discovery of predictive biomarker which can predict prognosis of kidney function is critical. In this study, exosome fraction was separated from 100 of CKD patients' plasma and quantitative proteome was investigated using high resolution LC-MS platform, called sequential window acquisition of all theoretical mass spectra–mass spectrometry (SWATH–MS). One of the advantages of SWATH-MS is high reproducibility and accurate quantitation and exosome proteome can reflect another features than plasma proteome in disease status. Extracellular vesicle fractions were isolated from plasma using commercial exosome isolation kit and digestion was performed using S-trap platform. SWATH-MS was conducted using Sciex 5600+ quadrupole time of flight mass spectrometry and data processing was done using DIA-NN with library free manner. From resulting 370 plasma exosomal proteome which were identified from this experiment, 274 proteins are known to belong to the exosome proteome. Among those proteins, plasma exosomal protein A-01 is one of DEPs which can distinguish normal and DM and also can reflect the albumin-to-creatinine ratio (ACR). In addition, plasma exosomal Protein A-02 can be applied to monitor the degree of CKD stage. In conclusion, plasma exosomal proteome analysis is effective to discover biomarker candidates for CKD monitoring.

PP03.105: Optimization of LC-MRM Method for the Quantification of Core-Fucosylated AFP Glycopeptide Using Evosep One and Triple Quad 6500+ system

Juri Park, Republic of Korea

Introduction: Early diagnosis of hepatocellular carcinoma (HCC) relies on tests such as AFP, AFP-L3, and PIVKA-II. The core-fucosylated glycoform of AFP is a well-known malignancy risk factor for HCC. However, the existing lectin-based AFP-L3 glycoform test method (Wako's uTAS) exhibits relatively low specificity and sensitivity. To address this, we developed and optimized an LC-MRM method for direct analysis of core-fucosylated AFP glycopeptides using Evosep One LC and Sciex Triple Quad 6500+ systems.
Methods: For LC-MRM analysis, the prepared peptide mixture was loaded into Evotip pure and analyzed by the 60SPD method. The Evosep performance column used was maintained at 40 °C with a temperature controller. Analysis was performed by optimizing the compound parameters and source/gas parameters of the mass spectrometer, and quantitative analysis was performed using SCIEX OS software.
Results: By using Evotip Pure, which has improved sample binding, the peak intensity of AFP peptides increased 1.5 to 3.0 times compared to the previous tip. The optimized EVOSEP LC method and column setting was used to separate of the non-fucolsylated and fucosylated peptides, resulting in increased the accuracy of quantification. In addition, optimization of each MS parameter value improved the sensitivity for analysis the fucosylated peptides. By employing these optimization, we confirmed the possibility to quantify fucosylated AFP up to a concentration level of 0.6 ng/mL in clinical samples.
Conclusions: Evosep One LC and Sciex Triple Quad 6500+ are systems equipped with both the high sensitivity and selectivity of MRM. The automated sample loading system provides high repeatability, allowing for consistent and efficient analysis of samples. Therefore, it is thought that this system can be used not only for analyzing samples present in low concentrations, but also as a powerful diagnostic tool in the clinical field.

PROGRAM

PP03.107: Compatibility of Plasma Collected Using Microsampling Devices with Olink® Proteomics Technology

Marijana Rucevic, Sweden

Biological sampling is essential for the screening, diagnosing, and monitoring of disease. However, standard sample collection methods such as tissue biopsy or venous blood draw are invasive and require the assistance of certified collection personnel at specialized facilities. Microsampling, however, is a minimally invasive procedure of capturing small volumes (typically 10 µL or less) of biological fluids and is amenable to home sampling, significantly reducing the challenges associated with patient follow-up and sample collection. Olink's unique proximity extension assay (PEA) technology enables high-throughput, multiplex immunoassays of up to 3000 proteins in a single sample while consuming minimal sample volumes. Olink PEA™ technology has been thoroughly validated using serum and plasma, and offers utility on alternative matrices, such as samples collected using microsampling devices or those derived from dried blood spots. This poster will highlight the utility of Olink's PEA technology for analyzing samples collected by microsampling techniques as dried blood spots or from interstitial fluid. The use of Olink® technology on such substrates combines massively-multiplexed biomarker detection with a convenient point-of-care and patient-centric approach for health monitoring. Such advances are particularly valuable for homecare, to monitor chronically ill patients via repeated sampling, for people averse to needles and blood collection, and pediatric settings.

PP03.109: FAIMS PRM: Sub Attomole Sensitivity for Plasma Proteomics Analysis

Simonas Savickas, Switzerland

Introduction: Parallel reaction monitoring (PRM) is known for its fast assay development turnaround time and high specificity that enables quantifying single amino acid exchanges. Here we looked into FAIMS-PRM, a gas-phase fractionation technology combined with targeted mass spectrometry, that has emerged as particularly sensitive. We compare instruments facility wide and characterize the method.
Methods: A set of 4 stable-isotope labeled peptides were spiked into trypsin-digested native human plasma. Human plasma was diluted 2-fold as a standard calibrant and inversely matched with peptides from chicken plasma as a matrix. One microgram of the peptide mix was loaded onto the column, eluted at 250 nl/min over 60 min, and analyzed using 9 independent FAIMS-enabled Thermo Fisher Orbitrap Exploris 480 mass spectrometers. We monitored four non-concurrent peptides with unique FAIMS compensation voltages in PRM mode using 240,000 resolution. The collected raw data was analyzed using FAIMS-supported SpectroDive 11.
Results: This study evaluates the potential of FAIMS-PRM as a sensitive parallelizable technology in mass spectrometry-based proteomics. The technical sensitivity of FAIMS-PRM is better than previously established MRM assays and is found to be comparable between different instruments. Multiple instruments can work in parallel. The limit of detection variation is manageable between worst and best performing instruments, and remaining quantitative batch effects can be easily corrected using SIS peptides. The quantitative precision is consistently under 15% before reaching Q-values above 0.01 or LOD. The study demonstrates that commercial ELISA sensitivity can be reached using FAIMS-PRM, with antithrombin-III limit of detection being 320pg/ml (0.04amol on column).
Conclusion: In conclusion, FAIMS-PRM is a parallelizable, stable, and sensitive technology for pre-clinical plasma proteomics analysis. Its low injection-to-injection variance, and ability to analyze native plasma with high sensitivity make it a promising tool for protein quantification studies.

PP03.111: Quantifiable Peptide Library Enable Rapid Development of Blood Test for Breast Cancer Detection

Hyeonseok Shin, Republic of Korea

Background: Mass spectrometry (MS) based diagnostic application to blood analyte is a growing field of proteomics. However, due to differences in analytical methodology and experimental conditions most of the biomarker candidates are discarded during the validation processes. To bridge the discrepancy between discovery and validation and speed-up the validation process, we generated a peptide library which allows the same analytical method and conditions to be used for both discovery and validation.
Methods: To generate a peptide library that can be quantified in a validation set-up, we synthesized 4,683 peptides that covered 3,393 proteins detectable in the human blood from public databases. The selection was performed by spiking the synthesized peptides to quantify target peptides in neat serum and plasma samples within a 10 min liquid chromatography-MS/MS run time.
Results and conclusion: The generated library composed of 852 quantifiable peptides that cover 452 human blood proteins. We next applied the PepQuant-library for breast cancer biomarker discovery and validation. A total of 215 breast cancer samples and 187 normal control samples were used which resulted in the identification nine biomarker candidates. We next trained a simple machine learning model on the quantification values of the nine candidate biomarkers. The trained model performed an average area under the curve of 0.9105 for the receiver operating characteristic curve. This data indicate that PepQuant-library can rapid up the process of discovery and validation which is shown with clinical trial of breast cancer prediction.

PP03.113: Investigation of Biomarkers in Osteosarcoma Chemoresistance Using Proteomics Technique

Nutnicha Sirikaew, Thailand

Introduction: Osteosarcoma (OS) is a malignant bone tumor, mostly found in children and adolescents. Although multi-drug neoadjuvant chemotherapy followed by surgical excision and adjuvant chemotherapy, has been the mainstay therapy for OS. Some patients develop treatment resistance, resulting in metastasis or recurrence. Proteomics has provided complimentary and contrasting data to their genomic counterparts, leading to a comprehensive understanding of the molecular mechanisms underlining the pathology of diseases. Thus, the identification and characterization of target therapy are necessary to improve the OS treatment. Here, we utilize sequential window acquisition of all theoretical fragment ion spectra mass spectrometry (SWATH-MS) for proteomic profiling of fresh frozen tissue from a cohort of OS patients.
Method: We created a comprehensive OS tissue spectral library of 2004 proteins by combining data-dependent acquisition (DDA) MS raw files from tissue samples of 12 patients well-responding to chemotherapy (good responders) or poorly responding to chemotherapy treatment (poor responders). We compared the proteome profiles of biopsy tissues vs. post-chemotherapy tissues of poor responders, and biopsy tissues of good responders vs. poor responders.
Results: A total of 1209 proteins were identified in biopsy tissues and post-chemotherapy tissues of poor responders. We found 48 upregulated proteins and 34 downregulated proteins in post-chemotherapy tissues compared to biopsy tissues of poor responders with p-value ≤ 0.05. These proteins are involved in RNA metabolism in upregulated proteins. We further identified the protein in good responder and poor responder OS tissues at diagnosis, resulting in identification of 27 upregulated proteins and 56 downregulated proteins in poor responders OS tissues.
Conclusion: Our research identifies critical molecular markers and related pathways that may contribute to OS chemoresistance. Such molecular signatures could be potential therapeutic targets to increase the effectiveness of current medicines, as well as having predictive/prognostic value in OS for treatment response.

PP03.115: Development of an Ultrasensitivie 2-Plex Immunoassay to Evaluate Serum Fucosylated PSA and GDF-15 for the Detection of Aggressive Prostate Cancer

Jin Song, United States

Background: Current PSA-based tests for the detection of prostate cancer (PCa) lack sufficient specificity, leading to significant overdetection and overtreatment. Our previous studies demonstrated that serum growth differentiation factor 15 (GDF-15) and fucosylated PSA (Fuc-PSA) had the ability to predict aggressive (AG) PCa. N70 glycosylation regulated GDF-15 function abolishing its inhibitory effect on EGFR in androgen receptor inhibitor-resistant PCa cells, suggesting that glycosylated GDF-15 may also be a potential biomarker for AG PCa. A high-throughput ultrasensitive assay format is needed to further evaluate the clinical utility of the corresponding glycosylated forms of these proteins.
Method: A magnetic bead-based 2-plex immunoassay for serum PSA and GDF-15 was developed and its analytic performance assessed using a Bio-Plex 200 system. Two proteins and their corresponding glycosylated forms were analyzed in sera from patients diagnosed with AG PCa, low risk PCa, non-PCa (biopsy negative), by the 2-plex assay either directly or after enrichment of fucosylated proteins using agarose bound Aleuria Aurantia lectin (AAL) beads. The clinical performance of these markers was evaluated in the detection of AG PCa.
Results: A 2-plex immunoassay was in-house developed with negligible cross-reactivity (<2%), wide dynamic range (>4 logs), recovery of 94-106%, LLOQ of 298 fg/mL (PSA) or 182 fg/mL (GDF-15), and intra-assay or inter-assay precision of 15.1% or 25.6%. There was a significant correlation (pearson R = 0.9767, p<0.00001) for PSA protein measurements using the 2-plex immunoassay compared to Access Hybritech PSA assay. Serum levels of tPSA and Fuc-PSA were significantly higher in PCa samples compared with non-PCa, and also significantly higher in PCa samples with higher Gleason scores [GS 7 (4+3) & 9] compared with low GS [6 & 7 (3+4)].
Conclusion: An ultrasensitive 2-plex immunoassay with appropriate analytical performance was developed to evaluate candidate biomarkers and their corresponding glycosylated forms for the detection of Ag PCa.

PP03.117: Comparative Study of LC-MRM Platforms for the Quantification of Core-Fucosylated AFP Glycopeptides in the Diagnosis of Hepatocellular Carcinoma (HCC)

Hanseul Suh, Republic of Korea

Introduction: Early diagnosis of hepatocellular carcinoma (HCC) relies on tests such as AFP, AFP-L3, and PIVKA-II. The core-fucosylated glycoform of AFP is a well-known malignancy risk factor for HCC. However, the existing lectin-based AFP-L3 glycoform test method (Wako's uTAS) exhibits relatively low specificity and sensitivity. In this study, we compared two LC-MRM methods for direct analysis of core-fucosylated AFP glycopeptides using Waters' TQ-S and M-Class UPLC, Sciex 6500+ and EvosepOne LC systems. We compared the analytical performance of these two instruments within the low concentration range of AFP (1~10 ng/mL).

Methods: LC-MS/MS conditions were established individually for the comparison between the instruments. We evaluated linearity, sensitivity, and reproducibility for AFP and AFP-Fuc%. Mutual comparison of AFP samples was conducted across concentrations ranging from 1 to 10 ng/mL. To assess the correlation between the two analysis platforms, we performed IP enrichment on 40 serum samples and compared the quantitative values of non-glycopeptides and glycopeptides.

Results: The quantitative values of AFP and AFP-Fuc% obtained from the two instruments did not exhibit significant differences. Both methods demonstrated excellent linearity, sensitivity, and reproducibility. The linear regression analysis of Waters TQ-S and Sciex 6500+ yielded R2 values of 0.990 and 0.991, respectively, within the AFP concentration range of 1-10 ng/mL. The correlation coefficient was confirmed through the analysis of 40 serum samples using the LC-MRM method.

Conclusions: We successfully established and compared two LC-MRM methods, namely Waters Xevo TQ-S LC-MS/MS and Sciex 6500+/Evosep LC system, for the quantification of core-fucosylated AFP glycopeptides. No significant differences were observed at the 1-10 ng/mL AFP level. Future investigations will focus on testing the two LC-MRM-based AFP assays at even lower concentrations to further validate their analytical sensitivity, aiming to achieve the lowest detection limits in one or both assay platforms.

PP03.119: Identifying Patients with Rapid Progression from Hormone-Sensitive to Castration-Resistant Prostate Cancer: A Retrospective Study

Yaoting Sun, China

Prostate cancer (PCa) is the second most prevalent malignancy and the fifth cause of cancer-related deaths in men. Androgen deprivation therapy (ADT) combined with androgen blocking is frequently beneficial to patients with locally advanced and metastatic PCa during the initial treatment stage. However, almost all hormone-sensitive prostate cancers (HSPC) progress to castration-resistant prostate cancers (CRPC) within five years, with only 5-10% of patients remaining alive ten years after initiating ADT. A crucial challenge is identifying the population at risk of rapid progression from hormone-sensitive PCa (HSPC) to the lethal castration-resistant PCa (CRPC). In this study, we collected 78 HSPC biopsies and measured their proteomes using pressure cycling technology and a pulsed data-independent acquisition pipeline. The proteomics data and clinical metadata were used to generate models for classifying HSPC patients and predicting the development of each case. We totally quantified 7335 proteins from the 78 formalin-fixed and paraffin-embedded biopsies. A total of 251 proteins were differentially expressed between patients with a long- or short-term progression to CRPC. Using a random forest model, we identified seven proteins that significantly discriminated long- from short-term cases, which were used to classify PCa patients with an area under the curve 0.873. Next, one clinical parameter (Gleason sum) and two proteins (BGN and MAPK11) were found to be significantly associated with rapid disease progression. A nomogram model using these three features was generated for stratifying patients into groups with significant progression disparities (p-value $=1.3 \times 10^{-4}$). In summary, we identified proteins associated with a fast progression to CRPC and an unfavorable prognosis. Based on these proteins, our machine learning and nomogram models stratified HSPC into high- and low-risk groups and predicted their prognoses. These tools may aid clinicians in predicting the progression of patients and guiding individualized clinical management and decisions.

PP03.121: SWATH-MS-based Proteomics for Osteosarcoma Surface Target Identification From Patient-Derived Tumor Tissues

Viraporn Thepbundit, Thailand

Introduction: Osteosarcoma, an aggressive bone cancer predominantly affecting adolescents and young adults, poses significant challenges, including chemotherapeutic resistance, tumor recurrence, and metastasis, despite the availability of standard treatments. Targeted therapy has been a highly effective strategy for treating cancer in recent years. Cell-surface proteins represent attractive targets due to their accessibility and involvement in cell survival and represent most FDA-approved drug targets. However, achieving clinically effective targeted therapy for osteosarcoma remains challenging, primarily due to the substantial tumor heterogeneity and the absence of applicable targets. Currently, DIA-MS is a next-generation proteomics strategy that provides better reproducibility and sensitivity over conventional DDA-MS in oncology studies. Accordingly, we performed SWATH-MS to identify overexpressed cell-surface proteins for further therapeutic target investigation for osteosarcoma target therapy.
Methods: Membrane proteins were extracted from patient-derived tumor tissues (including pre- and post-chemotherapeutic primary tumors and recurrent tumors) using a membrane protein-enrichment method. For proteomic analysis, a sample-specific spectral library was constructed by DDA-MS from 34 membrane protein-enriched samples, containing 1,733 proteins and 10,649 distinct peptides with a 1% critical FDR.
Results: 1,377 proteins were identified from SWATH data acquisition. Of these, 578 (42%) were plasma membrane proteins. Among primary tumors, 23 and 44 proteins were up-regulated and down-regulated, respectively, in post-chemotherapy. The up-regulated surface proteins were involved in clathrin-mediated endocytosis, membrane trafficking, and vesicle-mediated transport. When comparing recurrent tumors with primary tumors after chemotherapy, 126 up-regulated and 13 down-regulated proteins were observed. The up-regulated surface proteins play roles in AKT and Wnt signaling pathways, and senescence and autophagy in cancer. These proteins will be investigated for selecting candidate targets and experimentally validated further.
Conclusions: Proteomics analysis through SWATH-MS has provided valuable insights into potential therapeutic targets for osteosarcoma highlighting the involvement of important signaling pathways and cellular processes in tumor progression and treatment resistance.

PP03.123: Proteomics Assessment of Early Treated Adults With Phenylketonuria: A Perspective Sight to Personalized Medicine

Silvia Valentinuzzi, Italy

Introduction: Phenylketonuria (PKU) is an autosomal recessive disease which causes deficiency in the hepatic enzyme phenylalanine hydroxylase (PAH), it is expressed predominantly in the liver (but also kidney and pancreas) and is responsible for the conversion of Phe to Tyr. In untreated patients with PKU, blood Phe concentrations increase, resulting in phenyl pyruvic acid excreted in the urine, Tyr concentrations are low. Generally, individuals with severe phenotypes have classic PKU, and those with less severe PAH deficiency have moderate PKU. Nowadays, there are 0.45 million people worldwide with the inherited metabolic disorder PKU, which causes, if it is not treated, irreversible neurological damage like epilepsy, seizures, psychological behaviours, acquired microcephaly, people with PKU also have generalized skin hypopigmentation, and a musty sweat odor. Even if pharmaceutical therapies have been developed, a phenylalanine restricted diet is the only effective treatment. Our study aimed to investigate the protein cargo of lymphocytes of PKU early treated adult patients.
Methods: 3 million of lymphocytes were purified from plasma samples of 10 early-treated PKU patients compared to 10 healthy controls (HC). The protein lysates were tryptically digested by Filter-aided sample preparation (FASP) for label free proteomics. Peptides were acquired by nanoLC-Orbitrap-Fusion-Tribrid Mass Spectrometer. Quantitative proteomics data obtained from Proteome Discoverer were used for functional analysis through Ingenuity Pathway Analysis (IPA).
Results: Intriguingly, lymphocytes protein cargo revealed proteins involved in brain damage, oxidative stress, neurotransmitter and synaptic dysfunction, energy metabolism impairments. These data were corroborated by the modulation of proinflammatory cytokines levels. Our preliminary data confirm that PKU lymphocytes could be considered a useful platform for "liquid biopsy" in the clinical assessment to pave the way for a personalized diet and medicine. Future perspectives will include a correlation analysis between proteins markers and metabolites quantified by targeted metabolomics.

PP03.125: Multi-omics Precision Medicine Approach to Identify Effective Therapies for Advanced Cancer Patients

Juanjua Wang, China

Background:
In recent years, cancer treatment has evolved from "one strategy fits all" to a more personalized approach based on molecular profiling. This change, specifically tailoring therapies to match tumor-specific genomic variants, has boosted patient outcomes. Yet, the overall impact of such interventions isn't fully known. In Denmark, Rigshospitalet's Phase 1 Unit offers comprehensive genomic and transcriptomic sequencing to metastatic cancer patients participating in the CoPPO precision oncology trial. Our study will leverage this multi-omics tumor molecular profiling by integrating proteomics to improve prognosis and direct treatment decisions, aiming to provide targeted therapies based on extensive information.

Methods:
We aim to recruit 120 patients per year referred to the Phase 1 Unit at Rigshospitalet.
We will employ Deep visual proteomics (DVP) to ascertain the heterogeneity of proteomic signatures in selected patients on the tissue level. Proteomics will be used to identify potential drug targets, which will then be validated using drug screens on PDOs. Following drug treatments, DVP will be carried out on sections from PDOs. The proteomics samples will undergo analysis using a new high-resolution instrument with advanced scan modes.

Results:
The multi-omics strategy will enable a comparative analysis of proteomic data between patients showing positive responses and those with refractory lesions. This comparison could enhance our understanding of cancer signaling and resistance mechanisms. DVP will be useful in identifying "resistance signatures" and comprehending tumor etiology, resistance mechanisms, and treatment responses at the cellular population level.

Conclusion:
Our study will enhance the understanding of resistance mechanisms, potentially identifying new targets to combat resistance. By identifying and validating potential drug targets via proteomics on patient organoids, we aim to uncover potential "resistance signatures". We hope our work will offer new therapeutic alternatives for cancer patients and enrich our understanding of personalized cancer treatments.

PP03.127: Discovery of Urinary Proteins Correlating with HbA1c in Plasma by Quantitative Proteomics

Keiko Yamamoto, Japan

Background
Various complications occur in diabetes, and control of blood sugar levels has been shown to prevent the diabetic complications or delay their progression. Glycated hemoglobin (HbA1c) levels in plasma has been used as an index for clinical diagnosis of diabetes showing average level of blood sugar over the past 2 to 3 months.
In this study, we tried to find some urinary proteins which correlated with the HbA1c level.
Methods
Urine specimens of healthy volunteers and diabetic patients were measured by mass spectrometry with DIA mode and analyzed by DIA-NN. About 3800 proteins were quantified. Then, a multivariate analysis (SIMCA) was performed to search for the presence of urinary proteins that increased or decreased in correlation with the HbA1c levels.
Results and Conclusions
We could find several proteins which correlated well with the HbA1c values.
This shows that non-invasive urine tests for these proteins can be used as a screening tool to predict blood sugar levels for healthcare and diabetic disease monitoring.

PP03.129: Discovery of Urine Biomarkers for Early Detection of Kidney Injuries in Diabetic Patients by Quantitative Proteomics

Kengo Yanagita, Japan

Introduction
Diabetes mellitus (DM) is a lifestyle-related disease and easily progresses to cause damages of various organs and tissues throughout the body. Especially, kidney is one of the major organs impaired in DM and the kidney injuries are usually detected by microalbuminuria. Early detection of the onset of kidney injuries may facilitate improvement of patient lifestyle or medical interventions to prevent or arrest the progression of the diabetic kidney injuries.
Methods
In this study, urine samples were analyzed by quantitative DIA proteomics from healthy subjects and patients who developed microalbuminuria (an index of renal impairment) several years after trace of uncomplicated diabetic status for discovery of biomarkers of early diabetic kidney injuries. Tryptic peptides of urine samples were measured by mass spectrometry with DIA mode and analyzed by quantitative proteomics using DIA-NN. A multivariate analysis (SIMCA analysis) was performed to extract proteins that could be classified the samples into the three groups (healthy subjects, diabetic patients, and diabetic patients with microalbuminuria) with the identified proteins.
Results
The quantitative proteomics and multivariate analysis elucidated several proteins that could potentially classify the urine samples into the three groups. The statistical analysis of these proteins also demonstrated significant differences in the amounts among the three groups.
Conclusions
A quantitative proteomics platform for urine biomarker discovery using LC-MS in DIA mode was established to identify biomarker proteins that could potentially classify the three groups: healthy subjects, diabetic patients and patients with microalbuminuria.

PP03.131: In-Depth Urinary and Exosome Proteome Profiling Analysis Identifies Novel Biomarkers for Diabetic Kidney Disease

Linhui Zhai, China

Diabetic kidney disease (DKD) is a major microvascular complication of type 2 diabetes mellitus (T2DM). Monitoring the early diagnostic period and disease progression plays a crucial role in mitigating the risk of T2D in clinical practice, particularly for patients with a long course of DKD. In this study, to comprehensively elucidate the molecular characteristics of urinary proteins and urinary exosome proteins in type 2 DKD, we performed large-scale urinary proteomics (n = 144) and urinary exosome proteomics (n = 44) analyses on patients with T2DM in varying degrees of albuminuria. The dynamics analysis of the urinary and exosome proteomes in our study provides a valuable resource for discovering potential urinary biomarkers in patients with DKD. A series of potential biomarkers, such as SERPINA1 and transferrin (TF), were detected and validated to be used for DKD diagnosis or disease monitoring. The results of our study comprehensively elucidated the changes in the urinary proteome and revealed several potential biomarkers reflecting the progression of DKD, which provide a valuable resource for discovering potential biomarkers in patients with DKD, and improve the clinical diagnosis and monitoring of DKD.

PP03.133: Early Urinary Candidate Biomarkers and Clinical Outcomes of Intervention in a Rat Model of Experimental Autoimmune Encephalomyelitis

Yameng Zhang, China

Multiple sclerosis is a chronic autoimmune demyelinating disease of the central nervous system and is difficult to diagnose in early stages. Without homeostatic control, urine was reported to have the ability to accumulate early changes in the body. We expect that urinary proteome can reflect early changes in the nervous system. In this study, the early urinary proteome changes in a most employed multiple sclerosis rat model (experimental autoimmune encephalomyelitis (EAE)) were analyzed to explore early urinary candidate biomarkers, and early treatment of methylprednisolone were used to evaluate the therapeutic effect. Compare with controls, twenty-five urinary proteins were altered at day 7 when there were no clinical symptoms and no obvious histological changes. Among them, twenty-three have human homologs and fourteen were reported to be differently expressed in the serum/cerebrospinal fluid/brain tissues of multiple sclerosis patients or animal models. Functional analysis showed that the dysregulated proteins were associated with asparagine degradation, neuroinflammation and lipid metabolism. After the early treatment of methylprednisolone, the incidence of encephalomyelitis in the intervention group was only 1/13. This study demonstrates that urine may be a good source of biomarkers for the early detection of multiple sclerosis and early treatment can significantly delay disease progression. These findings may provide important information for early diagnosis and intervention of multiple sclerosis in the future.

PP03.135: Toxicoproteomics for Evaluating the Toxicity of Pharmaceutical By-Products of Furosemide, a Widely-Used Diuretics

Sandrine Bourgoin-Voillard, France

Introduction. Pharmaceutical by-products (PPs) including metabolites and transformation products of pharmaceuticals are a lot of concerns for both human and ecosystems health. Several studies pointed out PPs as emerging pollutants with a risk of toxicity exceeding the one of their parent molecules. Herein, we used toxicoproteomics to unveil toxicity effects of PPs of furosemide, a widely used diuretics. PPs of interest are pyridinium of furosemide (PYR), saluamine (SAL) and furfural (FRF) that were recently revealed as potential emerging pollutants in water.

Methods. A label-free proteomic approach and cell viability assays were performed on hepatocarcinoma Hep-G2 cell line exposed for 96h to PPs (PYR, SAL FRF), furosemide and their mixture (MIX) at a CL10 letal dose. Protein extracts were submitted to a trypsin/Lys-C enzymatic digestion before performing LC-MSE analysis by using a NanoAcquity-C18/SYNAPT-G2Si mass spectrometer system.

Results. Toxic effects were confirmed on Hep-G2 cell line with a higher toxicity after an exposure to PYR, SAL and FRF while more substantial toxic effects were observed after an exposure to MIX and FUR. Based on the quantification of 1379 proteins, we deciphered 71, 101, 138, 205 and 34 deregulated proteins after exposure to FUR, PYR, SAL, FRF, and MIX, respectively. A gene ontology enrichment analysis revealed that these proteins were involved in metabolism, immune response, biosynthesis or oxidative stress. For PPs exposure (PYR/SAL/FRF), these proteins were related to several diseases (neurodegenerative diseases/endocrine disruption/cancer) while, for MIX, these diseases were not retrieved, suggesting possible antagonistic effects.

Conclusions. This toxicoproteomic work contributes to improve the current understanding of furosemide by-products and their mixture impacts on human cells. Beyond the case of furosemide, our study underlined the need to better take into account drug by-products during toxicological risk assessment.

PROGRAM

PP03.137: Simultaneous Quantification of South Korea representative Allergenic Foods with Optimized HPLC-MS/MS Approaches

Minkyung Jun, Republic of Korea

Food allergy is an acute disease that can be caused by certain foods. However, there is no fundamental treatment for this, and it is best to avoid food that causes disease. Because of this, many countries are considering the labeling of allergenic foods as important. In this study, we optimized a method that allows simultaneous quantitative analysis of all 84 marker peptides with a single analysis using LC-MS experimental techniques for food labeling.

To select marker peptides for allergenic foods, species-specific marker peptides were identified using DDA analysis for 21 allergenic foods designated by Korea Food and Drug Administration. Followed by DDA analysis, PRM analysis was performed for verification of marker peptide candidates in more than 4 processed foods of each allergenic food. The final step of selecting marker peptides was validating all marker peptide candidates with MRM analysis. To quantify marker proteins, dynamic MRM method was optimized and the developed method was applied to 10 overseas processed foods.

In this study, we identified up to 2400 peptides using DDA mode in raw materials of 21 allergenic foods designated by KFDA. In addition, PRM analysis was conducted in consideration of the change in quantitative results due to the processing of raw materials, and peptides with strong detection signal strength were selected as marker candidates. Validation of all selected markers was conducted using MRM analysis, and the final simultaneous quantitative analysis method includes a total of 84 peptides and 252 transitions. The developed dynamic MRM method confirmed the limit of quantification and detection at the ppm level in a single analysis using 20ug of analyte within 25 minutes for 10 processed foods distributed overseas.

The dynamic MRM method developed through this study is considered to help establish an accurate labeling system and the standardization of food allergy labeling.

PP03.139: Investigation of Soluble Protein Retention in Donor Human Milk After Holder Pasteurization, High-Pressure Process and UV-C Treatment

Bum-Jin Kim, United States

Donor human milk (DHM) is recommended for preterm infants when a parent's own milk is unavailable or insufficient. To ensure microbiological safety, non-profit milk banks apply Holder pasteurization (HoP). However, HoP leads to the loss of several biologically active milk proteins. High-pressure process (HPP) and UV-C treatment (UV-C) can be alternative processes to inactivate neonatal pathogens. These processes may differentially affect retaining the bioactive proteins in DHM. In here, we applied LC-MS/MS-based proteomics to compare the detection of proteins in the soluble fraction of DHM after HoP, HPP and UV-C in comparison with raw DHM.

Aliquots of pooled DHM were sterilized by 1) HoP at 62.5 for 30 min, 2) HPP at 400–500 MPa for 1–9 min and 3) UV-C at 2,000–11,000 J/L. Soluble proteins were extracted by centrifuge, acidification and filtration from the DHM samples. Extracted proteins were denatured using DTT and IAA and digested using Lys-C/trypsin. Tryptic peptides were cleaned by C18-solid phase extraction, analyzed using C18 nano-LC/Orbitrap MS and identified using Proteome Discoverer.

Counts of the identified proteins were similar after HPP (on average, n=247) and UV-C (on average, n=259) compared to raw DHM (n=251) whereas, the total abundances (label-free relative quantitation) of proteins decreased in both HPP and UV-C than raw DHM. HoP further decreased protein retention than both HPP and UV-C. HPP for 9 min (regardless of pressure) and UV-C at 11,000 J/L of human milk samples decreased protein retention compared to the other HPP and UV-C samples. Representative DHM proteins including lactoferrin, bile salt-activated lipase, osteopontin, lysozyme and α-lactalbumin were highly preserved after HPP (2–74% decrease) and UV-C (1–48% decrease) than HoP (10–96% decrease).

Soluble human milk proteins were better preserved after HPP and UV-C compared to HoP. These results will guide DHM processing to preserve bioactive proteins and potentially improve infant outcomes.

PP03.141: Phospho-proteomic Analysis of Microbe-Associated Molecular Patterns (MAMPs) Signalling in Plant Immunity

Jianan Lu, United Kingdom

Vegetable Brassicas are important food crops in the UK but they have been suffering with serious challenges from various pests and diseases. The first layer in plant immunity to detect highly conserved components of microbes, such as flagellin and chitin, are called Microbe-Associated Molecular Patterns (MAMPs). These MAMP signalling pathways have been widely studied in the model plant Arabidopsis thaliana, but the overlap and differences among these mechanisms remain unclear in crop plants such as Brassica oleracea. Phosphorylation is an excellent post-translational modification to focus on because it can form the basis for physical enrichment of signal-transduction components and could be identified by high-throughput Mass spectrometry. Based on our newly established workflow including MAP kinases activation and phosphoproteomics, we have identified hundreds of phosphoproteins who changed their phosphorylation levels in plant defence both of Arabidopsis and Brassica. There were few interesting proteins from the shared dataset between the two species which have been reported involved in plant immunity and on their way to be validated specific to either flg22 or chitin signaling pathway. This project will draw a latest phosphoproteome map of Arabidopsis and Brassica which help offer candidate defence genes in genetic breeding.

PP03.143: Proteomics Analysis of Porcine Lens Epithelial Cells in Response to Lycium Barbarum Polysaccharide Against Oxidative Damage - iTRAQ Approach

Samantha Sw Shan, Hong Kong

Introduction: Cataract is the most common eye disease characterized by the presence of opacities in the crystalline lens. Oxidative stress induced by reactive oxygen species is a major pathogenic factor of cataract. Lycium Barbarum (LB) has been commonly used in traditional Chinese medicine because of its beneficial effects on improved vision. Lycium Barbarum Polysaccharide (LBP) is the major component responsible for the biological activities. In this study, we evaluated the protective effect of LBP against oxidative damage in lens epithelial cells (LECs).

Methods: Primary porcine LECs and intact porcine lens were used. Oxidative stress was induced by H_2O_2, either with or without LBP pretreatment. Differential protein changes (FC>1.2, 2 unique peptide match) after H_2O_2 either in the presence or absence of LBP treatment with 3 technical replicates were determined by iTRAQ labeled proteomics approach using a 6600 Triple-TOF LCMS.

Results: LBP pretreatment elicited a dose-dependent protective effect against H_2O_2-induced damage. At 800 mg/L, LBP increased the LEC's viability by 40% and increase the light transmittance in ex vivo pig lens. 1,707 proteins were identified in LECs. Comparing protein regulations between H_2O_2 and LBP, three proteins that showed opposite direction of expression with and without LBP [i.e. Thioredoxin domain containing 12 (Txndc12); Heterogeneous nuclear ribonucleoprotein (HNRNPF); and F-actin-capping protein subunit beta (CAPZB)] were selected for validation. Using semi-quantitative PCR, the results were consistent with the findings obtained from iTRAQ approach.

Conclusions: LBP exerts a protective effect against H_2O_2-induced oxidative stress in porcine LECs. Our results suggest that Txndc12, CAPZB, and HNRNPF are potential targets modulating the protective function of LBP. Additional functional studies are required to confirm the potential significance of LBP as a dietary supplement for cataract prevention.

PP03.145: Characterization of Potential Allergens in Different Forms of Krill and Whiteleg Shrimp by Shotgun Proteomics

Chantragan Srisomsap, Thailand

The differential expression of proteins from dried, fresh and powdered forms of krill (Acetes spp.) and whiteleg shrimp (Litopenaeus vannamei) were compared using shotgun proteomics. The LC-MS-MS and Mascot program were used to analyze and identify the protein expression. Allermatch and AlgPred were employed to characterize potential allergens and predict IgE epitope, respectively. The processed krill and shrimp revealed the high amount of both identified proteins and allergens. The intracellular and cytoskeletal protein binding and organelle organization were the major functions of identified proteins. The eleven common allergens were presented in both species. In-silico analysis of potential allergens of krill and whiteleg shrimp obtained tropomyosin fast isoform and tropomyosin isoform X19 and IgE epitopes on alpha-actinin sarcomeric-like isoform X1. The tropomyosin fast isoform revealed a peptide IQLLEEDLER which could be a potential allergen marker in fresh, dried and powdered forms of krill and whiteleg shrimp. Our work provides the potential allergenicity and its allergen biomarkers for the food safety, clinical and diagnostic aspects of krill and shrimp.

PP03.147:Effect of Postharvest Storage Conditions on Cellular Pathways in Kale (Brassica Oleracea)

Xin Ee Yong, Singapore

Once harvested, green leafy vegetables start to lose its freshness and nutritional properties. Postharvest losses are a major problem in agriculture, and is estimated to be as high as 25% in developed countries and 50% in developing countries. This poses a large economic, social, and ecological burden. Appropriate postharvest storage techniques, such as cold storage, are commonly used to reduce the rate of physiological degradation and microbial decay. However, the cellular pathways involved in vegetable deterioration have yet to be fully characterized. Thus, we elucidate the effect of various postharvest storage conditions on the cellular pathway in Kale, a popular green leafy vegetable, using proteomics analysis. We optimized protein extraction from Kale and compared proteomes from different storage conditions. Elucidating the cellular pathways involved in postharvest quality loss of green leafy vegetables improves the understanding of the plant's response to storage techniques, and can provide a framework to assess the effectiveness of storage techniques.

PP03.149: Development of Anion Exchange-mediated Glycopeptide Enrichment Methods for Characterization of Poly-LAcNAc-containing Glycoproteins in Malignant Melanoma Cells
Gege Xu, United States

• Introduction
Unbiased discovery of cell surface glycoproteins carrying polyLAcNAc remains challenging due to their abundance and increased size, rendering them difficult to detect by mass spectrometry-based glycoproteomics workflows. B3GNT2, an enzyme involved in polyLAcNAc elongation of N-glycans, has been identified as a driver of melanoma resistance to T cell-mediated cytotoxicity. Coincidentally, polyLAcNAc on PD-L1 leads to stronger interaction with its receptor PD-1 and enhanced T cell suppression and polyLAcNAc on LRP6 promotes Wnt/β-catenin signaling.
• Methods
We systematically evaluated a panel of 6 anion exchange-based matrixes for their ability to enrich intact glycopeptides from the A375 malignant melanoma cell line. Additionally, we compared the performance of intact glycopeptide profiling from whole cell lysates, crude membrane and plasma membrane preparations. All samples were run with a stepped HCD method optimized for intact glycopeptide analysis on a Thermo Orbitrap Exploris 480
• Results
Mass spectrometry analysis revealed 6-12% enrichment recovery yield and 1500-3000 unique glycopeptide IDs using selected anion exchange-based materials, demonstrating variability in the performance of anion exchange-based matrixes. Next, we compared the glycosylation landscapes of whole cells, crude membranes and plasma membrane fractions, detecting significant decrease in high mannose glycan content in plasma membrane fraction. Importantly, even though plasma membrane fraction resulted in as low as 40ug of total peptides, anion exchange-based enrichment enabled over 3000 unique glycopeptide identifications in a single MS run.
Finally, the application of an optimized protocol to melanoma cell lines overexpressing B3GNT2 enabled the identification of dozens of polyLAcNAc-containing N-glycoproteins implicated in signaling, antigen presentation, and tumor development.
• Conclusion
We developed approach for large scale mapping of polyLAcNAc-containing N-glycoproteins which represent key players in tumor development as well as potential targets for therapeutic intervention. Additionally, we provide evidence that plasma membrane fractionation is crucial to avoid possible biases resulting from cell-metabolic heterogeneity

PP03.151: Quantification Analysis of Site-specific N- and O-glycopeptides for Characterization of Fusion Protein Etanercept
Heeyoun Hwang, Republic of Korea

The fusion protein Etanercept is a novel drug for the treatment of various inflammatory disorders. Because multiple N- and O-glycosylation sites exist in Etanercept, there is a demand for in-depth analysis of dimeric regions at the tumor necrosis factor recep-tor 2 (TNFR2) and the Fc portion of human IgG1. We present here a method for LC/MS/MS analysis of TMT-labeled glycopep-tides with ZIC-HILIC enrichment and fractionation for simultaneous characterization of site-specific N- and O-glycosylation of a fusion protein. We also provide a comparative analysis of the originator and two biosimilars of Etanercept. A total of 87 N- and 67 O-glycopeptides from three N-glycosylation and 15 O-glycosylation sites in Etanercept were confirmed, respectively. At each glycosylation site, we found major glycan types including sialylation and fucosylation as well as minor O-acetylation in N- and O-glycopeptides. Specifically, in the TNFR2 region, afucosylated type of the N149 and fucosylated complex type of the N171 sites of Asparagine accounted for more than 70% of N-glycopeptides. We also report 12 O-glycopeptides, including two 27T and 62T sites of Threonine. In the IgG region, the asialo fucosylated complex and high mannose types were the main N-glycosylation types and more than 70% of the O-glycosylations were of the core1 type. In addition, a direct comparison of N- and O-glycosylation between three Etanercept products showed a similar pattern in major but differences in minor components. Finally, the TMT label-ing with ZIC-HILIC HPLC fractionation of N- and O-glycopeptides could provide more precise quantitation and site-specific characterization of glycosylation in a fusion protein as well as their biosimilar products. (Word Style "BD_Abstract"). All manu-scripts must be accompanied by an abstract. The abstract should briefly state the problem or purpose of the research, indicate the theoretical or experimental plan used, summarize the principal findings, and point out the major conclusions.

PROGRAM

PP03.153: Introducing the GlycoPaSER Prototype for Real-Time N-glycopeptide Identification on the PaSER Platform
ShinKwon Kang, Republic of Korea

Protein glycosylation is a key modulator of protein biology and has been shown to dynamically change in various inborn or acquired diseases. Glycoproteomics enables proteome-wide characterization of protein glycosylation at the level of individual glycosylation sites, which provides unique possibilities for understanding the biology underlying this complex modification class and subsequent biomarker applications. We developed GlycoPaSER, a set of software modules for the PaSER (Parallel Search Engine in Real-time) computational platform, which enables the real-time identification and quantification of glycopeptides from PASEF-DDA data. To expand the capabilities of PaSER to include glycopeptides, we decomposed the hybrid glycopeptide fragmentation spectrum into peptide- and glycan-moiety spectra by locating common glycopeptide fragments. The peptide moiety spectrum was identified by the database search engine available in PaSER (ProLuCID) and the glycan-moiety was identified by a new database-independent glycan identification module. Data from healthy control plasma glycopeptide samples were used to optimize the parameters for the GlycoPaSER modules. GlycoPaSER identified a glycopeptide spectrum every ~35 msec, on average, which is three times faster than glycopeptide data acquisition, indicating that GlycoPaSER can perform the analysis in real-time. To compare identification performance, we used 10 glycopeptide plasma samples of healthy controls and compared GlycoPaSER to MSFragger-glyco. Both software show excellent agreement in the overlap of peptide sequences (74%) and identified proteins (85%). Comparing the results at the PSM level showed that in 93% of the cases, both tools provided the same glycopeptide identification. Moreover, the identified glycoproteome is in line with available glycosylation data in the literature. The PaSER platform allows for the use of the real-time identification results to affect the data acquisition. We illustrated how this real-time results dependent acquisition (RDA) may improve glycoproteomics data quality by evaluating the effect of using glycopeptide-specific optimized collision energies.

PP03.155: Comparative Glycoprotein Analysis of Human and Porcine Red Blood Cells for Enhanced Xenotransfusion Compatibility
Jae Ho Kim, Republic of Korea

The current blood supply system heavily relies on allogeneic blood donations, which often fall short of meeting the demand. As an alternative, xenotransfusion using pig blood, known for its hematologic similarities to humans, has gained attention. Achieving compatibility between pig and human erythrocytes, particularly in oxygen-carrying capabilities, is crucial for the success of xenotransfusion. However, there is limited comprehensive information comparing the glycoproteins present in human and pig erythrocytes in terms of biomolecular characteristics. In this study, we employed stepwise approaches based on mass spectrometry to characterize membrane proteins in human and pig erythrocytes. Firstly, we utilized a proteomics approach to remove hemoglobin, which could interfere with protein identification, and identify low-abundance proteins. Subsequently, we selectively enriched glycoproteins in the erythrocyte membrane using the ERLIC-SPE method. Finally, a glycoproteomic approach was applied to compare site-specific glycosylation of target glycoproteins in human and pig erythrocytes. Specifically, the glycoproteins present in human and pig erythrocytes were compared for micro & macro glyco-heterogeneity at the glycopeptide level. The selective enrichment of glycoproteins was confirmed by comparing the results obtained from proteomics and glycoproteomics analyses. Interestingly, we found differences in glycosylation patterns of certain glycoproteins that were expressed identically in human and pig erythrocytes. These findings provide a valuable resource for understanding successful xenotransplantation. Moreover, this knowledge can aid researchers in modifying or removing these glycoproteins to enhance compatibility between pig blood and humans This knowledge can significantly impact the field of xenotransfusion and assist in the development of strategies to improve the compatibility and efficacy of pig blood in human transfusions.

PP03.157: Detection of Pancreatic Ductal Adenocarcinoma-Associated Proteins in Serum

Tung-Shing Mamie Lih, United States

Background:
Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal cancer types. Early detection using minimally invasive methods such as blood tests may improve outcomes. Previous studies have recognized the major genetic alterations that drive PDAC progression, including somatic point mutations in KRAS, as well as molecular heterogeneity of tumors based on multi-omics data. To improve the detection of PDAC, in this study, a quantitative glycoproteomic approach via data-independent acquisition mass spectrometry (DIA-MS) was utilized to detect glycoproteins in sera from 29 PDAC patients with matched tumor tissues, including 13 with case-matched normal adjacent tissues, in comparison to disease-free controls.
Methods:
DIA raw files of serum samples were searched against a UniProt/SwissProt human protein database via the direcDIA approach in Spectronaut. Abundance of each glycopeptide/protein must be two standard deviations away from the mean of the normal controls for each patient with PDAC. Receiver operating characteristic (ROC) analysis was conducted to demonstrate the clinical utilities of the glyco-signatures even if detected in a few samples.
Results:
Majority of the glyco-signatures were only highly expressed in one or few samples indicating the heterogeneity among the patients and their cancers. Many serum-detectable PDAC tissue-associated glycoproteins were involved in extracellular structure organization or immune-related processes. The ROC analysis suggested that combining glycopeptide of VNN1 with glycopeptides of ENPP2, APOB or F2 would improve the detection of PDAC.
Conclusions:
The main objective of the current study is to investigate the correlation between tissue and matched serum from the same patient with PDAC. The variations in expression profiles of the serum-detectable PDAC tissue-associated glycoproteins suggests a high variability in the glycoproteomic profile among serum samples from patients with PDAC. Although additional studies will be required to further determine the clinical utilities of these PDAC-associated proteins, the results suggests that detection is achievable using serum.

PP03.159: In-Depth Site-Specific O-Glycosylation Analysis of Glycoproteins in 3xTg-AD mouse model of Alzheimer's Disease

Jiyoung Mun, Republic of Korea

Introduction: Mapping site-specific O-glycoproteome in complex biological systems provides insights into the functional relationships between glycoprotein structure and their roles in physiological and pathological processes. However, limited analytical methods for large-scale analyses of intact O-glycopeptides hinder our understanding of O-glycoproteome heterogeneity and its biological contributions. In this study, we demonstrate the enrichment of site-specific microheterogeneity in O-glycoproteome through large-scale O-glycopeptide profiling using combined HCD (higher energy collisional dissociation) and CID (ion-trap-based collision-induced dissociation) methods, enabling the characterization of specific mucin-types of O-glycoproteins.
Methods: O-glycopeptides were extracted from six whole brain tissues of wild-type (n=3) and 3xTG-AD (n=3) mice using lysis buffer and digested with PNGaseF and trypsin. The resulting O-glycopeptides were enriched on hydrophilic interaction chromatography resin and analyzed using nanoLC/MS with HCD and CID.
Results: We quantified more than 2,000 unique O-glycopeptides from 1,150 O-glycoproteins in whole brain tissues of wild type and 3xTG-AD mouse, presenting the largest dataset on O-glycoproteins and O-glycopeptides for whole brain tissue in mice to date. Our study collected more than 8 million O-glycopeptide data as the mouse O-glycopeptide DB. Through our proprietary DB analysis, we identified 157 differentially expressed O-glycoproteins in this study with 70 up-regulated and 87 down-regulated in the 3xTG-AD mouse group. Gene ontology analysis revealed enrichment in cellular processes, biological regulation, localization processes, and molecular functions related to binding, catalytic activity, and molecular transducer activity.
Conclusions: We successfully optimized the pretreatment method for brain tissue, reducing N-glycopeptide interference, and implemented HILIC resin enrichment and orbitrap nanoLC/MS system for O-glycopeptides detection. Our data demonstrate differences in O-glycosylation profiles between whole brain tissues of wild type and 3xTG-AD mouse, as well as may O-glycosite heterogeneity on the protein level. Additionally, we are utilizing this large-scale O-glycoproteomic dataset to develop visualizations that will aid in the analysis of intact O-glycopeptides in future studies.

PROGRAM

PP03.161: Advanced Assessment Through Intact Glycopeptide Analysis of Infliximab's Biologics and Biosimilar

Yeseul Park, Republic of Korea

Originator and biosimilar products of antibody drugs represent heterogeneous variants characterized by differences in glycosylation, oxidation, glycation, aggregation state, and deamidation, where the glycosylation is an important post-translational protein modification that alters protein properties. Infliximab (brand name, Remicade®) is a representative mAbs that interacts with target tumor necrosis factor α (TNF-α) for treating autoimmune diseases. In this study, N-linked glycosylation of Remicade® and Remsima®, the originator and biosimilar of infliximab, respectively, were analyzed at the glycopeptide level with a focus on profiling the glycan composition through tandem MS spectra.

Based on the number of identified glycopeptide-spectrum matches (GSMs), the originator and biosimilar of Infliximab were compared quantitatively and the similarity was evaluated. Here, we demonstrated high-resolution tandem mass spectrometry with an ultra-high-performance liquid chromatography for characterization and comparison between originator and biosimilar mAbs at an advanced level. Focus on the infliximab, specifically, we compare the N- and O-glycopeptides profiles of originator and biosimilar products of Remicade® and Remsima®, respectively.

A total of 49 and 54 glycopeptides were identified in Remicade® and Remsima®, respectively, at two sites of N300 and N41ively. We also identified NeuGc and NeuAc-containing glycopeptides. At N41 site, only hybrid type and high mannose type glycosylation were expressed, unlike the N300 site. All of the top 10 peptides were expressed at the N300 site. The overall qualitative profiles for both the originator and biosimilar are very similar. The most frequently expressed glycan were G0F (3_4_1_0_0) and G1F (4_4_1_0_0) in N300, and is in agreement with the results of previous studies. 8 out of 10 glycopeptides were fucosylated, and 4 out of 10 sialylated glycopeptides were attached with NeuGc. Therefore, these results confirmed that the similarity between the originator and biosimilar of infliximab and between different batches was significant.

PP03.163: Identification of Intact Sialylated N-glycopeptides using TiO2 Chromatography in Combination with LC-MS/MS and Accurate Mass Matching Using the GPMAW Glyco-tool

Maria Kyung-Ah Petersen, Denmark

Sialylation on N-glycans is a post-translational modification (PTM) regulated by sialyltransferases (STs) and neuraminidases that adds or removes sialic acids (SAs) at the terminal end of the glycans. The most common SAs in vertebrates are N-acetylneuraminic acid (Neu5Ac) and N-glycolylneuraminic acid (Neu5Gc). However, humans are genetically deficient of the Neu5Gc nucleotide sugars compared to primates such as the chimpanzees, due to a 92 bp deletion in the cytidine monophospho-N-acetylneuraminic acid hydroxylase (CMAH) gene that generate the Neu5Gc from Neu5Ac [Varki A., 2017]. SAs have been speculated to have a large influence on many biological processes such as early brain development, human specific brain features, and virus-glycan interactions [Stencel-Baerenwald J.E., 2014].

Several mass spectrometry-based methods and informatic tools have emerged to allow the assignment of formerly N-linked glycosylated sites in proteins. However, the ability to perform intact glycopeptide analysis is still challenging, especially for intact sialylated glycopeptides [Matthies I., 2021].

In this study we present a strategy to for the characterization of intact sialylated N-linked glycopeptides using selective purification of sialylated N-linked glycopeptides by titanium dioxide (TiO2) chromatography in combination with liquid-chromatography tandem-mass spectrometry (LC-MS/MS). The identification of the intact sialylated N-linked glycopeptides was performed by accurate mass correlation between the formerly sialylated N-linked glycopeptides (deglycopeptides) and the intact sialylated N-linked glycopeptides using a novel software function in the GPMAW program. The aim was to create a spectral library over humane sialylated N-linked glycopeptides.

The strategy and preliminary results of the intact plasma N-linked sialiome is shown in this study. Further improvement of the novel software and the data analysis is still ongoing and more validating experiments and comparison with known software's (e.g., Byonics) will be conducted.

PP03.165: Effective Mass Spectrometry-Based Methods for Comprehensive Characterization of Surface Glycoproteins and Their Dynamics in Immune Cells during the Infection

Ronghu Wu, United States

Many important proteins on the cell surface are glycosylated, such as receptors, transporters and binding proteins, and they are essential to mammalian cells. Surface glycoproteins regulate nearly every extracellular event including cell-cell communications, cell-matrix interactions and cell-immune response. Aberrant protein glycosylation on the cell surface is directly related to human diseases such as cancer and infectious diseases. Comprehensive analysis of glycoproteins on the cell surface and their dynamics will help identify surface glycoproteins as effective biomarkers and drug targets. 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 glycoproteins site-specifically. One main advantage of this method is that the conditions are mild, which allows us to study the dynamics of surface glycoproteins. It was applied to quantify the dynamics of surface glycoproteins in monocytes and macrophages in response to bacterial infection. Many surface glycoproteins have different dynamics in between monocytes and macrophages. Further quantification of surface glycoproteins during the cell differentiation from monocytes to macrophages helped us better understand the response differences between the two types of immune cells. Besides well-known surface proteins changed during the infection, we also 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.

PP03.167: Search and Investigation of Potential Peptide Agents of Interaction Between Human Organism and Its Microbiome

Georgij Arapidi, Russian Federation

Symbiotic interaction between the human body and its microbiota is an important issue of modern biomedicine and personalized medicine. However, little is known on molecular mechanisms of that relationship. Bearing in mind the ubiquitous participation of peptides in biomolecular interactions and regulatory processes we attempted direct search of blood peptides originated from microbial proteins. LC-MS/MS analysis was carried out of blood serum and plasma samples taken from 20 healthy donors on Q Exactive HF-X Hybrid Quadrupole-Orbitrap mass-spectrometer.
Out of 13,625 identified peptides 912 were unique fragments of microbial precursors, which is about 6.69% of the total amount of detected bloodstream peptides. 30 peptide identifications were confirmed by synthetic peptide mass-spectra. Absolute quantification by MRM confirmed the presence of bacterial peptides in plasma and serum in the range of approximately 0.1nM to 1uM, which is comparable to physiologically significant hormone concentrations in human blood in normal conditions. The abundance of microbiota peptides reaches its maximum 5h after a meal. Most of the peptides correlate with the bacterial composition of the small intestine and are likely obtained by hydrolysis of membrane proteins with trypsin, chymotrypsin and pepsin — the main proteases of the gastrointestinal tract.
The isolated fraction of peripheral blood mononuclear cells showed increase secretion of proinflammatory cytokines, colony stimulating factors and chemoattractants as the response to the addition of the identified microbiotic peptides. Such peptides — identified both in blood plasma and serum — have properties of resistance to fibrinolysis, since the pool of peptides is preserved after passing through the digestive tract. Our approach to the identification of microbiota peptides in the blood derivatives may be useful for determining the microbiota composition of hard-to-reach intestinal areas, such as the small intestine, and for monitoring the permeability of the intestinal mucosal barrier.

PP03.169: The Proteomic and Metaproteomic Analysis of the Infant Gut and Gut Microbiome in African HIV Exposed Infants

Tara Miller, South Africa

Background: The new victims of the HIV pandemic are the HIV exposed but uninfected infants, who have avoided HIV infected but suffer an increased morbidity and mortality rate compared to their healthy counterparts. In Southern Africa, 90% of pregnant women are accessing ARVs to prevent Mother to Child Transmission. However, in resource limited settings Option B+ (exclusive breastfeeding on Highly Active antiretroviral treatment) is recommended by the WHO for 4-6 months for optimal infant outcomes. However, despite the precautions these infants are observed to have long term health deficits, including immunologic, metabolic, and neurological. In addition, a delayed immune development could be contributing to the vulnerability of these infants making them susceptible to opportunistic disease.
Aim: This study focuses on studying the perturbations in the infant gut and its microbiome which could correlate to the altered immune responses to childhood vaccinations. This will help understand the role of the microbial community's establishment in the infant gut with respect to early life immune system development.
Methods: To assess their development, the HIV exposed uninfected infants' gut and microbiome proteome are analysed using mass spectrometry and SWATH acquisition. Furthermore, deep data analysis into the pathways involved and proteins present was conducted. Consequently, providing insight into the functioning of the infant's gut and subsequent microbiome.
Results: Through this analysis a deeper understanding into the mechanisms involved in the infant's development and gut health was observed. The identification and investigation into the gut microbiome's proteome shows what affects the gut functioning and if HIV exposure results in a shift in the microbiome. Subsequently, how the shift in microbiome could affect the infants' immunity and development compared to their healthy counterparts.
Conclusions: The identification of proteins involved in the immune-microbiome interplay could lead to further research into how to improve the lives of these infants.

PP03.171: Accounting for Chimeric Spectra Boosts the Number of Identifications in Metaproteomics Without Impacting Sensitivity

Tim Van Den Bossche, Belgium

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 (doi:10.1080/14789450.2019.1609944). One of the underlying challenges is that traditional search engines stick to the one-spectrum-one-peptide paradigm. However, in highly complex biological samples, it is common to have different peptides of similar precursor masses that elute close in time and are co-fragmented simultaneously. To solve this issue, we applied the MS Amanda (doi:10.1021/pr500202e) chimeric search and Percolator (doi:10.1038/nmeth1113) to identify and validate multiple peptides per spectrum in several multi-species, metaproteomics datasets.

Methods
Here, the spectra are first searched with the database search engine MS Amanda resulting in peptide-to-spectrum matches (PSMs). After removing already identified peaks, a second search is applied on the new, cleaned spectra. The resulting multiple peptide-to-spectrum matches (mPSMs) are validated by the machine learning algorithm Percolator. As additional validation, we searched the original and newly created spectra with X!Tandem to verify that we obtain similar results without MS Amanda.

Results
We tested the performance of our workflow on the CAMPI benchmark datasets (doi:10.1038/s41467-021-27542-8), and on a publicly available metaproteomics dataset containing microbial gut cells from children with inflammatory bowel disease (doi:10.1038/s41467-018-05357-4). Our preliminary results show that by applying this workflow, there was an average increase of 26% of identified PSMs and a 16% increase to the number of proteins.

Conclusion
From these preliminary findings, we can conclude that chimeric search in MS Amanda can boost the number of identifications in metaproteomics without impacting sensitivity. In the near future, we will assess if this leads to a more in-depth taxonomic assignment.

See you in Busan!
BEXCO, Busan, Korea
🌐 2023.hupo.org
🐦 f 📷 in #HUPO2023

Together Through Proteomics

PROGRAM

PP03.173: Some Lessons Learned on the Impact of the Storage and Injection of Samples on the GC-MS Reproducibility

Ilya Kurbatov, Russian Federation

Metabolomics based on two-dimensional gas chromatography coupled with mass spectrometry is making high demands on accuracy at all stages of sample preparation, up to the storage and injection into the analytical system. In high sample flow conditions, good repeatability in peak areas and a list of detectable metabolites is sometimes challenging to obtain.

In this research, we successfully obtained good repeatability for the peak areas of MSFTA-derivatives of 29 core blood plasma metabolites. Six different strategies of storage and injection were investigated and evaluated for the reproducibility of the obtained data. As the essential factors, we considered popular GC-MS syringe washing solvents (methanol and pyridine); storage conditions (freshly prepared samples and stored for 24 h in ambient temperature or in the refrigerator); scheme of injection (one injection per intact vial or three sequential injections per vial).

Our GC×GC-MS results demonstrated that the usage of pyridine as a syringe wash solvent and triple injecting the sample from the same vial was the most appropriate for minimizing the coefficient of variation (CV) of the results obtained (in general, <10%). The prolonged storage of samples does not have a noticeable effect on the change in the areas of chromatographic peaks of metabolites, although it reduces CV in some cases. These storage and injection recommendations can be used in future study protocols for the GC×GC-MS analysis of blood plasma.

This work was funded by Ministry of Education and Science of the Russian Federation, grant number 075-15-2021-933 ("Avogadro" large-scale research facilities, unique project ID: RF00121X0004).

PP03.175: Chromosome-Centric Human Proteome Study of Chromosome 11 Team

Mina Park, Republic of Korea

As a part of the Chromosome-centric Human Proteome Project (C-HPP), we have developed a few algorithms for accurate identification of missing proteins (MP), alternative splicing variants (ASV), single amino acid variants (SAAV), and characterization of function unannotated proteins (uPE). 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. Here, we introduce the status of MP, ASV, SAAV and uPEs from the latest released neXtProt database (2023-04) which incorporates PeptideAtlas human (2023-01 build) and MassIVE. 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 uPE1 in chromosome 11, we have studied the function annotaion of CCDC90B (NX_Q9GZT6), SMAP (NX_O00193), and C11orf52 (NX_Q96A22). In addition, we found changes in protein entries again, where some proteins disappeared, and new proteins were added. Therefore, we always need to use the latest database of neXtProt.

PP03.177: Highly Selective and Reproducible Glycopeptide Enrichment using ZIC-HILIC Chromatography in Human Serum

Won Jun Yoo, Republic of Korea

Glycosylation is a process of post-translational modifications (PTM) that is important in affecting protein function and regulation. However, analyzing protein glycosylation is low abundances of glycoproteins and low mass sensitivities of glycopeptides, requiring glycopeptide enrichment processes. ZIC-HILIC (Zwitter Ion Chromatography-Hydrophilic Interaction Chromatograp) column has known advantages in the separation of hydrophilic substances and glycopeptide enrichment. In this study, we evaluated a glycopeptide enrichment method using a ZIC-HILIC column in high-performance liquid chromatography (HPLC). Here, we reduced the non-glycopeptide ratio by adjusting elution time and gradient in the HPLC condition. After fractionation, we analyzed glycopeptides enrichment, the characteristics of peptides and glycan compositions. Interestingly, sialylated glycopeptides eluted earlier in ZIC-HILIC chromatography, while fucosylated glycopeptides eluted subsequent. From the human serum, we identified 524 unique N-glycopeptides and 48 unique O-glycopeptides in 101 glycoproteins using the Glyco Proteome Analyzer (GPA) software with three replicates. In conclusion, this study has shown increased glycopeptide enrichment efficiency and reproducibility when using online ZIC-HILIC method.

PP03.179: A Novel, Deep, Unbiased and Scalable Nanoparticle-Based Proteomics Workflow from Model Organisms with Limited Plasma/Serum Sample

Shao-yung (Eric) Chen, United States

Background

Model organisms like mice are utilized to unveil insights to human biology research. However, due to the nature of these smaller organisms, the available blood sample volume is often reduced, which also makes repeated sampling over longitudinal studies more challenging compared to sampling from human donors.

Blood plasma and serum are rich, readily available sources of protein that are commonly used in clinical research studies. However, the large dynamic range in the plasma/serum proteome has hindered large scale plasma/serum proteomic research. The Proteograph™ Product Suite (Seer Inc.) enables high-throughput in-depth plasma proteome quantification, employing proprietary engineered nanoparticles (NPs), which improves protein identifications in terms of depth and breadth along with precise quantification.

Methods

For samples with lower than the standard Proteograph Assay processing volume (250ul), triplicates of 250, 125, 50, 25, and 10 μL of normal mouse serum and human plasma samples were first mixed with an appropriate amount of Reconstitution Buffer A (Seer Inc.) to yield a final volume of 250 μL. Samples are then ready to undergo protein corona formation and tryptic digestion to desalted and reconstituted peptides for LC -MS analysis.

Preliminary Data

Here we evaluate the current performance of Proteograph Product Suite coupled with Thermo Fisher Scientific Exploris™ 480 Mass Spectrometer with 60-minute Liquid Chromatography (LC) methods using Data Independent Acquisition strategies (DIA) and analyzed the data via Proteograph Analysis Suite 2.1, evaluating depth of proteome coverage, dynamic range, assay yield, and reproducibility of Proteograph Assay proteome profiling. Notably, with 5-fold lower than standard Proteograph Assay sample input volume, the Proteograph workflow still obtained ~2-fold increase in human plasma samples for protein group IDs (1246 v.s. 626, n=3) compared with traditional neat digestion.

Conclusions
Low volume sample processing with Proteograph workflows provides a solution for limited-sample applications while retaining performance advantages over direct digest approaches.

PP03.181: Automated Pipeline for Robust and High Throughput Analyses of Low Input Proximity-Labelling Samples

Therese Dau, Germany

Proximity-labelling has emerged as an important tool to study protein-protein interactions directly in-cell. Labelling time could be considerably reduced by optimisation of biotin ligases and has opened up the use for a wide variety of experiments, e.g., time-course analyses. Despite these advances sample preparation is still very labour intensive and limits experimental size. Here, we optimised the workflow for high throughput purposes by combining our low sample input pipeline with automated sample preparation on a liquid handler. A sample preparation workflow for enrichment and digestion of biotinylated proteins (Bartolome, A et al. bioRxiv 2022) was adapted and implemented on a liquid handler. Here, streptavidin beads are acetylated prior to loading of the samples. This decreases streptavidin contamination after on-bead digestion with LysC. In a second elution, a mixture of acetonitrile and trifluoroacetic acid is used to retrieve biotinylated peptides. As this step is time sensitive, automatization on a liquid handler improves reproducibility. In addition, the impact of the sample input as well as the LC gradient was analysed. The sample input could be reduced from 20 Mio to 8 Mio cells and the gradient shortened from 120 minutes to 21 minutes without compromising the efficiency of biotinylated protein enrichment. Taken together we were able to implement a new workflow that improves reproducibility and speeds up sample processing and measuring time for proximity labelling experiments considerably.

PP03.183: Protein and Peptide Signatures Separate Healthy Aging, Mild Cognitive Impairment and Alzheimer's Disease in a Paired CSF and Plasma Study

Yuehan Feng, Switzerland

Introduction
While aging is the primary risk factor for both AD and MCI, the biological pathways that are altered in healthy aging vs. pathologic aging remain to be elucidated and there is an unmet medical need for biomarkers in body fluids to yield biological insights, support therapy development and provide early detection. Here, we seek to address this knowledge gap by applying a novel mass spectrometry-based discovery workflow on a cerebrospinal fluid (CSF) and plasma paired cohort.

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). Plasma samples were depleted of the 14 most abundant proteins. The plasma and CSF samples were digested and analyzed using Biognosys' TrueDiscovery DIA-MS workflow.

Results
We analyzed 133 matched plasma and CSF pairs from young, old and MCI along with 21 CSF samples from Alzheimer's. Overall, we identified 5,736 proteins in CSF and 3,082 in plasma with about 2,800 quantified in both. Interestingly, only few proteins correlated strongly between plasma and CSF (median correlation 0.08) and consequently most proteins were regulated specifically in either CSF or plasma. Using machine learning we predicted both age and disease state. Firstly, age prediction of the samples was accurate in both body fluids with LTBP2, a heart failure marker, the most predictive plasma protein and strongly increased with age in both body fluids. Secondly, MCI/young/old classification was most accurate using the CSF dataset (>90%), while in plasma the peptide level information was important for achieving good accuracy.

Conclusion
Overall, peptide level information and the profiling depth enabled accurate disease classification as well as providing biological insights into the mechanisms of healthy and pathogenic aging.

PP03.185: onePOT, a Chaotrope-free Method for Near Single Cell Proteomics

David Goodlett, Canada

Recently, we reported on a comparison of three cell isolation and two proteomic sample preparation methods for single-cell and near-single-cell targeted, quantitative proteomics of glycated-hemoglobin (Eshghi et al. JPR 2023; PMID: 37093777). As a result of this targeted study we developed a chaotrope free proteomic sample preparation method for near single cell discovery-based proteomics. Circumventing use of chaotropes simplifies sample cleanup because no trap column is required to remove SDS and alkylating reagents; therefore adsorptive loss is reduced. This new method, which we refer to as onePOT, was inspired by autoPOTS (Liang et al. 2021; PMID: 33352054), but onePOT was carried out with typical instrumentation accessible in a standard proteomics core LC-MS facility. In our case this includes use of bespoke nanoLC columns and a ten year old Orbitrap Fusion (Thermofisher). The onePOT sample preparation method circumvented the need for solid-phase extraction by eschewing use of surfactant and alkylation, low-volume liquid handling instrumentation and humidified incubation chamber that are used with autoPOTS. Protein digestion was carried out in microliter wells of a 384 well plate by adding the sample in 10% acetonitrile. The plate was transferred to a ultrasonic cleaner water bath at 70°C for 15 min of sonication. After this a Rapid Trypsin digestion kit (Promega) was used. Samples were directly injected from the 384 well plate for nanoLC-MS/MS analysis. We are now using onePOT to characterize subsets of sorted ascites fluid cells (e.g. CD4 and CD8 T cells) from women being treated for ovarian cancer at BC Cancer (Victoria, British Columbia). In a pilot study use of onePOT allowed identification of 1000s of proteins from 5-10,000 cells. We will describe our efforts to characterize subsets of immune cells in ascites fluid of ovarian cancer patients with the goal of building a diagnostic model.

PP03.187: A Novel Online 3-Dimensional Separation for Comprehensive Phosphoproteome

Chaewon Kang, Republic of Korea

Protein phosphorylation is the most widely studied post-translational modification (PTM) and is crucial in regulating cellular processes associated with human disease. Recently, the utilization of high-resolution liquid chromatography technology coupled with extensive offline sample fractionation has become a widely accepted approach ("divide and conquer" approach) for phosphoproteome profiling. However, achieving a balance between the experimental throughput and the depth of profiling still presents a challenge.
In this study, we introduce an innovative method for online 3-dimensional separation, which combines online 2-dimensional reverse-phase/reverse-phase liquid chromatography (RP/RPLC) with online gas phase separation. The online 2-dimensional liquid chromatography employs an online non-contiguous fractionating and concatenating (NCFC) device, and we coupled it to online high-field asymmetric waveform ion mobility spectrometry (FAIMS) in a fully automated manner. The online 3-dimensional separation approach enables in-depth analysis of the phosphoproteome by synergistically combining DO-2D-NCFC-RP/RPLC and FAIMS. The novel online 3-dimensional separation methodology resulted in the identification of over 100,000 phosphopeptides (>60,000 phosphosites) from HeLa cells within a data acquisition time of 1.5 days, achieving the balance between the experimental throughput and the depth of profiling.

PP03.189: Discovery of Novel Bioactive Peptides in Plasma and Tissue Based on a High-Yield Peptide Extraction Method

Yoshio Kodera, Japan

Endogenous peptides in plasma play very important roles in the regulation of homeostasis in the body. However, they are much more difficult to analyze and identify than proteins. This is due to the fact that peptides are present in smaller amounts than proteins and are bound to carrier proteins, such as albumin. The differential solubilization (DS) method that we developed enables high-efficiency extraction of peptides from serum/plasma, including peptides bound to carrier proteins such as albumin. Based on DS method, we successfully identified more than 11,000 peptides from 0.2 mL plasma including well-known bioactive peptides such as insulin B chain, manserin, hepcidin, motilin, salusin-β, neurotensin, and somatomedin-B. From this 11,000 peptide library, we selected about 150 peptides derived from secreted proteins with an FDR of 0% and attempted to search for bioactive peptides. After investigating the response of various cultured cells and in vivo bioactivity, we discovered five bioactive peptides derived from precursor proteins of suprabasin, gastric inhibitory polypeptide and angiotensinogen.
Next to the analysis of peptides in plasma, we improved the DS method to establish an extraction method that is optimized for small amounts of tissue, and conducted peptide analysis of approximately 0.5 mg of hypothalamic tissue taken from frozen sections of cryopreserved brains. As a result, we identified 1,535 peptides derived from 297 proteins and detected 35 known peptide hormones. Furthermore, we have recently applied this DS method to the visceral ganglion of the shellfish. As a result, we have succeeded in discovering an entirely new peptide that induces spawning in the pen shell *Atrina pectinata*.
In conclusion, this highly sensitive peptide analysis technology based on the DS method is expected to accelerate the research on novel bioactive peptides and disease-related peptides in body fluids and tissues.

PP03.191: Microflow 4D-Proteomics for Robust, High-Throughput Sample Analysis

Kwangseon Lee, Republic of Korea

Introduction: The potential amount of information obtained from a sample is increased at lower LC flow rates, however nano-flow chromatography suffers from overhead times and relatively low reliability. Advancements in sensitivity of mass spectrometers enables higher flowrates while retaining high sensitivity at low sample amounts.

Methods: We evaluated a microflow LC coupled with the Bruker VIP-HESI source with respect to LC parameters and overall proteomics performance. Data for tryptic digests of cell lysate samples were acquired in dda-PASEF and dia-PASEF, respectively.

Results: By a single column setup, from only 2 ug of trypsin digested human cell lysate with a 35 min separation program, we consistently observe 3500-4000 proteins (standard PASEF workflow) and more than 5500 proteins, respectively (dia-PASEF workflow).

By a label-free quantitation approach (two proteomes mix, 1:1 ratio (human) and 1:4 (E.coli)) using dia-PASEF, we found the relative quantitation of the 4554 identified human protein groups to be centered at the expected log 2 ratio of 0 across the complete dynamic range. Additionally, we quantified 1386 E.coli protein groups with a measured protein regulation ratio of 1:3.7 being close to the expected ratio of 1:4.

Conclusions: Monitored by spiked-in synthetic peptides, the fast equilibration time, chromatographic separation performance and retention time stability over hundreds of injections were confirmed. Using a dedicated microflow ESI needle with 50 µm inner diameter provided efficient ionization at a long lifetime.

PP03.193: Quadrupole Isolation and Characterization of Specific Proteoforms and Protein Complexes at a High m/z Range Using Orbitrap Ascend

Rafael Melani, United States

Recent advancements in mass spectrometry (MS) and native MS have greatly enhanced the identification capabilities of proteoform and protein complexes. Improved ion transmission, multiple ion activation types, and high-resolution Orbitrap mass analyzers enable more accurate and sensitive detection and characterization of proteoforms. However, precise protein complex and proteoform-specific characterization require high isolation resolution in the high m/z range, especially for native MS. Therefore, we modified an Orbitrap Ascend Tribrid to perform quadrupole isolation with high efficiency and accuracy up to m/z 8,000. We report the characterization of NIST mAb (146 kDa), and tetrameric pyruvate kinase (232 kDa), statically sprayed, under native conditions. For NIST, single glycoforms of the most abundant charge state were quadrupole isolated, and after isolation, the proteoforms were fragmented with different ion activation techniques, including HCD, ETD, EThcD, and UVPD. Combining all fragmentation types, we achieve high sequence coverage for a native MS experiment and identified heavy chain fragments containing the different glycan moieties specific for each distinct proteoform. For pyruvate kinase, the most intense charge state of the homotetramer complex, four identical subunits (232 kDa), was isolated in the quadrupole, and the subunits were ejected using HCD. A single subunit proteoform was identified, and further fragmentation confirmed the amino acid sequence and the presence of acetylation at the N-terminus. Subsequently, we isolated the complex with one truncated monomer missing the first 22 N-terminal amino acid residues (229.5 kDa), corresponding to ~10-15% of the intensity of the main complex proteoform. MS^2 and MS^3 experiments annotated and characterized the two distinct proteoforms and confirmed their stoichiometry. The use of quadrupole isolation allows the isolation of closely spaced proteoforms and protein complexes, improves the MS2 signal-to-noise ratio, and reduces interference from other ions. It affords deep proteoform/complex characterization on the Tribrid platform in conjunction with different ion activation techniques.

PROGRAM

PP03.195: Development of PTMScan-HS Immunoaffinity Reagents and Standards for PTM Enrichment: High Sensitivity, High Specificity, Highly Simplified, and High Throughput

Alissa Nelson, United States

Protein activity, localization, turnover, and interactions are often regulated by post-translational modifications (PTMs). However, most PTM sites exist at low stoichiometry, so enrichment is required to achieve proteome-wide sensitivity in a mass spectrometry experiment. Antibodies raised against a PTM surrounded by a degenerate amino acid sequence have proved to be powerful and flexible tools to enrich a broad range of modified peptides (Rush et.al. Nature Biotechnology 2005). The immunoaffinity purification (IAP) technique works well for modifications such as phosphotyrosine (pY), acetyl lysine (AcK), monomethyl arginine (Rme), asymmetric- and symmetric- dimethyl arginine (ADMA, SDMA), succinyl lysine (SuccK), and the KGG remnant of ubiquitin left by tryptic digestion. We have developed a series of magnetic bead – conjugated antibodies, and optimized buffers for IAP experiments that exhibit substantial improvements over previous agarose-based reagents. We termed the new methodology "PTMScan-HS" referring to the "high sensitivity / high specificity" compared to prior techniques. The antibodies no longer co-elute with the enriched peptides and the optimized buffer formulations have reduced nonspecific binding by unmodified peptides. Sensitivity has improved by 1.5x-3x and specificity for the PTM of interest over the unmodified background improved 1.5x-7x. IAP performance can be monitored both within and across experiments by spiking in isotopically labeled peptides, which are available for each PTM antibody kit. High-throughput experimentation is now within reach since the magnetic bead formulation allows the reagents to be used with automated bead handlers. Additionally, many of the antibodies are compatible with multiplexed TMT labeling. All these improvements to the sensitivity and ease of use of the PTMScan IAP antibodies are enabling more comprehensive signaling studies in sample-limited biological systems.

PP03.197: One-STAGE Tip Method for TMT Based Proteomic Analysis of Minimal Amount of Cells

Narae Park, Republic of Korea

Liquid chromatography-tandem mass spectrometry (LC−MS)-based profiling of proteomes with isobaric tag labeling from low-quantity biological and clinical samples, including needle- core biopsies and laser capture microdissection, has been challenging due to the limited amount and sample loss during preparation. To address this problem, we developed OnM (On-Column from Myers et al. and mPOP)-modified on-column method combining freeze−thaw lysis of mPOP with isobaric tag labeling of On-Column method to minimize sample loss. OnM is a method that processes the sample in one-STAGE tip from cell lysis to tandem mass tag (TMT) labeling without any transfer of the sample. In terms of protein coverage, cellular components, and TMT labeling efficiency, the modified On-Column (or OnM) displayed similar performance to the results from Myers et al. To evaluate the lower-limit processing capability of OnM, we utilized 19 OnM for multiplexing and were able to quantify 301 proteins in a TMT 9-plex with 50 cells per channel. We optimized the method as low as 5 cells per channel in which we identified 51 quantifiable proteins. OnM method is a low-input proteomics method widely applicable and capable of identifying and quantifying proteomes from limited samples, with tools that are readily available in a majority of proteomic laboratories.

KEYWORDS: LC-MS/MS based proteomics, tandem mass tag, limited sample, sample preparation

PROGRAM

PP03.199: Improved SDS Depletion From Proteins With Automation and Minimal Sample Consumption by Transmembrane Electrophoresis.

Hammam Said, Canada

Sodium dodecyl sulfate (SDS) is a widely used surfactant in proteomics, however, it poses challenges in downstream processing. To deplete SDS from proteins, our group developed an electrophoretic approach called transmembrane electrophoresis (TME) that achieves efficient SDS depletion while maintaining a high protein yield (>95%). Nonetheless, the initial device required large quantities of both buffer and sample, lacked automation, and limited operation at higher voltages due to Joule heating. This project aims to miniaturize and automate the TME to reduce reagent and sample consumption while minimizing Joule heating.

Method: An applied biosystems capillary electrophoresis (CE) system was modified to perform a TME experiment. The modification included using a 70-micron ID capillary and a union containing a 1/16-inch cut of 3.5kDa dialysis membrane. Additionally, a method was developed where samples containing a mixture of 0.5% SDS and various protein types (BSA, myoglobin, and S. cerevisiae) were injected into the CE system via vacuum at 20mm Hg for 1 minute, consuming only 6 μL of the sample. 20kV was then applied to deplete the SDS, followed by -20kV to collect the sample in a new vial. The system utilized an outlet buffer reservoir with a capacity of 5 mL buffer, a buffer vial containing 3 mL of buffer, 500 μL sample vials. Protein recovery was measured using the BCA method, SDS depletion was measured using MBAS assay, and further verification of SDS-free proteins recovered from the CE system was conducted using LC/MS.

Results: The results demonstrated that with 30 seconds of applying the positive voltage, 92% of the SDS was depleted. The protein recovery was measured to be 72%. The MS analysis indicated the presence of BSA in the new collection vial.

Conclusion: The miniaturized version of TME has enabled the automation of SDS depletion with minimal sample consumption and high throughput.

PP03.201: SP3 vs. SP4: A Practical Comparison of Cost, Handling, and Performance in a Core Facility.

Marc Sylvester, Germany

The SP3 in solution digestion protocol („single-pot solid-phase-enhanced sample preparation", Hughes CS et al., 2014) has been adopted widely in the field of proteomics. It is robust, sensitive, and versatile. Johnston HE et al. (2022) recently described a version of this protocol that replaces the magnetic beads with glass beads (termed „SP4"). The authors described differential protein recovery, especially for membrane proteins. In order to verify the performance and practical aspects of SP4 we adopted the protocol for every-day proteomic work in a core facility setting.

We used various amounts of proteins from cultured C2C12 myoblasts (1 to 300 μg protein input) in order to cover the typical range of samples provided by core facility users. We adjusted the sample buffer to contain 1% SDS and 1% SDC instead of Triton, NP-40, or Tween. Acrylamide was used as alkylating reagent in order to avoid many of the problems seen with iodoacetamide or chloroacetamide. We used a peptide assay to compare yields and an Orbitrap Lumos Mass Spectrometer coupled to nanoflow-UPLC for in-depth proteomic analysis (1 and 100 μg inputs).

The SP4 protocol was easily adaptable, cheaper than SP3, and showed superior peptide yield with all conditions tested. Raw data showed a tendency toward higher complexity and a lower range between average and highly abundant peaks in total ion or base peak chromatograms of SP4 samples. PCA analysis showed similarity of data mostly within each protocol group and reproducible differences between the groups. The results for 1 μg input were remarkable for both protocols. In general, the SP4 protocol led to more protein identifications. Detailed analyses of the data revealed small but reproducible advantages of this protocol with regards to protein diversity without any apparent disadvantages. More details of the differential proteomes will be discussed.

PP03.203: High-Throughput Proteomics at All Chromatographic Flow Rates

Giorgi Tsiklauri, Germany

Efficient peptide separation and sensitive detection are vital in bottom-up proteomics analysis using liquid chromatography-mass spectrometry LC-MS. Therefore, optimizing liquid chromatography system carries a great importance.

In this study, we conducted a comparative analysis of dilution series from HeLa digest 1 ng - 20 µg, utilizing different chromatographic flow rates: 50 µL/min - Micro-flow, 5 µL/min, 1 µL/min - Capillary-flow, and 0.3 µL/min - Nano-flow. Our aim was to determine the optimal flow rates for sample loading based on sample quantity. Historically, Nano-flow systems have been most popular in bottom-up proteomics due to their high sensitivity. However, this sensitivity gain is sometimes redundant depending on the sample amount available to the researcher. Switching to higher flow rate methods offers a solution to avoid compromising system robustness and inefficient use of instrument analysis time caused by long overhead times in Nano-flow systems. This serves as a strong motivation to transition from low to high flow rate methods whenever feasible.

The results revealed that the micro flow system 50 µL/min exhibited excellent peptide separation, high identification rate, and robust performance for samples containing higher than 5 µg peptides. The capillary flow system 5 µL/min achieved a balance between sensitivity and system robustness for samples ranging from 1-5 µg. The 1 µL/min and 0.3 µL/min methods demonstrated superior sensitivity for samples with less than 1 µg peptides, albeit with compromised system robustness and longer overhead times.

This study aims to provide researchers with a comprehensive guideline for selecting optimal flow rates in proteomics experiments, considering sample quantity and available system capabilities. By optimizing chromatographic flow rates, researchers can enhance the efficiency and reliability of their proteomic analyses, leading to deeper insights and a better understanding of complex biological systems.

PP03.205: Preparation of High-Density and Scalable Protein Arrays for Comprehensive Single-Molecule Proteomic Studies

Sheri Wilcox, United States

Introduction: We have created a novel, scalable system with low sample input that allows for single-molecule protein interrogation with a wide dynamic range. This system has two components: a mono-dispersed DNA scaffold with a single protein attachment site and a nanoscale-patterned surface with billions of landing pads. This system creates super-Poisson single-molecule protein arrays that are interrogatable by multi-affinity probes or traditional affinity reagents.
Methods: Lysate is processed through a denaturing workflow followed by functionalization of amines with methyltetrazine, reduction of complexes, and alkylation of cysteines to prevent re-oxidation. The methyltetrazine is subsequently used to conjugate proteins to a DNA scaffold displaying a single trans-cyclooctene (TCO) moiety. In this manner, we efficiently create a population of conjugated scaffolds, each displaying a single protein from the lysate. The scaffold design facilitates the spatial isolation of individual proteins upon deposition onto a hyper-dense nanostructured array. The nanoarray is comprised of DNA binding sites surrounded by HMDS modified interstitial chemistry. To demonstrate single-molecule occupancy of the array, the scaffolds are dye labeled and loaded for imaging. By plotting a histogram of each nanoparticle's intensity, the single-molecule occupancy of the array is calculated.
Results: After processing low micrograms of human cell lysate with the workflow, we observe conjugation of the DNA scaffolds with a diverse set of proteins. The workflow disrupts model protein complexes and does not impair detection by multi-affinity probes. Minimal co-localization of two or more polypeptides is observed. An array loaded with model protein-scaffold conjugates shows approximately 98% single molecule occupancy.
Conclusions: We demonstrate a workflow for the preparation of protein samples that can be deposited on a dense patterned array for single-protein-molecule interrogation with multi-affinity probes. This workflow employs straightforward chemistry to functionalize diverse proteins, disrupt complexes, and conjugate individual proteins to a scaffold bearing a single attachment site.

PP03.207: Shredder: A New Way to Sequence

John Wilson, United States

Introduction
Protein sequencing remains at the heart of proteomics, especially in cases of new species, variants or PTMs, and has traditionally been performed via fragmentation by enzymes, chemicals or, length allowing, by various techniques of fragmentation. Each of these approaches has limitations: for full sequence coverage, often multiple enzymes and/or chemical fragmentations must be combined due to their specificity, lack thereof, or inappropriateness for a particular protein sequence of interest (e.g. submitting the basic tails of histones to tryptic digestion). Gas-phase fragmentation is limited not only by the size and length of peptide introduced, but also by the fragmentation behavior and specifically the lack of fragmentation, often in exactly the region of interest.

Methods
To solve these problems, we developed the Shredder, a new approach to bottom-up proteomics sample preparation that randomly cleaves all along the peptide backbone to yield peptides of widely varying length using a combination of low-specificity active sites and activated residues processed with rapid reaction times. These peptides are overlapping and generate overlapping identifications that verify each others, including the sites and presence of PTMS. The Shredder was applied to single proteins such as BSA and NIST mAb RM8671, Humanized IgG1κ Monoclonal Antibody, as well as other uncomplicated mixes of proteins.

We demonstrate the Shredder providing full sequence coverage in significant depth equivalent to "depth" in genomic sequencing: BSA alone produces >2200 peptides with significant depth of sequence coverage in 30 minutes. We show the Shredder's efficiency compared to multiple enzymatic digestions on both model proteins and those of pharmecutical interest, and subsequently propose possible uses in both academic and industry settings where verified PTM site localization may be essential. The Shredder offers a unique tool to quickly sample an entire protein at all residues, including sites of post-translational modification.

Session Date/Time: Tuesday, September 19, 2023 - 01:15 PM - 02:15 PM

ISS10: New Insights into Analyzing Glycopeptides on Tribrid Mass Spectrometers

13:15   **Speaker**

Nicholas Riley, United States

Session Date/Time: Tuesday, September 19, 2023 - 01:15 PM - 02:15 PM

ISS11: Seminar Supported by Bruker

13:15   **Throughput without compromise in Immunopeptidomics using timsTOF technology combined with EvoSep One**

Anthony Purcell, Australia

13:40   **Maximizing Information Content in Data-independent Acquisition using midia-PASEF**

Stefan Tenzer, Germany

Session Date/Time: Tuesday, September 19, 2023 - 01:15 PM - 02:15 PM

ISS12: A New Era in Proteomics, Introducing Olink Explore HT

PROGRAM

13:15   **Olink® Explore HT – A New Era in Proteomics**
Andrea Ballagi, Sweden

13:35   **Olink® Explore HT – Proven Technology, Validated by Scientists**
Jenny Samskog, Sweden

13:55   **Translating Millions of Datapoints to Actionable Insights**
Per Eriksson, Sweden

Session Date/Time: Tuesday, September 19, 2023 - 01:15 PM - 02:15 PM
ISS13: Disease Risk Visualization by SomaScan® Assay

13:15   **Speaker**
Iwao Waga, Japan

Session Date/Time: Tuesday, September 19, 2023 - 02:30 PM - 03:35 PM
CS17: Standardization and Harmonization

Chair
Sang-Won Lee, Republic of Korea

Chair
Lydie Lane, Switzerland

14:31   **CS17.01: Keynote Speaker - Exploring Public Data Repositories by Integration and Sharing of Proteome Data**
Yasushi Ishihama, Japan

14:56 **CS17.02: IesSDRF Is More: Maximizing The Value Of Proteomics Data Through Streamlined Metadata Annotation**
Tine Claeys, Belgium

Sharing data and resources has revolutionized life sciences, particularly in proteomics, where public data has enabled researchers to reanalyze and reinterpret data in novel ways. However, the lack of comprehensive metadata remains a significant challenge to unlocking the full potential of publicly shared data. In response, the Sample and Data Relationship Format (SDRF) Proteomics was developed, but its complex nature and the need for manual annotation present hurdles for users. This study investigated metadata annotations in 241 proteomics data sets from the PRIDE database and their corresponding publications. The analysis revealed identified major gaps in metadata provision, in the research articles as well as the metadata available in the public repository. Especially when there are discrepancies between both sources, this lack of (structured) metadata accession, makes it impossible to reuse data. To bridge this gap, we have developed lesSDRF, an online, user-friendly Streamlit application. The tool integrates various ontologies and controlled vocabularies, ensuring structured and standardized representation of metadata . With lesSDRF, researchers are guided through the annotation process using the SDRF format, without any of the struggles that come with manual annotation in spreadsheet software. lesSDRF aims to encourage researchers to provide more detailed metadata annotations, leading to greater insights and scientific advances in proteomics. By addressing this issue, we can facilitate more collaborative efforts and enhance our understanding of biological processes. LesSDRF is available via https://compomics-lessdrf-home-2rdf84.streamlit.app/.

15:09 **CS17.03: MassSpecPreppy - Cost-Effective End-To-End Solution for Automated & Flexible Sample Preparation for Proteome Profiling by Mass Spectrometry**
Stephan Michalik, Germany

Background

A versatile protocol and sample processing workflow are important key stones for successful high-throughput proteomics experiments. Here we introduce MassSpecPreppy, an end-to-end solution that provides a cost-effective workflow for high-throughput sample processing, including protein concentration determination and sample digestion up to Evotip loading.

Methods

We used a $20,000 OT-2 liquid handler package (Opentrons) with minor in-house modifications to the magnetic module, which allowed the transfer of a magnetic bead based SP3 sample cleaning and digestion protocol to the robot. We also ported the time-consuming BCA protein concentration determination protocol to the robot. Together with a shiny web application this forms MassSpecPreppy. The shinyApp allows user-friendly selection of experiment-associated parameters, enables the set-up of customized workflows and the OT-2 protocols and deck layouts are adjusted accordingly. To evaluate the performance of MassSpecPreppy in comparison to manual preparation, a BSA standard for protein concentration determination and species mixes (human - yeast - E. coli; HYE) for sample digestion were used.

Results

For the BCA assay, a CV below 5% was observed for the standard curve for manual as well as for OT-2 processed assays. Moreover, the mean recovery of the protein concentration was very comparable with 101.3% (SD: ±7.82%) for OT-2 and 96.3% (SD: ±9.73%) for manual handling.
The results of the sample digest benchmarking revealed a comparable number of protein identifications (OT-2: 9,604; manual: 9,567 protein groups (PG)). Comparison of ratios in the three-species mixes revealed 93.0% of PG for OT-2 digestion and 92.8% of PG for manual digestion passing the significance thresholds.

Conclusion

MassSpecPreppy is a versatile and scalable platform for automated protein concentration determination and digestion of samples resulting in injection ready samples for mass spectrometry and is expected to be a valuable platform for diverse proteomics experiments.

15:22   **CS17.04: Sample Preparation to Match Analytical Advances: 384-well S-Trap plates**

John Wilson, United States

Recent advances in analytical proteomics throughput, in some cases now requiring only minutes per sample to identify and quantify, necessitate concomitant progress in bottom-up sample preparation workflows. With the ability to handle extremely diverse sample types at varied operator skill levels and without the need for protocol modification, the S-Trap sample preparation system has found widespread adaptation in proteomics analyses. To date S-Traps have been available in spin columns of varying capacities and 96-well. To keep pace with advances in detection including ever-increasing throughput and single-cell analyses, we developed and present the new S-Trap 384-well plate suited for protein loads from single cells and sub ug quantities to 100 ug.

384-well S-Traps were manufactured to match the performance of S-Trap micros. Replicate sample preparations were performed on 384-well plates; S-Trap micro columns and 96-well plates were used as a baseline. The standard S-Trap steps were performed as per standard protocol. Well-to-well and plate-to-plate variation were compared based on contaminant removal, extent of recovery, extent of digestion and detection via analysis by LC-MS on a Bruker timsTOF Pro.

384-well S-Trap plates performed consistently with the results of spin columns and 96-well plates. Well-to-well and plate-to-plate variation was in essence equivalent to the reproducibility of replicate technical injections. In a 1 hr digestion at 47 C, an average sample processing speed less than 10 sec/sample could be attained, a speed compatible with or faster than current techniques of detection and quantification. Initial experiments in single-cell analyses indicate that in single cell proteomics, the 384-well plate affords more accurate representation of the underlying biological states by quenching biochemical reactions through direct dispensing of cells into 5% SDS.

Novelty:
Robust sample preparation suited for scales from single cell to 100 ug at an average of < 10 sec/sample.

Session Date/Time: Tuesday, September 19, 2023 - 02:30 PM - 03:35 PM

CS18: Glycoproteomics

Chair

Nicolle Packer, Australia

Chair

Morten Thaysen-andersen, Australia

14:31   **CS18.01: Keynote Speaker - Small but Giant Leaps towards Neuroglycomics: Spatial and Temporal Diversity of Glycome Expression in Mammalian Brain**

Hyun Joo An, Republic of Korea

14:56   **CS18.02: GlycopeptideAtlas: A Comprehensive Database and Analysis Tools for Glycoproteomic Characterization in Disease Studies**

Yingwei Hu, United States

Introduction: Protein glycosylation plays a pivotal biological function, which is underscored by the characterization of the intact glycopeptides (IGPs) presented in different tissues in elucidating protein glycosylation alterations and associated functions. With the advancement of mass spectrometry technology, a significant amount of glycoproteomic data has been generated, contributing to the understanding of the roles of glycosylation in disease status. However, it is worth noting the noticeable gap in accessible glycoproteomic data across diverse diseases. Addressing this gap by establishing comprehensive and up-to-date databases for IGPs would greatly facilitate research on protein glycosylation and its implications in disease states, ultimately leading to improved diagnostics and targeted therapies.

Methods: We addressed this gap by developing a database built on a consensus spectral library encompassing 90,795 glycopeptides from 2,195 glycoproteins. This library is generated from the tandem mass spectrometric analysis of ten cohorts from the Clinical Proteomic Tumor Analysis Consortium (CPTAC). We have further enriched this database by integrating knowledge from the existing and the newly developed tools for deep glycoproteomic investigations, which cover differential expression, IGP-based subtyping, survival analysis, post-translational modification (PTM) cross talk, and pan-cancer glycogene analysis.

Results: Our consensus library forms the backbone of the GlycopeptideAtlas, an online database committed to storing, analyzing, and investigating glycoproteomic data. We continuously update the GlycopeptideAtlas with new glycoproteomic data submissions, promoting a more exhaustive characterization of glycopeptides. We have validated the effectiveness of our tools and the database through a study on Pancreatic ductal adenocarcinoma (PDAC), where the findings present the potential of IGPs as reliable markers for cancer and its subtypes.

Conclusion: The GlycopeptideAtlas database represents a resource for researchers examining the role of protein glycosylation in relation to cancer or other disease phenotypes.

See you in Busan!
BEXCO, Busan, Korea
🌐 2023.hupo.org
🐦 f 📷 in #HUPO2023

Together Through Proteomics

PROGRAM

15:09   **CS18.03: Deep Quantitative Glycoproteomics Reveals Gut Microbiome Induced Remodeling of the Brain Glycoproteome**
Clement Potel, Germany

Introduction

Protein glycosylation is a highly diverse post-translational modification, modulating key cellular processes such as cell signaling, adhesion and cell-cell interactions. Its deregulation has been associated with various pathologies, including cancer and neurological diseases. Methods capable of quantifying glycosylation dynamics are essential to start unraveling the biological functions of protein glycosylation but are currently lacking.

Methods

Here we present Deep Quantitative Glycoprofiling (DQGlyco), a method that combines high-throughput and cost-effective sample preparation and glycopeptides enrichment, high-sensitivity detection, and precise multiplexed quantification of protein glycosylation.

Results

We used DQGlyco to profile the mouse brain glycoproteome, in which we quantified more than 50,000 unique glycopeptides per experiment, and identified in total 158,972 unique glycopeptides across all experiments. This amounts to 25-fold more glycopeptides identified compared to previous studies. We observed extensive heterogeneity of glycoforms and determined their functional and structural preferences. The presence of a defined gut microbiota resulted in extensive remodeling of the brain glycoproteome when compared to that of germ-free animals, particularly on proteins involved in neurotransmission and axon guidance, for which glycosylation has been shown to regulate activity. Finally, we show that glycosylation regulation is site-specific, suggesting complex post-translational mechanisms of regulation.

Conclusions

Alteration of the gut microbiome composition has been associated with behavioral changes and has been shown to impact brain development and function via nervous or chemical signaling along the gut-brain axis. However, underlying molecular mechanisms remain poorly characterized. This study exemplifies how the gut microbiome may affect brain protein functions. Moreover, this work constitutes a framework to address many unanswered questions, such as the kinetics of glycosylation changes in response to perturbations, or the impact of glycosylation microheterogeneity modulation on protein function.

15:22 **CS18.04: Distinctive Antibody Glycosylation Profiles in ESRD Patients with Divergent Immune Responses to COVID-19 Vaccination**
Yun-Jung Yang, Taiwan

Background: The COVID-19 pandemic has prompted the rapid development and distribution of vaccines worldwide. However, immunocompromised individuals, particularly those with end-stage renal disease (ESRD), often exhibit attenuated immune responses, including reduced antibody production, even after vaccination. Glycosylation of antibodies can modulate antibody functions and significantly impact their efficacy. Therefore, investigating antibody glycosylation profiles in ESRD patients with varying antibody levels after vaccination is crucial to understand the potential underlying mechanisms contributing to the heterogeneity of vaccine responses in this population.
Methods: Our study involved 66 ESRD patients, including 20 responders with high antibody levels, 26 non-responders with low antibody levels at 10 weeks after AstraZeneca vaccination, and 20 unvaccinated controls. Serum samples were prepared and subjected to LC-MS/MS analysis using multiple reaction monitoring (MRM) mode. We comprehensively analyzed three antibody isotypes: IgG, IgA, and IgM, six antibody subclasses, 10 Fc N-glycosylation sites, 49 glycosylation traits, and 105 glycopeptide analytes.
Results: We observed significant differences in six glycosylation traits between responders and non-responders. The responders exhibited higher trigalactosylation and sialylation of IgA1/2 N340/327, as well as increased fucosylation and sialylation of IgM. Conversely, the non-responders displayed higher digalactosylation of IgA1/2 N340/327 and high-mannose glycans of IgM. Notably, we found a positive correlation between IgM sialylation and the antibody levels in non-responders. Furthermore, using 10 glycopeptides that demonstrated significant differences between the two groups, we achieved a promising classification performance with an area under the ROC curve (AUC) of 0.82. These findings suggest antibody glycosylation profiles as biomarkers for assessing variations in vaccine response.
Conclusions: Our study provides valuable insights into the functional attributes of divergent antibody responses following COVID-19 vaccination. Our findings contribute to a better understanding of the unique immunological challenges faced by ESRD patients and may help guide future research and interventions to enhance vaccine responses in this population.

Session Date/Time: Tuesday, September 19, 2023 - 02:30 PM - 03:35 PM

CS19: Microbial Proteomics, Microbiome and Metaproteomics

Chair
Tim Van Den Bossche, Belgium

Chair
Uwe Völker, Germany

14:31 **CS19.01: Keynote Speaker - Proteomics of One Health**
Jennifer Geddes-McAlister, Canada

PROGRAM

14:56 **CS19.02: The Acetylome of Campylobacter jejuni Shows Lysine Acetylation of the CadF Adhesin Regulates Host Cell Binding**

Stuart Cordwell, Australia

Campylobacter jejuni is a Gram negative bacterium that is responsible for the majority of food- and water-borne gastroenteritis cases in humans. The molecular mechanisms underpinning C. jejuni human virulence however, remain poorly understood. Lysine acetylation (KAc) is a reversible post-translational modification (PTM) that is involved in transcriptional regulation in eukaryotes, however specific functions for KAc are only recently becoming clear in bacteria. In this study, acetyl-lysine immunoprecipitation and LC-MS/MS identified 5567 acetylated lysines on 1026 C. jejuni proteins (~63% of the C. jejuni proteome), showing that KAc is a widespread PTM in this organism. Acetylated proteins are enriched for major functions in metabolism, transcription and translation, and the stress response. KAc was identified on proteins from all subcellular locations, including outer membrane proteins (OMPs). Tandem Mass Tag (TMT) labelling and LC-MS/MS quantified acetylated peptides and KAc sites following growth in 0.1% sodium deoxycholate (DOC, a component of gut bile salts). 3571 acetylated peptides were quantified and 761 (409 proteins) were statistically differentially abundant following DOC growth. KAc changes involved proteins from many functional pathways suggesting a role for this PTM in regulating survival upon bile exposure. As observed in other bacteria, KAc in C. jejuni was primarily non-enzymatically mediated via acetyl-phosphate (AcP); however, reduced abundance of the SIRT-like deacetylase CobB also contributes to elevated KAc in DOC. We identified many acetylated OMPs and altered DOC abundance of acetylated peptides in the major fibronectin (Fn)-binding adhesin CadF. Changes in CadF KAc correlated with the production of processed variants that retain Fn binding but lose immunogenicity. Furthermore, in vitro acetylation of recombinant CadF with AcP reduced Fn binding in a dose-dependent manner, showing KAc can influence host binding. This study generated the first system-wide analysis of the C. jejuni acetylome and furthers our understanding of KAc as an emerging PTM in bacteria.

15:09 **CS19.03: Untargeted Data-Independent Acquisition for Metaproteomics of Complex Microbial Samples**

Laura Elo, Finland

Mass spectrometry-based metaproteomics is a relatively new field of research that enables characterization of the functionality of microbiota. We were recently the first to show that data-independent acquisition (DIA) mass spectrometry can be successfully applied to analyze complex metaproteomics samples by using a spectral library constructed from corresponding data-dependent acquisition (DDA) data. This allowed us to circumvent many limitations of the previously used fully DDA-based approach, especially the limited reproducibility when analyzing samples with complex microbial composition.

However, the requirement for having a DDA-based spectral library remained a drawback as it 1) consumes sample material, 2) may not represent well the content of all samples, and 3) still brings the DDA-related limitations of peptide identification to DIA, as only peptides present in the library can be detected. To overcome these limitations, we demonstrate here the utility of an untargeted DIA metaproteomics tool that does not require any DDA data, but instead generates a pseudospectral library directly from the DIA data, reducing the required mass spectrometry analysis to a single DIA run per sample.

To illustrate the feasibility and benefits of the DIA-only approach, we applied it to a laboratory-assembled microbial mixture containing twelve different bacterial strains and to human fecal samples from healthy donors. Our findings demonstrated the benefits of the new DIA-only metaproteomics approach in terms of high reproducibility and the ability to reveal individual-specific microbial functional profiles beyond existing methods.

To make the approach widely accessible, we have developed an open-source software package glaDIAtor, which is freely available at DockerHub (elolab/gladiator). Notably, glaDIAtor is implemented using container technology, which enables easy installation on multiple platforms, including support for server and workstation deployments. The tool also includes a modern web-based graphical user interface that simplifies the data analysis, facilitating its wide use by the community.

![HUPO BUSAN 2023 logo]

See you in Busan!
BEXCO, Busan, Korea
🌐 2023.hupo.org
🐦 📘 📷 in #HUPO2023

Together Through Proteomics

PROGRAM

15:22 **CS19.04: metaExpertPro: A Computational Workflow for Data-Independent Acquisition Mass Spectrometry-Based Metaproteomics Data Analysis**
Yingying Sun, China

Analysis of mass spectrometry (MS)-based metaproteomic data, in particular data-independent acquisition MS (DIA-MS) data, remains a great computational challenge. Here, we established a computational pipeline called metaExpertPro for effective analysis of DIA-based, including diaPASEF, metaproteomic data. It provides a complete pipeline for peptide and protein measurement, functional and taxonomic annotation, and generation of quantitative matrices for both microbiota and hosts. With ~15 min DIA data analysis, metaExpertPro can measure ~50,000 peptides and ~10,000 protein groups per human fecal sample using 60 min DIA-MS acquisition. Importantly, metaExpertPro maintains a low factual FDR (< 4%) of protein groups and delivers accurate estimation of diversity and relative abundance at the genus level in benchmark tests. Additionally, the quantitative results at the protein, taxonomy, and function levels exhibit high reproducibility and consistency across different public databases. Also, metaExpertPro-based metaproteomic analysis on dyslipidemia patients revealed characteristic alterations of microbial functions and potential interactions between the microbiota and the host. Together, metaExpertPro offers an effective computational method for analyzing DIA-based metaproteomics data.

Session Date/Time: Tuesday, September 19, 2023 - 02:30 PM - 03:35 PM
MS02: Mentoring Session 2 - Promoting Your Science

Chair
Daniel Garama, Australia

Chair
Mathieu Lavallée-Adam, Canada

MS02.01: Panelist
Alexey Nesvizhskii, United States

MS02.02: Panelist
Hyun Woo Park, Republic of Korea

MS02.03: Panelist
Stephen Pennington, Ireland

MS02.04: Panelist
Paula Burton, Australia

Session Date/Time: Tuesday, September 19, 2023 - 03:35 PM - 04:30 PM
PP03: Even Numbered Posters - Poster Viewing with Coffee Break

PP03.02: PandaNovo: An Efficient and Accurate Transformer-Based Model for de Novo Peptide Sequencing

Cheng Chang, China

Background
As the mainstream high-throughput method to identify protein sequences, tandem mass spectrometry (MS) plays an important role in proteomics research by generating mass spectra (MS1, MS2) and then analyzing the corresponding peptide sequences. However, current popular methods based on database search methodology are limited by the reference protein sequence database and cannot identify the protein sequences outside of the database, making them unsuitable for specific research, such as neoantigen discovery, antibody design, and vaccine development. De novo sequencing is a promising method to address the above shortcomings, but the current de novo sequencing methods cannot be industrialized due to its low accuracy and sensitivity. Therefore, improving the performance of the de novo sequencing model has become a challenging and important goal for the community.

Methods
A novel concept of the complementary spectrum is used to enhance the information of MS2, which has been proved useful in database search. In addition, we design suitable encoders to encode the experimental spectrum and complementary spectrum. Here, we propose PandaNovo, an efficient and accurate model for de novo peptide sequencing based on the Transformer architecture.

Results
A series of comparative experiments are applied to compare PandaNovo's performance with existing state-of-the-art de novo sequencing models (including DeepNovo, PointNovo and Casanovo). PandaNovo achieves the best performance in all the datasets. Furthermore, we trained PandaNovo with a larger dataset (4.3M PSMs) and observed that it exhibited stronger interpretation ability, even for the never-before-seen peptide sequences. Our results provide new perspectives for de novo sequencing.

Conclusions of the study
PandaNovo achieves the best performance as compared to existing state-of-the-art de novo sequencing models and takes de novo peptide sequencing a giant step forward.

PP03.04: Harnessing Machine Learning to Correct Peptides Intensity Heterogeneity and Enhance Mass Spectrometry Data Analysis

Daniel Hornburg, United States

The intensities of peptides measured by mass spectrometry (MS) instrument depend on the abundance of their source proteins, but also on the biophysical properties of individual peptides and complex signal processing by the MS software. This results in sequence-specific deviation of MS intensities from the original abundance and limits the direct comparison of absolute intensities between different peptides or proteins, as well as cross-platform integration.
Here we introduce PepCalibri, a novel deep learning-based method geared towards reconstructing absolute abundances by calibrating MS intensities of each peptide.
PepCalibri is implemented on top of the PyTorch framework, using AlphaBase for encoding sequences and post-translational modifications (PTMs). The model is based on the state-of-the-art transformer deep learning architecture leveraged by, e.g., ChatGPT and Google Translate. As input, the model receives the peptide sequence, including PTMs, and its MS identification patterns. The model returns the predicted intensity correction factor for each potential peptide charge state. To train the model, we used MS datasets comprised of human blood plasma samples processed with Seer Proteograph™ workflow and utilizing Seer's proprietary nanoparticles to increase proteins coverage and diversify their concentrations.
In explaining peptides intensity variation, PepCalibri achieved an R^2 of 74% on the validation data, an improvement from R^2 of 21% for the model that assumes peptide intensities only depend on protein group abundance. By visualizing the latent model variables that define intensity correction factors, we confirmed that these corrections are indeed based on biophysical properties of the peptides. Further exploration of these factors could help fine-tuning proteomic workflows.
By calibrating peptide intensities, we aim to provide actual protein concentrations for the biologists and clinicians, enhance inference of proteoforms, improve estimation of PTM occupancy, and allow combining intensities of variant peptides to amplify biological signal in and across large cohort studies.

PP03.06: Transfer Learning Model Based Nt-Arginylome Analysis Reveals Organelle Specific Novel Arg/N-degrons

Shinyeong Ju, Republic of Korea

N-terminal arginylation is best known not only as a degradation signaling modification for a single protein but also as a signal for macro-scale autophagic events. High cytosolic concentrations of the Nt-arginylated proteins could activate autophagic flux; however, accountable proteins of Nt-arginylation are yet understudied on a large scale, in part due to their extreme scarcity. Here, we constructed a deep-learning algorithm-assisted N-terminal peptide discovery workflow to identify Nt-arginylated peptides with the highest confidence, which is corroborated by both a diagnostic ion presence and a retention prediction with a transfer learning model that was trained by abundant non-endogenous Nt-arginylated peptides. With this strategy, we confidently determined 119 Nt-arginylated proteins, including previously known Nt-arginylated proteins. The Nt-arginylome landscape we unveiled was enriched with a combination of an ER-specific stress inducer, thapsigargin, and a proteasome inhibitor, MG132. By conducting a bioinformatics assessment of the Nt-arginylome landscape, we discovered Nt-arginylated proteins that were in various subcellular organelles, especially mitochondria. The discovered Nt-arginylated mitochondrial proteins were modified not just after the internal mid-chain site but also at the transit peptide cleavage site, which must be excreted after transportation, not during ER-related maturation. Using the Nt-arginylation-specific binding ZZ-domain-containing molecule, R-catcher, we further validated proteins of the Nt-arginylome landscape, suggesting a novel role of Nt-arginylation as an organelle quality-related signal. This improved Nt-arginylation identification approach would facilitate the detection of rare protein modifications using terminal peptide enrichment.

PP03.08: A Novel Denovo Model based on Transformer for Proteomics Research

Ziyi Li, China

The identification of proteins in proteomics is primarily achieved through the use of sequence databases. However, this standard methodology has proven insufficient in the face of the increasing prevalence of unknown sequences that arise during tumor and biopharmaceutical research and development. To address this issue, the AI field has developed several denovo algorithms, including DeepNovo and pNovo, that rely on deep neural networks. Despite these advancements, only a few methods have been developed based on the transformer framework. In this study, we present a novel algorithm based on the decoder module that utilizes MS2 for the prediction of amino acid sequences.

Our model effectively captures the positional relationship between different amino acid fragments by employing the attention module in the Transformer. This was achieved by inputting both positional and MS2 information, resulting in the successful acquisition of amino acid fragment spectrum information. In the decoder section, we utilized a coding scheme analogous to natural language processing, representing the 20 types of amino acids and their various ion forms. To address the variable lengths of amino acid sequences, start and end coding were included. To ensure the accuracy of our predicted results, we used the predicted amino acid's analytical quantity and spectral molecular weight to calculate ppm. This was combined with the likelihood of amino acid identification at a given location to produce a probability distribution for each position. By applying this filtering technique, we were able to maintain a high level of precision in the final predicted sequence.

The trained model files were then used for predicting new data, whereby only a mgf file was required for input. In the same sample set, Peaks detected 3634 peptides, while our model detected 3795 peptides with ppm<25. Approximately 61.2% of the detected peptides were found to be consistent across the two methods.

PP03.10: DeepMRM: Deep Learning-Based Object Detection Model for Targeted Proteomics Data and Its Extension to Untargeted Proteomics Data

Jungkap Park, Republic of Korea

Background

In clinical proteomics, targeted proteomics approaches often require manual data interpretation, which limits its transferability, reproducibility, and scalability. Here, we present DeepMRM, a software package leveraging deep learning for object detection, which can substantially reduce the manual inspection burden, even for noisy and complex data.

Methods

DeepMRM employs two neural network models: a peak detection model for identifying peak groups of targeted peptides and a transition classification model for selecting interference-free transitions. The peak detection model reframes the task as an object detection problem, detecting peak group instances within 1-D chromatograms, similar to traditional object detection models that identify instances of a specific class within a 2-D image. The transition classification model utilizes one-shot learning, training on non-interfered transitions and subsequently identifying quantitative transitions.

Results

The performance of DeepMRM was benchmarked against Skyline and validated using internal and public datasets generated by different acquisition methods. DeepMRM outperformed Skyline in quantification accuracy and demonstrated average precision (AP) of 98-99% and correlation coefficients of 0.96-0.99 across MRM, PRM, and DIA data. Furthermore, we extended DeepMRM with a new scoring model to identify peptides in untargeted DIA data, where convolutional features associated with candidate peak groups proved effective in scoring peptide matches. DeepMRM is packaged as a Windows desktop application and integrated into Skyline software as an external tool.

Conclusions

DeepMRM is a robust and highly accurate peak detection model designed for interpreting targeted proteomics data, which can be expanded for identifying peptides in untargeted proteomics data. Its deployment significantly enhances the interpretation of targeted proteomics data, enabling more efficient and reliable analysis in clinical proteomics studies.

PP03.12: Data Mining Antibody Sequences for Database Searching in Proteomics Data

Xuan-Tung Trinh, Denmark

Mass spectrometry-based proteomics is an important method for identifying and quantifying antibodies but suffers from challenges due to their vast variety. Common bottom-up proteomics approaches rely on database searches comparing experimental values of peptides to theoretical values derived from protein sequences in a database. Increasing the database size to millions of entries inflates the search space leading to very long search times and considerable difficulties with controlling the false discovery rate. This impedes antibody detection in complex samples like human plasma. Recent genomic studies have compiled millions of human antibody sequences publicly accessible through the Observed Antibody Space (OAS) database. However, these data have yet to be exploited to confirm these antibodies in human samples. In this study, we employed the extensive collection of antibody sequences available in the OAS database for conducting database searches in MS-based proteomics. After collecting 30.96 million heavy antibody sequences of 146 SARS-COV-2 patients from the OAS database, we performed in silico digestion (trypsin) and removed the peptides overlapping with reviewed UniProt human proteome (canonical and isoform) to obtain 18.42 million unique peptides. We then extracted a portion of the most common OAS peptides (i.e., peptides that appear in most of the antibodies) and combined them with UniProt human proteome (2023 March) and cRAP (a database of contaminant proteins) to form databases. We conducted tests using those databases and publicly available SARS-COV-2 human plasma samples in the proteomics identification database (PRIDE). To avoid false positives of antibody peptide identification, we tested the database search against negative controls (brain samples) and used different database sizes containing 100, 10000, and 1000000 OAS peptides. We expect that the newly discovered antibody peptides in the SARS-COV-2 samples can be further employed to develop therapeutic antibodies. The method will be broadly applicable to find characteristic antibodies for other diseases.

PP03.14: A Complete Software Suite for de Novo Sequencing and Predicting the Immunogenicity of Class 1 and Class 2 Neoantigens

Lei Xin, Canada

Background
Tumor-specific neoantigens are only expressed on the surface of cancer cells and represent ideal targets for the immune system to distinguish cancer cells from normal cells. Identifying neoantigens and predicting their T cell responses are essential for cancer immunotherapy and vaccine design.
Methods
We present DeepNovo-HLA, a collection of deep learning models for de novo sequencing of HLA-I and HLA-II peptides from MS-based immunopeptidomics data. The models were trained on a large, carefully curated dataset from over 3,000 MS runs, containing ~700K peptides and ~90 alleles for each HLA class . The models were also fine-tuned specifically for Orbitrap and timsTOF data to provide the best performance on each instrument. Once candidate neoantigens are identified, we further use DeepImmun, a personalized machine learning model to predict the T cell response of each individual patient to his/her neoantigens. DeepImmun models the central tolerance of T cells of each individual patient by considering his/her HLA self peptides as negative selection and immunogenic T cell epitopes as positive selection.
Results
We tested DeepNovo-HLA and DeepImmun on the MS-based immunopeptidomics datasets of ten cancer patients. DeepNovo-HLA identified ~50% more HLA peptides than existing immunopeptidomics search engines. More importantly, the number of novel HLA peptides that were not in the canonical protein database and represented the main source of neoantigens were increased by more than two folds. DeepImmun was further used to predict the immunogenicity of the novel HLA peptides and the predictions were validated against the T cell assay results of four patients. Our tool was able to rank 14 of 19 immunogenic neoantigens within its top 30% predictions, with 3-4 immunogenic neoantigens per patient.
Conclusions
DeepNovo-HLA and DeepImmun together form a complete software suite to identify and prioritize candidate neoantigens, which are essential for cancer immunotherapy and vaccine design.

PP03.16: MSFragger-WWA Coupled With Fragpipe Enables Fast and Easy Wide-Window Acquisition Data Analysis

Fengchao Yu, United States

The rapid evolution of data acquisition protocols poses unique challenges to peptide identification and quantification, especially in the field of single-cell proteomics. Recently, people proposed to use wide-isolation window coupled with data-dependent acquisition (DDA) for single-cell mass-spectrometry (MS) data collecting, while the progression of data analysis tools has not kept pace. There are a limited number of tools equipped to handle data from wide-window acquisition (WWA), with most facing limitations such as the need for cloud computing, which raises the concerns about data security and privacy. Furthermore, some commercial tools are not free to the research community, which added burdens to most laboratories.

To address these issues, we present MSFragger-WWA, a tool designed to facilitate peptide detection from WWA data. This tool, working together with our MS1-based quantification tool, IonQuant, is capable of efficiently identifying and quantifying co-eluted peptides resulting from wide isolation windows on a local desktop. These enhancements are part of the latest version of FragPipe, an easy-to-use proteomics data analysis suite. Our preliminary experiments show that MSFragger-WWA and IonQuant coupled with FragPipe have outstanding performance compared to state-of-the-art tools. This solution offers an accessible, cost-effective alternative due to local computing and being free of charge. Furthermore, given that peptide co-fragmentation also occurs in traditional DDA, we can utilize the analogous algorithms for peptide detection from Chimeric spectra, thereby enhancing both sensitivity and accuracy. Together, the MSFragger and FragPipe ecosystem are emerging as a leading solution for bottom-up proteomics analysis, accommodating a variety of applications. Its unmatched performance and user-friendly features have made it the go-to choice for laboratories and companies worldwide.

PP03.18: DeepFLR Facilitates False Localization Rate Control in Phosphoproteomics

Yu Zong, China

Protein phosphorylation is one of the most important post-translational modifications affecting the function of proteins and regulating many cellular processes. It is highly important to identify and quantify protein phosphosites accurately at the proteome-wide level. However, it is still a challenge of accurate phosphosites localization. Herein, we propose DeepFLR, a deep learning-based framework for false localization rate (FLR) control in phosphoproteomics. The framework is composed of a tandem mass spectrum (MS/MS) prediction module for phosphopeptides and an FLR assessment module based on a target-decoy approach. The MS/MS prediction module was developed based on bidirectional encoder representations from Transformers (BERT) trained using >467,000 MS/MS of >184,000 phosphopeptides and >165,000 non-phosphopeptides. Compared to existing tools for phosphopeptide MS/MS prediction, DeepFLR improves the prediction accuracy and can predict MS/MS for phosphopeptides at the quality close to experimental replication. Decoys for phosphopeptides are generated by randomly exchanging the phosphorylated amino acid residue with another amino acid residue in the sequence. MS/MS of both target and decoy phosphopeptides are predicted by DeepFLR and compared to the experimental spectra for FLR assessment. We demonstrated that DeepFLR can estimate FLR accurately using synthetic phosphopeptides datasets. The method was further applied to biological samples, and it was found that DeepFLR can localize more phosphosites than the probability-based phosphosite localization tools, and hence gain additional phosphorylation information for biological insights. DeepFLR is capable of handling datasets of different instrument types or organisms, performing analysis in combination with various database searching tools, and identifying both monophosphopeptides and multiphosphopeptides. Furthermore, DeepFLR can assist in phosphoproteomics data analysis by both data-dependent acquisition (DDA) and data-independent acquisition (DIA) approaches. We anticipate that DeepFLR can be a key metric for FLR estimation just as the target-decoy measure for FDR in peptide identification, and will expand the current toolbox for phosphoproteomics.

PP03.20: Omics Approaches to Right Ventricular Maladaptive Hypertrophy in Chronic Pulmonary Hypertension

Emilio Camafeita, Spain

The pathophysiological mechanisms that lead to right ventricular (RV) dysfunction in pulmonary hypertension (PH) are not yet fully understood, and therefore this condition lacks specific treatment. Pressure overload alone fails to explain the RV dysfunction associated with HP, where only a fraction of patients develop a maladaptative RV hypertrophy.

To investigate whether physiopathological factors triggered by the injured pulmonary vascular bed could account for the maladaptive RV response in PH, we have resorted to four different Yucatan pig models of RV pressure overload: chronic postcapillary PH by pulmonary vein banding (M1, n = 8); chronic PH by aorto-pulmonary shunting (M2, n = 6); RV pressure overload by pulmonary artery banding (hence without PH) (M3, n = 10); and a sham procedure (M0, n = 9). The animals were subjected to hemodynamics, imaging, histopathology evaluation, and blood withdrawal at different post-surgery times.

The plasma samples were analyzed by mass spectrometry (MS)-based proteomics and metabolomics. To ascertain differences across the different models, proteomics and metabolomics data were subjected to statistical analysis using the SanXoT software package and in-house built scripts run on Matlab, respectively. Pearson correlation coefficients were calculated between hemodynamic and imaging parameters and protein, lipid and metabolite measurements. Correlation networks between hemodynamic and imaging parameters and proteins, lipids and metabolites from M1, M2 and M0 were built with Cytoscape. This integrative analysis of clinical and omics outcomes revealed that the maladaptative RV hypertrophy associated with PH is linked to i) the alteration of energy metabolism and ii) increased oxidative stress and inflammation. Our findings open up new possibilities for the development of treatment for PH patients.

PROGRAM

PP03.22: Mitochondrial Creatine Kinase Rescues the Heart from Ischemic Injury

Maria Victoria Faith Garcia, Republic of Korea

Heart failure due to ischemic cardiomyopathy (ICM) is considered as one of the main causes of cardiovascular disease-related deaths worldwide. Patient mortality due to ICM still remains high. Ischemic preconditioning (IPC) has been found as an effective mitigator of ICM. This is done by short-time ischemia is applied before ischemia/reperfusion injury (I/R). The brief time applied for IPC may contribute to a rapid change in protein expression and regulation wherein protein function modulation by post-translational modifications is important. Mitochondria play a significant role in heart disease progression and so, it is a good target for ICM treatment. In this study, we focused on mitochondrial creatine kinase (CKMT2) under I/R injury. Ex vivo Langendorff system on Sprague-Dawley rat hearts were used to simulate normal perfusion, I/R, and IPC condition and used for phosphoproteomic analyses. In vitro study using human cardiomyocyte AC16 cells were used to determine the cardioprotective role of mitochondrial creatine kinase through overexpression and how CKMT2 site-directed mutagenesis can affect cardioprotection by CKMT2 protein activity, mitochondrial function, and protein expression. CKMT2 was dephosphorylated during ischemia and I/R but remained phosphorylated under IPC conditions. CKMT2 overexpression show increased cell viability and mitochondrial ATP level against hypoxia/reoxygenation confirming the cardioprotective effect of CKMT2. Conversely, there was decreased cell viability and increased ROS production during H/R when CKMT2 is phosphomutated, specifically in Y368. We also confirmed increased mitochondrial function via the proliferator-activated receptor γ coactivator-1α/estrogen-related receptor-α pathway during CKMT2 overexpression. CKMT2 regulation and phosphorylation may be used for future ICM therapeutics.

PP03.24: MDH2 Phosphorylation Renders Cardioprotection Against Hypoxia/Reoxygenation Injury

Jubert Marquez, Republic of Korea

Mitochondrial malate dehydrogenase (MDH2) regulates the conversion of malate/NAD+ to oxaloacetate/NADH. Proteomic studies show that MDH2 is involved in cardiovascular diseases wherein the expression of certain mitochondrial proteins were altered under ischemia/reperfusion conditions. Screening of phosphorylation sites of MDH2 revealed several posttranslational modifications occurring in the protein. However, it is still unclear how modifications of these sites lead to the altered activity and function of the system under pathologic models. This study focuses on the importance of MDH2 phosphorylation and how it regulates mitochondrial biogenesis and mitochondrial function. Phosphorylation occurs at various conserved sites, specifically Y56, Y80, Y161, and S246. Phosphorylation mutants based on these sites were manufactured using expression vectors and were transfected into cellular models for analysis. Phosphomutants decreased cell viability, ATP production and MDH2 activity. Mtochondrial biogenesis marker expressions were also altered by phosphomutants under hypoxia/reoxygenation conditions. The protective mechanism how MDH2 confers cardioprotection remains unclear. Further studies on the role of MDH2 phosphorylation in mitochondrial biogenesis can elucidate the importance of MDH2 in cardiovascular disease models and treatment modalities

PP03.26: Towards Deciphering the Molecular Signature Particular for Peripartum Cardiomyopathy Through a Data-Driven Multi-Omics Strategy

Carolin Sailer, Denmark

Peripartum cardiomyopathy (PPCM) is a rare form of acute heart failure affecting young women toward the end of pregnancy or in the early postpartum period. The diverse and non-specific symptoms make it a challenge for healthcare providers to diagnose the disease in an early stage and initiate prompt treatment. On the one hand, PPCM symptoms may overlap with non-pathological peripartum-associated physiological discomfort and on the other hand with more severe conditions such as dilated cardiomyopathy (DCM). Currently, little is known of how (and if) the molecular remodelling that drives the pathology differs between PPCM and DCM. To that end, we measured and compared the protein abundance profiles of hearts from women with end-stage heart failure (HF) to those of donor hearts. The HF cohort was split in females with PPCM and with DCM. We applied state-of-the-art TMT labelling in combination with high-resolution mass spectrometry-based proteomics which allowed us to quantify differences in the cardiac protein profiles across more than seven thousand proteins in PPCM, DCM and donor hearts. We observed significant regulation of canonical markers of end-stage heart failure in PPCM and DCM hearts, highlighting overall remodelling similarities. Further analysis and intersection with an independent transcriptomics dataset suggested a set of candidate proteins which are uniquely regulated in PPCM. To confirm the findings, we expanded the patient cohort and investigate the heart samples using quantitative proteomics, spatial transcriptomics and single nucleus RNA sequencing to gain deeper insights of the molecular remodelling distinct for hearts of PPCM patients.

PP03.28: Effects of Rice Germ on Chronic Unpredictable Mild Stress-Induced Depressive-like Behavior: Alleviation through Neuroinflammation Reduction

Sosorburam Batsukh, Republic of Korea

Chronic unpredictable mild stress (CUMS) is a known contributor to the development of depressive-like behavior, with neuroinflammation playing a crucial role in this process. This study aimed to investigate the potential of rice germ, enriched with 30% gamma-aminobutyric acid (GABA) (RG), in alleviating CUMS-induced depressive-like behavior through the reduction of neuroinflammation. Mice subjected to CUMS were administered different doses of RG (40, 90, and 140 mg/kg), and various parameters related to neuro-inflammation and depressive-like behavior were assessed.

Male mice were exposed to CUMS for a specified period, and RG was administered orally at different doses. Pro-inflammatory cytokines (TNF-α and IL-6) were measured in the serum and hypothalamus. M1-type microglia markers (CD86 and NF-κB) and components of the NLRP3 inflammasome complex (NLRP3, apoptosis-associated speck-like protein containing a caspase recruitment domain, and caspase-1) were evaluated in the hypothalamus. Additionally, the levels of IL-1β and IL-18 were measured. Depressive-like behavior was assessed using the forced swim test and tail suspension test.

CUMS led to elevated levels of TNF-α and IL-6 in the serum and hypothalamus, indicating increased inflammation. However, RG administration significantly reduced the levels of these pro-inflammatory cytokines. CUMS also induced M1-type microglia polarization, as evidenced by the upregulation of CD86 and NF-κB, whereas RG treatment attenuated their expression. Furthermore, CUMS increased the expression of components of the NLRP3 inflammasome complex, IL-1β, and IL-18 in the hypothalamus, which were markedly decreased following RG administration. Importantly, RG effectively mitigated depressive-like behavior in CUMS mice.

These findings demonstrate that RG supplementation attenuates neuroinflammation by reducing pro-inflammatory cytokines, modulating M1-type microglia polarization, and suppressing the NLRP3 inflammasome pathway. Moreover, RG administration alleviated depressive-like behavior in CUMS mice. These results suggest that rice germ, with its GABA content, holds promise as a potential therapeutic agent for reducing neuroinflammation and managing depression associated with chronic stress.

PP03.30: Comprehensive Characterization of Protein Turnover by Comparative SILAC Labeling Analysis in 3T3-L1 Adipocytes

Sunkyu Choi, Qatar

Background - A balance between the synthesis and degradation of proteins is referred to as protein turnover, which is crucial for cellular protein homeostasis. Proteome-wide analysis of protein turnover in adipocytes, which are well-known for their role in energy storage and their link to obesity and metabolism disorders, are yet to be conducted.

Method - Our investigation utilized a comparative analysis of time-dependent SILAC labeling to assess protein turnover in 3T3-L1 adipocytes, spanning a period of 0 to 144 hours. Half-life of each protein that was identified by MaxQuant search was calculated using equations adapted by the Schuman group.

Results - We confirmed the very long half-lives of the histone family and the lamin-B1. We also observed that fatty acid transport proteins, such as CD36, FABP4, and ACSL1, exhibited relatively longer half-lives and proteins associated with synthesis of fatty acid, such as FASN, ACLy and PLIN3, exhibited relatively faster protein half-lives in adipocytes. In this study, 26S proteasome non-ATPase regulatory subunit 4 (PSMD4) exhibited most faster protein half-life in the proteasome complex which has important functions by way of protein-protein interactions for stabilization of lid and base association of the 19S complex and binding ubiquitin chains. Subunits of the spliceosome complex, splicing factor 3A and 3B, which interact with other components also exhibited the most faster protein turnover compared to the other complexes. Proteins related to the Golgi apparatus and the ER displayed relatively faster protein half-lives, while lysosomal proteins exhibited comparatively slower protein half-lives in this study.

Conclusions - Our study indicated that carrier proteins of fatty acids exhibited relatively slower protein half-lives, whereas proteins associated with fatty acid synthesis have relatively faster protein half-lives in 3T3-L1. It was also noticed that subunit proteins in the complexes that interact with other molecules also tend to have faster half-lives.

PP03.32: Liver Proteomics Profile to Unveil the Biological Role of BAG3 through Tissue-specific BAG3 Knockout Mouse Models

Federica Di Marco, Italy

Background
BCL-2-associated anthanogene 3 (BAG3) is a multifunctional protein involved in several regulatory pathways, both in physiological and pathological condition: participation in cell stress response, regulation of insulin secretion, autophagy, and senescence. Greater knowledge about BAG3 activity has been obtained through studies on tissue specific BAG3 knockout mouse model, since whole body BAG3 knockout mice exhibited early lethality.

Methods
We generated ß-pancreatic cells BAG3 knockout (BAG3ßKO) and hepatocyte-specific BAG3 knockout (BAG3albKO) mouse model by Cre-mediated recombination and we performed a proteomics analysis on the livers of these mouse models to study the pathophysiological function of BAG3 both in ß-pancreatic cells and hepatocyte. The proteomics workflow consists of tryptic digestion with FASP protocol of samples, nanoLC-Orbitrap Fusion-MS/MS analysis, label-free quantitative proteomics, evaluation of differential proteins and Functional Gene Ontology term enrichment analysis through Ingenuity Pathway Analysis (IPA software).

Results
Proteomics analysis on the liver of BAG3ßKO mice (compared to WT) detects an over expression of cytosolic hydroxymethylglutaryl-CoA synthase coupled to the down regulation of the mitochondrial isoform, conditions that suggest respectively an increase in the synthesis of cholesterol and a decrease in the formation of ketone bodies. Functional proteomics analysis highlighted a metabolic modulation towards lipogenesis with down-regulation in fatty acid ß-oxidation, ethanol degradation and oxidative phosphorylation in BAG3ßKO mice livers. Functional proteomics analysis on the liver metabolism of BAG3albKO (the second model we generated), reveals an up-regulation in fatty acid ß-oxidation and ethanol degradation. It also reveals an inhibition of necrosis and cell death of liver such as hepatic steatosis with an activation of cell survival and viability. Moreover, by generating BAG3albKO, this condition seemed to preserve diethylnitrosamine (DEN)-induced hepatocarcinogenesis.

Conclusions
Our data demonstrate that BAG3 depletion in pancreatic β-cells increases the tendency to develop insulin resistance. BAG3 depletion in hepatocyte confirms autophagy, increases oxidative stress, and induces senescence.

PP03.34: LC-MS/MS-based Proteomic Analysis of Three-Dimensional Spheroids Derived From Human Primary Cells Exposed to Urban Particulate Matter

Young June Jeon, Republic of Korea

The rapid urbanization and industrialization have led to a significant threat to human health through air pollution, with urban particulate matter (UPM) playing a substantial role in this problem. Consequently, there is a growing demand for research to investigate the impact of UPM on human health. In this study, we attempted to pursue proteomic analysis of three-dimensional (3D) spheroids derived from human nasal epithelial cells, bronchial/tracheal epithelial cells, and microglia under the exposure of urban particulate matter (UPM) in order to understand how the UPM would have an impact on human health. 3D spheroids were first, respectively, formed from human nasal epithelial cells, bronchial/tracheal epithelial cells, and microglia, and were exposed to different concentrations of (0, 100, 200, 400 µg/mL) the UPM, a certified reference material obtained from Korea Research Institute of Standards and Science (KRISS) for 24 and 48 hours. After the viability of spheroids exposed to the UPM solutions was assessed, we performed proteomic analyses of the 3D spheroids exposed to UPM solutions based on liquid chromatography-tandem mass spectrometry (LC-MS/MS) in combination with tandem mass tag (TMT) labeling, which enabled simultaneous comparisons of relative protein abundances of multiple samples, in order to find differentially expressed proteins in the 3D spheroids under the exposure of UPM. Furthermore, we used the Ingenuity Pathway Analysis program to analyze the signaling pathways related to the differentially expressed proteins. The findings from this study will provide insight into the potential effects of the UPM on human health and contribute to identifying candidate biomarkers affected by the exposure of UPM.

PP03.36: Mitochondrial Intracristal Space (ICS) Proteome Was Revealed by Mass Detection of Isotope-Coded Post-translational Modification by APEX

Myeong-gyun Kang, Republic of Korea

The intracristal space (ICS) is a distinct sub-domain of the mitochondrial intermembrane space (IMS) where oxidative phosphorylation occurs. This place is expected to control dynamic demands to regulate mitochondrial respiration, however, the molecular components of the ICS have not been identified owing to the lack of an effective detection method. Since there is no known targeting sequence or protein for proximity labeling in this space, we developed isotope-coded desthiobiotin-phenol probes that enable differential mass analysis of post-translocated modifications by IMS-APEX2 and outer mitochondrial membrane (OMM)-APEX2. From this unique proximity labeling approach dubbed iSpot-ID, we identified TMEM177 is exclusively localized in the ICS and TMEM177 also offers a unique ICS targeting modality of various fluorescent sensor proteins for measuring local pH, redox states, and local temperature in the ICS under the uncoupling process. We also obtained an ICS proteome using TMEM177-APEX2 and the results showed that a surprisingly large portion of mitochondrial matrix targeting proteins and several known IMS localized proteases were found in this place. Since most of those matrix proteins (e.g., HSPD1, HSPE1) are self-oligomerized proteins, our data revealed that ICS is an important place to control import and homeostasis of those specific mitochondrial proteins under the dynamic metabolic demands.

PP03.38: Highly Expressed QSOX2 in Triple-Negative Breast Cancer Overly Stabilizes Integrin-β1 for Rapid Migration and Proliferation of Tumor Cells

A-In Kim, Republic of Korea

QSOX2 is reported as a secretory enzyme expressed in most types of human tissues and involved in disulfide bond formation. However, despite its general expression, the biochemical role of QSOX2 at a molecular level remains unclear. Here we analyzed the contribution of QSOX2 to breast cancer development and the mechanism of this progress. Bioinformatical data and our in-vitro validations revealed QSOX2 is overexpressed in breast cancer compared to normal conditions, and specifically, its mRNA and protein expressions were the highest in the "triple-negative" molecular subtype. Unfortunately, such aberrant expressions of QSOX2 led to clinically poor prognoses in breast cancer patients. But, the knock-down of QSOX2 by siRNA transfection in triple-negative breast cancer (TNBC) cells induced a definite decrease in cell proliferation, which was validated by cell counting and SRB assay. Our flow cytometric analyses indicated that the reduced cell proliferation was the outcome of increased apoptosis and decreased Ki-67 function. Furthermore, wound-healing assay and trans-well experiment verified that the migratory ability of TNBC cells significantly diminished when QSOX2 is knocked down. To investigate the in-depth mechanisms that cause all the observed conditions, we suggested integrin β1 as the main factor. Integrin β1 is associated with diverse cellular signals regulating cell migration and proliferation. Also, disulfide bonds are structurally enriched in the beta unit of integrins. Therefore, we hypothesized that highly expressed QSOX2 in TNBC integrates the molecular functions of integrin β1 by excessively forming disulfide bonds of it, so TNBC cells correspondingly migrate and proliferate rapidly. We confirmed the protein expressions of total integrin β1 get lessened when QSOX2 is knocked down in TNBC cells. Moreover, CHX or MG132 treatments demonstrated that QSOX2 stabilizes the expressions of integrin β1. As a result, inhibiting QSOX2 in TNBC patients is expected to suppress the cancer progression and lead to a better prognosis.

PP03.40: Analysis of Resistance Biomarkers after EGFR TKI Treatment on NSCLCs

Hyung Joon Kwon, Republic of Korea

Lung cancer is a leading cause of cancer-related death worldwide, especially non-small cell lung cancers (NSCLCs). While surgery is the most effective treatment, it is only viable for a small fraction of cases, and recurrence rates remain high. Platinum-based chemotherapy, the standard therapy for advanced or recurrent NSCLCs, has limited efficacy. Therefore, there is an urgent need for more effective strategies to prevent NSCLC recurrence.

EGFR mutations are common in NSCLC patients, leading to the development of targeted therapies such as first (Gefitinib, Erlotinib), second (Afatinib, Dacomitinib), and third (Osimertinib, Lazertinib) generation EGFR TKIs. These treatments have increased progression-free survival by approximately 19 months. However, recurrence remains a significant challenge.

This study aimed to identify a key biomarker associated with EGFR TKI recurrence. The PC9 cell line, representing EGFR exon 19 deletion, was utilized to assess three EGFR TKIs: ND20, Poziotinib, and a Takeda-developed drug. Phosphoproteome analysis was performed at 4 and 16 hours after treatment.

The 4-hour analysis aimed to capture immediate early responses, while the 16-hour analysis examined the cell's reactions to EGFR targeting. Proteogenomics analysis revealed time-dependent changes in phosphorylation. Anti-apoptotic signal molecules, including Her2 (ERBB2) and Braf kinase, were consistently inhibited by all three TKIs. However, proteins associated with anti-apoptotic activity, such as BAD, HDAC3, and SIRT1, exhibited increased phosphorylation over time.

The EGFR TKIs effectively inhibited specific phosphosites (ERRB2_s958, HDAC1_s409, CDKN18_s140, BRAF_T401, RAF1_s43). However, phosphorylation at other phosphosites (BAD_s118, BAD_s134, HDAC3_s424, SIRT1_s47, Sirt1_t719, CDKN1B_s178, CDKN1B_s10, FADO_s194) increased after 16 hours.

These findings suggest that targeting the rephosphorylation of specific biomarkers could be a promising strategy for concurrent therapy with EGFR TKIs to prevent NSCLC recurrence.

PP03.42: iTF-seq: Systematic Mapping of TF-mediated Cell Fate Changes by a Pooled Induction Coupled with scRNA-seq and Multi-omics Approaches

Muyoung Lee, United States

Transcriptional regulation controls cellular functions through interactions between transcription factors (TFs) and their chromosomal targets. However, understanding the fate conversion potential of multiple TFs in an inducible manner remains limited. To address this, iTF-seq was introduced as a method for identifying individual TFs that can alter cell fate towards specific lineages at a single-cell level. iTF-seq enables time-course monitoring of transcriptome changes, and with biotinylated individual TFs, it provides a multi-omics approach to understanding the mechanisms behind TF-mediated cell fate changes. Our iTF-seq study in mouse embryonic stem cells identified multiple TFs that trigger rapid transcriptome changes indicative of differentiation within a day of induction. Moreover, cells expressing these potent TFs often show a slower cell cycle and increased cell death. Further analysis using bioChIP-seq revealed that Gcm1 and Otx2 act as pioneer factors and activators by increasing gene accessibility and activating the expression of lineage specification genes during cell fate conversion. iTF-seq has utility in both mapping cell fate conversion and understanding cell fate conversion mechanisms.

See you in Busan!
BEXCO, Busan, Korea
2023.hupo.org
#HUPO2023

Together Through Proteomics

PROGRAM

PP03.44: The Role of Ferroptosis Progress and Some Related Genes in Psoriasis Disease.

Thien Nguyen Huu, Việt Nam

Introduction: Psoriasis is a chronic inflammatory skin disease with a strong genetic predisposition and autoimmune pathogenic traits. It characterizes by sharply demarcated, erythematous, pruritic plaques covered in silvery scales. The pathogenesis of psoriasis relates to immune cells and the increase of proinflammatory cytokines, especially in the IL-23/Th17 axis. Recently, there are many studies found the role of ferroptosis in the pathogenesis of many diseases related to the immune system such as acute kidney injury, intracerebral hemorrhage, and neurodegenerative diseases. So, in this study, we investigate the process of ferroptosis and some related genes in the Psoriasis model.

Material and methods: We induce the Psoriasis model on mice with 5% Imiquimod cream and induce the ferroptosis model on keratinocyte cells by erastin. Investigate some ferroptosis makers and related genes in these models. Skin parameters were measured and investigate skin lesions by hematoxylin and eosin staining. Western blot was used for protein levels evaluation. mRNA levels were measured by RT-PCR.

Results and discussion: There are proliferation and thickening of epidermal keratinocyte cells in a psoriasis model. Besides that, there are changes in ferroptosis makers such as GPX4, and ACSL4, and the increase of proinflammatory cytokines in vivo Psoriasis model. We also found that keratinocyte is sensitive to ferroptosis inducer and ferroptosis in this cell line has related to some genes that we investigate.

Conclusion: The study shows the role of the ferroptosis process and some related genes in the Psoriasis model.

PP03.46: Specific Tumor Immune Microenvironment(TIME) Profile Influences the Awakening of Dormant Cancer Cells

Jong Hyeok Park, Republic of Korea

Residual cancer cells, remaining in the body after treatment, are considered a cause of recurrence in many cancers, even after clinical remission. These cells are reported to be dormant, and it is noted that tumor microenvironment(TME) plays an important role in regulating their dormancy phenotype. However, the relationship between dormant cancer cells and the tumor immune microenvironment(TIME) remains poorly understood. Through this paper, we aim to investigate which TIME trend influence the awakening of dormant cancer cells. To examine the established microenvironment of reawakened dormant cancer cell in vivo, we use E0771 cell line that is induced dormancy through drug treatment and labelled by GFP+sLP mcherry+ referred by luigi ombrato paper for niche labelling. And inject via intravenous(IV) to C57BL6 mouse. Subsequently, using FACs, we examined the composition and ratio of immune cells in the microenvironment labelled with mcherry. When comparing it to the microenvironment established by proliferative cells through the same process, we observe distinct differences. These results suggest that this different composition and ratio of these immune cells may contribute to the reawakening of dormant cancer cells. Through further studies, we can potentially identify candidate targets that enable dormant cancer cells to maintain their dormant state by investigating these contrasting immune cell profiles.

PP03.48: The Use of Hydrophilic Magnetic Beads for Mass Spec Sample preparation as part of Complex Proteomic Workflows

Michael Rosenblatt, United States

Background: The need for mass spec sample prep technologies with widespread utility is needed. Historically, ultrafiltration and precipitation have predominated, but these approaches are technically challenging, tedious, costly, and not amenable to automation. Recently, the use of hydrophilic magnetic beads to selectively capture proteins, with the aid of organic solvents at concentrations of 50 % or greater, has shown utility for MS Sample prep. We have tested these beads for preparation of high quality MS samples. Specifically, we wanted to understand the selectivity for capturing a broad range of proteins. In addition, the ability to automate, as well as compatibility with processing small amounts of cellular protein will be presented.

Methods: Human cells (HEK and K562) were lysed with various lysis buffers   and then processed using hydrophilic magnetic beads (SP3 workflow). Samples were analysed by nanoflow LC-MS/MS using an Exploris 240 Mass Spectrometer (Thermo). Data were processed using PD 2.5.

Results: Magnetic beads, irrespective of surface chemistry are highly effective for the selective capture of proteins in surfactant or denaturant solutions. The beads are able to process protein mass as small as 5000 cells (0.5 mg) with high precision and capable of enrichment of dilute protein solutions, like conditioned media. The beads are also compatible with workflows involving quantitation and enrichment often save several days as desalting is not required. In addition, studies related to proteolysis of proteins adsorbed to beads versus solution digestion, protocol automation, use of multiple proteases, and compatibility with proteolysis of subcellular fractions will be presented.

Conclusions: Hydrophilic magnetic beads are amenable to selectively capture proteins in virtually any detergent or denaturant solution, over an unprecedented range of amounts/concentrations, thereby making then ideally suitable for mass spec sample prep. In addition, their magnetic properties make them ideally suitable for high-throughput applications.

PP03.50: Quantitative Analysis of Early Signaling Pathways in Lens-induced Myopia in C57BL/6J Mice Retina Proteome by SWATH-MS

Ying Hon Sze, Hong Kong

Background
Myopia (short-sightedness) has an exceptionally high prevalence rate in East Asia and increasing globally. Mice is an emerging model that can mimic myopia progression in humans using high powered optical lens-induced myopia (LIM). Retina is a light sensitive structural tissue composed of ten distinct layers connected by neuronal synapses that may perceive optical defocus signals leading to axial length elongation myopia. This study aims to explore the signaling of early myopic progression in mice retina, to identify potential therapeutic targets.

Methods
C57BL/6J mice were weaned on postnatal day 21, randomized eye was subjected to a customized -30 diopter PMMA spectacle treatment with contralateral eye as a control in each animal. Ocular measurement and refraction were performed on P21 (n=11), P28 (n=6), P35 (n=5) with optical coherence tomography (OCT) and eccentric infrared photorefractor. Differential retina proteins were quantified by SWATH acquisition with 1μg injection in a NanoLC mass spectrometry (ZenoTOF 7600, Sciex), analyzed with ProteinPilot (Sciex) and PeakView (Sciex) and pathway analysis by Ingenuity Pathway Analysis (IPA) Bioinformatics.

Results
SWATH-MS acquisition has quantified 3974 common proteins (1% FDR) in each biological samples (n=11, with technical duplicates) and significant axial elongation found in treatment eyes, with 58 up-regulated, 41 down-regulated proteins in P28 retina; 39 up-regulated, 84 down-regulated proteins in P35 retina respectively with $p<0.05$ and fold-change ≥ 1.2 or ≤ 0.83. Downstream pathway analysis with z-score > 2 and statistics showed that significant deactivation of Integrin signaling, RAC signaling, actin cytoskeleton signaling, senescence pathway and activation of HIF1α signaling in the early myopic time-point P28. In contrast, significantly deactivation of estrogen receptor signaling and activation of AMPK signaling in P35 using pathway enrichment analysis.

Conclusion
ZenoToF MS with pathway enrichment of retina treated with LIM C57BL/6J mice has shared novel insights and some previously reported pathways in other myopia studies.

PP03.52: Investigating the Insulin Secretion Mechanism Regulated by AC9 with Pancreatic β-cell-specific AC9 Knockout Mice Through Proteomics

Yanpu Zhao, China

Diabetes has become a serious threat to human health worldwide, however it is still remains incurable. One of the main obstacles is that the secretion mechanism of insulin, which is the only hypoglycemic hormones, is still not entirely clear. Our previous studies have shown that cyclic adenosine monophosphate (cAMP) plays an important role in the bypass secretion of insulin, and cAMP synthetase adenylyl cyclase 9 (AC9) plays an important role in this process. In this study, we constructed the islet β-cell-specific AC9 knockout mice (Ins-AC9loxp/loxp), and performed unlabeled quantitative proteomic analysis of mouse primary islet tissue to explore the genes and signaling pathways involved in insulin secretion. In total 1398 proteins were identified, out of which 94 proteins were differentially expressed between floxp and Ins-AC9loxp/loxp. By bioinformatics analysis, we found that membrane trafficking and vesicle-mediated transport were significantly dysregulated. Two of the canonical downregulated genes, Dync1i2 and Vamp2, were found, both of which are associated with vesicle trafficking. At the cellular level, after AC9 knockout, the expression of Ins2 was down-regulated and the secretion of insulin was decreased. These results suggest that AC9 may play an important role in insulin synthesis and secretion. This study enriched the theoretical mechanism of AC9 regulating insulin secretion, and laid a theoretical foundation for the treatment of diabetes through targeting AC9.

PP03.54: Unmasking Hidden Systemic Effects of Neurodegenerative Diseases: A Two-Pronged Approach to Biomarker Discovery

Sandra Anjo, Portugal

Blood biomarker discovery has been dominated by targeted analysis of disease-associated proteins or conventional untargeted proteomics strategies. However, these attempts have failed to identify high confidence biomarkers of Neurodegenerative Diseases (NDs), which we believe is in part due to: i) the use of conventional proteomics analyses in a very challenging sample, such as blood, and ii) the difficulties in classifying the patients' groups.

To evaluate our proposal, the same set of samples was subjected to the proteomics analysis of the whole serum and to fractionation using centrifugal ultrafiltration with 300 kDa molecular weight cut-off filters in a non-denaturing environment (henceforth referred to as HMW fractionation). This study used a cohort comprising of patients with neurodegenerative disorders, specifically Alzheimer's disease (AD) and Parkinson's disease (PD) patients, and healthy controls (CT) to test the significance of using HMW fractionation as a complementary tool for biomarker discovery. These disorders were selected as they are considered proteinopathies and may offer ideal targets for this purpose.

The combination of the two-analysis demonstrated to be highly effective in distinguishing AD and PD patients between themselves and between CT, in fact a strong diagnostic model comprising 10 proteins (5 from each approach) was created, also revealing clear evidence for the contribution of proteins from the apolipoprotein family for the diagnosis of NDs. Besides that, this approach also proves that the addition of the HMW fractionation analysis may reveal potentially altered macromolecular and macromolecular complex organization, thus being able to disclose hidden effects in serum, in the present work in the context of NDs.

PP03.56: Multi-omics Approach to the Identification of Biomarkers for Progression from Psoriasis to Psoriatic Arthritis

Annika Bendes, Sweden

Background:
Psoriatic Arthritis (PsA) is a chronic, inflammatory disease that severely impacts the quality of life. The disease develops in 20-30% of people with psoriasis (PsO). Known clinical and genetic risk factors are too insensitive or unspecific to predict or measure progression from PsO to PsA. The identification of molecular biomarkers would offer hope for the earlier diagnosis of PsA and the initiation of management and treatment strategies for people with the disease. In the IHI project HIPPOCRATES, multi-omics approaches are being used to initially identify molecular biomarkers that may predict or measure progression from PsO to PsA.

Methods:
Serum samples from 90 individuals participating in a prospective PsO study (BioCOM) at St. Vincent's University Hospital (Dublin, Ireland), were analyzed by HIPPOCRATES partners with expertise in LC-MS/MS-based proteomics, targeted affinity proteomics, metabolomics, lipidomics, and genomics. Of the 90 participants, 30 had established PsA, 30 had PsO with no clinical features to suggest musculoskeletal disease (MSK), and 30 had PsO and clinical signs of some MSK involvement.

Results:
Using LC-MS/MS, targeted proteomics (Multiple Reaction Monitoring) assays, and random forest algorithms, a panel of relevant peptides was identified, which distinguished PsO from PsA with an ROC AUC = 0.8. Targeted proteomics using Olink PEA assays, revealed quantitative differences in LASSO-selected inflammatory proteins between PsA and PsO groups.
Furthermore, lipidomics and metabolomics data for the samples were obtained using a combined approach for targeted and non-targeted analysis and indicated differences between the two groups for different lipid species.

Conclusions:
We have identified candidate molecular markers that may differentiate PsO from PsA. In ongoing studies, the multi-omics data is being combined and subjected to integrated Machine Learning analysis. These initial findings will require validation in additional PsO and PsA cohorts - ideally longitudinal cohorts.

PP03.58: Integrated Proteomic and Transcriptomic Analysis Uncovers CASP as A Novel Oncogenic Player in Colorectal Cancer

Ting Chen, China

Colorectal cancer (CRC) is the third most common tumor and the second cancer-related cause of death globally. While gene-signature based discoveries partly improved the individualized treatments and prognosis assessments, protein signatures evolving in CRC progression are largely unexplored. Therefore, we aimed to find novel protein-based carcinogenesis biomarkers in CRC. In this study, we re-analyzed the proteomic profile of FFPE tissue samples from patients with hyperplastic polyps (n=17), adenomas (n=22), CRC (n=30) and include normal negative margin (n=20) as control from our previous research using DIA-NN software to identify differentially expressed proteins during the evolving of the cancer. Integrated exploration by using Cancer Genome Atlas (TCGA) and Gene Expression Omnibus (GEO) datasets identified CDP/cut alternative spliced product (CASP) was significantly up-regulated and correlated with the prognosis of CRC. Further immunohistochemical (IHC) staining of a tissue microarray (107 CRC and matched adjacent tissues) had verified that the protein expression level of CASP was specifically higher in CRC than that in normal tissues. However, the role of CASP was barely characterized. We first identified CASP as an oncogenic player in CRC. Silencing of CASP inhibited the proliferation and migration of CRC cells, whereas expression of CASP significantly promoted the proliferation and migration of CRC cells in vitro and in vivo. Furthermore, CASP negatively regulated the protein level of E3 ubiquitin ligase triple domain containing protein 21 (TRIM21) and positively regulates the mitogen activated protein kinases (MAPK) signaling pathway. In summary, these findings suggest the potential oncogenic role of CASP and provide a promising target for intervention.

PP03.60: A Novel MALDI-TOF Platform for the Simultaneous Detection of Multiple Target Proteins in Clinical Microbiology

Donghuey Cheon, Republic of Korea

Introduction: MALDI-TOF MS has proven to be a highly effective tool for the routine identification of microorganisms in clinical microbiology laboratories. The application of a microbial identification platform in diagnosing infectious diseases provides significant advantages, such as rapid and accurate identification of target proteins. Carbapenem has been widely used as a class of antibiotics for bacterial infections. However, the emergence of carbapenemase-producing Enterobacteriaceae (CPE) has severely limited the effectiveness of these drugs. In this study, we have established a novel MALDI-TOF platform capable of identifying intact carbapenemases and their subtypes, thereby contributing to novel diagnoses in clinical microbiology.
Methods: We developed a novel MALDI-TOF platform involved the following techniques: (1) Optimization of MALDI-TOF MS parameters, (2) Internal mass calibration (In-Cal), (3) Adduct peak exclusion, (4) Multi-target detection. A total of 526 clinical isolates, including 414 CPE-producing bacteria (380 KPC, 30 OXA, 2 GES, and 2 IMP) and 112 non-CPE-producing bacteria, were used to evaluate our novel MALDI-TOF platform.

Results: Our novel approach, optimizes intact protein analysis and tuning the performance of MALDI-TOF MS, resulting in sensitive and consistent data acquisition. In addition, the In-Cal process and adduct peak exclusion improve mass accuracy and reduce errors. As a result, all clinical isolates exhibited 100% sensitivity and specificity of identification by our novel platform. Furthermore, our platform enables successful discrimination of their subtypes, such as KPC-2 or KPC-4, with high mass accuracy.
Conclusions: This study suggests a novel platform with high analytical sensitivity and diagnostic accuracy, which holds the potential for clinical diagnosis by providing more consistent and accurate diagnostic tools. Moreover, this platform may help with appropriate treatment, facilitate therapy selection, and support epidemiological investigations.

PROGRAM

PP03.62: Enhanced Performance of MALDI-TOF MS Analysis for Proteins by Graphene-coated Silicon Wafer Plate

YoonKyung Choi, Republic of Korea

Introduction: The reliable analysis using Matrix-assisted Laser Desorption/Ionization Time-of-flight Mass Spectrometry (MALDI-TOF MS) of proteins for clinical applications remains challenging due to their lower ionization efficiency and heterogeneous crystallization with the matrix on sample spots. Here, we investigate the potential of a customized graphene-coated silicon wafer (G/SiO2) plate for the analysis of clinically important proteins in comparison with a conventional stainless steel (SUS) plate.
Methods: To evaluate the performance of the G/SiO2 plates and its clinical applicability, we adopted four standard proteins covering a wide range of molecular weight (8 to 66 kDa) and the clinically important Klebsiella pneumoniae carbapenemase (KPC-2) protein as a main target protein. For each sample, five repeated measurements were made on SUS and G/SiO2 plates in all experiments. We further performed the inter-day experiment for five days to assess the reproducibility of KPC-2 protein identification.
Results: The G/SiO2 plate exhibits superior sensitivity, reproducibility, and mass accuracy/precision compared to the SUS plate for protein MALDI-TOF MS analysis across a diverse range of molecular weights, even in complex samples. Furthermore, a five-day robustness test confirms the practical applicability of the G/SiO2 plate for the reliable identification of target protein(s) in MALDI-TOF MS analysis.
Conclusions: Our findings suggest that the use of the G/SiO2 plate holds great potential for improving the sensitivity and reproducibility of MALDI-TOF MS analysis for the identification of pathogenic proteins, making it a promising tool for clinical applications.

PP03.64: Integrated Fast Analysis of Transplant Biopsy Tissues with Histopathology to Improve Patient Outcome

Garry Corthals, Netherlands

Background
Transplantation is the preferred treatment for end-stage kidney disease, but it requires lifelong medication to suppress rejection, which can have serious side effects. Analysis of biopsies by pathologists is essential for diagnosing transplant rejection and understanding patient response to medication. Deep Learning (DL), a form of machine learning, has shown promising results in improving the speed and accuracy of transplant diagnostics (Kers et al, 2022, Lancet. Digital Health). However, DL is challenging to understand. Additional molecular information offers humanly interpretable insights thereby enabling time-scale relevant intervention, treatment prediction, and improved patient stratification.

Methods
A cohort of 300 patient tissues was examined, following the development of a new SOP. Molecular profiles of kidney tissues were collected using Bruker timsTOF HT in dia-PASEF mode. A new SOP was developed to ensure consistent quantitative molecular accuracy of kidney tissue biopsies, and seamless alignment with the pathology workflow in hospitals. SWATH-MS and DDA (Sciex) were used for method development and quality control, while the timsTOF HT enabled high-quality analysis of the cohort in 21 minutes/sample using minimal biopsy amounts. Data analysis was performed using DIA-NN in library-free mode.

Results
The aim was to support histological decision-making following kidney transplantation by combining quantitative molecular analysis with new ML tools. Key findings include the development of a new protocol based on FFPE samples with highly reproducible values. The Bruker timsTOF HT system demonstrated remarkable speed, sensitivity, accuracy, and reproducibility for quantitative analysis on 300 patient samples, providing insights into classifications across different patient categories within days. Additionally, we will also discuss sample size considerations and analytical speed.

Conclusion
With the Bruker timsTOF HT operating in dia-PASEF mode only ~1/10th of the sample (0.2 mm2) analysed in 20 min (5x faster) was necessary to quantify >6500 proteins (5x more) compared to existing methods.

22nd Human Proteome Organization World Congress

See you in Busan!
BEXCO, Busan, Korea
🌐 2023.hupo.org
🐦 f 📷 in #HUPO2023

Together Through Proteomics

PROGRAM

PP03.66: Bench, Bedside, and Beyond: Navigating Ethics in Large-Scale Clinical Proteomics Research

Ina Devos, Belgium

BACKGROUND: The identification of biomarkers for precision medicine relies on high-throughput omics research, including proteomics. Health-related research can have significant impact on participants, patients, further research, the medical system, or society at large. Thus, it is crucial to reflect on the bioethical aspects of large-scale omics research. Genomics research has already sparked extensive ethical discussions on various issues, including privacy, consent, ownership, and control. However, there has been little research or guidance on ethical issues in other omics fields, including proteomics. Specific properties of proteomics research, such as the link to genotype and phenotype or the spatio-temporal variability of data, might shift the relevance and scope of existing ethical discussions and introduce new issues.

METHODS: We have explored the ethical literature on proteomics research in a systematic literature review. Following PRISMA guidelines, we systematically searched PubMed and Web of Science using broad search terms (ethic* AND proteomic*). We have categorized issues and arguments in the 18 selected papers into relevant themes (e.g., privacy, societal benefits, and justice). Results are compared with discussions in the broader biomedical research context.

RESULTS: The existing ethical literature predominantly addresses aspects of data sharing such as privacy, identifiability, discrimination risks, informed consent, ownership, intellectual property, return of results, and research promotion. Several articles also include justice concerns surrounding unequal database access for researchers and unequal distributions of benefits. In contrast, little attention is paid to evaluating the implications of the hypothesis, problem setting, methods, or purposes of proteomics research.

CONCLUSION: Current ethical discourse on proteomics research mainly revolves around participant and research interests regarding data sharing, resembling discussions in the genomics field. However, this focus on external effects of data sharing might obscure equally relevant, internal ethical aspects of proteomics research, such as underlying assumptions, decisions, and methods, both shaped by and shaping societal contexts.

PP03.68: Mass Spectrometry-based Profiling of Small FFPE Tissue Areas

Marius Fraefel, Germany

Formalin-fixed and paraffin-embedded (FFPE) tissue is the basis for clinical and biomarker research, representing the most valuable diagnostic asset in hospitals. FFPE tissue is stable over time and is associated with clinical data, patient response, and histology. Although mass spectrometry-based proteomics results are promising when clinical FFPE samples are measured, capturing the cellular heterogeneity of such samples is challenging. It is therefore necessary to provide further proteomics workflows which can analyse smaller tissue areas and thereby enhancing the spatial resolution.

The presented proteomics workflow enables the analysis of minute amounts of FFPE material (1 mm²). The workflow utilizes heat extraction, physical disruption, SP3 clean up and digestion of the input material. Afterwards, the sample is directly loaded onto Evotips which can be further subjected to LC-MS/MS analysis. A special alignment of Evosep LC FAIMS-Exploris 480 allows for in depth proteomic profiling of clinical samples. Throughout the method optimization, homogeneous mouse liver FFPE tissue was used. To showcase the applicability on real world samples, human myocarditis needle biopsies were profiled. Optimization steps encompassed multiple parts of the sample preparation as well as adjusting LC-MS parameters.

Following this approach and label-free DDA data acquisition enabled the identification of 4000 protein groups in mouse liver and 5000 proteins in human myocarditis samples. This protocol is well suited for large cohorts, as it can be easily upscaled with little sample handling time. Furthermore, it demonstrates considerable reproducibility (CV <15% for 90% of all proteins in mouse FFPE replicates).

This protocol is envisioned for future applications to other clinical entities. DIA data acquisition for needle biopsies is also being considered to generate more consistent data sets. Additionally, the measurement of multiple layers of a needle biopsy in a 3D manner, from the top layer to the bottom layer, presents an interesting task for exploration.

PP03.70: Viewing Amyloid Through the Proteomic Microscope: Characterization of Two Protein Misfolding Diseases Targeting the Heart

Juliane Gottwald, Germany

Introduction: Amyloidosis is a group of diseases where misfolded proteins form amyloid fibrils, which are deposited in different organ- and tissue-sites. Among these, transthyretin (ATTR) and immunoglobulin light-chain derived amyloid (AL) affect most commonly the heart. Untreated, they cause devastating tissue damage, ultimately leading to fatal heart failure. In some cases, heart transplantation could be the only treatment option. However, drug therapy may slow or even stop disease progression in ATTR cases, whereas in AL amyloidosis patients frequently receive chemotherapy. Although diagnostics and treatment options have improved, they are still limited, because we have not yet fully understood the disease-related molecular mechanisms.

Methods: To address this issue, we performed an in-depth quantitative proteomic analysis[1] of lambda-AL (n=6 cases) and ATTR (n=2) amyloidosis. For each case, tissue microarrays were sectioned from cardiac tissues. Congo red fluorescence scanning guided the selection of 8 samples with variable amyloid load per case. The samples were then subjected to bottom-up liquid chromatography tandem mass spectrometry proteomics. The case-specific amyloidogenic DNA and amino-acid sequences were added to the database search and label-free intensity profiling was used to correlate the abundance of constituents with the amyloid protein.

Results: Initial results provided >100 proteins correlating significantly with the amyloid protein. These proteins were enriched in, e.g., high-density lipoproteins and proteins associated with lipoprotein metabolic processes, activation of the complement system and the immune response. Further, we will investigate the differences between AL and ATTR and combine our findings with structural information on the respective amyloid fibrils and the occurrence of the same amyloid type at different tissue sites.

Conclusion: Summing up, our study connects multi-layered pieces of information on amyloid deposition in the heart and will hopefully initiate discovery of new biomarkers for early diagnosis and novel targets for therapy.

[1] Treitz, C., et al. 2021 Amyloid 29(2)

PP03.72: An In-Depth Plasma Proteomics Workflow Powered by Orbitrap Astral Mass Spectrometer

Amirmansoor Hakimi, United States

Introduction

Mass spectrometry-based plasma proteomics remains the promising method of understanding human molecular pathophysiology and the discovery of disease biomarkers. However, it has been a challenging workflow for many years due to the large dynamic range in protein expression and the current capabilities of analytical methods, especially with regard to the throughput and depth of proteome coverage. Here we evaluate the performance of the novel Orbitrap Astral mass spectrometer for plasma proteomics using three of the most common plasma sample preparation methods.

Methods

The neat and depleted plasma samples were prepared using Accelerome, and the enriched plasma sample was prepared on Seer's Proteograph Product Suite. Samples were analyzed using multiple different workflows including high-throughput (180 SPD) to Max-ID (4 SPD) enabling the user to choose depending on the project. EasySpray PepMap Neo and µPAC-Neo columns were utilized with a Vanquish Neo UHPLC system coupled to an Orbitrap Astral mass spectrometer. Data analysis was done using Proteome Discoverer software 3.1.

Results

Neat plasma suffers from dynamic range issues resulting in lower coverage compared to depleted plasma. Enrichment/fractionation with the Seer methodology offers more depth of analysis while balancing the need for high throughput. The high throughput method of 180 SPD delivered a depth of coverage of 643 protein groups. By running a longer gradient using the 24 SPD method, 1137 protein groups were identified. For depleted plasma, the 18 SPD method was employed for greater depth of coverage, resulting in 2,702 protein groups being identified. The multi-nanoparticle enrichment of plasma proteins with the Seer Proteograph Product Suite resulted in a record number of protein identification (6341 protein groups) from plasma samples to date.

Regardless of the sample preparation method chosen for plasma proteomics, the Orbitrap Astral mass spectrometer delivers the highest proteome coverage and throughput compared to current mass spectrometers.

PP03.74: Proteomic Landscape Reveals Potential Protein Signature Independent of Helicobacter Pylori Infection in Gastric Cancer

Hsiang-en Hsu, Taiwan

Gastric cancer is the fifth most common neoplasm and remains one of the leading causes of cancer-related mortality worldwide. Helicobacter pylori (H. pylori) infection is a well-known environmental risk factor and its eradication by screening-to-treatment has effectively improved gastric cancer control. However, 30% of patients are not etiologically related to H. pylori. Although several genomic studies had identified molecular signatures associated with pathogenic phenotypes, information beyond the genomic data is still necessary to improve the understanding of cancer biology of non-H. pylori patients. To gain more insights into gastric cancer pathogenicity and therapeutic drug discovery, we performed a deep proteomic and phosphoproteomic profiling of paired tumor and adjacent normal tissues (NAT) from a prospectively collected gastric cancer cohort to identify the molecular subtypes and association with clinical features and lifestyle. Global proteome and phosphoproteome profiles were conducted by TMT 10-plex-based proteomic workflow using pooled NAT and tumor as an internal standard to ensure long-term analysis reproducibility. The PCA analysis revealed distinct normal and tumor profiles and different gastric cancer subtypes. Unsupervised hierarchical clustering of proteomics profile stratifies patients into 4 subtypes (P1-P4). Significantly, subtype P1 is associated with female with non-smoking, and P2 is related to H. Pylori infection. Interestingly, a unique proteomic subgroup (P4) which is independent of H. pylori enriched with younger male (<60 yr) with smoking and frequent fish intake. Further pathway and functional analysis results indicated that this subgroup without H. Pylori infection might be associated with the response of immunity (B cell receptor signaling) and inflammation (NF-kappa B signaling and Leukocyte transendothelial migration). Moreover, we also observed that the EB-virus infection signaling was activated in this subtype. Taken together, the proteomic profiling (ongoing) revealed new insight into the etiology of gastric cancer patients without H. Pylori infection, and may provide potential mechanisms for drug treatment.

PP03.76: Optimization of MALDI-TOF MS for CTX-M Subtype Detection From Clinical Isolates

Heejung Jang, Republic of Korea

Introduction: Due to the abuse of antimicrobiotics, there has been an increasing number of CTX-M-type enzymes known to be the class A extended-spectrum β-lactamase (ESBLs) producing Enterobacteriaceae resistant enzyme. For effective antimicrobial treatments, CTX-M subtype detection is required since they exhibit different resistances to several antibiotics. Here, we have developed the MALDI-TOF MS method to differentiate CTX-M subtypes which is faster and cost-effective technique than the traditional antibiotic susceptibility test, a disk diffusion method which takes at least 48 hours.

Methods: For this study, we first made the clones of CTX-M subtypes, expressed, and purified their protein products from bacterial system. The proteins served as reference materials for mass correction in our analysis. To ensure accurate mass measurement and identification of CTX-M subtypes originated from clinical isolates, we optimized various MS parameters.

Results: We confirmed that the standard proteins could be distinguished within 28 to 102 m/z by applying MS optimization. Of the 82 clinical isolates, we identified 78 (95%) single subtypes of CTX-M protein and 4 (5%) multi-types of CTX-M proteins. Furthermore, we obtained accurate mass of CTX-M subtypes that was within the range of ±10 m/z.

Conclusion: We optimized the MS parameter using the standard protein mixtures to detect and differentiate CTX-M subtype proteins with adjacent mass. Using this technique, we expect that it may not only enhance the antibiotic resistance detection but also would help effective antimicrobial treatments.

PP03.78: Membrane Proteogenomics Analysis of CRISPR-9 Edited Non-small Cell Lung Cancer Cells

Yu Teng Jheng, Taiwan

Dysregulated membrane proteins, particularly those harboring onco-driver somatic mutations, are vital biomarkers and therapeutic targets in various cancers. However, detection of mutated membrane proteome is challenging due to their low abundance and hydrophobic properties. For this reason, we presented a data independent acquisition mass analysis (DIA-MS)-based proteogenomics platform to improve the detection coverage of membrane proteome and their harboring mutations. We applied the pilot study on the CRISPR-edited H1975 cells with an additional drug-resistant C797S mutation in EGFR gene. A twofold increase in protein identification was obtained with our platform when compared to traditional data-dependent acquisition methods. Interestingly, the CRISPR-editing in EGFR gene did not affect the expression of EGFR protein itself. However, the expression levels of EGFR-interacting protein network were significantly influenced. Based on the functional enrichment analysis of differentially expressed proteins, H1975C797S cells showed downregulated glycolysis and enhanced oxidative phosphorylation in accordance with reported drug resistance mechanism. Our study identified down-regulated CAPN2_K490Q in H1975C797S cells which have been reported to associate with gefitinib resistance. An up-regulated single nucleotide variant (K469E) on ICAM1, a marker associated with poor prognosis in lung cancer, were identified as well. Overall, our developed platform could systematically elucidate the dysregulated membrane proteome and variant expressions in H1975 cells. We expect that this proteogenomics platform will serve as a powerful tool for studying the single-nucleotide variant-directed drug resistance mechanisms in cancer therapy.

PP03.80: Avian Model of Experimental Keratoconus: A Preliminary Proteomic Analysis of Corneal Limbus in Chicks

Byung Soo Kang, Hong Kong

Backgrounds: Keratoconus is a degenerative corneal disease characterized by corneal thinning, softening, and protrusion. Despite its visual impacts, the complex etiology of keratoconus is yet to be fully understood, necessitating an effective animal model to determine its pathogenesis. This study aimed to develop an avian model of experimental keratoconus and investigate proteomic profile in the corneal limbus using a nanoLC integrated ZenoTOF 7600MS.

Methods: Six chicks were anesthetized and had their corneal epithelium debrided bilaterally on post-hatching day 6. Right eyes were treated with collagenase, while left eyes served as control. After treatments, ocular dimensions and corneal stiffness were measured. Corneal limbus digested (500ng) were analyzed by data-dependent acquisition, and protein IDs were searched against Uniprot database using ProteinPilot. Afterwards, equal amounts of digests were loaded for quantitation using Zeno-SWATH acquisition.

Results: Collagenase-treated eyes had significant corneal thinning, softening, and anterior chamber deepening. A total of 3666 proteins were identified from the corneal limbus, which is significantly more than the number of proteins we previously reported chicken corneal proteome (2096 proteins from 1µg digest) under typical DDA with peptide fractionation in a TripleTOF 6600 system. Comparative analysis identified 48 differentially expressed proteins in the treated corneal limbus (23 up-regulated and 25 down-regulated; FC>1.5 and p<0.05). Specifically, Beta-defensin antimicrobial peptide, Leukocyte associated proteins, alpha-2-macgroglobulin-like protein, Filamin B, and Myosin heavy chain were upregulated, indicating that active immune responses may be taking place to counter the effects of collagenase and preserve the integrity of the corneal collagen structures.

Conclusions: This study demonstrated that collagenase can induce keratoconus-like structural changes in chicken corneas, including corneal thickness and biomechanics. Proteomic analysis of the corneal limbus provides preliminary results on the protein homeostasis regulation upon corneal degradation. Further studies are warranted to develop an effective regimen for keratoconus treatments using this avian animal model.

PP03.82: Discovery of MDR-TB Biomarker Signature by Secretome Analysis and Quantitative Proteomics

Hye-Jung Kim, Republic of Korea

This study collected MDR-TB patients' sequential serums undergoing anti-TB treatment. Tuberculosis (TB), caused by the Mycobacterium tuberculosis (M.tb) complex has further complicated the major health problems in the world (10 million were infected, and 1.5 million died in 2020). Especially multi-drug resistant tuberculosis (MDR-TB) means resistant to the two most potent first-line drugs, isoniazid and rifampicin, making it more challenging to treat and control. Where there are insufficient indicators to confirm treatment progress and failure in TB patients, proteomics-based analysis of the MDR-TB patient's serum is expected to help understand the treatment course. High abundant protein depletion is a common strategy applied to increase analytical depth in global plasma proteomics experiment setups. Here we evaluate the performance of top 14 spin columns for serum depletion with high resolution LC-MS/MS run on a Q-Exactive Plus mass spectrometer, followed by label-free quantitation. We identified 235 proteins out of which 226 proteins were differentially expressed (≥ 2-fold). The bioinformatics analysis showed that the functions of differential serum proteins during the MDR-TB treatment were significantly correlated to the complement coagulation cascade, suggesting a coagulation disorder in TB. Our study may contribute to elucidating the mechanisms underlying MDR-TB.

PP03.84: LC-MS based Discovery of Plasma Phosphopeptide Markers for Alzheimer's Disease Staging and Clinical trials

Kyungdo Kim, Republic of Korea

Alzheimer's disease (AD) is a neurological progressive disorder and is commonly caused by extracellular plaque deposits of amyloid-beta (Aβ) and abnormal accumulation of tau which is called neurofibrillary tangles (NFT). Mass spectrometry technique was suitable for screening post-modification (i.e. phosphorylation) of proteins and simultaneously detecting diverse isoforms of p-Tau and phosphorylated peptides belonging to hyper-phosphorylated proteins. Conventional in-solution digestion was conducted for plasma protein tryptic digests. Plasma samples were purified through Sep-Pak columns and dried samples were reconstituted with Fe-IMAC buffer A (50% ACN, 0.1% TFA) and injected into Propac IMAC-10 column (4   50 mm). Phosphopeptides were eluted with Buffer B (0.5% NH4OH) and eluate fractions were collected. For PRM analysis, the Q Exactive Plus was operated with nanoflow LC and 35 phosphopeptides were included in the inclusion list. Quantification results were interpreted with Skyline software. Based on prior research which revealed that 44 AD-related proteins containing tau were detected in AD patients pooling plasma, we analyzed 4 groups (control-, control+, MCI+, AD+) pooling plasma sample according to discovery cohort with LC-MS/MS PRM analysis and a total of 16 phosphopeptides were identified that could be differentiated by AD stage. Among 16 phosphopeptide candidates, one phosphopeptide increased more than 2 times in preclinical AD compared to healthy control and the number of phosphopeptides changed in MCI AD compared to healthy control more than 2 times was 10. The above 11 phosphopeptides corresponding to tau protein could be selected as biomarker candidates for AD early diagnosis. The remaining five phosphopeptides, which correspond to brain tissue-specific proteins such as NEFM, showed a more than 2-fold increase in MCI AD compared to healthy control. Finally, the potential of classification by stage of Alzheimer's disease could be confirmed using LC-MS/MS.

PP03.86: Targeted Mass Spectrometry-based Validation Race-specific Disease Diagnostic Protein Profiling in Laboratory Developed Tests

Woojin Kim, Republic of Korea

Introduction
The occurrence and progression of diseases are influenced by the physiological and genetic diversity among individuals. These variations, especially across different races, underscore the importance of considering race-specific target proteins for disease diagnosis. However, the active pursuit of considering racial differences in selecting protein targets for disease diagnosis is currently lacking. In this study, we have developed a Laboratory Developed Tests (LDTs) that incorporates racial differences in the selection of protein targets for disease diagnosis.

Methods
The effectiveness of the Laboratory Developed Test (LDT) targeting specific proteins was validated using Liquid Chromatography-Mass Spectrometry (LC-MS) analysis. Blood samples were collected from individuals of diverse racial backgrounds including Black, White, and Hispanic individuals. A total of 92 disease-diagnostic-related protein targets were selected for LC-MS analysis. The aim was to validate these targets and assess the differences in protein expression among the various racial groups.

Results
Blood samples from diverse racial backgrounds were analyzed to investigate the expression levels of the target proteins and identify significant differences among races. Method validation was also conducted to confirm the significance of the analysis for the target proteins. Calibration curves, stability, specificity, precision, accuracy, dilution integrity, matrix effect, and reproducibility were evaluated for each target protein to establish the analytical robustness and determine potential interethnic variations in protein expression levels.

Conclusion
Our study contributes to the understanding of interethnic differences in the expression of disease diagnostic target proteins. By identifying disparities in the expression of disease diagnosis proteins among races, we aim to establish a personalized approach for effective disease diagnosis that considers individual racial characteristics. This research holds promise for improving the accuracy and efficacy of disease diagnosis process.

PP03.88: Discovery, Verification, and Validation of Walnut Protein Marker Peptides Using LC-MS Approaches

Dain Lee, Republic of Korea

A key requirement of liquid chromatography-mass spectrometry (LC-MS)-based allergenic food protein analysis methods is to use protein marker peptides with good analytical performances in LC-MS analysis of commercial processed foods. In this study, we developed a multistage walnut protein marker peptide selection strategy involving marker peptide discovery, verification and LC-MS validation of chemically equivalent stable isotope-labeled peptides. This strategy proposed three walnut protein marker peptides, including two new marker peptides. Our LC-MS-based walnut protein analysis method using the three stable isotope-labeled peptides showed acceptable linearity (R2 >0.99), matrix effects (coefficient of variation <±15%), sensitivity (limit of detection >0.3 pg/μL, limit of quantification >0.8 pg/μL), recovery (85.1–103.4%), accuracy, and precision (coefficient of variation <10%). In conclusion, our multistage marker peptide selection strategy effectively selects specific protein marker peptides for sensitive detection and absolute quantification of walnut proteins in LC-MS analysis of commercial processed foods.

PP03.90: Development of an Efficient Detection Method for Monoclonal Protein using MALDI-TOF Mass Spectrometry

Jikyo Lee, Republic of Korea

Introduction: Monoclonal protein (M-protein) is an abnormal increase of monoclonal protein found in the bloodstream that is produced by one or more clonal cells in patients with plasma cell disorders (PCDs). Recently, detection methods based on mass spectrometry (MS) are emerging as highly sensitive for detecting M-protein in smaller amounts. The aim of this study is to develop and evaluate efficient method for screening and detecting monoclonal protein using mass spectrometry in a clinical laboratory.
Methods: Residual samples were used after performing routine tests at Seoul National University Hospital (SNUH). Based on IFE results, normal serum and abnormal serum samples including different subtypes of M protein were selected. To isolate the immunoglobulin and the light chain, six isolated beads (IgG, IgA, IgM, kappa, lambda, and mixed kappa lambda) were used to prepare by using CapturesSelect nanobody affinity beads (Thermo
Fisher Sc ientific Inc., MA, USA). Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) MS (Bruker Daltonics, Germany) was performed. High sensitive resolution MS, liquid chromatography combined with Synapt G2 quadrupole time-of-flight (qTOF) MS (Waters, U.K.), was performed to confirm the m/z difference occurs in MALDI-TOF MS. For analytical performance, limit of detection (LoD) was evaluated. MALDI-TOF MS combined with NB serum preparation (NB-MALDI-TOF) was performed for 25 normal and 25 abnormal IFE samples.
Results: Reduced light chains were observable as a peak at a certain mass-to-charge ratio (m/z ratio) range in the mass spectrum. Monoclonal peaks were observed in abnormal samples, while polyclonal peak was observed in normal ones. Comparing the NB-MALDI-TOF and IFE results among 50 samples, NB-MALDI-TOF showed 92% sensitivity and 92% specificity. NB-MALDI-TOF showed the LoD at 0.1g/dL.
Conclusions: In conclusion, NB-MALDI-TOF was sensitive and useful as a qualitative method to detect M-protein. NB-MALDI-TOF might be helpful to identify M-protein in false negative IFE and confirm ambiguous result of IFE.

PP03.92: Serum Proteome Profiling of Sarcopenia Through Integration of ProteographTM Workflow

Jiyeong Lee, Republic of Korea

Sarcopenia, a condition characterized by muscle loss, lacks effective treatment strategies and a clear understanding of its underlying causes. Early diagnosis and the identification of pathophysiology and reliable markers are crucial for addressing these challenges. However, current plasma proteomics approaches have limitations in capturing the complex and dynamic proteome involved in Sarcopenia. To overcome these limitations, the ProteographTM offers a precise and unbiased proteome profiling solution by utilizing nanoparticles with diverse surface functionalities and physicochemical properties. In the study, serum proteome profiling was performed on Sarcopenia patients before and after treatment intervention using the Proteograph coupled with LC-MS/MS analysis. A total of 1,450 proteins were identified, including 169 differentially expressed proteins (DEPs) between the two groups, consisting of 132 increases and 37 decreases. In contrast, the conventional depletion method only detected 487 proteins, including a mere 7 DEPs. Overall, the Proteograph platform detected more than three times the total number of proteins and 24 times the number of DEPs compared to the conventional method. Notably, while the depletion method failed to identify any low-abundance DEPs with concentrations below 100 pg/mL, the Proteograph technology successfully identified 12 such proteins. Functional enrichment analysis using GO/KEGG revealed new candidate proteins related to actin cytoskeleton regulation, lipid oxidation, and lipoprotein-related proteins. In conclusion, this study using LC-MS/MS with the Proteograph platform to provide comprehensive analysis of the global proteome of Sarcopneia patients before and after intervention enabled improved proteome coverage, enhanced detection of low abundance proteins, and increased reproducibility, providing valuable insights into identification of novel candidate proteins associated with Sarcopenia pathophysiology.

PP03.94: Candidate Protein Biomarkers Associated with Glomerular Filtration Rate in Chronic Kidney Disease: a Proteomics Cohort Study

Zhalaliddin Makhammajanov, Kazakhstan

Introduction: Proteinuria is a marker of chronic kidney disease (CKD) progression and cardiovascular complications. The toxic effects of filtered proteins on the tubules cause more rapid disease progression in individuals with higher levels of proteinuria, suggesting "high-risk candidate" proteins that could contribute to CKD progression. Therefore, we aimed to study the relationship between urinary proteomic profiles and estimated glomerular filtration rate (eGFR) levels in CKD and healthy individuals.

Methods: The urinary proteomics was investigated using liquid chromatography-mass spectrometry in 88 CKD patients with stages 1-3 and 49 healthy individuals. Data analysis was performed by Mascot-SwissProt and STATA software. The association between proteomic data and eGFR was performed using linear regression. The Exponentially Modified Protein Abundance Index (emPAI) was used to estimate the peptide count of proteins.

Results: Overall, 714 urinary proteins were identified in the whole cohort. Of them, 446 proteins were detected in the control group with median (IQR) emPAI 41.7 (18.8–54.8) and 360 proteins in CKD stage-1 with median (IQR) emPAI 61.4 (35.6–80.7), 251 proteins in CKD stage-2 with median (IQR) emPAI 49.8 (30.3–101.7), and 202 proteins in CKD stage-3 with median (IQR) emPAI 63.6 (39.5–89.8).

The incidence of many proteins substantially decreased in the patient groups, whereas the incidence of some proteins was increased compared to the control group. Specific urinary proteins, including UROM, KNG1, CD59, RNAS1, CD44, FBN1, OSTP, CERU, LMAN2, and VMO1, were positively associated with eGFR. In contrast, B2MG, FETUA, IGHA1, IGKC, VTDB, IGK, ALBU, PLMN, IGLC2, and ZA2G had significant negative associations with eGFR.

Conclusions: The number and type of urinary proteins were markedly different between the control and patient groups. Several urinary proteins showed significant positive or negative associations with eGFR increase or decrease, respectively. The association of the proteins merits further validation studies for potential kidney function biomarkers and CKD progression.

PP03.96: Identification of Prognostic Protein Biomarkers in Tears From Non-infectious Uveitis Patients Undergoing Biologic Treatment as a Prelude to Personalized Medicine.

Jesus Mateos, España

Introduction
TNF inhibitors (TNFi) keep ocular inflammation under control in patients with non-infectious uveitis (NIU) that do not respond to classical treatment with corticosteroids and immunosuppressants, but in some patients the inflammation persists. The identification of biomarkers for prediction of treatment response is of outmost importance to individualize treatment and increase clinical benefits. Blood is widely used as a source of biomarkers since it is easily accessible and minimally invasive, but it may not accurately reflect the pathological state of the affected areas in ophthalmic diseases, for which the use of ocular fluids may be more representative of undergoing biological processes.
Methods
Tear samples from 35 NIU patients under TNFi treatment (Adalimumab, 6 months minimum) were collected using glass microcapillary tubes and stored in ProteinLoBind tubes at -20ºC until analysis. Tear proteins were quantified using a NanoDrop One spectrophotometer and analyzed by Data-Independent Acquisition (DIA/SWATH) using a Triple TOF 6600 system. Acquired data were processed using Spectronaut TM 16.0.
Results
In total, 399 proteins were quantified in tear samples with at least one unique peptide, of which 37 were upregulated in responders and 14 in non-responders to TNFi treatment. A major finding was the consistent upregulation of proline-rich protein 4 (PROL4) in non-responders (1.98-fold change, Q=0.018) compared with responders, a protein related to protective functions in the eye. Our preliminary results await further validation through targeted proteomic techniques such as ELISA in an independent cohort of patients with NIU.
Conclusions
The use of DIA/SWATH enables protein profiling in small and complex samples such as tears, thus achieving a deeper molecular characterization of ophthalmic pathologies, and contributes to identify potential biomarkers for prediction and monitoring of treatment response that could help to implement personalized medicine.

PP03.98: Single-Glomerular Spatial Proteomics Profiles Glomerular Heterogeneity in Chronic Kidney Disease

Liyuan Meng, China

Background   The spatial expression of proteins is very important for determining their localization and function in cells and tissues. In kidney disease, all the glomerulus are affected to different extent. Decay in individual glomerulus and morphology determines organ function and clinical prognosis, the heterogeneity of single glomerulus is very important for the diagnosis, classification, and prognosis of the disease. Spatial proteomics can provide a new perspective in tissue heterogeneity study. However   there are technical bottlenecks in using such trace FFPE samples for proteomics research.

Method: Single glomerulus was separated by LMD (Laser micro-dissection) from renal biopsy FFPE samples. Then, the glomerulus was pretreated using micro-sample method and analyzed with MS-spectrometry using data independent acquisition mode. Finally the single glomerulus spatial proteomics data was analyzed with location information by LMD.

Results: We provided a efficient and economical method for trace FFPE samples to analyze the proteome, using this method 3090 proteins were identified from one glomerulus FFPE sample with single MS run. By constructing a proteomic database of a single glomerulus, construct a machine learning model and train the model to distinguish the heterogeneity of pathological damage with glomerulus. It is prospective to investigate the single glomerular heterogeneity with spatial proteomics and its application in the study of the mechanism of glomerular injury of chronic nephropathy.

Conclusion: We establish the proteomic database of single glomerulus, and analyze single glomerulus heterogeneity from spatial dimension by correlating with the information of clinical pathology and morphology. This technology can capture intra-individual variability in kidney diseases and other tissues at sub-biopsy scale. We can use spatial proteomics techniques that based on the basic functional structural units level to explore the glomerular damage mechanisms and to find heterogeneity markers of the diseases.

PROGRAM

PP03.100: Deeper Plasma Proteome Coverage Enables Identification of Novel Biomarkers and Classification of Diseases
Jonathan Moss, Australia

Background
Blood plasma is a valuable specimen for clinical research. However, access to the proteome is limited by the highly dynamic nature of protein abundance, which spans approximately 10 orders of magnitude and with only 22 proteins accounting for 99% of the whole protein mass. To address this challenge, we developed a novel workflow for LC-MS-based plasma proteomics that enriches low abundant proteins and enables an improved coverage of the plasma proteome.
Methods
The described workflow achieves efficient dynamic range compression by capturing and thus enriching low abundant proteins on paramagnetic particles (called ENRICH technology, PreOmics). The particles were separated from the residual plasma and briefly washed to remove residual low-affinity interactors. For LC-MS sample preparation, samples were processed with our iST-BCT protocol (PreOmics) including on-bead reduction and alkylation, digestion and peptide clean-up. Resulting peptides were analyzed by nanoC18 separation (nanoElute LC) coupled to a timsTOF HT mass spectrometer using a dia-PASEF acquisition cycle (Bruker). Measured peptides were identified and quantified by Spectronaut 17 software (Biognosys).
Results
We applied our novel workflow to plasma samples derived from lung cancer patients and age matched healthy donors. From neat plasma samples, we recovered 350 protein groups per sample, covering 405 protein groups represented in the PQ500 panel. From ENRICH-processed samples, we obtained on average 1000 protein groups per sample and were able to recover 800 protein groups in the majority of the two plasma conditions. Statistical analysis of quantified proteins demonstrated a clear separation of healthy donors from patients with lung cancer diagnosis. Strongly upregulated factors in lung cancer samples include S110A8 and S100A9 which have previously been reported to be upregulated in lung cancer and other types of cancer.
Conclusion
By combining enhanced proteome coverage with workflow robustness, the ENRICH technology improves and simplifies plasma proteomics in clinical research studies.

PP03.102: Unravelling the Pathomechanisms of Uterine Fibroids and Associated Heavy Menstrual Bleeding through Systems Biology
Darragh O'Brien, United Kingdom

Uterine fibroids (UFs) are benign tumours affecting up to 80% of women of reproductive age, with 30% of patients suffering from severe symptoms such as painful heavy menstrual bleeding (HMB). Although mutations in MED12 or HMGA2 account for the majority of UF occurrence, the processes by which these lead to UFs and HMB remain poorly understood. In the largest study of its kind, fibroid, myometrium, and endometrium tissues were collected from 137 donors undergoing hysterectomy, myomectomy, or transcervical resection. Of these, 91 donors were profiled by genome wide SNP arrays and their fibroids genotyped for known mutations. Tissues were analysed by RNA-sequencing and quantitative proteomics and a systems level analysis was performed using the MultiOmics Factor Analysis (MOFA+) package for R. The frequency of common UF mutations in our cohort was substantially lower than other reports, potentially reflecting a bias towards more complicated surgeries during tissue collection. Genotyping nevertheless revealed multiple novel exonic and intronic variants. Systems level analysis of genotype, transcriptomic, and proteomic data between myometrium and UF donors identified multiple interrelated gene sets involved in fibroid pathophysiology, including extracellular matrix deposition and remodelling, protein glycosylation, and sulphate biology. Equivalent analysis of endometrium stratified by donor HMB status revealed novel gene sets implicated in the condition, in particular RNA splicing in MED12 mutant fibroids. A paradigm is proposed and supported by a mouse model of HMB, whereby aberrant production of signalling molecules by MED12 mutant fibroids influence isoform expression in the endometrium, resulting in HMB. By merging clinical, genetic, transcriptomic, and proteomic information, we reveal multiple pathways which may underlie the pathomechanisms of UF biology, facilitating the development of novel therapeutic strategies to treat associated pain and HMB.

PP03.104: Development of Diagnostic Multimarkers for Glioblastoma at Early Stages Using Quantitative Proteomic Method

Hyeonji Park, Republic of Korea

Glioblastoma multiforme (GBM) is a fast-growing glioma that develops from star-shaped glial cells supporting the health of the nerve cells within the brain. And it can be very difficult to treat and a cure is often not possible. So glioblastoma cancer-specific biomarker is need due to the lack of specific method for early screening, diagnosis, and prognosis of the patients with glioblastoma. In this study, we have conducted a comprehensive proteome study using human brain tissue from patients with glioblastoma (Grad 1 - Grade 4). In the discovery stage, we have identified 10,910 glioblastoma-specific proteins (Protein Groups), where 2,886 proteins (ANOBA test, P value < 0.01) were quantitated using Tandem Mass Tag (TMT) method. In order to select reliable biomarker candidates, we have carried out the clustering analysis based on the expression pattern in 5 groups, whrere 5 clusters were showed. In the verification stage (Selected target proteins: 159), we developed quantitative targeted method using stable isotope standards (SIS) peptide such as multiple reaction monitoring (MRM) assay which are capable to target peptide fragments very selective and sensitive in complex sample. The expression of 4 proteins were significantly differed between Normal (N: 22) and cancer (G1&G2: 34). Further, we performed a multiplex assay using logistic regression and the 4-protein marker panel (Kbio 1, Kbio 2, Kbio 3, Kbio 4) was constructed, which resulted in a merged AUC value of 0.910. Although we acknowledge that the model requires further validation in a large sample size, the 4-protein marker panel can be used as baseline data for the discovery of novel biomarkers of the glioblastoma.

PP03.106: Machine Learning Applied to Molecular Protein Patterns for Diagnosis of Motor Neuron Disease

Livia Rosa-fernandes, Australia

Motor Neuron Disease (MND) is a general term frequently referring to amyotrophic lateral sclerosis (ALS), but that also includes rarer conditions such as primary lateral sclerosis and progressive muscular atrophies. Disease progression varies, and diagnosis is often difficult owing to the heterogeneity of symptoms that may be present in different patients. In addition, MND can exist as a familial form in about 10% of cases in which a gene mutation can be traced in all affected individuals of a family. Still, in most of MND, it occurs as a sporadic form, in which identifying the underlying causes of the disease poses an extra challenge. Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) MS is a technology first developed in the '80s that is broadly used in clinical microbiology laboratories nowadays, especially for the rapid identification of bacteria. MALDI-TOF has the benefit of requiring a low sample amount, less pre-analysis preparation and being a robust, high-throughput, and cost-effective system. This study focuses on developing an analytical and computational platform for diagnosing MDN patients by combining MALDI-TOF MS and machine learning. During methodological development, different sample preparation methods and analytical parameters are compared, including peak quantity, peak intensity, and noise, before database construction. Acquired data is used to train multiple machine learning algorithms and compare performances. The result is a sample classification methodology that will be expanded to predict the disease status of familial and sporadic disease and non-affected controls.

PP03.108: Optimized Methods to Investigate the Human Lysosomal Proteome by Targeted Proteomics

Shigeru Sakamoto, Japan

Lysosomes fulfil crucial roles for the degradation and recycling of intra- and extracellular macromolecules and play essential roles in metabolic signaling. These functions are facilitated by >340 lysosome-related proteins, whose loss of function can result in a variety of disorders. Proteomic analysis of such diseases is complicated by the low abundance of lysosomal proteins. An ideal method to address this is data-independent acquisition, which enables the reproducible and accurate protein identification and quantification across large sample cohorts. dia-PASEF merges the benefits of DIA with the advantages of ion mobility. Here, we use dia-PASEF to develop a large-scale targeted quantitation assay for the lysosomal proteome.

Samples were analyzed by coupling a nanoElute online to a timsTOF Pro 2 (both Bruker Daltonics). Peptides were separated on a 25cm reversed-phase C18 column using a 60-minute gradient. For dia-PASEF a method consisting of sixteen TIMS ramps with 2 mass ranges in each 100ms scan was applied. Data were processed with Spectronaut v18 (Biognosys) using either a targeted lysosomal protein library (297 proteins) or a full human proteome fasta file.

We identified on average 97,528 peptides and 8,623 protein groups from whole cell lysate and 70,453 peptides and 7,912 protein groups for lysosome-enriched samples, using a 60-minute linear gradient. To investigate the coverage of lysosomal proteins in both sample types, we used targeted data processing by applying a library consisting of 297 lysosomal proteins. On average, we identified 3013 peptides and 269 protein groups from whole cell lysate and 3078 peptides and 276 protein groups from lysosome-enriched samples. Even though the total number of identified proteins doesn't differ significantly, the overall signal intensity for peptides originating from lysosomal proteins was ~30% higher in the enriched sample set. Our results show that dia-PASEF is well-suited for analyzing the lysosomal proteome.

PP03.110: Quantitative Analysis of Endogenous Native Peptides in Urine by DIA-NN after Generating a Comprehensive Spectral Library from DDA Mascot Data.

Amr shalaby, Japan

In peptidomics research, the accurate identification of endogenous peptides is crucial for understanding biological systems and disease mechanisms. Data-dependent acquisition (DDA) is a commonly used mass spectrometry-based approach for peptide identification, but it has limitations, including incomplete sampling of the sample and biased selection of peptides for fragmentation. Data-independent acquisition (DIA) approach overcomes these limitations by acquiring data for all endogenous peptides in a given mass range. However, DIA data analysis requires a spectral library to match the acquired spectra with known peptides. In this study, we generated high-quality, non-redundant, and comprehensive endogenous peptide spectral library from DDA Mascot data using a combination of data extraction and filtration. We then used this library to search against DIA data, resulting in more accurate endogenous peptide and protein identification. Our results demonstrate the utility of creating a comprehensive endogenous peptide spectral library from DDA Mascot data for use in DIA data analysis, providing a comprehensive and unbiased view of the sample, and facilitating reproducible and accurate quantitation of the endogenous peptides.

PROGRAM

PP03.112: Comparative Proteoinformatics for Discovering the Potential Pathogenic Mechanisms of Oligozoospermia

Timur Shkrigunov, Russian Federation

Male infertility affects about 23% of men of reproductive age and is the sole or contributory factor in half of assisted reproductive treatments. Male infertility is caused by a variety of factors, including lifestyle (smoking, alcohol consumption), environmental factors, endocrine disruption, genetic and epigenetic modifications. The oligozoospermia is one of the conditions related to male infertility which associated with abnormal spermatozoid count, motility and morphology. Many cases of male infertility remain unexplained (idiopathic), and therefore the combination of appropriate sample preparation, mass-spectrometry and bioinformatics can be a valuable contribution to semen proteome research.

We analyzed the proteome of semen samples containing cellular (spermatozoa) and non-cellular (seminal plasma) components. Ejaculate samples were treated by SDS-based solubilization combined with the 1DE-gel concentration procedure (truncated SDS-PAGE) and in-gel digestion prior to LC-MS/MS. The comparative proteoinformatics was applied for identifying the proteins responsible for revealing sperm disfunctions associated with oligozoospermia. There were 303 common proteins between normal and oligozoospermia semen samples, including 183 downregulated identifications. Only four upregulated proteins were revealed. The top-level gene ontology biological processes of down-regulated proteins were those: detoxification (GO:0098754), response to stimulus (GO:0050896) and developmental process (GO:0032502). We have noted that detoxification-related proteins CAT, SOD1 and GST play important roles in spermatogenesis and normal sperm function. Indeed, we observed that spermatogenesis (GO:0007283) was significantly decreased regarding oligozoospermia unique proteins compared to normal semen identifications.

We concluded that stress response and cellular detoxification can be the most common aberrant cellular mechanisms among infertile males. Besides, we speculate that semen samples without fractionation can be beneficial for revealing the proteins and/or pathways associated to the occurrence of male infertility.

PP03.114: Investigating the Role of Proteins in Regulating Breast Density in Premenopausal Breast Cancer Patients

Minsoo Son, United States

Breast density is a strong risk factor for breast cancer in premenopausal women. Premenopausal women have denser breasts than postmenopausal women as mammographic breast density decreases slowly with age. Through a systematic and rigorous proteomic analysis, we aimed to provide a comprehensive understanding of the biological mechanisms involved in the regulation of breast density in premenopausal women diagnosed with HR+ or HR- breast cancer.

Formalin-fixed and paraffin embedded (FFPE) specimens from 50 premenopausal breast cancer patients were used for the study. Tumor region and adjacent normal region, as evaluated by pathologists, were included in the proteomics analysis. Samples were analyzed on Orbitrap Eclipse Tribrid coupled with Vanquish Neo LC system.

A total of 3,571 proteins were identified from all the samples analyzed. The protein modules were defined using 'wgcna' package in r and they showed significant correlation and connectivity between modules and with clinical variables such as breast density and hormone status. The regression analyses were performed to find the differentially expressed proteins (DEPs) between tumor region versus adjacent normal tissue which are also associated with breast density, and hormone status after controlling the confounding effects from age, body mass index (BMI), and parity. The biomarker candidates for predicting breast cancer were selected by classification model development using 10 times repeated 5-fold cross validation. Through this investigation, we elucidated the proteomic profiles associated with breast density depending on hormone receptor status in premenopausal women diagnosed with breast cancer. The findings from this study offer novel avenues for the identification of targeted risk management, which may help to optimize prevention regimens in a manner that is tailored to individual patient characteristics, such as breast density and hormone receptor status.

PP03.116: Utilizing HRAM Orbitrap MS to Quantify Therapeutic Monoclonal Antibodies (mAbs) in Human Serum for Clinical Research

Yvonne Song, United States

Background: In clinical testing, the presence of endogenous immunoglobulins with almost identical structures from patients' samples adds another challenge to the accurate quantitation of therapeutic mAbs. Accordingly, mass spectrometry has gained substantial popularity for therapeutic mAb monitoring in clinical laboratories due to its great versatility to detect both tryptic peptides and intact light and heavy chains quantitatively. Here we present the intact light chain quantitation approach for measuring concentrations of therapeutic mAbs in human serum using Orbitrap Exploris 240 MS for clinical research.

Methods: T-mAbs used in this study include adalimumab, bevacizumab, camrelizumab, daratumumab, golimumab, nivolumab, and vedolizumab. Samples were purified by Protein L magnetic beads followed by IdeS digestion and reduction. LC-MS was performed using Thermo Scientific™ Vanquish™ HPLC system interfaced to Thermo Scientific™ Orbitrap Exploris™ 240 mass spectrometer (OE240).

Results: The OE240 MS fully resolved isotopic clusters of different charge states of three subunits by operating at a resolution > 120k. Analytical performance was evaluated by generating the calibration curve, resulting in LOQs between 1 to 5 µg/mL of the mAb concentration in human serum. Excellent linearity was observed with R² values higher than 0.99. The % RSD of the peak areas was less than 15%, which supports the reproducibility of the entire process from sample preparation to LC-MS analysis. Also, the variation of the detected retention time of two IS mAbs was determined to be less than ± 0.05 minutes. Through a quick column reproducibility evaluation, reproducible data were generated over two different column lots showing a 0.2-minute shift with less than 20% peak area differences. This report demonstrates that the MAbPac column generates coherent intact protein data for clinical research including reproducible retention times and peak areas.

Conclusion: Providing highly confident detection and accurate quantitation of therapeutic mAbs in human serum by HRAM Orbitrap MS

PP03.120: Quantitation of Clinically Approved Breast Cancer Biomarkers Utilising Selected Reaction Monitoring

Erin Sykes, Australia

Current diagnosis and treatment selection for breast cancer patients relies heavily on immunohistochemical (IHC) techniques which suffer from issues with specificity, lack of quantitative standardisation and both inter- and intra-laboratory variation. Mass spectrometry, particularly selected reaction monitoring (SRM), is a highly specific, robust, reproducible technique that is capable of high-throughput rapid screens at relatively little cost.

We have developed an SRM assay for breast cancer biomarkers that are currently used in the clinic, specifically oestrogen receptor, HER2 and proliferative markers; Ki-67 and PCNA. SRM quantitative results in a breast cancer cell line panel (n = 22) and breast cancer patient sample (n = 40) were compared to the known mutational status and patient IHC diagnoses to assess the clinical utility of the SRM assay.

For oestrogen receptor and HER2, the SRM assays showed a high level of concordance with the IHC diagnosis. The SRM assay accurately diagnosed 26 of the 28 cases with oestrogen receptor IHC results, with 2 false negatives. For HER2, SRM accurately diagnosed 26 of the 28 cases with HER2 compared to IHC which only accurately diagnosed 13 cases and required further testing for confirmation. Additionally, SRM was able to differentiate 9 cases with low to very low HER2 levels that could be high priority test cases in clinical trials for anti-HER2-chemotherapy conjugates. While a high concordance was observed between the SRM quantitation of the 2 proliferative markers Ki-67 and PCNA, the SRM showed no correlation with Ki-67 IHC results in patient samples.

Our findings demonstrate the feasibility and benefits of SRM assays for protein biomarker quantitation. Accurate quantitation of biomarkers which are already well known to clinicians and pre-approved for clinical use, is an easy pathway in facilitating the uptake of proteomics in the clinic.

PP03.122: Quantitative DIA Proteomics of Urinary Proteins for Identification of Gender and Ages, and for Biomarker Discovery in the Future

TOMOHIRO UCHIMOTO, Japan

Introduction and Methods :
We used DIA method of proteomics for precise quantitation of urinary proteins. Proteins were purified from urine of healthy children (4 -13 years old) and adults (19-60 Years old) and 500 ng of tryptic peptides of each sample was measured in SCIEX 6600 TTOF MS with DIA method. Keratins contaminated during the sample preparation were monitored to omit for this analysis and few other proteins were used for validation of gender. DIA data was processed by all MS/MS with SWATH add-in PeakView software and DIA-NN tool both. The quantity of each protein was calculated by sum of intensity of the Extracted Ion Chromatogram (XIC) respectively.
Results:
Over 1,200 proteins were identified in urine from children and around 2,000 proteins from adults. Some proteins were different in quantity between males and females or boys and girls, were increased or decreased with age.
Conclusions :
As the protein profiles were variable between genders and among ages, the profiles from the same gender and the same ages should be compared for biomarker discovery.

PP03.124: Exploring Sample Preparation Methods for Plasma Proteomics

Iolanda Vendrell, United Kingdom

One of the major aims of clinical proteomics is to identify biomarker panels in body fluids that can help in disease diagnosis, prognosis and patient stratification. Plasma is one of the most used body fluids due to its accessibility, however, it presents some challenges as a result of its high protein dynamic range (>12 orders of magnitude) where fourteen proteins accounts for 95 % of all plasma proteins. The traditional untargeted proteomics approach (non-depleted, 1h gradient and data dependent acquisition) confidently identify around 200-250 proteins. Improvements in sample preparation workflows and depletion strategies, together with advances in mass spectrometry technology (normal throughput versus high-throughput), implementation of new data acquisition methods (data-dependent -DDA- vs data-independent -DIA- acquisition) and data analysis software/strategies (DIA library free/library based) are having a positive impact on the depth of the detected plasma proteome. These allow to look beyond the classical plasma proteome into the realm of tissue leakage products and cytokines/interleukins.
In our lab, we have performed a direct comparison on non-depleted plasma pools from SARS-CoV-19n patients plasma using two different mass spectrometer platforms: Ultimate 3000 Orbitrap-Fusion Lumos (13, 24 and 32 samples per day -spd-) and the high-throughput Evosep tims-TOF-Pro (15, 30, 60, 100 and 200spd) in both DDA and DIA. Furthermore, we have explored two different depletion strategies: top 64 antibody based off-line HPLC depletion (Seppro IgY14 column + Seppro Supermix column) to build a library (for DIA-library-based) and a bead-based plasma enrichment technology (ENRICH-iST kit by PreOmics). We show that, for large cohorts of clinical samples, the 60spd method is most efficient (IDs/time) with a 25-30% increase on proteins quantified in DIA (library-free) compared to DDA. A further 300% increase was observed when using the bead-based plasma depletion strategy (60spd, DIA-library free).

PROGRAM

PP03.126: Proteomic Profiling of Extracellular Vesicles and Particles From Prostate Cancer Cell Lines and Plasma Samples for Diagnosis and Risk Stratification

Qi Wang, Australia

Prostate cancer (PCa) is the second leading cause of cancer-related death in men. The current standard marker PSA cannot accurately differentiate among different PCa stage. Therefore, non-invasive methods detecting novel biomarkers with higher sensitivity and specificity are in great demand. Extracellular vehicles and particles (EVPs), secreted by all kind of cells, existed in all kind of body fluid and enriched in proteins from parent cells, including the large extracellular vesicles, small extracellular vesicles, exomeres and supermeres. EVPs hold promise for PCa diagnosis, risk progression stratification.
Aims: We aimed 1) to investigate the proteomic profile of EVPs in a panel of PCa cell lines and human plasma samples from different stages of PCa patients.and; 2) to identify PCa-specific EVP protein profiles form cell lines and plasma samples for PCa diagnosis, progression risk stratification.
Methods: PCa cell lines (PC3, LNCaP and 22Rv1), and a normal prostate epithelial cell line (RWPE-1) were cultured to isolate EVPs. PCa patients were divided into four groups (control, low risk, intermediate risk, high risk, metastasis. N=3 in each group). All EVPs were isolated by ultracentrifugation and then characterised by transmission electron microscopy, nanoparticle tracking analysis and western blotting, atomic force microscopy, and nano-flowcytometry. LC-MS/MS proteomics was carried out to profile the EVPs protein profiles from cell lines as well as PCa patients plasma samples, respectively.
Results: We have identified distinct protein profiles from 4 EVP subpopulations for further validation and functional study.
Conclusion: We have successfully established a method for isolating EVP sub-populations from a group of PCa cell lines and human PCa plasma samples and comprehensively characterised their properties. Several panels of new EVP protein biomarkers have been identified. The new proteins identified hold promise for the application in liquid biopsy for PCa early diagnosis, risk stratification, monitoring metastasis progress as well as for therapeutic purpose.

PP03.128: Discovery of Urine Biomarkers for Diabetic Nephropathy Detectable Earlier Than Microalbuminuria Development

Tadashi Yamamoto, Japan

INTRODUCTION: Diabetes is a lifestyle-related disease that causes various complications and worsens life prognosis. Among the complications, kidney injuries such as diabetic nephropathy in particular progress to end-stage of chronic renal failure requiring dialysis therapy or kidney transplantation. The diabetic nephropathy is diagnosed by microalbuminuria, however, if it can be detected at an earlier stage, the progression may be suppressed or cured by improving lifestyle habits or medical interventions. In this study, we aimed to search for urinary biomarkers that can detect renal injuries earlier than microalbuminuria.
METHODS: Plasma and urine proteomes of healthy volunteers were analyzed quantitatively by LC-MS and ratios of each protein/serum albumin in plasma and urine were compared to select plasma proteins which ratios in urine were larger than plasma since their reabsorption in proximal tubules is considered less than serum albumin as ones which were leaked at glomerulus with serum albumin but were not reabsorbed efficiently at proximal tubule.
 Then, a system to measure amounts of the selected urine proteins was constructed by surface plasmon resonance method using antibodies against the plasma proteins selected and was used to examine in urine from diabetic patients with or without microalbuminuria and healthy volunteers. The diabetic patients with microalbuminuria were selected who were initially negative but turned to positive in several years for microalbuminuria. The change of their urine levels was examined by using urine samples collected every 4 months thereafter the time point of microalbuminuria
RESULTS: We selected the plasma proteins which ratios in urine were larger than in plasma. Some of those proteins were significantly increased in urine of diabetic patients with microalbuminuria compared to those from healthy controls or diabetic patients without microalbuminuria. Based on this, those urinary proteins were considered to be biomarkers that can detect renal injuries at an earlier stage than microalbuminuria.

PP03.130: Quantitative Proteomics Approach Reveals ARCN1 as Potential Therapeutic Target of LUAD

Chunhua Yang, China

Lung adenocarcinoma (LUAD) is one of the main types of non-small cell lung cancer, represents about 40% of all lung cancers. The prognosis of patients with LUAD is very poor. To obtain potential therapeutic targets for the treatment of LUAD, a high-resolution label-free mass spectrometry was performed on LUAD tissues and adjacent normal tissues from eight patients. A total of 3070 proteins were identified and 241 proteins showed significantly different expression between LUAD and normal tissues ($p < 0.05$, log2(fold change) 1 or <-1). GO and KEGG analysis demonstrated that majority of the proteins were involved in endoplasmic reticulum to Golgi vesicle- transport mediated by COPI complex. All the subunits of COPI complex present upregulated expression level in LUAD tissues compared with adjacent normal tissues. Among them, high ARCN1 level strongly association with poor prognosis in LUAD patients. The upregulated expression of ARCN1 in LUAD tissues were validated by WB, RT-PCR and IHC analysis. Future research suggests that ARCN1 knockdown in LUAD cells inhibits cell proliferation, migration, invasiveness and tumorigenesis. Thus, ARCN1 may be considered as a possible therapeutic target for LUAD.

PP03.132: Exploration Towards the Novel Biomarkers of Human Cystic Echinococcosis Based on Coupling of LC-MS/MS and Immunoassay

Congmin Zhang, China

Cystic Echinococcosis (CE) is a zoonotically parasitic disease and is mainly diagnosed by ultrasonography, which favors the patients at advanced CE but fails to detect those at early/mid-term stage. Serology to detect the CE antigens in blood provides another clinical scenario to improve diagnostic efficiency. There has been a slow progress in recent decades, however, in finding of the CE biomarkers that are accepted in clinical diagnosis, because of lack of a global approach to screen the high antigenic candidates of native CE proteins. In this study, protein identification using LC-MS/MS in the CE surgery tissues revealed that protoscolex and hydatid fluid of active hydatid cysts contained rich CE proteins, while the immunoblotting bands of SDS-PAGE with the patient plasmas as primary antibodies possessed approximately 1100 CE proteins. Data filtration through the frequency of protein detection and score of B-cell epitope prediction, 25 candidates were selected and expressed in the system of E. coli, and then 8 recombinants were found in relatively higher immune-responses to the patient plasmas. These recombinants were employed to the ELISA validation in a large cohort with 600 patient and 600 health plasmas. The survey unveiled 5 CE recombinants, CST, HSPG, MG2-1, MG2-2 and PSAP-3, to show higher sensitivity ranged from 92% to 97% and specificity ranged from 95% to 97%, whereas Egr, a commercial biomarker, to exhibit sensitivity at 85% and specificity at 88%. The ELISA with randomly combined CE recombinants indicated 19 of dual combinations with the exceeded sensitivity and specificity, with sensitivity range from 95% to 99% and specificity ranges from 96% to 99%. Furthermore, combination of these CE recombinants could well discriminate the CE patients at early or later stage. Thus, coupling of LC-MS/MS and immune-recognition with the CE patient plasmas is favorable to explore novel CE biomarkers for serological diagnosis.

PP03.134: Characterization of the Epigenetic Profile of Epidermis in Response to Co-exposure to Ultraviolet Radiations and Benzo[a]pyrene

Sandrine Bourgoin-Voillard, France

Human skin is daily exposed to environmental stressors that can significantly impair skin homeostasis and trigger different disorders including aging and cancers. Ultraviolet radiations (UVR) and polycyclic aromatic hydrocarbons such as Benzo[a]pyrene (BaP) are two main pollutants that can harm the skin. UVR is a complete carcinogen while exposure to BaP is known to trigger pathologies such as immunotoxicity. Co-exposure to UVR and BaP increases skin tumor incidence compared to BaP- or UVR-exposure alone. However, few data are available to explain the biological processes that are altered following co-exposure of skin to BaP and UVR. To get insight into the epigenetic alterations triggered in epidermal cells in response to co-exposure, a comparative mass-spectrometry-based proteomic analysis was performed on skin explants following exposure to either no pollutant, UVR or BaP+UVR. The epidermis was separated, and epidermal proteins were extracted from different cellular fractions before an LC-MS analysis based on a label-free quantitative approach was done. Bioinformatic analyses using partial least squares discriminant analyses and differential expression using the EpiFactors database were used to highlight proteins involved in epigenetic processes that were differentially expressed following the co-exposure. Among all the proteins differentially expressed upon the co-exposure, we identified 12 proteins playing a role in epigenetic functions. 7 of these were in the cytosolic fraction (BRE, CTPB1, CUL2A, DDB1, HMGN5, SRSF1, SRSF3), 4 in the membrane fraction (HMGB1, PRKDC, SFPQ, UBE2N) and 1 in the nuclear fraction (SF3B1). A better understanding of these epigenetic alterations after co-exposure to UV and BaP is of special interest to devise topical treatments that potentially maintain skin homeostasis.

PP03.136: Exposure of Aged Microplastic Induces Changes in the Proteome of Daphnia Magna – A Comprehensive Ecotoxicoproteomic Study

Thomas Fröhlich, Germany

Microplastic particles (MP, < 5 mm) are ubiquitous not only in marine- but also in freshwater. An important freshwater model organism to investigate biological effects of MP is the cladoceran Daphnia, which inhabits a central role in freshwater ecosystems and has been established in ecotoxicology for many years. As plastic particles change their morphological and chemical properties through aging processes, the aim of this study was to investigate effects induced by the ingestion of aged compared to pristine microplastics in D. magna. Therefore, we analyzed proteomes of D. magna exposed to pristine and artificially aged microplastic of different polymer types (PS, PP and LDPE). Using a data-independent (DIA) mass spectrometry-based approach, we were able to quantify around 4500 proteins. Using a customized bioinformatic workflow, we detected differentially abundant proteins for all comparisons between aged and pristine MP. Compared to PS and LDPE, PP displayed the lowest number of altered proteins. Since Daphnia ingest particulate matter, the gut is particularly exposed to MP. Therefore, we additionally examined the proteome of dissected D. magna guts from the same experiment. We found a similar pattern, with PP causing the lowest number of altered proteins, but could identify significantly more differentially abundant proteins for PS and LDPE as compared to the analysis of entire daphnids. Bioinformatic analysis of the proteins significantly altered between pristine and altered MP, revealed interesting candidates (e.g. cuticle related proteins) that were consistently altered in two or more polymer types. Overall, this ecotoxicoproteomics study improves our understanding of molecular mechanisms underlying adverse effects of MP in freshwater ecosystems.

PP03.138: Identification of Cadmium-Responsive Proteins as Potential Biomarkers for Environmental Hazard Exposure

Hee-Gyoo Kang, Republic of Korea

Urine cadmium, a harmful factor found in the environment, has been associated with adverse health effects. However, the specific changes in the body resulting from increased urine cadmium levels remain unknown. This study aimed to identify proteins that exhibit altered expression associated with increased urine cadmium levels, focusing on individuals exposed to environmental harmful factors. The study included a control group (Kimhae) and individuals from areas exposed to environmental harmful factors(Goseong, Goseong-Sangchon, and Janghang). Urine cadmium levels were measured in both groups, and correlation analysis was conducted to identify proteins that showed a significant association with urine cadmium levels. Proteins exhibiting altered expression in the environmentally hazardous areas compared to the control group were further examined. A total of 18 proteins were found to have a statistically significant correlation with urine cadmium levels. Among these proteins, 8 proteins showed significant increases or decreases in expression in the environmentally hazardous areas compared to the control group. Ultimately, these proteins were selected as final candidates representing cadmium-related protein alterations. To validate the expression patterns of these proteins, multiple reaction monitoring (MRM) analysis was performed, revealing that 4 proteins displayed similar tendencies. In this study, 8 proteins that exhibit altered expression in response to increased urine cadmium levels associated with exposure to environmental hazards could serve as potential biomarkers for cadmium exposure. Research results suggest that cadmium has a significant effect on the expression of certain proteins in the body. Understanding the underlying mechanisms and pathways involved in cadmium-induced protein alternation can help develop preventive and therapeutic strategies for individuals exposed to this deleterious factor.

PP03.140: Proteome Profiling of Alternative Proteins in Food Safety

Qifeng Lin, Singapore

The global food supply landscape is facing a crisis with production struggling to meet demand. However, increasing production results in substantial strains on environmental resources, raising ecological and sustainability concerns. Consequently, novel food products which are viable alternative protein sources are rapidly gaining traction. However, these alternative proteins present unknown risks to food-sensitive individuals. Deep understanding of the alternative protein foods and their components will therefore be critical for assessing food safety. To this end, we developed a generalized protein extraction procedure for proteomics analysis of diverse alternative protein food matrices. We applied the protocol in four main categories of alternative proteins: plant-based meats, insect proteins, microbial proteins and cultured meats. Preliminary bioinformatics analysis of the alternative protein proteomes showed that putative allergens from the major ingredient sources could be detected, which will be highly useful for informed allergenic potential risk assessment. Moreover, the comprehensive proteome data could be a valuable resource to aid in driving product innovation and optimization.

PP03.142: Proteomic Mechanisms Underlying Lung Injury in Aging Rat Model Exposed to Fine Particular Matter Air Pollution

Manh Quan Nguyen, Taiwan

Particular matter (PM) air pollution, especially the fine-sized fraction PM2.5, is one of the significant risk factors for various pulmonary diseases, including chronic obstructive pulmonary disease (COPD). Due to the unclear mechanisms of PM-caused COPD, the treatment efficacy of COPD remains further improved. To study the molecular mechanisms underlying PM2.5-caused lung damage in the elderly population, we conducted a quantitative proteomic analysis on lung tissues of 1.5-year-old male Fischer 344 rat models exposed to traffic-related PM2.5 air pollution via whole-body exposure system for three months with and without high-efficiency particulate air (HEPA) filtration. Our study identified 530 and 593 differentially expressed proteins in PM2.5 and HEPA groups. The functional enrichment analysis of the differentially expressed proteins showed the dysregulations of oxidative stress, cell stress and injury, immune response, and cytokine signaling in rats exposed to HEPA and PM2.5. Our findings showed that under PM2.5 exposure, the STAT3 pathway was inhibited by Ptpn2, which further down-regulated a number of proteins involved in the coagulation system in comparison with HEPA. PM2.5 elevated high oxidative stress from mitochondrial oxidative phosphorylation in the lung, which activated Nrf2-mediated oxidative stress response to promote antioxidant gene expression and protection from oxidative stress. Down-regulation of LXR/RXR activation was observed in both PM2.5 and HEPA groups, which resulted in the dysregulation of the complement system. Furthermore, the CXCR4 signaling was decreased in HEPA, while it was increased in PM2.5. In summary, our study proposed PM2.5-regulated molecular mechanisms in aging rats. We expect that the proposed PM2.5-caused pathogenesis mechanisms could be a valuable source to discover potential targets and drugs for treating the elder COPD population.

PP03.144: Crosslinking Mass Spectrometry for Rabbit Calicivirus Receptor Identification

Elena Smertina, Australia

Introduction
In Australia, the European rabbit is one of the most damaging environmental and agricultural vertebrate pest species. The Rabbit haemorrhagic disease virus (RHDV) is a highly pathogenic hepatotropic virus that causes haemorrhages, liver failure and death within 72 h post infection. Since the mid-1990s, the virus is used as a key biocontrol agent, but due to the lack of a robust cell culture system, many aspects of the RHDV life cycle remained unknown, e.g., the entry receptor. Here, I used a recently established liver organoid cell culture system and crosslinking mass spectrometry to identify the RHDV entry receptor.
Methods
Rabbit liver organoids were incubated with RHDV virus-like particles labelled with a heterobifunctional crosslinker. The cell-bound particles were exposed to UV light. Crosslinked virus-receptor complexes were then purified using a biotin group on the crosslinker and receptor candidates were identified using label-free quantification.
Results
A list of the potential receptor candidates has been identified. The list includes liver-specific cell membrane proteins such as transporters, growth factor receptors and membrane proteins involved in endocytosis.
Conclusions
Crosslinking mass spectrometry can be used as an efficient tool for virus receptor screening. Further validation of candidate receptors will be performed to confirm the specificity of binding and biological significance.

PP03.146: Bee Metaproteomics as the Toolbox for Assessing Microbial Dynamics of One-Health Relevance.

Bruno Tilocca, Italy

Cheese is an important component of the Mediterranean diet, commonly appreciated for its health-promoting features and unique taste. Here, among the variety of cheeses, a distinct importance is attributed to raw goat cheese. A pivotal role in the development of these characteristics is attributed to the cheese-associated microbiota and its continuous remodeling over space and time. To date, no thorough metaproteomics study of the raw goat cheese-associated microbiota has previously been conducted and a comprehensive picture of such microbiota, in terms of both composition and activity, is desirable to guide the onset and enhancement of both gustatory and nutraceutical properties. To fulfill this knowledge gap, we employed 16S rRNA gene sequencing and metaproteomics to explore the functional microbiota of a typical raw goat milk cheese at various ripening time points. Also, structural and functional features of the raw goat cheese microbiota were assessed in the rind and core of the cheese mass. The outcomes of this first explorative survey portrayed a stable architecture of the microbial community over the selected ripening time points, providing evidence of a stepwise, unsteady, fermentation of the cheeses.
The thorough characterization of the composition and activity of the microbiota at different cheese-wheel depths described a rind microbiota struggling to maintain the biosafety of the cheese through competition mechanisms and/or by preventing the colonization of the cheese by pathobionts of animal or environmental origin. On the other hand, the core microbiota was focused on third biochemical processes, supporting its role in the development of both the health benefits and the pleasant gustatory nuances of goat cheese.

PP03.148: Algorithm Development for Analysis of O-GlcNAcylated Protein using LC-MS/MS

Dasom An, Republic of Korea

O-GlcNAcylation is a post-translational modification that involves the addition of a single GlcNAc residue to the Ser or Thr of a protein. This process is catalyzed by O-GlcNAc transferase and hydrolyzed by O-GlcNAcase (OGA). The O-GlcNAcylation is related to cellular activities, the stress response, gene transcription, protein translation, cell signaling, and cell cycle regulation. We tried to find the O-GlcNAcylated proteins by comparing OGT knockdown or not in OGA inhibitor treated at cancer cell. After protein extraction and digestion from cancer cells treated with OGA inhibitor, HILIC fractionation and LC-MS/MS analysis combined with HCD, triggered HCD and ETD method were performed. Using the LC-MS/MS raw files, we developed an identification algorithm coded by python 3.9 including calculation steps as follows: 1. Selection of spectra with oxonium ions from raw MS data was obtained using LC-MS/MS; 2. Spectral matching was performed with 20 ppm tolerance against a tryptic and O-GlcNAcylated peptide DB generated from proteins identified using IP2 proteomic pipeline; 3. In the protein candidates, matching score was calculated between b/y and c/z theoretical ion m/z list, and experimental HCD and ETD spectra, respectively; 4. The best calculated score was selected in each O-GlcNAcylated peptide spectrum; As a result, we identified 45 O-GlcNAcylated proteins of 66 distinguishable peptides in a total 92 spectra from control cells treated with OGA inhibitors. On the other hand, we confirmed only 21 O-GlcNAcylated proteins of 30 distinguishable peptides in a total 38 spectra from cells treated with OGT shRNA and OGA inhibitor. Finally, we discovered the interaction between O-GlcNAcylated proteins and transcription related proteins such as MED20, SMAD4, FOXP1 and ID1.

PP03.150: Identifying Glycan Profile Differences of CD33 Expressed in HEK293 and CHO Cells

Kyle Hoffman, Canada

Protein glycosylation is one of the most common and complex post-translational modifications (PTMs) and plays a critical role in protein folding, stability, localization, function, and interactions. Expression of a recombinant glycoprotein in different host cell lines can result in different glycoforms, altering the properties and function of the protein. For this reason, careful consideration must be made when producing glycoproteins for research or therapeutic use. Here, we use our new software platform, PEAKS GlycanFinder, to identify major differences in the glycan profiles and abundances at six positions in myeloid cell surface antigen CD33. CD33 protein samples were purchased from R&D Systems and Sino Biological vendors. Samples were reduced, alkylated, and digested with trypsin in 50 mM TEAB buffer. Peptides were separated using a nanoElute® UHPLC then analyzed on a timsTOF Pro (Bruker) instrument. All raw data was analyzed using PEAKS GlycanFinder. Precursor, glycan, and peptide fragment ion mass errors were set to 15 PPM, 20 PPM, and 0.05 Da, respectively. We identified six highly glycosylated sites across CD33, decorated with over 200 different N-linked glycans in both the Sino Biological and R&D Systems samples. While each protein had the same glycosites and a similar number of glycans, only 53% of glycan were shared. Moreover, the glycan profiles at each of the six sites significantly varied between samples. There was a higher representation of fucosylated glycans attached to CD33 from Sino Biological, likely a result of expression in HEK293 cells. Each of the glycopeptides were supported by high-quality spectra that passed a 1% false discovery rate threshold for both glycan and peptide identifications. Taken together, PEAKS GlycanFinder accurately identifies differences in glycan moieties at specific sites of a protein expressed in different host cell systems.

PP03.152: Site-Specific Glycan Microheterogeneity Evaluation of Aflibercept Fusion Protein by Glycopeptide-BasedLC-MSMS Mapping

Seoyoung Hwang, Republic of Korea

The evaluation of the protein glycosylation states of samples of aflibercept obtained from three different regions was conducted by site-specific N-linked glycan microheterogeneity profiling. Glycopeptide-based nano-LC MSMS mapping of tryptic-digested samples of each aflibercept lot provided site-specific information about glycan microheterogeneity on each of the five N-glycosylation sites (two sites in the VEGFR-1 region, two sites in the VEGFR-2 region, and one site in the human IgG Fc region). Next, the glycopeptide-mapping results obtained from the three different aflibercept lots were compared to evaluate the similarity between the samples. Three aflibercept lots showed a high degree of similarity in glycan composition, fucosylation level, sialylation level, and branching, when all five N-glycosylation sites were assessed together as a group. On the other hand, noticeable variations between lots in the glycan types and sialylation levels on the two sites of the VEGFR-2 domain were observed when each of the five N-glycosylation sites were assessed using the glycopeptide-based method. The presence of N-glycolylneuraminic acid (NeuGc) glycans, which may mediate adverse immune reactions in antibody therapeutics, were also detected on the sites of VEGFR1 and VEGFR2 domains, but not on the IgG Fc domain site. These results imply that analyses of the glycosylation profiles of fusion proteins containing multiple N-glycosylation sites, such as aflibercept, being done as a part of quality control for the therapeutics manufacturing process or for biosimilar development, can be done with a more applicable outcome by assessing each site separately.

PP03.154: A New Strategy for Glycopeptide Enrichment Using Combining ZIC-HILIC and Molecular Weight Cut-off

Ji Hyun Kang, Republic of Korea

Glycosylation, the most common protein post-translational modifications (PTMs), plays a key role in biological and disease processes. However, glycosylation takes place with low abundance and exhibits a broad dynamic range across different glycoproteins. Additionally, large amounts of non-glycosylated peptides in complex mixtures would hinder the detection of low-abundant glycopeptide signals during LC-MS/MS analysis. These characteristics present significant difficulties in N-glycosylation analysis and require an efficient approach, such as a specific glycopeptide enrichment method. For glycopeptide isolation, zwitterionic hydrophilic interaction liquid chromatography (ZIC-HILIC) has been widely used. In this study, we show that the glycopeptide enrichment step was improved by a combination of ZIC-HILIC and molecular weight cut-off (MWCO) filter. MWCO filter-aided method increased the number of identified glycopeptides compared to method and decreased the percentage of non-glycopeptide-matched spectra by 27.8 % compared to ZIC-HILIC method without MWCO filter step. These results indicate that removal of non-glycopeptides was effectively achieved using MWCO. These findings indicate that the site-specific characterization of glycoproteins from complex sample can be improved using the MWCO-aided intact glycopeptide enrichment method.

PP03.156: N-glycomic Identification of Novel Soft Tissue Prognostic Biomarkers for Oral Cancers

Enikő Gebri, Hungary

Introduction: The mortality rates of oral cancers have increased six-fold in the last 50 years. As the number of malignant diseases proliferates, more than 377,000 oral squamous cell carcinoma (OSCC) new cases and consequently 170,000 deaths are diagnosed worldwide annually. In addition, OSCC is an aggressive disease with a glycoproteomically unmapped disease progression and disheartingly low five-year survival rate. Besides the most commonly known risk factors such as alcohol consumption, tobacco, poor oral hygiene, HPV infection, long-term immunosuppressant therapies may also increase the risk and change the therapeutic response of secondary malignancies. Alterations of protein N-glycosylation have a pivotal role in tumorigenesis and metastasis formation. Thus, the aim of our study was to identify novel glycobiomarkers to predict more precise prognosis suggesting more efficient therapeutic alternatives for oral cancers.
Methods: Oral mucosal soft tissue samples were obtained by using incisional biopsy from five patients with OSCC, both from the malignant and the opposite healthy gingival sides, as well as from seven age-sex matched healthy controls with the appropriate Ethical Permissions and Informed Patient Consents (DE RKEB/IKEB: 6152-2022). 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: Ten out of the 39 identified N-glycan structures showed significant (p<0.05) differences between the malignant tissue samples of OSCC patients and the healthy controls. Comparing the healthy and the positive control oral mucosal samples two significantly different N-glycan structures have been revealed, while there were no differences between the N-glycan profiles of the malignant tumor and the positive control samples.
Conclusions: The high-resolution CE-LIF-based glyocoanalytical method reported in this presentation proved to be an efficient and sensitive workflow for glycobiomarker-based molecular diagnostics of oral malignant lesions.

See you in Busan!
BEXCO, Busan, Korea
2023.hupo.org
#HUPO2023

Together Through Proteomics

PROGRAM

PP03.158: Characterizing Degradation Products from Bacteroides Thetaiotaomicron to Understand Bacterial Dextran Utilization in the Gut.

Wai-Chi Man, United Kingdom

Background:
The gut microbiota and metabolism of a diversity of colonic symbionts help break down otherwise undigestible carbohydrates. We know the importance of bacteria colonizing the gut broadly, there is much to learn about carbohydrate-active enzymes that allow the bacteria to enact function. By characterizing dextran as function of both molecular machinery available for dextran transport and the size of dextran for metabolism.

Method:
Bacterial cultures were grown until mid-exponential in minimal media with 0.5% dextran as a sole carbon source. Culture supernatant was filtered, and metals were removed by treatment with Chelex resin. After supernatants were purified with porous graphitized carbon resin, then analyzed using a Thermo Scientific™ Dionex™ CarboPac™ PA300-4µm analytical column with Dionex™ ICS-6000 HPIC™ system with an electrochemical detector and a Q Exactive™ HF-X hybrid quadrupole-Orbitrap™ mass spectrometer. Prior to MS injection, column effluent was passed through a Dionex™ ERD 500 electrolytically regenerated desalter for salt removal. Data were processed with Chromeleon™ Chromatography Data System and Xcalibur™ software. Mass spectra were annotated manually using GlycoWorkbench.

Results:
With high-performance anion exchange chromatography (HPAE) with electrochemical detection in pulsed amperometric mode (PAD) hyphenated to mass spectrometry (HPAE-PAD-MS, as IC-MS) for identification and characterization of dextran samples recovered from bacterial culture supernatants. The IC-MS method capitalized derivatization-free samples with the power of HPAE to resolve heterogeneous mixtures of oligosaccharides, including isomeric structures. With CarboPac PA300-4µm column, we demonstrated the power of the platform to resolve the dextran sizes from DP2 to DP31 for subsequent structural characterization. Glycosidic backbone fragmentation and cross-ring fragmentation enabled both sequence and linkage characterization of dextran samples, to elucidate some molecular boundaries for the transport of dextran via the PUL degradation and utilization by Bt.

Conclusion:
Ion chromatography hyphenated to mass spectrometry can be used for structural analysis of dextran degradation products from bacterial cultures.

PP03.160: Proteomic Analysis of Human Tissue and Organoids Derived From Hepatocellular Carcinoma

Ye Eun Park, Republic of Korea

Organoids have emerged as a promising ex vivo 3D culture engineering in medicinal research. However, the proteomic profile of organoid has not been fully studied, and their proteome and glycoproteome have yet to be compared to the original tissue. additionally, the limited sample amount of organoid can be challenging to obtain high-quality mass spectrometric data. In this study, we generated proteomic and glycoproteomic profiles with the microgram-scale (1-5 µg) of protein amount from hepatocellular carcinoma organoids. We identified a total of 2,099 proteins in global proteomics, which were associated with the metabolism of RNA, nucleobase-containing small molecule metabolic process, and carbon metabolism. These functions were consistent with the major metabolic functions of liver tissue. Interestingly, important liver cancer biomarker, Alpha-fetoprotein (AFP) were identified with high sequence coverage. In the glycoproteomic approach, we identified 41 glycoproteins from 159 glycopeptides comparing glycan compositions between HCC tissue and HCC-derived organoids, where we found that they were very similar. Consequently, These proteomic and glycoproteomic analysis suggest that organoids share similar characteristics with actual tissues

PP03.162: High-resolution MS-based Glycoproteomics Analysis of Esophageal Cancer Lines Reveal Potential Link Between Estrogen Signaling and Esophageal Cancer

Chao Peng, China

Esophageal cancer (EC) is the fourth leading cause of cancer-related deaths and one of the most aggressive cancer types with poor prognosis in China.The 5-year overall survival (OS) rate of EC remains in the range of 15–25%. There is a clear male gender bias in the incidence that cannot be fully explained by known risk factors. To benefit the drug and biomarker discovery, we perform proteomics and N-glycoproteomics analysis on Esophageal cancer cell lines with PEAKS online platform to provide additional clues into tumor biology that cannot be deciphered by genomic analysis.

We developed a straightforward and high effective glycopeptide-enrichment and detection methods with the art of state MS equipment Orbitrap Tribrid eclipse and timsTOF Pro2.A novel HILIC-type material was applied for high yield glycopeptide enrichment and Stepped CE mode was used for effective glycopeptides fragenment both in the HCD mode using Orbitrap and the CID mode using DDA-PASEF.

Finally, a total of 5047 proteins and 46596 peptides were quantified and 8670 intact N-glycopeptides in 624 N-glycoproteins, and 558 N-glycans were identified . 6183 intact glycopeptides from 520 glycoproteins and 5789 intact glycopeptides from 493 glycoproteins were identified in TE-1 and HET-1A respectively , of which 4688 (64.4%) intact glycopeptides from 401 (65.5 %) glycoproteins were shared between the two samples. Additionally, we identified 453 and 369 N-glycans in TE-1 and HET-1A, for a total of 286 (53.4 %) in both samples.

KEGG analysis of DE proteins revealed that DNA repair, microRNAs in cancer, small cell lung cancer, O-glycan biosynthesis and central carbon metabolism in cancer were over-represented in proteins that were upregulated in TE-1. Whereas, metabolism and Estrogen signaling pathway were over-represented in proteins that were down-regulated. The down-regulation of estrogen response-related proteins was consistent with previous studies, suggesting a potential link between estrogen signaling and esophageal cancer.

PP03.164: Improvements in Glycoproteomics Through Architecture Changes to the Tribrid MS Platform

Nicholas Riley, United States

Recent hardware changes introduced on the Orbitrap Ascend Tribrid MS include dual ion routing multipoles (IRMs) that can be used to parallelize accumulation, dissociation, and mass analysis of three separate ion populations. Here we explore how this architecture improves N- and O-glycopeptide characterization by increasing scan acquisition speeds without sacrificing spectral quality. The balance between scan speed and MS/MS product ion signal-to-noise is especially important in glycoproteomics. Complexities of glycopeptide fragmentation necessitate large precursor ion populations, and consequently, long ion accumulation times, for quality MS/MS spectra. To compound matters further, dissociation methods like electron transfer dissociation (ETD) that benefit glycopeptide characterization come with overhead times that also slow down scan acquisition. Conversely, heterogeneity inherent to glycosylation means that any given retention time during an LC-MS/MS analysis may contain numerous glycopeptide species to target through data-dependent acquisition. Often duty cycle is sacrificed to some degree, which results in higher quality spectra of abundant species but leaves other precursor ions under-sampled. We analyze mixtures of N- and O-glycopeptides to show that 20-30% more MS/MS scans can be acquired when parallelizing three ion populations using the dual IRMs of the Orbitrap Ascend. This translates to 10-20% gains in glycopeptide identifications depending on dissociation type(s), scan acquisition schemes, and method parameters (e.g., precursor ion accumulation and reaction times). Focusing on O-glycopeptide analysis with ETD-based methods, we also explore how acquisition rates and ion-ion reaction times affect identifications and product ions generation. We show what parameters need to be considered in O-glycopeptide characterization to generate c- and z-type ions that can be used for O-glycosite localization while also maximizing scan acquisition rates to improve total site-localized O-glycopeptide identification. In all, we show how architectural changes to the Tribrid MS platform benefit glycoproteomic experiments by parallelizing scan functions to minimize overhead time and improve sensitivity.

See you in Busan!
BEXCO, Busan, Korea
2023.hupo.org
#HUPO2023

Together Through Proteomics

PROGRAM

PP03.166: Proteome-Wide Discovery of Human Brain Protein Glycoforms and Glycan Modification Changes in Alzheimer's Disease

Qi Zhang, United States

Glycosylation is the most prevalent and complex form of protein modification which produces vastly diverse arrays of glycosylated proteoforms or glycoforms to regulate a variety of physiological processes, including synaptic function and brain homeostasis. We and others have previously shown that altered N-glycosylation may play a role in Alzheimer's disease (AD). However, our knowledge of system-wide changes in site-specific N-glycans and glycoforms in AD is still limited. In this study, we developed an integrated approach that combines high-resolution mass spectrometry-enabled, intact glycopeptide-based glycoproteomics and systems biology for large-scale, in-depth analysis of protein glycoforms and site-specific N-glycans in human brain and their changes in AD. We used this approach to analyze human brain tissue samples from neuropathologically confirmed AD cases and their age-matched controls. In total, we identified 12,176 unique intact N-glycopeptides with attached N-glycans at the N-X-S|T (X ≠ P) sequon, corresponding to 10,731 unique N-glycoforms with 164 distinct N-glycan compositions at 2544 unique N-glycosites in 1184 unique N-glycoproteins. Our analyses reveal previously unknown changes in protein glycoforms and site-specific glycans in AD and uncover glycoform co-regulation networks in human brain and their alterations in AD. Together, our findings provide novel insights into the roles of glycan modifications in brain dysfunction in AD and establish a new framework of glycosylation-based networks and pathways for understanding and treating AD.

PP03.168: Unveiling the Performance of a Novel High-Resolution Accurate Mass Platform for Proteomics Applications

Tabiwang N. Arrey, Germany

LC-MS-based proteomics has proven to be a powerful tool for the deep profiling of proteins in biological samples. Despite its advances, obtaining comprehensive protein profiles remains challenging due to the complexity and wide dynamic range of proteomes. Here we investigate the capabilities of a novel HRAM mass spectrometer for the qualitative and quantitative single-shot based LC-MS analysis of different proteome samples with different complexity and dynamic range such as yeast cells, mammalian cells or microbiome.
Yeast and mammalian cell line digests were resuspended in 0.1 % Formic acid to a concentration of 200 ng/ul. Microbiome proteins were extracted and digested using AccelerOme platform. All samples were separated with the Vanquish Neo and analyzed on a novel HRAM mass spectrometer, operated in either DIA or DDA mode. The raw data were processed with a beta version of Proteome Discoverer 3.1.
We evaluated the novel HRAM platform by analyzing 3 samples of different complexity: (1) low complexity yeast digest, (2) medium complexity mammalian cell line digest, and (3) high complexity Gut microbiome digest. The results show that complete yeast proteome (4.400 protein groups) can now be analyzed three times faster and with less sample compared to previous published data [A. S.Hebert & A. L.Richards at al. 2014].
From the mammalian cell line, an average of 8,000 protein groups and over 60,000 unique peptides were identified with 18min gradient (60 SPD) and 50 ng sample load. From the Gut Microbiome standard digest over 18,000 protein groups and >100,000 unique peptides were identified from all 21 organisms using a 90 min LC gradient. This is an increase of about 46% protein groups and about 25% unique peptides compared to an Orbitrap Exploris 480 MS system.
In-depth evaluation of novel HRAM mass spectrometer for bottom-up proteomics.

PP03.170: Generation of Amino Acid Sequences of Unknown Species for Metaproteomics Using Phylogenetic Relationships of Known Species

Nobuaki Miura, Japan

Background: Metaproteomics using mass spectrometry is a powerful method to simultaneously profile species and proteins in the intestinal microbiome [1]. Protein amino acid sequence (hereafter simply referred to as sequence) databases for intestinal bacteria have been actively developed, such as MetaHIT (Metagenomics of Human Intestinal Tract)[2] and HMP (Human Microbiome Project)[3]. The fundamental problem is that the diversity of the database cannot represent the diversity of the bacterial flora. Therefore, we have developed a method to generate sequences of unknown species probabilistically based on phylogenetic relationships in sequence databases of known species.

Methods: The sequence of H. Pylori F16 strain was generated using the known sequences of 29 closely related strains not including F16. Leaves of the phylogenetic tree were randomly selected, and a branch of species X located at a randomly set evolutionary distance was inserted. Position and amino acid substitution probabilities were calculated from the frequencies in the 29 species to produce a sequence that satisfied the conditions based on evolutionary distance.

Results: Compared to the 29 closely related species, the coverage of the original F16 sequences improved from 91% to approximately 97% for the generated sequences using random branching. In conventional target decoy search analysis of mass spectrometry data, PSM increases in large databases using random branching, but the proportion of hits for peptides other than the F16 original sequence also increases significantly.

Conclusion: The test of sequence generation by random branching using H. pylori F16 strains suggested that this method is useful. However, it was also seen that there is a problem peculiar to the analysis with a large database such as these generated by random branching.

[1] Comput Struct Biotechnol J. 2023;21:1140-1150.
[2] Nature. 2010;464:59–65.
[3] Nature. 2012;486:215–221.

PP03.172: A Complete and Automated End-To-End Sample Preparation Strategy for High-Throughput and Standardized Proteomics With High Sensitivity

Dorte Bekker-jensen, Denmark

Proteomics is evolving at a rapid pace and it's becoming a more standardized and high throughput technology allowing larger samples cohorts to be analyzed. It is a significant paradigm shift, which calls for standardized and automated end-to-end workflows to reproducibly process the increasing number of samples. It is also timely to challenge and rethink the traditional proteomics sample preparation strategy, to only digest and process the amount of sample needed for one injection to minimize consumable and enzyme costs, which becomes significant as sample cohorts increase. Here we present a generic Evotip Pure loading strategy as a cornerstone technology for a standardized and modular automation concept where functionality can be included to customize the workflow to include digestion, enrichment, depletion, etc. We show the Evotip Pure loading strategy in an automated end-to-end sample preparation workflow on the Opentrons OT-2 liquid handling robot.
HeLa cells, harvested in boiling 5% SDS, were used as input material for all digestions, ranging from 1 ng to 1 µg starting material. The automated sample preparation utilizes protein aggregation capture on magnetic particles (Resyn Biosciences), followed by on-bead digestion and direct sample loading of peptides. All automated protocols are available in user friendly HTML format, that generate complete ready-to-load scripts for the Opentrons app.
Automated tip loading resulted in effective peptide binding with stable storage on the Evotip for more than three weeks. The protocol was optimized for short digestion time at ambient temperature and sample loading of up to 192 samples in less than 8 hours, enabling a throughput of up to 384 samples in a workday. By keeping the digestion to low starting amounts, the protocol offers a highly cost-efficient sample preparation strategy for large sample cohorts, while still attaining a deep proteome coverage of more than 7,000 identified proteins.

PP03.174: Operating, Maintaining, and Troubleshooting the Sensitivity and Robustness of timsTOF Platforms for Proteomics Studies

Xianming Liu, China

Background
Proteomics applications going from sample preparation to data analysis have significantly improved the sensitivity and robustness. The development of the Parallel Accumulation Serial Fragmentation (PASEF) technology increases peak capacity, sensitivity, and acquisition speed for confident identification and quantification. However, high throughput sensitive proteomics analysis requires a reliable quality control (QC) to maintaining the best performance and minimize down time. Here, we study factors that affect instrument performance and methods to monitor for these effects.

Methods
Commercial Human protein extracts (HeLa and K562) were analyzed by coupling nanoElute I or Evosep One to 3 different trapped ion mobility – QTOF mass spectrometers (timsTOF Pro2, timsTOF SCP, and timsTOF HT). Several LC columns, sample quantities, separation and MS methods were evaluated. Raw data were processed with PaSER, DIA-NN, or FragPipe to estimate the number of peptides and proteins. Selected "target peptides" were extract with DataAnalysis and Skyline.

Results
More than 5,000 proteins and 40,000 peptides were identified in dia-PASEF from 4ng K562 with Evosep One and timsTOF SCP and more than 100,000 peptides from 200ng of K562 with timsTOF HT. Based on results, we generated a list of 12 "target peptides" which are detected in K562 and HeLa. We evaluated the total number of proteins, peptides, and quantitative effect from the "target peptides" in 9 different MS methods. For example, 4 or 20 PASEF ramps decreased the peptide number to respectively 17% and 30% compared to the reference method. Furthermore, a 6% duty cycle resulted in the lowest identifications but the highest intensity of the ion chromatograms and mobilograms extracted from the "target peptides". Additionally, the influence of chromatographic performance on the number of peptides and proteins was studied.

Conclusions
It is important to establish a reliable LC-MS QC strategy, that is based on identifications, whereas selected targets give additional information.

PP03.176: S-Trap Turbo: From Sample Prep to Analysis in Record Time

John Wilson, United States

With its high levels of ease of use and reproducibility, the S-Trap sample preparation system has found widespread adaptation in proteomics analyses. However, recent advances in proteomics throughput necessitate concomitant advances in sample preparation. The elimination of any and all removable workflow steps increases both throughput and robustness. Sample dry-down is tedious, and often time-variable. We present the new S-Trap turbo that yield minimal elution volumes of highly concentrated peptides suited for immediate analysis by injection on LC-MS or spotting on MALDI.

New snap-cap S-Trap turbo micro plates and snap cap columns were constructed via plastic injection molding containing newly developed, compressed polymeric traps derivatized with new surface modifications. The S-Trap protocol was performed and analyzed by LCMS. 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 serum (most hydrophilic), HeLa or HEK cell lysate (both hydrophilic and hydrophobic) and rabbit brain acetone powder (most hydrophobic); between 1 ug to 100 ug of protein was processed. S-Trap turbo elutions as low as 5 uL 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 to 2 hrs at 47 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; acidification was observed to produce peptide precipitation with some samples. No significant loss of hydrophobic peptides between the standard S-Trap protocol and S-Trap turbo was noticed, likely due to mostly-aqueous buffer A to solubilize peptides followed by hard centrifugation.

PP03.178: A Unified Approach for Comprehensive Characterization of Intracellular and Extracellular Proteome Remodeling, Specifically Thiol-based, in Response to Oxidative Stress

Sandra Anjo, Portugal

In the present work, we apply our new approach, the oxSWATH[1] to perform an exhaustive characterization of the intra- and extracellular proteomic alterations of cells exposed to oxidative cells. OxSWATH allows integrating the information regarding relative cysteine oxidation with the analysis of the total protein level. Thus, in a single analysis, it was possible to evaluate the alteration considering protein redox status and performed a generic differential protein expression analysis of the cells exposed to an oxidative stress condition caused by an acute stimulation with hydrogen peroxide. Moreover, to completely characterize the cellular response, both the cells and the secretome were analyzed, covering the intracellular and extracellular responses, respectively. A total of 915 proteins were altered upon oxidative stress from which 90 were altered in both intra- and extracellular space. Moreover, a clear tendency for a remodelling of the extracellular space was observed with near 80% of the altered proteins found altered in the secretome. The analysis of the overall redox status of the proteins reveals a tendency to have a reduced environment in the extracellular space, while an equilibrium between the reduced and oxidized proteins is achieved in the intracellular environment. Again, a higher number of secreted proteins present an alteration of their redox status upon oxidative stress when compared with the intracellular protein (250 and 61 proteins, respectively). From those, only 4 were commonly altered between the two cellular spaces. Overall, these results indicate that there is a differential adaptation of the intracellular and extracellular proteomes, with the extracellular space being particularly affected by the oxidative stress. Moreover, the potential of the oxSWATH method was confirmed in this work, since a truly comprehensive evaluation of proteomics changes upon the oxidative stimulus was achieved using a single approach.

[1]Anjo, Sandra I et al. 2019 Redox biology 22: 101130. doi:10.1016/j.redox.2019.101130

PP03.180: Utilization of the Speed, Sensitivity and Accuracy of the ZenoTOF 7600 to Enhance Protein Identifications from Packed Emitter Columns

Jenna Cowen, Australia

The field of proteomics demands high-performance mass spectrometry tools to achieve comprehensive protein identifications, sensitive analysis, and reliable quantitation. IonOpticks' Aurora Series SX packed emitter columns have been specifically designed to optimize peptide and protein analysis on the ZenoTOF 7600 mass spectrometer, enabling researchers to unlock a new level of sensitivity. This enhanced sensitivity allows for the identification and quantification of proteins from complex samples with unprecedented accuracy, significantly expanding the depth of proteome coverage. Researchers can now confidently explore the complex proteomic landscape and uncover novel biomarkers or protein interactions that were previously challenging to identify.

Furthermore, the combination of IonOpticks' Aurora Series SX columns and the ZenoTOF 7600 mass spectrometer ensures stable quantitation across multiple samples. The Aurora Series SX columns exhibited excellent reproducibility, providing consistent results and minimizing variability in quantitative analyses. The high-sensitivity analysis facilitated by the Aurora Series SX columns on the ZenoTOF 7600 mass spectrometer, coupled with stable quantitation, represents a powerful approach for comprehensive proteomics research. We were able to confidently identify more than 7300 proteins per run on a 45 min gradient with a majority of proteins having a CV <10%. The ability to maximize protein identifications, accurately quantify protein expression, and maintain analytical reproducibility empowers scientists to unravel complex biological processes and gain deeper insights into disease mechanisms, drug responses, and cellular signaling pathways.

In summary, the integration of IonOpticks' Aurora Series SX packed emitter columns with the Sciex ZenoTOF 7600 mass spectrometer offers a transformative solution for maximizing protein identifications, achieving high-sensitivity analysis, and ensuring stable quantitation in proteomics. This powerful combination enhances the capabilities of mass spectrometry and paves the way for breakthrough discoveries in the field of protein research.

PP03.182: A Comparative Investigation of Human Plasma Proteome Profiling With State-Of-The-Art Mass Spectrometry and Affinity-Based Assays

Yuehan Feng, Switzerland

Introduction
Plasma/serum is the liquid component of blood containing a plethora of biomolecules and a wealth of information. Unlocking this complex matrix requires large-scale analytical techniques that provide robust quantification, reliability, as well as reproducibility, thereby revealing valuable biological data. There are two major classes of technologies that are widely used in high-throughput proteomics studies: mass spectrometry and affinity-based proteomics. Here, we performed a comparative assessment of two leading state-of-the-art proteomics approaches to profile human plasma samples from an oncology case control cohort.

Methods
Fifteen plasma samples from late-stage prostate cancer patients and fifteen matched healthy controls were analyhed with Biognosys' TrueDiscovery DIA-MS platform and with Olink's Explore panels.

Results
Using Biognosys' TrueDiscovery platform, a total of 3532 proteins could be identified in the plasma samples, compared to 1497 proteins that were detected with Olink's Explore 1536. Both data sets were comparable in terms of technical reproducibility, with a median CV of less than 15% across replicate samples. The different proteomics approaches were partially complementary; while both techniques uniquely identified a set of proteins, a considerable share of the found proteins (865) was detected with both platforms. When extrapolating to the more recent Olink Explore 3072 assay, this number would increase to 1461 overlapping proteins. The overall Spearman correlation for the overlapping proteins was 0.604 (median rho). This is higher than the recently reported quantitative correlation between Olink's PEA and another affinity-based proteomics platform, SomaScan (median rho 0.454; Katz et al., 2022).

Conclusion
Taken together, this technical comparison of two cutting-edge platforms for blood plasma proteome profiling revealed the robustness and complementarity of both approaches for biomarker discovery. While the unbiased nature of mass spectrometry allows for hypothesis-free and disease-agnostic discovery of novel biomarkers with proteoform-resolution, affinity-based approach offers a high-throughput solution with comprehensive coverage of predefined protein panels.

PP03.184: Analysis of Peptides and Proteins by Native and SDS Capillary Gel Electrophoresis Coupled to Electrospray Ionization Mass Spectrometry

Enikő Gebri, Hungary

Background: Capillary electrophoresis (CE) is one of the frequently used liquid phase separation methods for the analysis of peptides and proteins, mostly utilizing UV or fluorescent detection both in zone (CZE) and gel electrophoresis (CGE) modes. Hyphenation of CE with electrospray ionization mass spectrometry (ESI-MS), however, provides additional structural information about the separated sample components. In the past decades, various CE-MS interfaces have been developed including sheath flow, sheathless and liquid junction based approaches, but none of them supported CGE-ESI-MS for SDS-proteins.

Methods: Coupling capillary gel electrophoresis to a mass spectrometer via the Coaxial Sheath Flow Reactor Interface only required a cut-to-size rugged bare fused silica separation capillary filled with the corresponding gel-buffer compositions for native- and SDS-CGE modes. The setup could be readily connected in just a few minutes to any commercial ESI-MS via the closed-circuit flow reactor tube.

Results: The excellent potential of this novel setup was shown by comparing UV and MS detections under identical separation conditions for peptide and protein analysis. This arrangement also offered the option of the application of post column reactions in the flow reactor section, e.g., to remove non-MS friendly background electrolyte components. In SDS-CGE mode, inclusion complexation was utilized in the flow reactor section having γ-cyclodextrin in the sheath liquid to remove the SDS content from the sample and the background electrolyte before entering the MS unit. While maintaining good separation efficiency, the decreased ion suppression made possible the long sought MS detection after separating SDS-proteins by CGE.

Conclusions: A simple and widely applicable Coaxial Sheath Flow Reactor Interface was designed and implemented for easy and robust connection of liquid-phase microseparation methods to mass spectrometric detection, especially for capillary gel electrophoresis analysis of proteins and peptides, including SDS-CGE-ESI-MS.

PP03.186: Critical micelle considered Filter-Aided Sample Preparation Method Combined with LC-MS/MS Allows Comprehensive Global Proteomic Profiling of Adipocytes

Jiwon Hong, Republic of Korea

Obesity has been merging as a major health problem not only from the abruptly growing number of patients but also exposing patients to risk of other diseases such as diabetes, high blood pressure and cardiovascular diseases. Adipocytes, also known as lipocytes or fat cells, are what primary composes adipose tissue where energy is stored as fat. Contrary to the previously acknowledged role of simple energy storage, more evidence shows that adipocytes play dynamic roles in metabolism beyond mere fat storage proposing the need of a more comprehensive study of their working mechanisms. In particular, browning, where fat storing white adipocytes differentiate to energy consuming brown(beige) adipocytes, has long been suggested as a solution for obesity treatment. However, due to the presence of lipid, technical limitation has restricted extensive proteomic studies of adipocytes, where those already done were mainly studied by in-gel digestion of specified proteins.

Here, we have developed an optimized critical micelle considered FASP (Filter-Aided Sample Preparation) method for lipid abundant samples allowing efficient digestion possible for adipocytes. Combining this modified sample preparation method with 10-plex TMT labeling and our DO-NCFC-RP/RP-MS/MS platform, 117,824 global peptides were identified corresponding to 7,691 protein groups (≥2 hits). With our comprehensive profiling data, we plan to analyze proteins showing significant change between pre, beige and white adipocytes. By investigating the differentiation mechanism of pre to white adipcoytes and the browning mechanism of white to beige adipocytes, we expect finding hints from target markers for application in obesity treatment.

PP03.188: An Approach Using Peptide Barcodes for Quantification of Duplicated Gene Products With Identical Sequence to Study the Evolutionary Significance

Keiji Kito, Japan

Gene duplication has a significant role in evolution of a variety of organisms. Duplicated gene pairs have sometimes undergone function divergence, since at least one counterpart can often accept dynamic mutations. In contrast, duplicated pairs having sequences with high similarity are also observed in genome, some parts of which are believed to contribute expression levels of a such group of gene products. Mass spectrometry-based analysis is useful to quantify protein abundance with high accuracy, however, protein pairs with highly similar or identical sequences are quite difficult to distinguish and detect by this analytical technique. Recently, we have developed an approach that overcome this type of problems, in which unique peptide-tags with high sensitivity in MS analysis are introduced into individual proteins to be analyzed and used as barcodes for protein detection. This strategy can also achieve highly sensitive detection of proteins even if those are very low abundance. In this study, peptide-tags are used to quantify the abundance of duplicated protein pairs with conserved sequences in budding yeast. We successfully detected each abundance of duplicated proteins with identical sequence. In order to understand the evolutionary significance, it was investigated what extent phenotype and protein abundance are affected in mutant of genetic deletion of one counterpart of duplicated pairs. In the case of certain pairs, cell growth was largely decreased when deletion was not compensated by increased expression of remaining counterpart gene. This result suggests that, in this case, need of their protein abundance generated from both genes is a possible reason why duplicated pair with identical sequence are evolutionary conserved without natural selection. We will also repot the effect of deletion of one counterpart gene when exposed to different types of stresses and discuss the evolutionary significance of duplicated genes remaining in current living organisms.

PP03.190: Single Drop Microextraction for Enhanced Detection in Glycan Analysis by Capillary Electrophoresis

Enikő Gebri, Hungary

Introduction: Capillary electrophoresis (CE) handles complex samples with excellent resolution, but it has poor sensitivity due to the small detection volume and suffers from destacking of high-conductivity samples. Therefore, sample preparation is often required to clean up the matrix and enrich the analyte(s). Despite advancements in high performance analytical instruments, sample preparation remains indispensable for extracting the analyte(s) of interest in a concentrated form to facilitate high sensitivity analysis. In this paper, single drop microextraction (SDME) is evaluated for sample preparation, aiming to enhance sensitivity in CE analysis of glycans.

Methods: A single drop hanging at the inlet of the separation capillary was used as the acceptor phase in SDME of carbohydrate samples ranging from monomers to oligomers of up to a dozen units. The enriched sample in the single drop was injected into the separation capillary for subsequent high resolution analysis. This in-line coupling of SDME and CE demonstrated the advantages of reducing sample loss and providing convenience.

Results: SDME was first evaluated for preconcentrating the neutral fluorophore carbohydrate tag, 2-aminoacridone (AMAC). Following the successful enrichment of the neutral fluorophore, standard carbohydrates such as maltose and maltooligosaccharides up to a degree of polymerization of 12 with AMAC label were used to optimize the SDME method for glycans. Then, released and AMAC labeled IgG glycans were analyzed by in-line SDME-CE.

Conclusions: This paper presents an operationally-simple sample preparation approach that can be easily adopted without modifying the commercial CE instrument with complex interfaces. SDME has proven to be a powerful tool for analyzing glycan samples, especially for trace analysis in complex biological matrices.

PP03.192: Improving Mass Accuracy in MALDI-TOF MS Analysis of Pathogenic Proteins Using 6xHIS-Tagged Internal Calibration

Saeyoung Lee, Republic of Korea

INTRODUCTION: Linear mode of MALDI-TOF MS has been routinely used for bacterial identification in clinic, depends on the pattern analysis of spectral library rather than accurate mass measurement of ribosomal proteins (10~15 kDa). However, a demand for more accurate mass analysis of pathogens (e.g., KPC-2 carbapenemase) is more recently increasing for diagnostic purpose.
METHODS: We introduced a 6xHIS-tagged KPC-2 (i.e., hKPC-2) and used it as an internal mass calibrator for the mass calibration of target proteins. After internal mass calibration (In-Cal), we evaluated the observed mass of KPC-2 against theoretical mass of hKPC-2, which has 823 Da mass difference from the target protein. We further assessed the accuracy and precision of our calibration method regarding the identification of KPC-2 and other pathogens in clinical isolates (n=42).
RESULTS: Among several candidates for internal mass calibrators, the internal mass calibration using a 6xHIS-tagged protein on the target showed the highest mass accuracy and precision in the detection of target proteins (e.g, KPC-2). The application of hKPC-2 as an internal calibrator resulted in significant enhancements in mass accuracy, mass precision, linearity and repeatability of KPC-2.
CONCLUSIONS: These findings highlight the effectiveness of employing hKPC-2 as an internal mass calibrator for robust and reliable detection of KPC-2 and other target proteins.

PP03.194: Approaching Single-Shot, Full Proteome Coverage Using Packed Emitter Columns

Yanxiang Meng, Australia

Full proteome analysis is a long-standing aspirational goal in the field of proteomics. The advent of high-resolution mass spectrometry, coupled with advancements in chromatographic separation, is bringing us closer to achieving this objective. IonOpticks' Aurora Series packed emitter columns, when combined with Thermo Scientific's Orbitrap mass spectrometers, provide a powerful platform for achieving deep and comprehensive proteomic profiling. When used to analyze complex proteome samples, the IonOpticks Frontier column (60 cm x 75 μm i.d.), specifically optimized for deep proteome analysis, exhibited exceptional resolving power and robustness. It enabled highly efficient chromatographic separations, resulting in improved peak capacity and enhanced identification and quantification of proteomic samples.

The integration of IonOpticks' Aurora Frontier column with Thermo Scientific's Orbitrap mass spectrometers allowed the identification of greater than 10,000 proteins from a single-shot - which is approaching full proteome coverage. This achievement was attributed to the unique features of Aurora Series columns, including their elimination of dead volumes and unique fittings capable of withstanding >1700bar. These attributes contributed to increased separation efficiency, reduced peak broadening, and enhanced detection sensitivity, ultimately enabling the identification of a greater number of peptides and proteins in a single-shot analysis. Moreover, the robustness and reproducibility of IonOpticks' Aurora Series columns on Thermo Scientific's mass spectrometers ensured consistent results across multiple experimental runs.

The implementation of IonOpticks' Aurora Series packed emitter columns on Thermo Scientific's Orbitrap mass spectrometers represents a significant breakthrough in proteomic research. Achieving single-shot full proteome coverage will open new avenues for in-depth exploration of complex biological systems.

PP03.196: Quantifying 1000 Protein Groups per Minute of Microflow LC Gradient Using Data-Independent Acquisition (DIA)

Jason Neo, Singapore

High-throughput proteomic analysis has long been the goal of many researchers, and this workflow has been shown to work well using the Evosep One HPLC system when coupled to a variety of mass spectrometry systems. Data-independent acquisition (DIA) has become the standard method of analysis with many researchers. Using DIA on a hybrid quadrupole time-of-flight (QTOF) system gives incredible speed and sensitivity with very high quantitative precision, especially when the quantitation is performed on the MS2 transitions. It has been previously shown using a combination of the Evosep One and a QTOF system that impressive numbers of proteins can be identified and quantified from standard cell lysate digests using DIA. Here, we present this workflow using a conventional UPLC system at 5 L/min. The gradients used were designed to approximate the active gradient used on the Evosep One system, and cell lysate digests were separated on a 150 mm x 0.3 mm microflow column in trap/elute mode. A trap/elute method was designed using a 5-minute active gradient and 11 minutes total run time to mimic the Evosep 100 SPD method. Using human cell lysates of K562 and HeLa and a 200 ng on-column load, approximately 5,000 protein groups could be quantified using DIA, which is approximately 1,000 protein groups per minute of active gradient. These analyses were very robust with about 90%-95% of proteins being quantified with a CV <20%. These analyses were not restricted to human cell lines, as impressive data were also obtained with a yeast proteome sample where over 3,100 protein groups could be quantified in 5 minutes. To visualize the data obtained from DIA-NN software, we used a cloud-based software suite to import the .tsv output file, followed by statistical analyses to show differential protein expression.

PP03.198: AbMap: A High-Throughput Platform for Antibody Decoding

Huan Qi, China

Antibodies are crucial molecules in life sciences and biomedical researches. Numerous antibodies have been produced for various targets in both academia and industry (Nat. Biotechnol., 2020, 38, p. 1234-1239). The presence of antibodies in sera is closely related to health conditions and can be easily collected. Antibodies carry their functions through the bindings to the epitopes of other molecules, mostly, proteins. We define these bindings as binding capacity. However, a high-throughput platform for profiling antibody/sera binding capacity is currently lacking. To overcome this limitation, the AbMap platform was developed, integrating phage display, DNA barcoding, next-generation sequencing, and bioinformatics. To optimize and validate AbMap, ~10,000 antibodies have already been analyzed, including therapeutic antibodies, such as Sintilimab (Acta. Bioch. Bioph. Sin., 2021, 53, p. 628–635; Mol. Cell. Proteom., 2021, 20, p. 10059). Moreover, AbMap was employed to investigate the critical residues of neutralizing antibodies and vaccine-induced antibodies to evaluate their protective effects against emerging mutant strains of SARS-CoV-2 (Cell. Mol. Immunol., 2021, 18, p. 1067-1069). Additionally, AbMap was utilized to profile sera for identifying biomarkers in systemic lupus erythematosus (Mol. Cell. Proteom., 2019, 18, p. 1851-1863). Given the versatility of the AbMap platform, other libraries constructed using display technology, such as the human peptidome, could be readily incorporated. In the near future, AbMap may be applied to explore other classes of antibodies, including IgE, IgA, and IgD, and a variety of other diseases. In summary, AbMap is a powerful, high-throughput, and universal platform for addressing antibody-related questions.

PROGRAM

PP03.200: Unlocking the Full Potential of the timsTOF Platform Using Packed Emitter Columns

Jarrod Sandow, Australia

The timsTOF mass spectrometer has revolutionized proteomics research with its exceptional capabilities in speed and sensitivity. However, the full potential of this platform can only be realised by partnering this technology with high-performance chromatography and peptide separation. Aurora Series packed emitter columns are designed to optimize peptide separations and ion transmission efficiency by eliminating dead volumes from the analytical column, ultimately leading to dramatically improved sensitivity, dynamic range, and robustness. By incorporating these columns into the timsTOF workflow we were able to significantly enhance the depth and quality of proteome coverage.

Aurora Series columns offered superior resolving power compared to other commercially available columns, allowing for enhanced separation of complex peptide mixtures. This resulted in increased identifications and quantification of peptides and proteins with more than 6500 proteins identified in 30 mins. The high ion transmission efficiency of Aurora Series columns led to improved signal intensity and sensitivity. This was particularly advantageous for single-cell applications with more than 3000 proteins identified from true single-cell samples. Moreover, the compatibility of Aurora Series columns with the timsTOF system ensures seamless integration and optimal performance. The combination of Aurora Series columns and timsTOF technology allows for robust and reproducible proteomic analyses, allowing scientists to discover more proteins from a large variety of research samples.

The utilisation of IonOpticks' Aurora Series columns on the timsTOF platform offers substantial advantages for proteomics research. Researchers can expect enhanced peptide separations, increased sensitivity, and improved robustness, leading to deeper insights into complex biological systems. These advancements in performance will undoubtedly accelerate discoveries and drive breakthroughs in the field of proteomics.

PP03.202: Specific Pupylation as IDEntity Reporter (SPIDER) for the Identification of Protein-Biomolecule Interactions

Sheng-ce Tao, China

Protein-biomolecule interactions play crucial roles in nearly all biological processes. Identifying the interacting protein(s) for a biomolecule of interest is vital. Although numerous assays exist, there is always a demand for highly robust and reliable methods. We developed the Specific Pupylation as IDEntity Reporter (SPIDER) method for identifying protein-biomolecule interactions by combining substrate-based proximity labeling activity from the pupylation pathway of Mycobacterium tuberculosis and the streptavidin (SA)-biotin system. With SPIDER, we verified the interactions between known binding proteins of protein, DNA, RNA, and small molecules. We successfully utilized SPIDER to construct the global protein interactome for m6A and mRNA, identifying various uncharacterized m6A binding proteins, and confirming SRSF7 as a potential m6A reader. We also determined the binding proteins for lenalidomide and CobB on a global scale. Furthermore, we pinpointed SARS-CoV-2-specific receptors on the cell membrane. In conclusion, SPIDER is a substrate-based proximity labeling system. Due to its enzymatic catalytic nature, which converts noncovalent interactions to covalent ones, it allows for the efficient and specific identification and validation of biomolecule-protein interactions, especially weak, transient, and membrane-localized interactions, as long as the biomolecule can be biotinylated.

PP03.204: Novel Tandem Nano and Capillary Flow LC-MS Based Approach for Ultra-High Performance Proteome Profiling With Near 100% MS Utilization

Alec Valenta, United States

Low-flow UHPLC (<5 μL/min) coupled with high-resolution accurate-mass (HRAM) mass spectrometry (MS) is the gold standard for deep quantitative profiling of complex proteomes. However, the unmatched sensitivity of low-flow LC-MS typically comes at a cost of low MS utilization and sample throughput. The high proportion of idle MS time results from the long sample injection and loading and column washing and equilibration cycles. Here we present a novel, facile and robust workflow that eliminates these pockets of idle MS time by employing a tandem LC-MS analysis regime compatible with nano and capillary flow separations.

The LC-MS platform comprises a Thermo Scientific™ Vanquish™ Neo UHPLC system configured for tandem direct injection workflows coupled to an Orbitrap Astral™ MS. The configuration is easy to assemble using finger tight nanoViper™ fittings. Optimized method templates for nano and capillary LC gradient separation ensure high MS utilization, negligible carryover, and high separation quality.

The tandem LC-MS platform was tested using 8-65 min gradients (180 and 22 samples/day, respectively) and found capable of delivering 24/7 profiling of complex protein digests. For example, a 65-min gradient with a 75 μmx75 cm (2 μm particles) column at 300 nL/min enabled the identification of more than >100,000 peptides and >10,000 protein groups on column 1 and column 2 in each replicate with > 94% MS utilization. Greater than 90% of protein groups overlapped among runs for excellent reproducibility. We show how the resolving power of the tandem nano and capillary LC-MS create a new performance standard in the speed and proteome depth. It is a promising alternative to conventional low-flow LCMS-based shotgun and targeted proteomics analysis in complex matrices combining robustness and ease of use with maximum MS utilization, sample throughput and minimum column carryover.

A novel LCMS configuration for high-throughput and high-sensitive proteomics analysis with high MS utilization

PP03.206: BCA-no-more: Seamless, High Throughput Protein Quantification Directly on S-Trap Plates

John Wilson, United States

Recent advances in proteomics throughput necessitate concomitant workflow improvements. One standard step in sample preparation is protein concentration determination, typically performed using the BCA assay. However, these assays are time-consuming, prone to interference, and may exhibit edge effects in a 96-well plate format. To overcome these challenges, we introduce a new concept: direct quantification of cleaned, surface-bound protein on S-Trap 96-well plates using intrinsic protein fluorescence, eliminating the need for an additional destructive assay.

Proteins applied to S-Trap columns load at the head, progressing deeper into the trap as affinity sites become occupied. This surface-concentrated presentation of intrinsically fluorescent residues enables quantification through top excitation and emission detection, removing the need for separate protein assays and sacrificing additional samples.

S-Trap plates and columns are specifically designed to trap proteins and eliminate interfering contaminants, such as buffers, reducing agents, detergents, and small molecules. These interfering molecules affect various protein assays, including BCA assays and fluorescence-based quantification. This approach allows the use of reagents during sample prep that could interfere with subsequent protein assays, ensuring compatibility with various lysis buffer reagents. It is a general-purpose technique with broad applicability and compatibility.

Our patent-pending direct-determination method significantly reduces protein quantification time compared to BCA assays, while maintaining compatibility with standard workflows. The S-Trap sample preparation workflow effectively removes matrix contaminants, simplifying protein concentration determination without additional steps. This on-plate protein concentration determination is ideal for high-throughput clinical settings with automated fluid handlers.
S-Trap 96-well plates were prepared following standard protocols. Samples were bound, washed, and standard BSA curves were loaded. Protein fluorescence was measured and compared to BCA for limit of detection, reproducibility, and dynamic range assessment.

This patent-pending direct determination of protein concentration with intrinsic clean-up eliminates the need for additional costly assays and seamlessly integrates into automated sample preparation workflows.

PP03.208: AccelerOme Automatic Sample Preparation Enables Highly Reproducible Quantitative Proteomics Analysis in Velocity Data-independent Acquisition (DIA) Workflow

Kevin Yang, United States

Data-independent acquisition (DIA) coupled with label-free quantification (LFQ) has emerged as a powerful tool for quantitative proteomics. The sensitivity and reproducibility of DIA analysis for proteomics makes it attractive for large-scale biological investigations. Despite recent advances in liquid chromatography and mass spectrometry, LFQ-DIA is hampered by sample preparation uniformity, a critical step for robust protein quantitation. Manual sample preparation is time-consuming, labor-intensive, and lacks reproducibility due to its inherent error-prone processes. In this study, we coupled a previously developed Velocity DIA workflow with an AccelerOme platform, an automatic sample preparation solution to ensure uniformity across different samples and minimize variation. As a comparison of the reproducibility in automated sample preparation, proteins extracted from HeLa cells were processed with AccelerOme platform or through commonly used manual preparation methods, including EasyPep, suspension trapping (STrap), and urea denaturation followed by C18 clean-up (urea-C18). The Velocity DIA workflow was operated by directly injecting the ensuing samples into a 50-cm µPAC Neo column and the peptides were resolved in a 30-min gradient operated by a Vanquish Neo UHPLC system. The eluted peptides were analyzed on an Orbitrap Exploris 480 mass spectrometer operated in DIA mode. The results demonstrated that AccelerOme outperforms manual preparation, where it affords the lowest median coefficient of variance between replicates. Furthermore, we were able to identify over 6000 proteins within a 30-min gradient for all sample preparation methods except urea-C18, suggesting an exhaustive protein extraction and a minimal sample loss provided by AccelerOme. In conclusion, we demonstrate that AccelerOme together with a Velocity DIA workflow provides an end-to-end platform for high-throughput and reproducible DIA analysis that can improve the quality and efficiency of proteomic analysis.

Session Date/Time: Tuesday, September 19, 2023 - 04:30 PM - 05:35 PM

CS20: AI and MS Analysis

Chair

Nuno Bandeira, United States

Chair

Laura Elo, Finland

16:31 **CS20.01: Keynote Speaker - Towards Sensitivity and Quantitative Accuracy in DIA Proteomics**
Vadim Demichev, Germany

16:56 **CS20.02: Deep Neural Networks for Combining Heterogeneous Features of Peptides in Data Independent Acquisition Mass Spectrometry**
Namgil Lee, Republic of Korea

Data-independent acquisition (DIA) has gained much attention in mass spectrometry (MS)-based proteomics for its improved reproducibility and unbiased data acquisition. In DIA-MS, the spectral library is crucial in peptide identification. However, this method is limited to peptides previously identified via data-dependent acquisition (DDA) MS experiments. This study proposes a deep learning approach for generating spectral libraries, even for previously unseen peptides. While most deep learning-based methods rely on one-hot encoding representation for peptides, the proposed method incorporates physicochemical features, including atomic composition, hydrophobicity, flexibility, fractional surface probability, and aromaticity. We introduce sparsity regularized neural network layers to facilitate the selection and combination of important high-dimensional physicochemical features and improve prediction performance. Furthermore, we suggest a transfer learning strategy for training the proposed deep neural networks having multiple heterogeneous input channels. Numerical experiments using benchmark MS data demonstrated that the proposed deep learning model outperformed other benchmark deep learning models, such as Prosit and DeepDIA. And it was demonstrated that the proposed models with sparsity regularization yielded larger numbers of identified peptides than the other deep learning models.

PROGRAM

17:09   **De Novo Peptide Sequencing With Instanovo: Highly Accurate, Database-Free Peptide Identification for Large Scale Experiments**
Timothy Jenkins, Denmark

Mass spectrometry-based proteomics has revolutionized the study of proteins and their abundance in different matrices on a large scale. Bottom-up proteomics is the most widely used approach and relies on identification of peptides by comparing recorded MS2 spectra to theoretical peptide fragmentation spectra generated from in silico digestion of a protein database. Here, the target-decoy (TD) database strategy is almost exclusively used for both spectrum-centric and peptide-centric experiments to evaluate false discovery rates (FDR) of the resulting peptide-spectrum matches. However, TD searches struggle with the identification of protein isoforms, alternative splicing events, and proteins from organisms that are not included in the dataset. Additionally, the inclusion of post-translational modifications (PTMs) exponentially increases the computational time and the complexity of TD searches, thereby allowing searches of only a few PTMs and limiting semi-tryptic or open searches. An alternative approach is de novo peptide sequencing, which identifies peptides through precursor fragmentation and fragment ion fingerprinting. Modern de novo sequencing algorithms still suffer from high computational costs and FDRs, rendering de novo sequencing for large scale experiments infeasible. Here, we introduce InstaNovo, a deep learning model trained on one of the largest mass spectrometry datasets generated to date, containing all human peptides and tens of millions of mass spectra. InstaNovo achieves leading-edge performance in de novo peptide prediction, approaching fidelity rates comparable to TD approaches. We benchmark InstaNovo against state-of-the-art algorithms, showcasing the benefits of human-inspired, iterative refinement of de novo prediction. Our results demonstrate the applicability of InstaNovo to both established and emerging fields. InstaNovo appears to be a powerful approach for system-wide de novo sequencing experiments, without the need for prior sequence information and not limited by the same constraints as TD strategies. Thereby, InstaNovo presents a powerful tool for scientists for easy adoption of de novo sequencing with unprecedented precision.

17:22   **CS20.04: MAGPIE: Using Machine Learning to Assess the Confidence of Protein-Protein Interactions in Human Plasma**
Emily Hashimoto-Roth, Canada

Immunoprecipitation coupled to mass spectrometry (IP-MS) methods are often used to identify protein-protein interactions (PPIs) in biological samples. While these approaches are prone to false-positive identifications through contamination and antibody non-specific binding, their results can be effectively filtered by combining the use of negative controls and computational modelling. Unfortunately, these filtering methods do not bode well when IP-MS is performed on human plasma samples because, among other reasons, of the notably higher fraction of non-specific interactions. Therein, proteins cannot be overexpressed or inhibited, and existing modeling algorithms are not adapted for execution without such controls.

To address this challenge, we introduce MAGPIE, a novel machine learning-based approach for identifying PPIs in human plasma using IP-MS, which leverages negative controls that include antibodies targeting proteins not known to be present in human plasma. To first identify a high-quality set of negative controls that can be used for false-positive interaction modelling, unsupervised learning algorithms are applied to label-free MS quantification data. In doing so, outliers are identified, yielding a set of high-quality controls for modeling antibody non-specific protein bindings. MAGPIE then uses a logistic regression classifier to assess the confidence of PPIs detected in IP-MS experiments using antibodies targeting known plasma proteins.

When applied to 5 IP-MS experiments, our algorithm identified 68 PPIs with an FDR of 20%, outperforming approaches using Z-score and fold-change thresholds. MAGPIE also significantly outperformed the state-of-the-art PPI discovery tool SAINT (Choi et al., 2011), detecting 3 times more interactions at half the FDR. Further support for MAGPIE's PPI identification is confirmed by known or predicted interactions in the STRING repository (Szklarczyk et al. 2015).

Ultimately, MAGPIE is a new software tool to fill an unmet need in the analysis of PPIs in human plasma, facilitating the characterization of biological processes and molecular pathways mediated by such interactions.

Session Date/Time: Tuesday, September 19, 2023 - 04:30 PM - 05:35 PM

CS21: Food/Environmental Impact

PROGRAM

Chair

Tae-Young Kim, Republic of Korea

Chair

Eva Csosz, Hungary

16:31   **CS21.01: Keynote Speaker - Proteomics on the verge of One Health Approach**
Paola Roncada, Italy

16:56   **CS21.02: Predicting the Impact of Chemical Mixtures Through Proteome Integral Solubility Alteration and Intrinsic Properties of Protein Targets**
Susana Cristobal, Sweden

Our understanding and characterization of the exposome, which includes the chemical mixtures encountered through air, water, food, and consumables, is continually improving. However, evaluating the effects of exposure to these mixtures remains a major challenge in toxicology. Current methodologies for assessing mixture hazards often rely on prior knowledge of individual compounds, which is limited in the case of complex and unidentified mixtures. To address this, we propose integrating proteomics-based methods for chemical risk assessment of chemical mixtures. In this study, we applied a two-dimensional proteome integral solubility alteration (2D-PISA) approach to identify protein targets in a model chemical mixture (TCDD, alpha-endosulfan, and bisphenol A) using HepG2 cells. We analyzed 2,886 proteins with 2D-PISA, identifying 24 targets with significant alterations in solubility across temperature and concentration dimensions. Structural validation of these targets was performed using nanoDSF. Additionally, we introduced the analytical hierarchy process to prioritize protein targets based on their intrinsic properties and potential impact on cellular function. Our result shows that this unbiased methodology allows the evaluation of previously unexplored targets, bypassing the need for preexisting knowledge of protein interactions. We anticipate that ranking the targets based on their intrinsic properties associated with cellular functionality will facilitate inter-species extrapolation of toxicity predictions, even for non-model organisms. Therefore, it will become feasible soon to predict the impact of chemical mixtures on any species with available genome or proteogenomic information. In summary, our study presents a framework to predict the impact of chemical mixtures on human health and the environment. By integrating proteomics-based methods and considering the intrinsic properties of protein targets, we could advance the field of predictive toxicology for chemical mixtures.

17:09 **CS21.03: The Proteomes that Feed the World**
Genc Haljiti, Germany

Plants form the basis for almost all life on Earth. Protein-rich plant-based alternatives to animal-derived foods not only constitute a worldwide megatrend but are also necessary for supporting a growing human population and counteracting climate change. Although crop genomes are being increasingly elucidated, less is known about crop proteomes. Thus, we have assembled an international team of experts in the fields of plant biology, proteomics, and bioinformatics, whose aim is to map out the proteomes of tissues and organs from the 100 crop plants that are most crucial for human nutrition. This team constitutes the Crop Proteome Engine – the core component of the "The Proteomes that Feed the World" doctoral program launched at TUM and funded by the Elite Network of Bavaria.

A three-step sequential workflow including TCA/acetone precipitation, SDS solubilization, and phenol protein extraction was used, followed by tryptic digestion and SP3 sample clean-up. A robust and reproducible microflow LC-MS/MS workflow on high pH reverse-phase fractionated peptide samples was tested on various tissues, including seeds, roots, and leaves. MaxQuant and Prosit rescoring were used to identify and quantify the proteins.

The workflow described above resulted in high protein yields for various crops, including quinoa, sorghum, potato, and tomato. We were able to identify and quantify more than 10,000 proteins in the tomato plant <i>Solanum lycopersicum. Further, we generated the first thorough proteome of the 18 quinoa plant, <i>Chenopodium quinoa tissues, for which we could detect more than 20,000 proteins in 6 h/tissue of instrument time. The raw data for all studied crops, alongside the results of protein identification and quantification, will be routinely made available to the public via the resources such as PRIDE and ProteomicsDB.

This will be the first proteome atlas of the 100 most significant crop plants used in human nutrition.

17:22 **CS21.04: Proteomic Study on the Physiological Responses of Marine Ectotherm Under Temporal and Spatial Variation of Temperature Stress**
Woo-young Song, Republic of Korea

Global climate change is having a significant impact on the survival and growth of marine invertebrates. In South Korea, there have been frequent incidences of mass mortality of aqua-cultured ectotherms, most prominently in summer. Interestingly, the mortality of ectotherms is significantly dependent on both the degree of temperature fluctuation and the zonal distribution of the habitat. This finding suggests that ectotherms have different physiological responses to temporal and spatial temperature variations in tidal zones.
To reveal the mechanism of acclimation, two marine ectotherms, abalone and oyster, were subjected to isobaric tag-based quantitative proteomics. To understand the physiological responses of abalone under fluctuating temperature, a time-course exposure of abalones to a thermal fluctuation chamber was conducted and their oxygen consumption and ammonia excretion rates were measured. When exposed to thermal fluctuations (20-26°C), energy metabolism was downregulated and muscle constituent protein expression was upregulated compared to that at a constant temperature (26°C). This result suggested that the diminished demand for amino acid catabolism during the low-temperature period (20°C) of thermal fluctuations may buffer abalone from the lethal consequences of extreme temperatures in summer.
To investigate the mechanism of acclimation of Pacific oysters depending on zonal distribution, intertidal and subtidal oysters were transplanted into each other's habitat, cultured for two weeks, and sampled. Without transplantation, the gills of intertidal oysters exhibited upregulation of heat shock proteins compared to subtidal oysters. The fold changes of heat shock proteins were greater in summer than fall. However, heat shock proteins were not significantly higher in the transplanted intertidal oysters compared to the transplanted subtidal oysters, implying that the short-term transplantation did not lead to a phenotypic change against environmental stress.
In summary, the differential protein expression of marine ectotherms provides clues to the stress response mechanism to meet the increased energy demand and repair cell damage.

Session Date/Time: Tuesday, September 19, 2023 - 04:30 PM - 05:35 PM

CS22: Clinical Proteomics

PROGRAM

Chair

Polina Prokofeva, Germany

Chair

Stephen Pennington, Ireland

16:31 **CS22.01:Multi-Omics Analyses Reveal Novel Regulators for Age-Associated Deterioration of Musculoskeletal System**

Eun-Soo Kwon, Republic of Korea

Aging is associated with a progressive loss of musculoskeletal system, leading to progressive disability and loss of independence in human. To gain insight into these changes, we performed multi-omics analyses including proteomics and transcriptome analyses using muscle and blood ; Our studies have uncovered multiple factors that modulated the function of musculoskeletal system with age. In skeletal muscles, fatty acid-binding protein 3 (FABP3) mediated membrane lipid saturation alters fluidity and induces ER stress with aging. Plasma proteomic profiling of young and old mice reveals cadherin-13 prevents age-related bone loss. Transcriptomic analyses identified multiple miRNA whose expressions are age-associated. Among them, miR-431 promotes differentiation and regeneration of old skeletal muscle by targeting Smad4. Further, a subset of microRNAs in the Dlk1-Dio3 cluster regulates age-associated muscle atrophy by targeting muscle E3 ligase, Atrogin-1. Taken altogether, our study provides multiple therapeutic targets for musculoskeletal diseases including sarcopenia and osteopenia.

16:44 **CS22.02: Proteomic Signatures of Drug Susceptibility in Cancer**

Priya Ramarao-milne, Australia

Introduction: Proteomics is emerging as a promising field for discovering cancer biomarkers predicting treatment efficacy. Recently, ProCan and the Wellcome Sanger Institute published the world's largest pan-cancer proteomic dataset of 949 cell lines, treated with 625 anti-cancer drugs. This dataset is a powerful tool for identifying proteomic biomarkers of drug sensitivity and resistance, which can be used to build drug susceptibility profiles for tumours. This capability could be useful as a clinical adjunct to determine the optimal treatment for a patient, while avoiding administration of ineffective and toxic drugs.
Methods: Recent studies have identified single proteins in cell line proteomic data correlating with drug susceptibility. However, due to computational demand and lack of suitable algorithms, identifying pair-wise (doublet), and higher-order (triplet and quadruplet) combinations that synergistically modulate drug susceptibility are beyond the scope of current methods. Here, we use a novel machine learning algorithm to identify pathway synergies, uncovering higher-order proteomic signatures underlying drug response.
Results: We present a comprehensive catalogue of proteomic signatures in cancer correlating with drug susceptibility, enabling insight into biologically relevant pathways with predictive value. Our method uncovers "global" baseline signatures predicting drug susceptibility that recurrently appear across all cell lines, and "local" signatures that exclusively predict susceptibility to specific drug classes. For example, high baseline expressions of MCM family proteins are highly predictive of low IC50 values, or increased sensitivity to microtubule inhibitors only. On the other hand, we identify protein hubs centred around PAIRB and LMNB2 which confer "global" resistance to most drug classes.
Conclusion: This study lays the foundation for developing diagnostic and predictive panels which can help to identify signatures of sensitivity and resistance in a patient's tumour. Our findings contribute towards the goal of leveraging 'omic data to guide cancer precision medicine, leading to more effective, personalised treatments for cancer patients.

PROGRAM

16:57   **CS22.03: Clinical Proteogenomics, a Powerful New Tool for Understanding Response and Resistance to Therapies for Breast Cancer**
Shankha Satpathy, United States

In clinical settings, limited material poses a significant bottleneck to translational proteogenomics. We previously reported the Biopsy Trifecta EXTraction (BioTEXT) method suited for tissue-sparing specimen processing and microscaled proteogenomic analysis (Satpathy, Nat Comm. 2020). We applied BioTEXT to two BRCA clinical trials with pathological complete response (pCR) as an end point: a TNBC cohort (Anurag, Cancer Disc., 2022; n=59), where patients were treated with neoadjuvant chemotherapy (Carboplatin and Docetaxel), and a HER2-positive cohort (n=54) where patients were treated with targeted anti-HER2 therapies. Biopsies were collected at baseline (both trials) and post-treatment (TNBC). In TNBC patients, chemo-response was marked by elevation of DNA repair, E2F targets, interferon-gamma signaling, and immune-checkpoint components. Proteogenomic analyses of copy-number aberrations highlighted a resistance-associated loss of the 19q13.31–33 locus, where LIG1, POLD1, and XRCC1 are located. LIG1 (DNA ligase I) shallow deletion and/or low mRNA expression levels were associated with non-pCR cases, poor prognosis, as well as carboplatin-selective resistance in TNBC clinical and preclinical models. We also observed progressive loss of LIG1 in a PDX treated with carboplatin that eventually developed resistance. In the HER2-E cohort, we observed targetable proteomics-only driven pathways that were elevated in non-pCR cases, while the pCR cases showed higher levels of immune and cell cycle activities. Using phosphoproteomics, we identified PAK1, a regulator of cytoskeletal remodeling, as a potential therapeutic target in HER2+ BRCA that is refractory to anti-HER2 therapy. The proteomics data from this study and our initial study revealed a signature that was elevated in non-pCR tumors in two independent targeted anti-HER2 clinical studies, and 5/6 proteins with good mRNA-protein correlation were validated with RNA-seq data from a third trial. Our studies highlight that clinical proteogenomics is proving to be a powerful tool to dissect biological principles of resistance and response within clinical trials.

17:10   **CS22.04: Tear Proteomic Profile in Response to Fenofibrate Treatment in Diabetic Corneal Neuropathy**
Lei Zhou, Hong Kong

Background
Diabetic corneal neuropathy (DCN) is a common microvascular complication. However, there is very limited therapeutic agent available. In this study, the effects of a peroxisome proliferator-activated receptor-alpha (PPAR)-α agonist, fenofibrate, on patients with DCN and diabetes mellitus (DM) mouse model were investigated.
Methods
Thirty DCN patients (60 eyes) were recruited for this single-arm, open-label, interventional study. Tear samples were collected prior to and after oral fenofibrate treatment for tear proteomic analysis and tear neuromediator analysis. Quantitative proteomic analysis was carried out using data independent acquisition (DIA) on an Orbitrap Exploris™ 480 Mass Spectrometer. Other clinical assessments including in vivo confocal microscopy, clinical neuropathic ocular surface assessments, and anterior segment optical coherence tomography (ASOCT) were also performed to evaluate the corneal nerves and corneal epitheliopathy. The results were further verified using Ins2Akita mice under topical and oral fenofibrate treatment.
Results
Typically, more than 2000 tear proteins were quantified using DIA. When comparing tear proteomic profiles before and after oral fenofibrate treatment, several proteins (SMAD1, ST6GAL1, RAB5A, and TTC9) related to neural functions were found to be significantly upregulated after treatment. Gene set enrichment analysis (GSEA) also revealed that neurotrophin signalling pathway was one of the dysregulated pathways. This was further echoed by the evidence of significantly increased substance P levels in tears after the treatment (p=0.03). Quantitative tear proteomic analysis in animal experiments also showed that neurotrophin signalling and anti-inflammation reactions were significantly associated with the therapeutic effects, whether applied topically or orally.
Conclusions
Tear proteomics results suggested that fenofibrate could modulate neurotrophin signalling pathway as well as lipid metabolism and anti-inflammation process. Both human and animal studies demonstrated that fenofibrate could potentially be a novel treatment for patients with DCN.

17:23 **CS22.05: In-Depth Serum Proteomics Reveals the Trajectory of Hallmarks of Cancer in Hepatitis B Virus-Related Liver Diseases**
Xiaobo Yu, China

Hepatocellular carcinoma (HCC) is a prevalent cancer in China, with chronic hepatitis B (CHB) and liver cirrhosis (LC) being high-risk factors for developing HCC. Here, we determined the serum proteomes (762 proteins) of 125 healthy controls and Hepatitis B virus-infected CHB, LC, and HCC patients and constructed the first cancerous trajectory of liver diseases. The results not only reveal that the majority of altered biological processes were involved in the hallmarks of cancer (inflammation, metastasis, metabolism, vasculature, coagulation), but also identify potential therapeutic targets in cancerous pathways (i.e., IL17 signaling pathway). Notably, the biomarker panels for detecting HCC in CHB and LC high-risk populations were further developed using machine learning in two cohorts comprised of 200 samples (discovery cohort=125, validation cohort=75). The protein signatures significantly improved the area under the receiver operating characteristic curve (AUC) of HCC (CHB discovery and validation cohort = 0.953 and 0.891, respectively; LC discovery and validation cohort = 0.966 and 0.818, respectively) compared to using the traditional biomarker, alpha-fetoprotein (AFP), alone. Finally, selected biomarkers were validated with parallel reaction monitoring (PRM) mass spectrometry in an additional cohort (n=120). Altogether, our results provide fundamental insights into the continuous changes of cancer biology processes in liver diseases and identify candidate protein targets for early detection and intervention.

Session Date/Time: Tuesday, September 19, 2023 - 04:30 PM - 05:35 PM

CS23: Cardiovascular

Chair
Rebekah Gundry, United States

Chair
Anthony Gramolini, Canada

16:31 **CS23.01: Keynote Speaker - Identifying Protein Targets to Mitigate Pathological Remodeling in Hearts by Proteomics Investigations**
Alicia Lundby, Denmark

16:56   **CS23.02: Disruption of Protein Complexes involved in Ca+2-Handling and Mitochondrial Function is an Early Hallmark of R14del-Phospholamban Cardiomyopathy**

Brian Foo, Germany

Background: Multi-protein complexes underlie many critical cellular functions and their disruption often carries clinical consequences. For example: disruption of the cardiac Ca+2-handling supercomplex, a large (~5MDa) sarcoplasmic-reticulum membrane complex containing Ca+2-cycling, membrane-tethering and phospho-regulatory machinery, is associated with multiple cardiopathies. Phospholamban (PLN) is a key regulatory component of this complex. The R14del-PLN mutation causes severe cardiomyopathy and untreatable heart failure via unknown mechanisms. We hypothesize that R14del-PLN may disrupt the Ca+2-handling supercomplex and/or other multi-protein complexes. Methods: We developed and applied novel complexome profiling (CP) workflows to systematically analyze multi-protein complexes in cardiac tissues. Samples from WT and R14del-heterozygous mice at 28w of age (significantly prior to the onset of cardiomyopathy at >60w of age) were homogenized under non-denaturing conditions and fractionated by size-exclusion chromatography (SEC). Fractions were then subject to mass-spectrometry (MS) analysis. Proteins with altered molecular weight (MW)-distribution profiles, consistent with altered protein-complex integrity/composition, were identified using both targeted (candidate-based) and untargeted (screening-based) approaches. Parallel global proteomics analysis facilitated comparison between MW-distribution profiles and protein-expression levels. Results: Targeted analysis of known PLN-interactors showed alterations to key Ca+2-handling supercomplex components. Additionally, untargeted analysis via novel protein-centric workflows identified 290 proteins (out of >3600 detected) with disrupted MW-distribution profiles. Bioinformatics analysis revealed an overrepresentation of mitochondrial proteins within these hits. Global proteomic analysis and mitochondrial activity measurements confirmed mitochondrial disruption at the protein-expression and functional levels, respectively. Conclusion: Our findings implicate mitochondrial dysfunction as an early R14del-disease event; treatment with established mitochondria-targeting small-molecules represents an intriguing therapeutic strategy. Future work will translate these findings into human stem-cell and patient-tissue models. Lastly, this study represents, to our knowledge, the first application of SEC-CP-workflows to cardiac samples. Enhanced resolution at high-MW ranges makes this workflow ideal for studying large (>5MDa) complexes underlying critical cardiac functions such as membrane excitation and gap-junction formation.

17:09   **CS23.03: Insights Into Mode of Action of Empagliflozin to Prevent Heart Failure Using Large-Scale Proteomics in Participants in the EMPEROR Trials**

Marijana Rucevic, Sweden

Sodium-glucose co-transporter 2 (SGLT2) inhibitors improve cardiovascular outcomes across a wide range of high-risk patients, but the underlying mechanisms by which SGLT2 inhibitors improve heart failure remains incompletely understood. This study investigated the effect of empagliflozin on the circulating levels of intracellular proteins in patients with heart failure, using large-scale proteomics. Olink® Explore 1536 platform was used to measure the levels of 1250 circulating proteins at the baseline, week 12 and week 52 in 1134 patients participating in the EMPEROR-Preserved (NCT03057951) and EMPEROR-Reduced (NCT03057977) trials (sponsored by the Boehringer Ingelheim & Eli Lilly). Statistical and bioinformatical analyses identified differentially expressed proteins (empagliflozin vs. placebo), which were then linked to demonstrated biological actions in the heart and kidneys. At week 12, nine from 32 differentially expressed proteins demonstrated the largest treatment effect of empagliflozin: insulin-like growth factor-binding protein 1, transferrin receptor protein 1, carbonic anhydrase 2, erythropoietin, protein-glutamine gamma-glutamyltransferase 2, thymosin beta-10, U-type mitochondrial creatine kinase, insulin-like growth factor-binding protein 4, and adipocyte fatty acid-binding protein 4. Of these nine differentially expressed proteins, 6 were found to promote autophagic flux in the heart, kidney or endothelium, 3 improve heart mitochondrial health, 3 renal integrity. Changes in circulating protein levels in patients with heart failure are consistent with the findings of experimental studies that have shown that the effects of SGLT2 inhibitors are likely related to actions on the heart and kidney to promote autophagic flux, nutrient deprivation signaling and transmembrane sodium transport. Our findings suggest that the results of these experimental studies are likely to be highly relevant to the clinical setting (Zannad F. et al. Eur Heart J. 2022 ehac495).

17:22 **CS23.04: High Throughput Label-Free Quantitative Plasma Proteomic Profiling for Abdominal Aortic Aneurysm Biomarker Discovery in a Large Patient Cohort**

Santosh Renuse, United States

Background
Patients with abdominal aortic aneurysms between 3.5 and 5.0 cm in maximal transverse diameter were followed for up to 2 years as part of a clinical trial. AAA diameter change rate by CT scan was characterized as high or no/low growth. Here, we analyzed plasma samples from these patients obtained at enrollment, 1 year and 2 years for label-free quantitative proteomics using high-resolution Orbitrap mass spectrometry.

Methods
Plasma samples from AAA patients were obtained with prior consent and processed on Hamilton MicroLab Starlet liquid handling system for reduction, alkylation and in-solution trypsin digestion using EasyPep™ kit. Data acquisition was performed on a Orbitrap Exploris 480 Mass Spectrometer coupled to Vanquish™ Neo UHPLC system. ~1 µg peptides were separated using trap and elute set up on a 15 cm analytical column using 60 min gradient. Data analysis was done using SequestHT search engine, INFERYS and CHIMERYS® in Proteome Discoverer software 3.0. Identified proteins and peptides were filtered at 1% FDR.

Results
The integration of high-throughput sample preparation, robust UHPLC system and high resolution Orbitrap Mass spectrometer resulted in identification of >1,000 proteins from 1 µL plasma samples. A total of 570 AAA samples were analyzed by LC-MS/MS including 168 with no/low growth and 65 with high growth rates. A total of 1,077 proteins were identified across all the samples with 722 proteins identified in >300 samples. There were 47 proteins upregulated in high growth vs. no/low growth group (all p<0.05) while 10 proteins were downregulated in high growth vs. no/low growth group (all p<0.05). Partial Least Squares Discriminant Analysis (PLS-DA) showed separation between the two groups. Further analysis is ongoing to identify growth specific biomarkers.

Conclusions
In this study, we developed a proteomics platform that allowed identification of protein biomarkers which are associated with more rapid AAA growth.

Session Date/Time: Tuesday, September 19, 2023 - 05:45 PM - 06:30 PM

PL05: Plenary Session: Kathryn Lilley, UK - The Orchestration of Subcellular Processes through RNA and Protein Interactions

Chair

Jin Han, Republic of Korea

17:47 **PL05.01: Plenary Speaker - The Orchestration of Subcellular Processes through RNA and Protein Interactions**

Kathryn Lilley, United Kingdom

The biological intricacy of a cell is only partially explained by protein expression. Added layers of complexity are provided by non-coding RNA mediated control mechanisms, post-transcriptional and post-translation processing, and the subcellular localisation and interactions of both proteins and RNA. It is crucial to our understanding of biological processes to be able to capture these additional layers of complexity.

In this talk, I will discuss how we can map the dynamics of RNA-protein interactions during key cellular processes. I will also introduce a new approach for the simultaneous mapping of the both the subcellular spatial proteome and transcriptome on a cell-wide scale. I will demonstrate how we have used this approach to show the orchestrated re-localisation of RNA and protein upon the induction of the unfolded protein response (UPR) giving essential insights into the rules governing re-localisation of RNA to stress granules (SGs).

Session Date/Time: Wednesday, September 20, 2023 - 09:15 AM - 10:00 AM

PROGRAM

PL06: Plenary Session: Hee-Sup Shin, Korea - Neural Mechanism Underlying Observational Fear, a Rodent Model of Affective Empathy

Chair
Uwe Völker, Germany

09:17   **PL06.01: Plenary Speaker - Neural Mechanism Underlying Observational Fear, a Rodent Model of Affective Empathy**
Hee-Sup Shin, Republic of Korea

Empathy, the ability to understand and share emotions of others, is crucial for social animals. It forms the foundation of diverse social behaviors, including emotional contagion, prosocial behavior, theory of mind, and perspective-taking. Observational fear, a form of emotional contagion, is conserved among diverse animals including humans. The observational fear assay offers an opportunity to study the neural mechanisms and circuitry underlying empathy. In my talk I will provide an overview of the status of current research on the neurobiology of empathy.

Session Date/Time: Wednesday, September 20, 2023 - 10:15 AM - 11:45 AM

CS24: Structural Proteomics

Chair
Dina Schuster, Switzerland

Chair
Francis O'Reilly, United States

10:16   **CS24.01: Keynote Speaker - Structure and Funation of TMEM87A, A Unique Voltage-Gated Cation Channel in the Golgi**
Ho Min Kim, Republic of Korea

10:41   **CS24.02: Keynote Speaker - Developing Structural Interactomics and its Application in Cell Biology**
Fan Liu, Germany

11:06

CS24.03: Integrative Approach for Large-Scale Protein-Protein Interaction Analysis Using Deep Learning and Proteomics

Martin Garrido Rodriguez-Cordoba,
Germany

Background: Proteins function through complex networks of interactions that regulate physiological processes. These interactions can vary in nature, including stable physical interactions forming complexes, transient physical interactions mediating signaling, and functional interactions of proteins participating in the same biological processes. To investigate and predict such interactions, a range of proteomics methods are available, differing in reliability and throughput. Additionally, recent advancements in deep learning have shown impressive performance in predicting protein-protein interactions from protein sequence. In this study, we propose a protocol that combines three deep learning methods with five proteomics technologies to comprehensively characterize protein-protein interactions at scale.

Methods: We employed various proteomics techniques, including cross-linking proteomics, thermal proteome profiling, perturbational proteomics, size-exclusion chromatography, and blue native gels. These techniques were combined with state-of-the-art deep learning methods that leverage protein language models and network topology to predict interactions. To integrate the diverse data modalities, we employed a network-based approach that consolidated information from different predictors into a single output.

Results: We applied our protocol to study the protein-protein interaction landscape of two human gut microbes, Bacteroides uniformis and Phocaeicola vulgatus, which possess extensive genomic information but limited phenotypic insight. We compared the performance of each predictor in recovering interactions that could be inferred using conventional computational approaches such as sequence similarity or genomic context prediction. Additionally, we validated our findings by demonstrating that the number of protein interactions in the predicted networks correlated with gene essentiality.

Conclusions: This study presents the first comprehensive overview and evaluation of integration strategies for diverse proteomics technologies in profiling large-scale protein-protein interactions. Our findings also shed light on understudied gut microbes and provide a map to unveil protein-protein interactions with significant implications for the relationship between these microorganisms and the host.

11:19

CS24.04: Validation of a Zero Degrees Celsius Capillary Electrophoresis Platform for Hydrogen Exchange Mass Spectrometry

Jordan Aerts, Sweden

Hydrogen deuterium exchange mass spectrometry (HDX-MS) has been a valuable tool for structural proteomics studies for more than 30 years. Labeling of proteins with deuterium in solution is a straightforward experiment, but downstream sample handling steps should be conducted under quench conditions (low temperature and pH) to maximize the structural information obtained from protein and peptide measurement. Traditional HDX-MS workflows utilize low-temperature liquid chromatography (LC) with short gradients for protein and peptide separations. However, operating an LC system at low temperatures generally results in sub-optimal separations, increased sample carry-over, and the need for expensive system components. Previously, we reported the development of the first cold capillary electrophoresis (CE) platform for separating deuterium-labeled proteins and peptides in solution. Cold CE separations offer a low cost alternative for achieving separations at quench conditions for HDX-MS workflows. In this study, we present, for the first time, a direct comparison at the peptide level using bovine hemoglobin analyzed on both the laboratory-built CE platform and a fully automated Waters HDX-2 system. In both cases, a Waters Synapt G2Si ion mobility mass spectrometer was used, and data analysis was performed using HDExaminer. The quenched, and digested protein (10,000 fmol on column for LC, 50 fmol on capillary for CE) was measured after labeling in D2O for 0, 50, 500, 5000, and 50,000 s. Preliminary results demonstrate similar deuterium uptake curves for proteolytic peptides detected across both separation methods, at significantly lower sample amounts. These findings validate the use of cold capillary electrophoresis as a promising alternative to HPLC in HDX-MS workflows.

11:32   **CS24.05: In-Cell LiP-MS: Detecting Protein Structural States in their Native Environment**

Franziska Elsässer, Switzerland

Protein structures are highly dynamic and adapt to their environment and interaction partners. Limited proteolysis-coupled mass spectrometry (LiP-MS) has been successful in capturing structural alterations on a proteome-wide scale. In LiP-MS, proteins are cleaved by an unspecific protease for a short period of time, which results in structure-specific peptide patterns. Mass spectrometry is used to identify these structural fingerprints. The standard LiP-MS workflow captures protein structural states in native cell lysates. However, cell lysis may expose proteins to non-native conditions and dilution may disrupt labile interactions.
This is particularly detrimental for structures such as phase separated protein assemblies, that are held together by weak interactions. An example of this are stress granules that are labile macromolecular assemblies of proteins and RNA that form under stress conditions and are poorly characterized at the structural level. Because of their transient nature, established methods are insufficient in capturing stress granule formation.
To circumvent disruption of protein structures that may occur upon cell lysis and study protein structures in their cellular context, we developed in-cell LiP-MS. We are using electroporation to introduce a low-specificity protease, proteinase K into mammalian cells. We observed a significant increase in peptide fragments after electroporation in the presence of proteinase K, while electroporation itself did not fragment proteins. We show that in-cell LiP-MS captures not only known specific structural changes like rapamycin-binding of FKBP1A, but also downstream effects of pathway activation.
We applied in-cell LiP-MS to study stress granule formation in mammalian cells. We observed structural changes of known stress granule components like G3BP1. Moreover, we provide the first in situ dataset on the global structural alterations of proteins during stress granule formation that we used as basis for functional studies. In future applications, in-cell LiP-MS may enable structural studies of other labile protein assemblies in their native environment.

Session Date/Time: Wednesday, September 20, 2023 - 10:15 AM - 11:45 AM

CS25: PTMs

Chair

Jennifer Geddes-McAlister, Canada

Chair

Minjia Tan, China

10:16   **CS25.01: Keynote Speaker - Revving an Engine of Human Metabolism: Activity Enhancement of Triosephosphate Isomerase via Hemi-Phosphorylation**

Neil Kelleher, United States

10:41   **CS25.02: Keynote Speaker - N-Terminal Formyl-Methionine: a Critical Determinant for Ribosome and Protein Quality Control in Eukaryotes**

Cheol-Sang Hwang,

11:06   **CS25.03: decryptM: Decrypting Drug Actions and Protein Modification by Dose- and Time-resolved Proteomics**

Matthew The, Germany

Introduction
Most drugs act on proteins and all drugs exert their effects in a dose-dependent fashion. Meanwhile, drug mechanism of actions are often not well understood. Here, we present decryptM, a method to quantitatively characterize proteome-wide changes on the level of post-translational modifications (PTM) for drugs in live cells in a dose- and time-dependent manner.

Methods
By encoding each dose or time point by tandem mass tags, perturbations in PTM-peptide (phosphorylation, acetylation, ubiquitinylation) abundance are quantified at high precision. 31 drugs representing 6 drug classes in 14 human cell lines were profiled with decryptM. Each of the resulting 1.8 million drug-response curves represent the modulation of a PTM-peptide by a drug in a cellular system. 10-100s PTM-peptides were found regulated per experiment in a background of 20,000 unregulated PTM-peptides. Most PTM-peptides were only regulated in one or a few experiments, reflecting common and drug-specific effects. This data is integrated into ProteomicsDB (https://www.proteomicsdb.org/decryptm), allowing interactive exploration and cross-referencing to UniProt/PhosphoSitePlus.

Results
An illustration of how decryptM assists in drug/PTM characterization is the comparison of 10 kinase inhibitors applied to the A549 lung cancer cell line. Regulated phosphosites clearly clustered by pathway, e.g. mTOR/PI3K versus MAPK, in accordance with the drugs' known targets. The dose-dependent dimension allowed deconvolution of pathways for drugs with multiple targets, e.g. the mTOR/PI3K inhibitor Dactolisib exhibited a bimodal distribution of high (mTOR/PI3K) and low potently (off-target ATR/ATM/PRKDC binding) regulated phosphopeptides. Typically, only 10-20% of phosphosites in such clusters had annotations. Using EC50 and kinase motif information, hundreds of previously unannotated phosphopeptides were thus linked to kinases and pathways through guilt-by-association.

Conclusions
decryptM improves our understanding of drugs and should become a standard tool in drug discovery, particularly for MoA elucidation, compound prioritization, clinical candidate selection and drug differentiation and repurposing.

11:19   **CS25.04: Mining the Dark Proteome: Uncovering Wide Lactylation in Human With Cyclic Immonium Ions**

Hui Ye, China

Lactylation is a new modification discovered on histones. However, whether it can be installed on non-histone proteins remains unclear. Here we report the formation of a signature cyclic immonium ion of lactyllysine, together with the characteristically changed chromatographic behavior, enabling confident protein lactylation assignment by mass spectrometry. This identification strategy was confirmed by affinity-enriched lactylation proteome and revealed lactylation on nuclear non-histone proteins such as nucleolin and YBX3. Subsequent exploitation of the approach to mining unenriched, deep proteome resources unveiled an understudied lactylation landscape. From the draft map of the Human Proteome, we identified widespread lactylation on DHRS7 among human tissues, and demonstrated site-directed mutagenesis of the lactylated site affects previously unannotated proteinaceous association. Additionally, the Meltome Atlas showed lactylation frequently occurs on glycolytic enzymes and concomitantly induces thermal stability changes on carrier enzymes. Collectively, the identified signatures of protein lactylation enable confident assignment and allow for the discovery of lactylation proteome expanding beyond histones, representing a step to further understand how lactylation governs cells.

11:32 **CS25.05: PTMeXchange : Globally Harmonized Re-Analysis and Sharing of Data on Post-translational Modifications**
Juan Antonio Vizcaino, United Kingdom

Background

Post-translational modifications (PTMs) play an important role in e.g. cell signalling, protein function and disease processes. The aim of PTMeXchange is to re-analyse public enriched PTM datasets, focusing on accurate PTM localisation, for human and the main model organisms, integrating data across studies and disseminating the data to UniProtKB, linking it to the original MS evidence in PRIDE and PeptideAtlas. The overall goal is to make PTM data FAIR (Findable, Accessible, Interoperable and Reusable).

Methods

An open data analysis pipeline using the Trans Proteomic Pipeline (TPP) was used. We developed a statistical method to ensure confident PTM localisation. We tested several decoy amino acids for false localisation rate (FLR) control. We also developed an empirical method for controlling FLR inflation when combining multiple datasets. The final step was to integrate the "PTM build" into public databases, including UniProtKB, PRIDE and PeptideAtlas.

Results

We first focused on phosphoproteomics studies. We have multiple PTM builds completed for phosphorylation in different species: Human, Mouse, Plasmodium, Rice and Arabidopsis. We have also prototyped PTM builds for more challenging modifications including ubiquitin, SUMOylation and lysine acetylation.

The first "proof of concept" build, for the rice phosphoproteome, is already available in UniProtKB, with others planned to be included in later releases. This allows users to visualise the PTMs in the Protein Record pages, including the feature viewer, where sites can be observed alongside SNPs, domain and other proteomics evidence and linked to protein structures/AlphaFold2 predictions. Each build is also integrated into PRIDE and/or PeptideAtlas. Universal Spectrum Identifiers are available for all spectra.

We are currently working on community guidelines to allow users to generate PTM builds which can be integrated in the PTMeXchange framework, for making PTM build data available to the community.

Conclusions

PTMeXchange provides the infrastructure and resources for making PTM data FAIR.

Session Date/Time: Wednesday, September 20, 2023 - 10:15 AM - 11:45 AM

CS26: Cancer Proteomics

Chair
Hui Zhang, United States

Chair
Cheolju Lee, Republic of Korea

10:16 **CS26.01: Keynote Speaker - Immunological Impact on Internal Proteomics Cancer Heterogeneity**
Tami Geiger, Israel

PROGRAM

10:41   **CS26.02: Keynote Speaker - Functional Studies on Melanoma Cancer: Assessing Drug Impact and Correlation with Survival**
György Marko-Varga, Sweden

11:06   **CS26.03: Extensive Proteogenomic Analysis on Human Pancreatic Ductal Adenocarcinoma (PDAC) In an Asian Population Identified Six PDAC Subtypes**
Dowoon Nam, Republic of Korea

We report a proteogenomic analysis of pancreatic ductal adenocarcinoma (PDAC). PDAC is a devastating disease with poor prognosis, and the situation has not improved despite extensive clinical and scientific research. To understand PDAC pathogenesis and prognosis prediction, effective integration of genomic, transcriptomic, and proteomic data from large cohort of PDAC patients are required. Therefore, we conducted extensive phosphoproteome and global proteome profiling using novel dual-online noncontiguous fractionating and concatenating reverse-phase/reverse-phase liquid chromatography (DO-NCFC-RP/RPLC) technology with high resolving mass spectrometry. Combined with multiplexing capability of TMT and efficient enrichment of "one-pot" IMAC, we were able to identify 171,272 peptides and 49,651 phosphopeptides which were mapped to 11,599 protein-coding genes, 95.5% of the expressed transcriptome size. Based on the phosphoproteome and global proteome profiling data, integrated clustering of mRNA, protein, and phosphorylation data from 196 patients were performed and six PDAC subtypes were identified. Six PDAC subtypes were characterized as classical progenitor (TS1), three types of squamous (TS2–4), immunogenic progenitor (IS1), and exocrine-like (IS2) subtypes using cellular pathways analysis.

11:19   **CS26.04: Analysis of Therapeutic Target Networks of Cancer Stem Cells by Proteomics-Based Multi-Omics**
Norie Araki, Japan

Recently, the existence of cancer stem cells has been demonstrated, and they have attracted attention as the root cause of cancer resistance to chemotherapy and recurrence. However, due to the lack of easy cancer stem cell models and technical difficulties, information on functional molecules of cancer stem cells is very limited. In order to elucidate key factors for the reciprocal induction of cancer stem cells into cancer cells and specific signals for therapeutic targets, we have established dozens of cancer stem cells (GSC) from malignant brain tumor tissue cells of multiple patients, and have studied the transcriptome-proteome-metabolome of these cells. We have succeeded in obtaining multilevel of molecular information integrated by origincal iPEACH (Araki et al MCP 2013) of the Transcriptome-Proteome-Kinome-Metabolome of these models. Using these 25,000-molecule fusion data from glioma stem cells, we analyzed the dynamic variation of clusters and networks over time and examined in detail the molecular network dynamics involved in cancer stem cell maintenance and differentiation-induced switching, and identified important key networks. Among them, networks related to fat and glucose metabolism, which are specific signals in cancer stem cells, were detected at the top level, and validation experiments with 250 inhibitors focused on these networks were attempted. Based on the molecular information of various detailed stem cell differentiation switching dynamics, there is a possibility to design novel drugs targeting the dynamics of cancer stem cells and cancer cells, and to apply them to the medical treatment of refractory cancers such as malignant glioma with poor prognosis.

11:32   **CS26.05: Pan-Cancer Proteogenomics Expands the Landscape of Therapeutic Targets**

Jonathan Lei, United States

Background: Proteins are the primary targets of molecularly targeted therapies, but only 5-10% of the potentially druggable proteins are targeted by FDA-approved drugs. We integrated Clinical Proteomic Tumor Analysis Consortium (CPTAC) proteogenomics data from 1,039 cancer patients spanning 10 cancer types with other public datasets to delineate the landscape of therapeutic targets.

Methods: We curated druggable proteins from four public databases. Proteomic data from tumor specimens was integrated with genetic screening data from cancer cell lines to identify common and cancer-type-specific druggable protein dependencies. We implemented a strategy to target "undruggable" tumor suppressor gene loss to identify synthetic lethality based on proteogenomic data from human tumor specimens and genetic screen data from cancer cell lines. To identify tumor associated antigens, proteins overexpressed in human tumors compared with adjacent normal tissues were filtered with GTEx RNASeq data from normal tissues, followed by experimental validation.

Results: We identified >3,000 druggable proteins and classified them into 5 tiers to facilitate different applications such as companion diagnostics, drug repurposing, and new therapy development. 618 druggable proteins showed both overexpression in tumors compared to normal and significant dependency in cell line CRISPR-Cas9 screens of the same lineage. Notably, 68 proteins demonstrated both overexpression and dependency in five or more cancer types. A similar analysis of phosphoproteomics data focusing on known activating sites of druggable proteins further revealed targetable dependencies driven by protein hyperactivation. We identified synthetic lethality for difficult to target tumor suppressor losses, revealing TP53 mutations as a candidate biomarker to select endometrial cancer patients for treatment with doxorubicin. Our tumor associated antigens analysis followed by experimental confirmation nominated peptides as promising immunotherapy targets.

Conclusions: Our analyses create a comprehensive landscape of protein and peptide targets, paving the way to future development of companion diagnostics, drug repurposing, and new drugs for cancer treatment.

Session Date/Time: Wednesday, September 20, 2023 - 10:15 AM - 11:45 AM

CS27: Single Cell

Chair

Jennifer Van Eyk, United States

Chair

Takeshi Masuda, Japan

10:16   **CS27.01: Keynote Speaker - Is it Possible to Analyze 5000 Proteins from a Single Human Cell?**

Karl Mechtler, Austria

10:41   **CS27.02: Keynote Speaker - Interfacing Optics, Microfluidics, and Mass Spectrometry to Advance Single-cell and Spatial Proteomics**

Ying Zhu, United States

PROGRAM

11:06 **CS27.03: Single Cell Proteomics Study of Drugs Responses**

Bogdan Budnik, United States

The use of single-cell technologies, such as single-cell RNA sequencing (scRNA-seq), and associated computational tools are transforming drug discovery and development. Using cell subtyping to identify targets offers new opportunities for improving how we understand the mechanism of disease. The lack of correlation between RNA and protein abundances necessitates more and better proteomics data at single cell resolution.

Traditionally, the use of single-cell proteomics (SCP) has been used to study cancer cell monocultures and their response to drug treatments. In our lab, we have demonstrated unique cell responses from different classes of drug treatments, and our results have shown that single-cell proteomics is a useful technique to study complex biological systems that provides essential data that can be used to uncover novel biology processes that can lead to better treatments.

To validate our technique, we used three different drugs to treat human induced pluripotent stem cells (hiPSCs) derived from healthy and bipolar-disorder diagnosed patients. These cells were grown in both mono-culture and co-culture. Single-cell proteomics analysis showed inherent heterogeneity of cell population and the differences in drug response that were not present in bulk proteomics. We observed subpopulations of cells with unique drug response profiles. Thus, we show that SCP analysis is necessary to understand the effect of drugs under different disease states, and discoveries made from data at single-cell resolution can be used to develop much needed effective treatments for this disease.

11:19 **CS27.04: Platform for Single Cell Science (PSCS) Repository Enables No-Code Single Cell Proteomic Data Analysis and Sharing**

Alexandre Hutton, United States

Background
Single cell proteomics (SCP) is evolving rapidly and new data analysis tools are needed. A major challenge for this fledgeling field is reproducibility, which is often complicated by uncertainty in the data analysis workflow where the choice of parameters and order of processing steps can profoundly impact results and their interpretation. To make SCP data processing more accessible and reproducible, we are developing the web Platform for Single-Cell Science (PSCS, "pai-seez").

Methods
PSCS is a web application written in Python and JavaScript using Flask, and hosted on Amazon EC2. The core data structure uses the anndata Python package. Pipeline execution is distributed to the Open Science Grid, and results are returned and registered on PSCS.

Results
ProteomeXChange, MassIVE, and PeptideAtlas have focused on distributing data; PSCS instead aims to facilitate access to post-quantification methods, and to serve as a repository for quantitative datasets, interactive results, and their exact data processing pipeline to be co-published with their scientific articles. By enabling the distribution and modification of published computational analytical pipelines, PSCS enables verification of published results and adaptation of existing effective pipelines to data from new projects. Key features of PSCS include (1) a no-code interface for designing arbitrary data analysis pipelines, (2) multi-site collaboration on projects and results, (3) version-control of analysis pipelines and published datasets. To demonstrate the utility of PSCS to the proteomic community, currently-available analyses include dimension reduction of proteomic profiles and clustering of cell types. As the platform matures, we expect that the collection of pipelines will grow to include processing of raw mass spectrometry data, statistical comparisons between groups, live comparison of your data to published projects, and multi-omic data integration.

Conclusion
By enabling reproducibility through accessibility, PSCS can supplement scientific publications by incentivizing reliable results and making analytical methods fully transparent.

PROGRAM

11:32   **CS27.05: Spatial Proteomic Approaches for Triple-Negative Breast Cancer on Single-Cell Resolution**

Gangsoo Jung, Republic of Korea

The tumor microenvironment has diverse landscapes of cell types including cancer-associated fibroblasts, endothelial cells and immune cells and the non-cellular components of extracellular matrix such as fibronectin, collagen, hyaluronan among others. Typical proteomic studies with bulk tissue would lose spatial information for explaining the heterogeneity of different cell types in these complex tissue architectures. Among the various kinds of cells in breast cancer, especially, the cancer stem cell (CSC) is being emerged as the main target to cure and inhibit TNBC (triple-negative breast cancer).

In this study, we analyzed of the proteome profiling in a single cell scale to sort populations of TNBC patient. Regions of interest (ROIs) as small as single cells was defined by immunofluorescence-stained tissue for CSC-related proteins of ALDH and CD44, captured by laser capture microdissection (LCM). Proteomic analysis was conducted with single cell from the isolated ROI using NanoLC-timsTOF SCP system and DEP and GO term analysis was carried out on bioinformatic study.

Consequentially, the combination of LCM and ultra-sensitive mass spectrometry is a powerful tool to identify and quantitate the tissue-specific proteins. The spatial proteomics with the method allowed proteomic mapping of TNBC at the single cell resolution and help to accurately pinpoint markers for TNBC therapeutic development strategies.

Session Date/Time: Wednesday, September 20, 2023 - 11:45 AM - 01:00 PM

PP05: All Posters - Poster Viewing with Coffee Break

PP05.01: Enhanced Insight Generation Through Automated Transformation of Historical Experiments Into Quantitative Knowledge Base

Mark Condina, Australia

Following recent technological advances in proteomics, there has been a dramatic increase in the volume of proteomics datasets. As this wealth of data increases, it is plausible to suggest that new analyses will share facets with previous studies, such as design, cell lines, or tissue types, whether these studies are public or reside with lab ownership. However, the responsibility of querying these past experiments and harnessing potential insights for interpretation relies heavily on the researcher's personal knowledge and predispositions, thus potentially overlooking significant results that could otherwise be of relevance.

The Mass Dynamics platform is already engineered to build a quantitative knowledge-base from a user's or a lab's historical data as they analyze their experiments. It also provides the capacity to interrogate and explore the analysis of proteins across the user's past experiments and those publicly available on the platform. This combination of features facilitates extensive exploration and effective utilization of data.

Here, we present a novel set of features within the application, enabling users to merge multiple experiments into a unified analysis. This capability enhances the results interpretation of single experiments by combining multiple datasets within the application and their re-analysis using various statistical methods. Simultaneously, it provides users with the means to conveniently validate the merged dataset's quality through interactive visual tools and undertake multi-experiment evaluations. The platform also supports result interpretation in the context of the researcher's historical and public experiments, and annotation using external knowledge bases such as Reactome, STRING, and Gene Ontologies. Crucially, this set of tools creates an unprecedented capability that achieves comprehensive data exploration and insight generation.

To demonstrate the platform's capabilities, we have employed datasets from public repositories to illustrate not only the platform's ability to seamlessly merge and analyse experiments but also its facilitation of insightful data interpretation.

PP05.02: Potein Language Models for Phosphorylation Site Prediction from LC-MS/MS data

Sven Degroeve, Belgium

With a regulatory impact on numerous biological processes, protein phosphorylation is one of the most studied post-translational modifications. Effective computational methods that provide a sequence-based prediction of phosphorylation sites are desirable to guide functional experiments. Currently, the most successful methods train neural networks on amino acid composition representations. However, recently proposed protein language models provide enriched sequence representations that contain higher-level pattern information on which more performant phosphorylation site predictions may be based.

We explored the applicability of different protein language models (ESM-1small and ESM-1b, ESM-2, ProtT5-XL-U50, CARP-640M, Ankh-base, and Ankh-large) to general phosphorylation site prediction. We constructed several training sets that were compiled from many publicly available LC-MS/MS experiments. We found that training prediction models on top of protein language models yield a relative improvement of up to 68.4% in terms of area under the precision-recall curve over the state-of-the-art predictors. Model interpretation and model transferability experiments reveal that protease-specific cleavage patterns in the LC-MS/MS experiments give rise to a protease-specific training bias. This can result in an overly optimistic estimation of phosphorylation site prediction performance, an important caveat in the application of advanced machine learning approaches to protein modification prediction based on proteomics data. We show that improving data quality by negative sample filtering using experimental metadata can mitigate this problem.

PP05.03: The 2023 Report on the Human Proteome from the HUPO Human Proteome Project

Eric Deutsch, United States

Background
Since 2010, the Human Proteome Project (HPP) home of the flagship initiative of global HUPO has pursued two goals: (1) To credibly identify the protein parts list and (2) To make proteomics an integral part of multi-omics studies of human health and disease methods.

Methods
International collaboration, data sharing, standardized reanalysis of MS data sets by PeptideAtlas and MassIVE with HPP Guidelines for quality and assurance, plus extensive use of antibody profiling by the Human Protein Atlas.

Results
According to the neXtProt release of 2023-03, protein expression has now been credibly detected (PE1) for 18,397 of the 19,778 predicted proteins coded in the human genome (93%). Of these PE1 proteins, 17,453 were detected with mass spectrometry in accordance with HPP Guidelines and 944 by a variety of non-MS methods. Conversely, the number of neXtProt PE2, PE3, and PE4 missing proteins has been reduced to 1381. These numbers represent experimental progress on the Human Proteome parts list across all of the chromosomes with significant re-classifications. Meanwhile, there are several categories of predicted proteins that have proven resistant to detection. Applying proteomics with a large array of biological and clinical studies ensures integration with other omics platforms as reported by the Biology and Disease-driven HPP teams.

The HPP has now launched its Grand Challenge to find the functions of every protein.

Conclusions
The global proteomics community has made remarkable progress in detecting and characterizing protein expression and protein functions in pathways and networks critical to understanding human health and disease.

See you in Busan!
BEXCO, Busan, Korea
2023.hupo.org
#HUPO2023

Together Through Proteomics

PROGRAM

PP05.04: Improved Library-Free Proteomics Analysis for dia-PASEF Using directDIA+ in Spectronaut

Sira Echevarria, Switzerland

Introduction
Data-independent acquisition (DIA) is increasingly being adopted as the default method for label-free discovery proteomics. This is supported by the possibility to skip the creation of project-specific libraries. Here we present an improved library-free workflow for dia-PASEF projects in Spectronaut. We compared analyses of dia-PASEF data with and without a library. Overall, library-free directDIA outperformed a deep project-specific library in finding true candidates.

Methods
We prepared a 4-species mixed proteome sample with two conditions (H. sapiens 1:1, S. cerevisiae 1:2, E. coli, 1:10, C. elegans 1.3:1). The library (~19,500 protein groups across all species) was generated using Pulsar, with a deeply fractionated pooled sample acquired in PASEF mode (timsTOF Pro) using a 120 min gradient. For the quantitative experiment, each condition was acquired in triplicates using dia-PASEF with 30, 60, and 120 min gradients. The library and library-free directDIA+ were used for the analysis in Spectronaut with 1% FDR. Comparison was based on overall identifications, precision, accuracy, and number of true candidates.

Results
While directDIA+ resulted in slightly less identifications than the library-based workflow, it outperformed in true candidate discovery. We performed an unpaired t-test at protein group level. Upon counting the number of true candidates with less than 5% error rate, directDIA+ performed better than the library-based analysis in all 3 gradients, providing in average 10% more true candidates than the library-based method.

Conclusions
Overall, directDIA+ was better in true candidate discovery than a deeply fractionated project specific library across all 3 gradients. This highlights the advantage of this approach as it does not rely on acquiring matching DDA runs for every single project which significantly increases the cost and complexity of DIA.

PP05.05: Synchronizing Analysis With Acquisition for Significantly Faster Library Free Analysis of DIA

Tejas Gandhi, Switzerland

Introduction:
Increasingly "library free" analysis of DIA data has become a popular alternative to analyzing DIA data. Being able to skip DDA acquisition for library generation greatly simplifies the workflow. The "library free" approaches still involve creating a library, albeit in silico using machine learning. A downside of this approach is that it can be significantly slower than performing a classical DIA analysis with a library in hand. In certain cases, the analysis step can be as slow as acquisition time. We hypothesize that the trend towards larger raw files will further continue and hence there is a need to synchronize acquisition with analysis.

Methods:
We believe that synchronizing library free analysis of DIA data with acquisition should fulfill the following requirements to be beneficial: 1) You should be able to process data at a single run level, but still be able to combine at project level. 2) The combining at the project level should give same results, including false discovery rate control.   3) The sum of acquisition plus synchronized library-free analysis should be equal to the sum of acquisition plus analysis with a library in hand, and 4) The workflow should be fully functional for a user with no programming knowledge.

Results:
With these requirements in mind, we implemented a new workflow in Spectronaut that allows users to define a job that monitors samples being acquired by the MS and automatically performs a library-free search of that run based on the job definition. The output of this individual jobs can then be combined very quickly so that the additional time needed for the full analysis is 75% less than doing the analysis fully after the acquisition.

PP05.06: Evaluating Network Methods to Understand Cellular Signaling From Phosphoproteomics Data

Martin Garrido Rodriguez-Cordoba,
Germany

Background: Recent technological advancements have greatly expanded our knowledge of cellular signaling, revealing its intricate complexity beyond traditionally taught linear pathways. Although current phosphoproteomic methods allow us to measure numerous phosphosites and their abundance, there is a challenge in connecting perturbation targets to the most significantly altered phosphosites using standard pathway analyses. Thus, there is a need for innovative computational strategies to decipher and interpret the vast amount of phosphoproteomic data.

Methodology: In this study, we investigated different computational methods capable of integrating perturbational phosphoproteomics data with existing knowledge through network modeling. To address this, we developed multiple iterations of our method, PHONEMES, which utilizes Integer-Linear programming to construct discrete models of signaling networks based on kinase-substrate interactions. Through cross-validation, we demonstrated how modifications to parameters, and the incorporation of alternative sources of kinase-susbtrate knowledge can improve the identification of omitted phosphorylated proteins.

Results: We applied our computational model to the recent study DecryptM, describing the response of various cancer cell lines to drugs with known protein targets. We evaluated three different sources of prior knowledge regarding kinase-substrate interactions: PhosphoSitePlus, OmniPath, and a recently released kinase-substrate specificity atlas. Our analysis revealed notable differences in data coverage and in the ability to recover omitted measurements across different configurations. Moreover, we found that incorporating kinase-substrate specificity into the model helped to prioritize more informative phosphorylation events while penalizing promiscuous ones (e.g. phosphosites regulated by many different kinases).

Conclusion: This research offers valuable guidelines for interpreting human phosphoproteomics data by leveraging state-of-the-art computational frameworks and knowledge resources. The insights gained from this study contribute to a better understanding of signaling on a larger scale and provide a means to unravel biological processes such as drug mechanisms of action, signaling pathway crosstalk, and drug resistance mechanisms using proteomics data.

PP05.07: Normalization to External Reference for Reduction of Technical Variation

Yolanda Hagar, United States

Introduction: Mitigation of technical variability is a key aspect in relating proteomic biomarkers to clinical endpoints. A primary source of variability comes from inherent properties of the technology used to measure protein levels in a sample, but additional variability can come from many different sources, including sample handling and study differences. Bridging samples have historically been used to remove variation between studies, but increase costs and can be difficult to obtain across longitudinal studies or between different research groups. Here, we present results for normalization to an external reference population, which allows us to combine datasets without the need for bridging samples.

Methods: SomaLogic performs a series of steps to standardize the SomaScan assay data. The last step is adaptive normalization by maximum likelihood (ANML), which normalizes SomaScan EDTA plasma measurements to a healthy U.S. population reference. Because this normalization technique does not require bridging samples, it can be used to combine data from different times and sources. The impact of this type of normalization is quantified through CV calculations over multiple runs and time points. As an example, we demonstrate the benefits of this method on age and sex as clinical endpoints.

Results: The median coefficient of variation (CV) on raw SomaScan data is 22.4%, which drops to 5.3% after all standardization steps and ANML normalization are applied. Longitudinally, the median CV after ANML normalization remained steady at 5.3% across multiple cohorts that were run between June of 2020 and May of 2023. Examination of analytes related to age and sex confirmed that known biomarkers were more easily identified after ANML normalization was applied.

Conclusions:
Use of a reference population allows for bridging across varied cohorts and studies, which allows for increased use of SomaScan data among researchers using proteomics for biomarker discovery and clinical development.

PP05.08: Proteogenomics Data Integration to Assess Clinical Potential of Protein Sequence Variants

Peter Horvatovich, Netherlands

Introduction: The variability of protein sequences can significantly impact various aspects of protein biology, including protein 3D structure, activity, post-translational modifications, and cellular localization. Living organisms utilize this property to fine-tune protein activity through splicing. However, somatic and germline variants may contribute to disease susceptibility, such as development of COPD, or play an important role in the onset and progression of cancer. To address this, we have developed a proteogenomic pipeline, which enables the prediction of protein sequence variants from transcriptomics and genomics next generation sequencing data, including single amino acid variants (SAAV), effects of indels and single and multi-nucleotide polymorphism and splicing events, and provides support for assessing the reliability of variant-specific peptide identifications.
Methods: Our proteogenomic pipeline, implemented in Python/Nextflow, follows the FAIR principles for data, code, and workflow management. The pipeline incorporates modules for mRNA/DNA data quality control, alignment to the Ensembl human genome, de novo assembly, and annotation of known variants with population frequency and clinical effects. We have assessed the reliability of variant peptide-spectrum matches obtained with MSFragger/DIAUmpire using a workflow that integrates tools for confirming spectral evidence of single amino acid variant, evaluating alternative spectrum interpretations, and comparing with in silico predicted spectra.
Results: We demonstrate the application of our proteogenomic pipeline using mRNAseq and proteomics DDA/DIA LC-MS/MS data obtained from human lung tissue samples of COPD patients and controls, and from melanoma and head and neck cancer tissue samples.
Conclusions: Our approach successfully identified several disease-specific protein variants, including peptides derived from a novel exon encoding a new protein domain for SORBS1 gene in COPD patients, as well as SAAV enriched in specific melanoma subtypes. The pipeline provided evidence of newly translated proteins predicted from the dark genome identified in a longevity study involving elderly healthy volunteers of South American origin.

PP05.09: PaxDB 5.0: Curated Protein Quantification Data Suggests Adaptive Proteome Changes in Yeasts

Qingyao Huang, Switzerland

The "Protein Abundances Across Organisms" database (PaxDb) is an integrative meta-resource dedicated to protein abundance levels, in tissue-specific or whole-organism proteomes. PaxDb focuses on computing best-estimate abundances for proteins in normal/healthy contexts, and expresses abundance values for each protein in "parts per million" (ppm) in relation to all other protein molecules in the cell. The uniform data re-processing, quality scoring, and integrated orthology relations have made PaxDb one of the preferred tools for comparisons between individual datasets, tissues or organisms.

In describing the latest version 5.0 of PaxDb, we particularly emphasise the data integration from various types of raw data, and how we expanded the number of organisms and tissue groups as well as the proteome coverage. The current collection of PaxDb includes 831 original datasets from 170 species, including 22 Archaea, 81 Bacteria and 67 Eukaryota. Apart from detailing the data update, we also show a comparative analysis of the human proteome subset of PaxDb against the two most widely-used human proteome data resources: Human Protein Atlas (HPA) and Genotype-Tissue Expression (GTEx). Lastly, through protein abundance data, we reveal an evolutionary trend in the usage of sulfur-containing amino acids in the proteome of Fungi.

PP05.10: MOLE: MultiOmics Learning to Extrapolate Proteome Expression

Ekaterina Ilgisonis, Russian Federation

Understanding the relationship between gene expression at the RNA level and protein abundance poses a significant challenge in comprehensively analyzing biological processes. However, a substantial disparity exists between the number of genes identified at the RNA level and those that are translated into proteins. To address this discrepancy, we developed a 10-channel convolutional model that integrates label-free LC-MS/MS data, RNA-seq data and database annotations into a single tensor, enabling the prediction of protein abundance based on known RNA expression levels.

Our solution was designed as an extrapolating tool for online public web usage, employing lightweight deep neural architectures for fast inference. The model was optimized using a validation set comprising 20% of genes from the datasets used.

Evaluation on a healthy human tissues dataset yielded promising results, with a coefficient of determination (R^2) of 0.61 and a Spearman correlation of 0.75 for all samples. We observed up to 0.7 R^2 for experiments per tissue, indicating the model's accuracy and tissue-specific predictability. Similarly, in the tumor cell lines dataset, our best model achieved an R^2 of 0.57 and a Spearman correlation of 0.72 for all validation samples, with up to 0.65 R^2 for experiments per cell line.

To optimize our model, we incorporated translatomic data, including ribosome and polysome profiling. By leveraging protein half-life times and translation efficiency scores, we enhanced the biological relevance and accuracy of our predictions.

In conclusion, our convolutional model presents a promising approach for bridging the gap between transcriptomics and proteomics. Its efficiency, flexibility, and integration of translatomic data and protein-related features contribute to its utility for comprehensive molecular analysis.

PP05.11: Reaching Methodological Agreement in Proteomics – A Thematic Analysis Scoping Downstream Data Analysis Workflows

Somya Iqbal, United Kingdom

Analysis and pre-processing steps have not been formally standardised or taxonomised in downstream workflows for mass spectrometry (MS) based proteomic data. The aim of this work is to move towards standardisation and methodological agreement in the field. We conducted a scoping review from proteomic studies in human health from the past 10 years. The results we have begun to identify include the parameters of existing workflows and potentially suitable tasks for machine learning (ML) applications.

The proteome is crucial to functional outcomes in our physiology, making it the focus of research interests in human health. However, unlike its counterparts in OMICs, proteomics is in its nascent stages when it comes to fully standardising the handling of large-scale data from (MS) methods. Various approaches have been introduced to manage the high dimensional data, which forms a complex landscape when selecting biomarkers for therapeutic applications in disease. There is also an interest in ML/AI applications and automated processes for improved data mining, biomarker selection and reproducible findings from publicly available datasets.

Our review includes a thematic analysis from text extracts, to capture the trends in bioinformatics pipelines from the proteomics community. We conducted a qualitative content analysis (QCA) and a thematic analysis to group methodology concepts. These analyses are used to highlight where to focus standardisation efforts, suitable fits for ML, and how to evolve workflows.

An outline of the outputs include, identification of: 1) converging approaches to proteomic data handling, 2) ML usage, gaps, and possible future steps in proteomics, 3) some areas where standardisation is necessary for reproducibility. Finally, 4) thematic categorisations of the main issues, and warranted new methods identified by the proteomics community. These analyses can point to potentially viable pathways for designing bioinformatics pipelines suited to proteomic data analysis for discovery driven biomarker selections.

PP05.12: Enhanced TMT Data Analysis with AI-driven Workflows Utilizing CHIMERYS and INFERYS Algorithms

Anas Kamleh, Netherlands

Introduction:
Significant progress has been made in proteomics data processing leveraging deep-learning and AI. However, few studies exist which examine the use of cutting-edge AI-driven algorithms for labeled proteomics data analysis. Here we demonstrate the application of deep learning algorithms to increase identified and quantified TMT labeled peptides.

Methods:
Data from Fürtwangler et al, MCP, 2022 available via PRIDE (PXD029320) were used for this evaluation. Briefly the authors labeled single cell(SC) digests with TMTPro(TM) reagents and compared three acquisition methods: MS2, SPS MS3 and RETICLE (real time search triggered MS2). CHIMERYS(TM) and INFERYS (TM) algorithms were used to analyze the data, implemented as nodes in the Proteome Discoverer software 3.1.

Preliminary results:
For the single cell SPS MS3 data SEQUEST HT identified and quantified 4863, 3490 peptide groups and 1347, 1111 protein groups, respectively. Adding INFERYS rescoring improved quantified protein groups by 11%.
Using CHIMERYS, the intelligent search algorithm, resulted in 5824 (+20%) peptides and 1632 (+21%) proteins identified and 3662 peptides and 1169 proteins quantified. For the single cell MS2 data, SEQUEST HT alone identified 4073 peptides and 1135 proteins, quantifying 3854 peptides and 1081 proteins. Adding INFERYS rescoring results in 4008 peptides and 1150 proteins identified while the numbers of quantified peptides and proteins were 3769 and 1085, repsectively.
Finally, CHYMERIS identified 5037 peptides and 1658 proteins while quantifying 3518 peptides and 1168 proteins. The significant increase in identifications from chimeric spectra indicates the high degree of co-isolation explaining why CHYMERIS produced a modest increase in quantified proteins for the SC MS2 dataset.

Conclusion:
Based on these preliminary results MS2 based acquisition strategies benefit most from inclusion of CHIMERYS in the data analysis workflow while MS3 based strategies show greatest benefit from inclusion of SEQUESTHT+INFERYS rescoring during data analysis. Further studies on more complex datasets are currently ongoing.

PP05.13: dia-PASEF Tools: a Shiny App for Data Visualization and Exploration of dia-PASEF Data

Kyoko Kato, Japan

dia-PASEF has matured into a powerful and widely used analytical technique in the biological and clinical field. The throughput of sample acquisition and data analysis has increased drastically due to the improvements in TIMS technology and data analysis software tools. To quickly inspect the results of dia-PASEF, we developed a dia-PASEF Tools Shiny App. This app is a user-friendly tool designed to streamline the process of exploring and visualizing dia-PASEF results. The app uses the reports from common software tools, DIA-NN, Spectronaut and tims DIA-NN, and offers a user-friendly interface and a variety of interactive charts and graphs to help users better understand their data. We illustrate its use with a dilution series experiment acquired using dia-PASEF.

Heavy and light non-human peptides were spiked into a commercially available human digest (Promega K562) to generate a dilution series sample set with peptide concentrations ranging from 0 to 50 fmol. The samples were analyzed using dia-PASEF on a Bruker timsTOF HT coupled to a nanoElute system. The peptides were separated on a PepSep C18 column using a 75-minute gradient. DIA was used covering the range of 350-1200 m/z and 0.7-1.3 1/k0 with a cycle time of 1.5 seconds. The real-time data analysis was performed by PASER (Parallel Database Search Engine in Real-Time, Bruker) and post-acquisition analysis done using Spectronaut (Biognosys).

The app provides a streamlined and efficient solution for the visualization and analysis of complex quantitative proteomic results from across several DIA software tools. The inputs for the app is the output report from DIA-NN, tims DIA-NN or Spectronaut, and experimental metadata. The user can also input a list of target proteins or targets, giving them the ability to generate hypotheses and uncover hidden patterns in the data.

![HUPO BUSAN 2023 SEP 17-21 — 22nd Human Proteome Organization World Congress]

See you in Busan!
BEXCO, Busan, Korea
🌐 2023.hupo.org
🐦 f 📷 in #HUPO2023

Together Through Proteomics

PROGRAM

PP05.14: PPIAT: Targeted Mass Spectrometry-Based Analysis Tool for Protein-Protein Interactions

Hyunsoo Kim, Republic of Korea

Introduction
Targeted mass spectrometry-based Cross-linking mass spectrometry (XL-MS) is a powerful method for mapping PPIs. To apply targeted mass spectrometry for understanding a target protein's interaction with a specific disease, it is necessary to profile all proteins involved in the disease beforehand.
However, profiling of all proteins related to disease requires a lot of time and resources for researchers to perform, which is a major factor hindering research efficiency. In order to solve this problem, various analysis tools are required, but due to the shortage of analysis tools for XL-MS, there are still challenges to be solved. In this respect, the development of analysis tools for XL-MS is still needed.

Methods
We proposed PPIAT, a targeted mass spectrometry-based protein-protein interaction analysis tool to overcome these hurdles. PPIAT is a web-based analysis tool that can search interaction information about human proteins and calculate mass values given the properties of cross-linkers, enzymes, charges, and modifications. PPIAT is a web-based analytics tool that utilizes STRING and UniProt databases to search for theoretical interacting proteins for target proteins and calculate their mass in peptide and ion levels, taking into consideration all possible interactions with each protein complex. Its primary function is to facilitate the identification of physical protein-protein interactions through targeted mass spectrometry.

Results
We confirmed that the results of PPIAT calculated by considering various conditions of XL-MS matched those calculated directly by Skyline. The results can be summarized by results from other analysis tools and can be used as input data for target mass spectrometry analysis.

Conclusion
PPIAT provides a solution for the search of protein-protein interactions involving target proteins and calculation of the mass values of all theoretical interaction cases between proteins at both peptide and fragmented peptide ion levels, overcoming a significant obstacle in XL-MS analysis.

PP05.15: FLASHViewer: a Web Application for Visualizing Deconvolved Signals in Top-down Mass Spectrometry-based Proteomics

Jihyung Kim, Germany

Despite significant recent advancements in top-down proteomics (TDP) based on mass spectrometry (MS), data analysis is still a bottleneck. Deconvolution, a crucial initial step in TDP data analysis, tackles the inherent complexity of the data. Although several tools have been developed to automate the deconvolution process, manual validation is still much needed for quality control of deconvolved masses due to a lack of evaluation methods. This highlights the pressing demand for an effective and interactive deconvolution viewer.

To address this, we present FLASHViewer, a web application for visualizing deconvolved MS signals. FLASHViewer first applies a processing step to the deconvolution results and then displays them. This two-step approach allows FLASHViewer to accommodate different deconvolution tools' results. Currently, FLASHDeconv (Jeong et al., 2020) is employed to perform the deconvolution and generate input files for FLASHViewer. Displaying part consists of modularized components; therefore, can be configured based on the protocols or specific needs. FLASHViewer is written in Python and javascript and leverages the Streamlit and pyOpenMS libraries, allowing open-source and platform-independent software development.

FLASHViewer processes uploaded input files and generate various informative plots. Initially, users are presented with two heat maps displaying the feature maps of raw MS1 signals and deconvolved masses, providing a birds-eye view of the experiment. Also, a scan table presents the deconvolved MS scans with essential information. Selecting a specific scan triggers a drawing of the raw and deconvolved spectra, accompanied by a mass table that showcases detailed information about deconvolved masses from the scan. Furthermore, users can select a deconvolved mass and explore a 3D plot depicting the signal and noise peaks calculated by FLASHDeconv, offering a comprehensive understanding of the data.

A prototype version of FLASHViewer is available at https://github.com/JeeH-K/FLASHViewer. Future plans include dedicated support for native MS and additional parsers for other deconvolution tools.

PP05.16A: Single-cell Proteomics Methodologies on the Orbitrap Tribird Platform for Increased Throughput and Extended Coverage

Amanda Lee, United States

Multiple techniques are in development for the study of single-cell (or limited sample amount) proteomics. Data Independent Acquisition (DIA) is one popular method to prevent missing values for better quantitation while achieving high identifications. There are limitations to the number of samples run per day. 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 adding a carrier channel. However, the long ion accumulation times required for sampling enough ions from the single-cell channels limit the number of quantified peptides during the LC-MS analysis. The Orbitrap Ascend Tribrid MS was compared to the Orbitrap Eclipse Tribrid MS using a diluted standard, measured using a DDA MS2 OT/OT and DIA-based methods. For the comparison of the Eclipse MS to the Ascend MS, the same chromatographic setup was used (Evosep Whisper100 20SPD, 1h gradient), µPAC Neo Low Loads column (flowrate of 65 nL/min with direct injection on the Vanquish Neo). Real single-cell samples were measured using the µPAC Neo Low Loads column on the Ascend MS with a throughput of 272 cells per day to compare the performance of classical MS2 acquisition to a real-time search-assisted MS2 method. The results show a similar performance between Eclipse and Ascend and a considerable improvement when using the µPAC Neo Low Loads column, visible as higher spectra identification rates and signal-to-noise ratios in the single-cell channels. Furthermore, the results show a large improvement in proteins per cell using real-time search-assisted acquisition (RETICLE), due to higher spectra identification rates and improved S/N distribution across the measured proteins.

PP05.16: Increasing the Depth of Single Shot Proteomics with Enhanced Data Acquisition and Processing Strategies Using Orbitrap Ascend MS

Amanda Lee, United States

Advances in proteomics workflows, including ultra-high resolution separation columns, high-field asymmetric waveform ion mobility spectrometry (FAIMS), and new Orbitrap Ascend Tribrid MS architecture, enable deeper proteome mining with single-shot methods using Data Independent Acquisition (DIA) methods. In addition to the improvements described above, 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. Here we employ all these strategies together in a single-shot proteomics workflow for improved speed, sensitivity, and depth of coverage compared to current acquisition methods and previous search strategies. HeLa protein digests were evaluated at various gradient lengths ranging from 8 to 60 minutes, sample loads from 10ng to 4ug, and quadrupole isolation widths. Data were collected using a Thermo Scientific™ FAIMS Pro Duo™ interface and a Thermo Scientific™ Orbitrap Ascend™ Tribrid™ mass spectrometer in data-dependent acquisition mode. Data files were processed with Thermo Scientific™ Proteome Discoverer™ 3.0/3.1 software using CHIMERYS. Using an isolation width of 4 Th resulted in an average of 6,621 proteins and 56,175 peptides, a 15% increase in protein groups, a 34% increase in peptides, and a 32% increase in the number of MS2 scans acquired compared to the Orbitrap Eclipse. Using a 30-minute gradient an average of 6,137 proteins and 46,992 peptides were identified, which is comparable to the performance of the previous generation of instrumentation using the 45-minute gradient, equating to a 33% increase in sample throughput at the same coverage. These results demonstrate that coupling advanced capabilities in online separation and a new Orbitrap Ascend to enhanced data acquisition and intelligent data processing allows for substantial improvements in single-shot proteomics performance to yield a more thorough coverage of biological pathways with higher throughput and minimal offline sample preparation.

PP05.17: Retention Time-Free (RT-free) Using Featured Ion-Guided Stoichiometry (FIGS) Enables Peptide Identification and Quantification by Data-Independent Acquisition

Qingrun Li, China

Introduction
The data-independent acquisition (DIA) coupled to liquid chromatography-tandem mass spectrometry has become a mainstream proteomic acquisition method. However, the current DIA data processing workflow, including chromatographic ion current preprocessing, elution peak construction, and chromatographic feature extraction, is complex and susceptible to errors under varying liquid chromatography conditions or retention time shifts. These vulnerabilities can compromise peptide identification and quantification accuracy. To overcome these limitations, we propose a deep learning approach for direct peptide identification and quantification independent of chromatographic information (retention time free, RT-free).

Methods
Unlike traditional DIA data processing approaches relying on chromatography, our method disregards chromatographic dimension (retention time information) for peptide identification and quantification. The preprocessed mass spectra are input into two models based on convolutional neural networks. Briefly, these models extract fragment ion intensities, ion mobility, and other chromatography-independent information from the mass spectra and solve qualitative classification and quantitative regression problems.

Results
We conducted experiments using human plasma samples and other datasets. Compared to the benchmark results from Spectronaut, our method significantly improved qualitative reproducibility and increased peptide identifications by at least 10%. Furthermore, our method achieved over 80% overlap in identified peptides with Spectronaut. We also assessed the accuracy and general applicability of our RT-free quantitative model. Finally, experimental results showed our method accuracy was comparable to Spectronaut.

Conclusions
Our results demonstrate that peptide identification and quantification independent of chromatographic information (RT-free mode) are feasible. Moreover, the amount of identifications and the accuracy of quantification can also be validated by the results.

PP05.18: A Target-decoy Competition Approach to Control for False Discoveries in Nautilus PrISM Data

Parag Mallick, United States

Introduction: The Nautilus proteomics platform is powered by a single-molecule analysis method named Protein Identification by Short-epitope Mapping (PrISM). Here, we present methods to control the false discovery rate within PrISM experiments. Using these methods, a researcher can constrain the rate of false protein identification to a desired threshold (e.g. to < 1%). Controlling the error rate in this manner is a critical step to generating confident biological insight. These methods employ a target-decoy competition strategy leveraging a decoy protein database to model the null hypothesis.

Methods: PrISM experiments were simulated where model lysates were deposited on single-molecule arrays and measured using up to 300 multi-affinity probes targeting short linear epitopes. The observed binding data was processed by a decoding algorithm to assign the best scoring protein to each single-molecule. Next, a database of decoy proteins was generated. Decoys were generated to reflect the likelihood that one protein is mis-identified as another protein by the decoding algorithm. The observed binding data was then processed by the decoding algorithm using the decoy database to generate a null distribution of scores. This null distribution allowed for estimation of false discoveries. Various experimental confounders were modeled to assess the robustness of the target-decoy approach. Lastly, we applied these methods to evaluate the target-decoy approach on experimental data from the Nautilus platform.

Results: The target-decoy approach controls for false discoveries within a PrISM experiment. The decoy score distribution accurately reflects the score distribution of incorrect protein identifications. These outcomes held true even when PrISM was provided with an inaccurate binding model. Importantly, false discoveries can be controlled regardless of how many multi-affinity reagents are included in the experiment. Future work will focus on increasing sensitivity while still accurately controlling for false discoveries.

Conclusions: A target-decoy approach can control for false discoveries in PrISM data.

PP05.19: A Novel Deep Learning Approach to Predict Protein O-GlcNAcylation Sites

Jia Mi, China

O-GlcNAcylation is an important post-translational modification involved in major cellular processes and associated with various diseases. Even though several predictors were developed, computational prediction of O-GlcNAcylation sites remains a significant challenge. Advances in deep learning (DL) algorithms and availability of O-GlcNAc-proteomics data over the last decade have opened up opportunities to decipher the O-GlcNAcylation system with DL-based prediction tools. This study aims to develop the first O-glycosylation predictor based on deep learning approach. A hybrid multilayer deep learning model based on CNN, Attention, and LSTM is constructed with current validated O-GlcNAcylation database after a comprehensive comparison with various classifiers including deep learning and traditional machine learning methods. Encouragingly, this model achieved 93% accuracy, 91% area under the curve (AUC), 97% average precision (AP), and Matthew's correlation coefficient (MCC) over 0.83 in an independent data set. It outperforms current existing O-glycosylation prediction online servers, indicating that it could offer more instructive guidance for further experimental research on O-GlcNAcylation. Moreover the predictor (DeepO-GlcNAc) is implemented as a web server to facilitate the query of high-accuracy O-GlcNAcylation prediction for experimental validation.

In general, the developed predictor achieved a remarkable performance in the O-GlcNAc prediction. The success of DeepO-GlcNAc proved the feasibility of deep learning in O-GlcNAc prediction, and the online predictor service provides a new tool for future research.

PP05.20: The PBMC Methylome Landscape in CMTs Reveals the Epigenetic Regulation of Immune Genes and its Application in Predicting Tumor Malignancy

Areum Nam, Republic of Korea

Genome-wide dysregulation of CpG methylation accompanies tumor progression and characteristic states of cancer cells, prompting a rationale for biomarker development. Understanding how the archetypic epigenetic modification determines systemic contributions of immune cell types is the key to further clinical benefits. In this study, we characterized the differential DNA methylome landscapes of peripheral blood mononuclear cells (PBMCs) from 76 canines using methylated CpG-binding domain sequencing (MBD-seq). Through gene set enrichment analysis, we discovered that genes involved in the growth and differentiation of T- and B-cells are highly methylated in tumor PBMCs. We also revealed the increased methylation at single CpG resolution and reversed expression in representative marker genes regulating immune cell proliferation (BACH2, SH2D1A, TXK, UHRF1). Furthermore, we utilized the PBMC methylome to effectively differentiate between benign and malignant tumors and the presence of mammary gland tumors through a machine-learning approach. This research contributes to a better knowledge of the comprehensive epigenetic regulation of circulating immune cells responding to tumors. It suggests a new framework for identifying benign and malignant cancers using genome-wide methylome.

PP05.21: iDeepLC: A Deep Learning-based Retention Time Predictor for Unseen Modified Peptides with a Novel Encoding System

Alireza Nameni, Belgium

Retention time prediction of peptides has previously been shown to be able to rank PSMs and flag incorrect identifications in open modification searches. Previously we introduced a novel method that is also capable of predicting retention times for even unseen peptide modification. This ability is achieved through a novel feature encoding of the atomic composition. However, this method is unable to capture differences between structures with the same composition and has limited extrapolation capabilities. In the iDeepLC, we even further improved the accuracy of predicting the retention time of modified peptides. This improvement was achieved by using chemical descriptors to encode amino acids and modifications.
iDeepLC uses a straightforward neural architecture consisting of convolutional layers followed by dense layers. the less complex architecture of iDeepLC makes it easier to interpret, adapt and retrain, moreover it needs less time for the training and predicting phase.
By encoding new chemical descriptors, this architecture achieves better retention time prediction performance, especially with isomeric structures. To evaluate this improvement, iDeepLC is evaluated on a wide variety of 19 data sets and outperforms previous state of art models in terms of pearson correlation and mean absolute error.
Encoding chemical descriptors instead of atomic composition results in better performance on 19 datasets. alongside the more accurate retention time prediction, iDeepLC shows its true potential in distinguishing isomeric peptides. iDeepLC better understands the peptides' physicochemical characteristics, this is observed in predicting the retention time of modified without prior using them in the training phase.
Encoding chemical descriptors instead of atomic composition results in better performance on 19 datasets. alongside the more accurate retention time prediction, iDeepLC shows its true potential in distinguishing isomeric peptides.
In conclusion,iDeepLC implements a less complex neural architecture, can more easily be interpreted, and has faster model inference.

PP05.22: Updates to FragPipe Computational Platform: New Capabilities, Tools, and Workflows

Alexey Nesvizhskii, United States

FragPipe is a complete proteomics data analysis pipeline (https://fragpipe.nesvilab.org), powered by the fast database search engine MSFragger. Direct analysis of data-independent acquisition (DIA) data can be done with MSFragger-DIA or DIA-Umpire followed by hybrid (combined) spectral library building using EasyPQP and DIA quantification with DIA-NN. Peptide validation can be performed with either PeptideProphet or Percolator, followed by PTM site localization by PTMProphet, and protein inference by ProteinProphet. Philosopher extracts isobaric labeling-based quantification and generates multi-level reports. PTM-Shepherd provides summarization and characterization of possible post-translational modifications (PTMs) found from MSFragger open searches. Label-free quantification (with FDR-controlled match between-runs) can be accomplished with IonQuant, and TMT or iTRAQ data can be analyzed with TMT-Integrator. FragPipe provides workflows for traditional (closed) search; open or mass offset searches for PTM discovery; non-specific (HLA or peptidome) searches; labeling and label-free quantification; glycopeptide (N- and O-linked) analysis; analysis of protein-RNA crosslink data, chemical proteomics workflows (e.g., isoTOP-ABPP for cysteine activity-based protein profiling), FPOP, and for profiling of labile modifications such ADP-Ribosylation.

We have further extended and improved FragPipe with new features, including visualization module FragPipe-PDV. MSFragger-Labile improves a variety of PTM searches including phosphorylation and ADP-ribosylation, as well as analysis of labile chemoproteomics probes and other applications. The glycoproteomics toolkit in FragPipe, already containing MSFragger-Glyco search and glycan composition assignment and FDR in PTM-Shepherd, has been expanded with the addition of the O-Pair tool for localizing O-glycans. Support for visualizing spectra of glycopeptides and peptides bearing other modifications from MSFragger-Labile search has been expanded in the integrated FragPipe-PDV viewer. The FDR filtering and reporting has been updated to add the group FDR for proteogenomics and rare PTM searches. To facilitate the downstream analysis, we developed FragPipe-Analyst webserver that takes FragPipe output to perform downstream analysis, including imputation, differential expression analysis, enrichment analysis, and various data visualization.

PP05.23: Search Engine Optimization for midiaPASEF Data Independent Acquisition (DIA)

Robin Park, United States

In a recent publication, Distler et al. presented midiaPASEF, an innovative data-independent acquisition (DIA) scanning mode. This strategy employs diagonal scanning of the quadrupole with overlapping windows, enabling efficient coverage of the precursor ion cloud in both ion mobility and m/z dimensions.

While midiaPaSEF retains the advantages of data-independent acquisition (DIA), such as reproducibility and a high-duty cycle, the inclusion of deconvoluted data-dependent acquisition (DDA)-like tandem mass spectrometry (MS/MS) spectra enables users to employ database search engines for identification. The procedure for identifying characteristics and establishing associations between precursor ions and fragment ions is accomplished through the utilization of 4D-Proteomics, which incorporates collision cross section (CCS), retention time, m/z (mass-to-charge ratio), and MS/MS fingerprint.

In contrast to conventional data-dependent acquisition (DDA) method, wherein each fragment ion is characterized solely by its mass-to-charge ratio (m/z) and intensity, midiaPASEF introduces an additional parameter known as a confidence score for every fragment ion. The confidence score generated by midiaPASEF can be effectively employed within the search engine algorithm to enhance the identification of peptides.

A weight function was developed to standardize the score and integrate it into the existing cross-correlation score in the real-time ProlucidGPU search engine. This weight function serves to enhance or penalize individual fragment ions accordingly. A weight function has been formulated with the purpose of normalizing the score and incorporating it into the pre-existing cross-correlation score of the real-time ProlucidGPU search engine. This weight function effectively adjusts the influence of individual fragment ions by either enhancing or penalizing their contribution.

The optimized ProlucidGPU search engine has been integrated into the ProteoScape platform to facilitate real-time as well as offline searches.

See you in Busan!
BEXCO, Busan, Korea
2023.hupo.org
#HUPO2023

Together Through Proteomics

PROGRAM

PP05.24: Analyzing Data-independent Acquisition (DIA) Data One Experimental Spectrum at a Time

Patroklos Samaras, Germany

Background: DIA spectra are inherently chimeric as they contain fragment ions from multiple peptide precursors, some of which will be shared. Peptide-centric approaches typically remove these shared fragment ions based on correlations with precursor ions over retention time. We have previously developed a technique to deconvolute chimeric DDA spectra that does not rely on such correlations and can distribute shared fragment ion intensities between precursors instead of discarding them. We demonstrate that this approach extends to any chimeric MS2 spectrum, enabling the consistent analysis of DDA and DIA data.

Methods: Our CHIMERYS™ algorithm is a spectrum-centric and library-free method for analyzing any chimeric MS2 spectrum. It deconvolutes spectra by distributing shared fragment ion intensity to relevant precursors, quantifies peptide contributions to spectra over retention time and controls false discovery rates using Mokapot. Post-processing was performed using Thermo Scientific™ Proteome Discoverer™ software.

Results: We compared our algorithm to DIA-NN and Spectronaut® on public DIA data. It performs on par with state-of-the-art software in terms of identified peptide precursors while entrapment experiments demonstrate well-calibrated error rates. Shared peptides show high quantitative similarity between the different software solutions. Missed identifications by our algorithm are primarily low-abundance peptides with limited fragment ions. In terms of quantitative precision, we demonstrate very comparable performance recovering known ratios from mixed-species samples. The algorithm achieves low coefficients of variation even with independent searches due to its ability to utilize shared fragment ions for quantification without the need for harmonization across multiple files. Finally, for the first time, we can properly perform head-to-head comparisons of DDA and DIA measurements of the same sample processed with the same algorithm.

Conclusion: A novel, spectrum-centric algorithm for the analysis of DIA data and head-to-head comparison of DDA and DIA data.

PP05.25: Identification of Senescence Signatures and Senescence Characterization in High-grade Serous Ovarian Carcinoma

Dongjun Shin, Republic of Korea

Cellular senescence is a state of permanent cell-cycle arrest which influence various age-related diseases including cancer. It has known to play a role in chemotherapy-induced resistance in cancers such as high-grade serous ovarian carcinoma (HGSOC), through secretion of immune-related secretomes. Therefore, selective elimination of senescent cells, named as 'senolysis', has emerged as a potential therapeutic method for cancers. However, the heterogeneous nature of senescence poses a challenge in identifying relevant markers, resulting in a limited understanding of senescence characteristics in cancer patients. Here, we identified 17 common as well as 27 inducer-specific senescence signatures through meta-analysis of various senescence RNA-seq expression datasets from public database. Using machine learning approaches, we constructed a senescence prediction models with identified genes, as well as four pre-defined senescence gene sets from various studies. Model performance on test sets were compared, and our model outperformed pre-defined senescence genesets from other studies. Next, we performed an analysis of single-cell RNA-seq data of HGSOC patients collected from GEO database (GSE165897) to characterize senescence in HGSOC using our senescence genes. When applying our therapy-induced senescence geneset to single-cell HGSOC data, we found the presence of senescence features in cancer-associated fibroblasts. Notably, among the paired patients in the pre- and post-chemotherapy groups, we identified an increase of senescence-related characteristics in the cells of the post-treatment patient group. Collectively, we identified 44 senescence signatures by meta-analysis of senescence RNA-seq data and machine learning approaches. The model using these genes was able to discriminate various senescence status than other previous models. Moreover, we characterized senescence in single-cell RNA-seq data of HGSOC patients.

PP05.26: A Cloud-based QC Platform for Comprehensive Examination of MS-based Proteomics Data

Yourae Shin, Republic of Korea

Introduction: Quality control (QC) is an integral part of omics data acquisition and analysis. A proper QC of omics data can guide researchers to determine whether and what kind of batch effect is present, and whether a re-experiment or post-hoc data handling is necessary. While streamlined QC procedures are well-established for NGS data, a standardized QC protocol is absent in proteomics. This often leads to suboptimal data acquisition, inappropriate data handling, and misinterpretation of biological significance. Existing proteomics QC tools suffer from limitations such as a lack of user-friendly interfaces, limited customization options, incomplete QC components, and restricted to a handful of input data types. We developed a novel QC platform to address these unmet needs.
Methods: Our platform operates on an AWS cloud server, through which it accepts MS data and search files and seamlessly runs QC protocol.
Results: The versatility of our platform is highlighted by the compendium of input data types it accepts; users can pre-define characteristics of input data including organism, sample types, MS acquisition and quantification mode, and omics level. Additionally, users can select normalization methods. Based on these pre-defined parameters, our platform performs a comprehensive QC encompassing raw data quality, identifications, peptide characteristics, intensity distributions, and sample clustering. These QC outputs are presented through interactive figures and data tables, allowing users to explore the quality of their data both intuitively and quantitatively. Furthermore, a highly customizable graphic styling toolkit further aids data exploration. Finally, users can determine whether a batch effect is present, in which case they can return to the initial parameter setting to choose an alternative normalization method.
Conclusions: By employing our platform, researchers can conduct a comprehensive QC of their proteomics data, maximizing biological signals while minimizing technical noise. We envision our platform to constitute a streamlined analysis protocol for proteomics.

PP05.27: Identifying Protein Products of Germline Variation Using Mass Spectrometry

Dafni Skiadopoulou, Norway

Adapting health care to the individual profiles of patients is the main goal of precision medicine. Monitoring the unique proteome of patients, shaped by the genetic variants they carry, can shed light on the different factors that drive the cause and progression of diseases. While mass spectrometry-based proteomics has proven very effective at identifying and quantifying proteins, it still remains very challenging to accurately monitor products of genetic variation. The main problems faced in such analyses are: (i) the expansion of the search space that increase the number of peptides than can possibly match a spectrum, and (ii) the high similarity between variant peptides that contain an amino acid substitution and canonical or modified peptides.

We implemented a novel proteogenomic pipeline featuring multiple identification algorithms and executed it on publicly available datasets of human proteomic data. We demonstrated that even after including variant protein sequences, a decrease in the analysis performance can be avoided with a careful curation of the protein sequence database . We show how the integration of peptide characteristic predictors improves identification rates for variant peptides. The combination of predictors of the retention time and fragmentation pattern of a peptide with the machine learning-based post-processing tool Percolator increased the discriminative power between correct and incorrect peptide identifications. In addition, when accounting for a wide list of chemical and post-translational modifications, we found a high prevalence of ambiguous cases where a spectrum could be explained either by a modified or a variant peptide. Our results prove that using the proposed proteogenomics pipeline, proteomic searches can effectively be extended with germline variation in order to obtain a better coverage of the proteome and thereby improve patient care.

PP05.28: Precise and Accurate Real-time de Novo Sequencing of TimsTOF Data with the Novor Algorithm on the Bruker ProteoScape Platform

Tharan Srikumar, Canada

Bruker ProteoScape (BPS; formerly PaSER) has been transforming into a comprehensive proteomics data analysis platform that can integrate 3rd-party tools while utilizing the concept of data-streaming to realize customizable real-time processing workflows including on-the-fly decision making. However, database searching is only the preferred solution when canonical proteins are being investigated. To expand the capabilities of the BPS platform for immunopeptidomic, metaproteomic, and other applications, we integrated a newly timsTOF optimized de novo sequencing engine from Rapid Novor Inc., called BPS Novor.

BPS Novor was trained on a variety of timsTOF acquired data, where ground truth is taken from ProLuCID-GPU database search results filtered to 1% PSM FDR. Here we compare BPS Novor against other de novo tools across multiple datasets, including various enzyme digests, mixed species and immunopeptidomics timsTOF data. MGF files were utilized to remove the confounding effects of any pre-processing and to allow direct comparison between algorithms based on scan number matching.

We first evaluated the processing speed of Novor, average processing time ranged from 86-199 seconds, or an average processing speed of 1338±226 spectra/second. PSMs with an FDR less than 1% were used to define the 'ground truth' for each dataset. The datasets were processed with the standard Novor model that was not trained on timsTOF data, as well as the BPS Novor model retrained on timsTOF data. For comparison, we analyzed the data with the de novo module from Peaks Studio 10.6.

BPS Novor consistently outperformed both standard Novor and Peaks Studio on all datasets. On amino acid level, at 75% precision, PaSER Novor achieved between 40-60%, whereas standard Novor achieved between 25-50%, and Peaks Studio achieved between 25-55% recall (pepsin and trypsin, respectively). Taken together, BPS Novor is a fast, precise and accurate engine that allows real-time de novo sequencing of timsTOF data.

PP05.29: TRIMQuant: Precise and Scalable MS1 Quantification for DDA and DIA Using Transfer Learning, Targeted Analysis and Semi-supervised Machine Learning

Tharan Srikumar, Canada

MS1-based approaches have been established for label-free DDA and DIA experiments. DDA-based implementations typically rely on prior feature finding, which are then mapped to identified or aligned PSMs. DIA-based methods are typically based on MS2 peptide detection to guide the extraction of ion chromatograms (XIC). Recently, it has been demonstrated that XIC-based methods can also improve MS1-level quantification for DDA data, however, the challenge of inter-run alignment persists and represents a computational bottleneck. New approaches are required that can alleviate these limitations for increased scalability and robustness of quantification.

Here we present TRIMQuant (TRansfer learning and Ion Mobility-guided Quantification), a novel approach tailored for the Bruker timsTOF platform that makes use of a retention time (RT) and ion mobility (IM) prediction and transfer learning module to predict the precise location and extraction window widths in RT and IM dimensions for individual runs. These coordinates are then used for targeted data extraction of peptide precursor isotopes and subsequent scoring. Using a null model, representing coordinates of mutated -decoy peptide sequences, semi-supervised machine learning trains an XGBoost-based classifier that allows statistical validation of identified and predicted peak groups. Protein-level aggregation is conducted by the MaxLFQ.

Our workflow is fully parallelized without workflow bottlenecks and thus allows experimental designs with much increased scalability. Semi-supervised learning identified mass and IM accuracy and isotope error as most important scores for classification of true and false signals. Using an LFQbench-like dataset, we show competitive performance compared to established, timsTOF-optimized algorithms, with benefits regarding data completeness. Next, we use a large dda-PASEF dataset of >1000 samples to illustrate the scalability of the TRIMQuant workflow.

We developed a timsTOF-optimized MS1-based quantification algorithm that provides increased scalability for large DDA and DIA-based experimental designs integrated into Bruker ProteoScape. We demonstrate validity of quantification values and competitiveness with state-of-the-art competing solutions.

PP05.30: Reproducibility-optimized Multi-group Statistic and Survival Analysis

Tomi Suomi, Finland

Bioinformaticians are faced with increasingly large and complex datasets as the costs of performing high-throughput omics are constantly coming down. Selecting differentially expressed features (e.g. genes or proteins) from these data remains vital to address biological questions, and for example to discover viable biomarkers. The methods used in differential expression analysis of omics datasets remain important, as it has been shown that the traditionally used classical methods are suboptimal when applied to high dimensional data.

We have previously introduced a widely used Bioconductor R package ROTS for calculating reproducibility-optimized test statistic. It has been successfully applied to multiple types of data, including quantitative mass spectrometry proteomics and both bulk and single-cell RNA sequencing data. To further encourage the use of reproducibility-optimized test statistic in more complex settings, we have now added support for performing analysis between multiple groups and to perform survival analysis. In two-group analysis, ROTS adjusts a modified t-statistic according to the underlying data. In multi-group and survival settings, ROTS uses modified f-statistics and Cox score test, respectively.

The new statistical tests were benchmarked using gold standard spike-in datasets and clinical gene expression data of breast cancer patients. ROTS offered better or comparative performance to other state-of-the-art methods. The updated software is freely available from Bioconductor.

PP05.31: XMass: XGBoost-based Peptide Spectral Library Prediction Integrated into MaxQuant for DDA and DIA Data Analysis

Shamil Urazbakhtin, Germany

Spectral prediction by deep learning using recurrent or convolutional neural networks has gained popularity for peptide spectral library prediction due to its excellent predictive performance. Here we present an alternative approach called XMass, which is amino acid sequence window-based with XGBoost as the underlying machine learning method.
Previously, we introduced an alternative spectral prediction method, wiNNer, utilizing an amino acid sequence window approach to generate a fixed-size feature space from variable-length peptide sequences, and a fully connected neural network to predict fragment intensities. In XMass we use XGBoost regression instead, which improves the prediction performance over that of the recurrent neural network approach.
We simplify the task of predicting peptide fragmentation spectra from amino acid sequences by predicting the ratio of fragment peak intensities to the base peak intensity separately for each peptide bond. The feature space includes one-hot encoded amino acids in a symmetrical window of length 12 around the bond, with modified amino acids treated as additional amino acids. Features describing the termini, bond position, and peptide charge are also included. The hyperparameters of XGBoost were tuned once using cross-validation on HCD and CID models respectively and then fixed for subsequent analyses. We extended this approach to the TMT, mTRAQ, and dimethyl labeling data with promising results and applied it to analyze DDA and DIA data in MaxQuant.
Compared to the dominant recurrent neural network deep learning approaches, XMass is faster to train and requires less data. Therefore, it is ideal for creating project-specific prediction models to capture the experimental conditions at hand. XMass is integrated into MaxQuant in two ways: as a source of predicted intensities for the PSM ranking in the Andromeda 2.0 search engine, improving identification rates for tryptic and non-tryptic peptides, and for on-the-fly prediction of in silico libraries in the MaxDIA workflow.

PP05.32: BatchDesigner: Streamlining Metadata Management for Efficient Experimental Design and Data Quality Control in Mass Spectrometry Analysis

Dhonggeon Won, Republic of Korea

Introduction
Consistent and streamlined management of sample- and experiment-related metadata are crucial in mass spectrometry (MS) analysis. However, varied practices among researchers often lead to errors and inefficiencies in data sharing and analysis. To address these challenges, we developed BatchDesigner, an open-source software package designed to streamline metadata management, assisting in experimental design, and facilitating data quality evaluation.

Method
BatchDesigner consists of three components: BatchNamer, BatchRandomizer, and BatchChecker. BatchNamer provides standardized templates and guidelines for metadata entry, and stores metadata in a structured data format. BatchRandomizer determines the sample processing order using block randomization, enabling effective batch effect correction during data analysis. BatchChecker assesses data integrity through outlier detection and batch discrepancy detection. Implemented with the PyQt6 framework, BatchDesigner provides a user-friendly graphical interface for seamless utilization.

Results
The implementation of BatchDesigner significantly enhanced data organization and quality control in our research laboratory. The introduction of BatchDesigner resulted in a reduction in input errors in metadata, leading to significant time and effort savings in data organization and sharing. The integration of block randomization addressed concerns about batch effects. BatchChecker facilitated seamless data verification and quality control. As a result, BatchDesigner ensures the quality and integrity of data by providing consistent and effective management of metadata.

Conclusion
BatchDesigner streamlines experimental design and ensures data quality through efficient metadata management. In future developments, we plan to further enhance BatchChecker's capabilities by introducing machine learning techniques that utilize metadata.

PP05.33: Accurate in Silico Functional Annotation for Entire Proteomes

Gong Zhang, China

With the rapid expansion of known proteomes, the functional annotation of proteins becomes a bottleneck of understanding the proteome. Biological experiments are too costly and time-consuming for functional annotations. Computational predictions are usually limited by the poor alignment to known proteins and the unresolved structures. Taking the advantage of the deep learning approaches, here, we propose a functional annotation strategy combining the similarities of consensus coding sequence (CCDS) alignments and the deep learning-predicted structures. With this strategy, we provided functional annotations for 19,129 proteins in human proteome, which comprises 97% of the known PE1~4 proteins. Our computational method provided near-correct gene ontology (GO) predictions for all proteins with known functions. We also applied the strategy to uPE1 proteins and new proteins (protein products of the protein-coding "non-coding" RNAs) to provide functional hints for 98% of these unstudied proteins, facilitating further experimental investigations. This strategy can be also expanded for other non-human species. In sum, this functional annotation strategy may serve as a powerful tool to understand the functional nature for the entire protein universe.

PP05.34: Fully Automated and Spectrum-centric Processing of Parallel Reaction Monitoring (PRM) Data

Daniel Zolg, Germany

Background: Targeted mass spectrometry assays are vital for the sensitive quantification of low-abundant peptides and biomarkers. Parallel reaction monitoring (PRM) is a targeted technique where full fragmentation spectra from predefined isolation windows are monitored over retention time. Identification is typically performed by correlating data with a spectral library, and quantification uses area under the curve from extracted ion chromatograms (XICs) of chosen fragment ions, requiring expert knowledge and frequent manual refinement. Here, we present an automated algorithm for PRM data analysis, abstracting manual fine tuning in the development or execution of targeted assays.

Methods: Chimerys is our acquisition-type and analyzer agnostic algorithm that compares predicted and experimental MS2 spectra using intensity-based scores. It performs model refinement to enhance prediction accuracy and considers all promising peptides in each MS2 isolation window simultaneously. The algorithm aims to explain experimental intensity the minimal number of peptides and deconvolves chimeric spectra by distributing shared fragment ion intensity to peptide precursors based on their proportional contribution. Quantification is performed by integrating the area beneath these contributions across retention time, while FDR-control is executed using Mokapot.

Results: Our algorithm can deduce peptide fractional contributions from single MS2 spectra without generating fragment ion XICs. When applied to a PRM run targeting 18 proteins with 52 peptide precursors, it provided identification and quantification results comparable to Skyline 22.2. Both methods identified 47 targeted peptides, but our algorithm additionally identified nearly 2000 co-isolated peptides. This allowed to perform target/decoy competition using Mokapot and calculate PSM FDR for any given spectrum. Resulting quantification demonstrates strong correlation (R=0.99) with Skyline data. These findings validate our algorithm as a viable tool for automated PRM data processing.

Conclusions: With the new functionalities, CHIMERYS can be employed as an automated, spectrum-centric data analysis pipeline for PRM data.

PP05.35: Comprehensive Proteomic Analysis of FFPE Specimens in Hepatocellular Carcinoma for Investigating Recurrence Mechanism

Yuki Adachi, Japan

[Background]
Hepatocellular carcinoma (HCC) is the third most common cause of cancer-related deaths worldwide. One of the clinical challenges in HCC treatment is the high recurrence rate of 80% within five years post-surgery, however, the underlying mechanisms remain poorly understood. Establishing accurate predictors of recurrence is crucial as early intervention for recurrent lesions can potentially improve patient prognosis. Recent advancements in protein extraction protocols and mass spectrometry technologies have enabled accurate and detailed proteomic analysis of formalin-fixed paraffin-embedded (FFPE) samples. This study aims to perform a comprehensive proteomic analysis of FFPE samples from HCC to elucidate the mechanisms of recurrence and identify novel prognostic biomarkers.

[Methods]
A total of 250 patients who were diagnosed with untreated primary HCC and underwent curative liver resection at Asahikawa Medical University Hospital between 2015 and 2022 were included in the study. 200 Patients with insufficient clinicopathological and prognostic data or those who received preoperative treatment were excluded. The remaining 50 patients were divided into two groups: 25 patients who developed recurrence within one year (recurrence group) and 25 patients who had no recurrence for five years (no recurrence group). The macrodissection and peptide extraction was performed on the sectioned FFPE tissues. Mass spectrometry analysis compared the protein expression profiles between the recurrence and non-recurrence groups.

[Results]
Proteomic analysis revealed altered expression of cancer-related proteins in the recurrence group compared to the non-recurrence group. Detailed analysis to investigate the impact of these protein changes on the mechanisms of recurrence is currently underway.

[Conclusion]
The Proteins identified in this study are implicated in the recurrence of HCC, showing promise as a novel predictor of recurrence. These findings contribute to a better understanding of HCC recurrence mechanisms and offer potential insights for developing a novel therapy.

PP05.36: Global Proteome Expression Study of Patient-derived Pleomorphic Sarcoma Cell-lines Toward Optimization of Therapeutic Strategy Using Trabectedin and Eribulin

Taro Akiyama, Japan

[Introduction]
Pleomorphic sarcomas are the most common malignant tumors of mesenchymal tissues, and harbor genetic variability. The prognosis of pleomorphic sarcoma is dire, and the gold-standard therapy is not yet established. Although the clinical use of trabectedin and eribulin have been approved toward pleomorphic sarcomas treatment, their effectivity varies due to the genetic variability of the diseases. Therefore, the predictive biomarker of trabetedin and eribulin is required. The purpose of this study is to explore proteins that are related to the anti-tumor effects of trabectedin and eribulin to establish their therapeutic strategy by developing predictive biomarkers toward pleomorphic sarcomas treatment.
[Method]
We exploited the 11 cell-lines which were derived from patients with pleomorphic sarcoma. The efficacy of trabecedin and eribulin was evaluated by 50% inhibitory concentration (IC50). Mass spectrometric protein expression profiling was performed in all cell-lines. The linear regression analysis between each protein quantity and IC50 values of both trabectedin and eribulin was conducted. We selected proteins whose absolute R value > 0.6 as candidates for predictive biomarker.
[Result]
We generated mass spectrum protein expression profiles of all 11 cell lines examined in this study. The response to the treatment with trabectedin and eribulin was considerably different among cell lines, which was consistent with the clinical observation. We identified proteins whose expression level is remarkably associated with the response to trabectedin or eribulin. Although the identified proteins are multifunctional protein and associated with cancer development, their predictive utility has not been reported in pleomorphic sarcomas.
[Discussion]
The experiments using patients derived cell-lines are competent for searching the protein that may be associated with drug effect. This study gives potent clues to elucidate the new predictive biomarker as well as a mechanism of response to trabectedin and eribulin.

PP05.37: Secretome Landscape of Triple Negative Breast Cancer Unravels Subclass-specific Functional Intricacies

Asfa Alli-Shaik, Singapore

Background: Triple-negative breast cancer (TNBC) is the most aggressive subtype among all breast cancer types. The intrinsic molecular heterogeneity of TNBC complicates design of effective therapeutics, and with the lack of defined targets, TNBC remains a major clinical challenge. While numerous studies have focused on the intracellular heterogeneity within TNBC, secreted proteins can also profoundly influence tumour behaviour. However, knowledge of this facet is limited, and hence we interrogated the secreted protein landscape of TNBC to delineate subtype-specific aberrations.

Methods: The secretome from a wide panel of TNBC cell lines including both aggressive and non-aggressive types as well as receptor positive subtypes were profiled using multiplexed tandem mass tag (TMT)-based mass spectrometry analysis. This was followed by intensive computational analysis to gain insights into the underlying heterogeneity among different TNBC subtypes, based on alterations in secreted or shed protein profiles.

Results: The secretome snapshot from the different subtypes of breast cancer showed distinct protein profiles with the receptor-negative type further showing subclass-specific differences that conform to the less aggressive and more aggressive TNBC subclasses. Systematic interrogation of the shed proteins allowed identification of several pathways functioning in invasive processes including extracellular matrix remodeling and EMT to be consistently dysregulated in the aggressive TNBC type. Functional inferences from the secretome profiles showed correlation with the molecular rewiring associated with the different subclasses within TNBC. The secretome landscape also unraveled unique proteolytic events involving various receptor and ECM components within TNBC, allowing appreciation of novel protease-based vulnerabilities that can be exploited as targets for aggressive TNBC types.

Conclusion: Our study provides new understanding of breast cancer heterogeneity from the secretome perspective and sheds novel insights on subtype-specific molecular perturbations for exploring new age functional markers and novel targeted therapeutics for aggressive TNBC types.

PROGRAM

PP05.38: Proteomic Discovery of the Correlation Between Anticancer Drug and NK Immunotherapeutic Resistance in hABCB1-expressing A549 Cells

Geul Bang, Republic of Korea

During lung cancer chemotherapy, the development of drug resistance occurs. Overexpression of multidrug resistance (MDR) transporter is known as a mechanism that leads to resistance against anticancer drugs. The hABCB1 gene encodes the p-glycoprotein, a member of the ATP-binding cassette transporter superfamily, which is one type of MDR transporter. However, an in-depth understanding of the mechanism at the proteome level remains unclear. In this study, we investigated the mechanism of drug resistance associated with overexpression of hABCB1 using proteomics analysis. In total, 465 differentially expressed proteins were identified and quantified from TMT 11-plex experiments. Compared to wild-type A549 cells, we observed an increase in hABCB1 protein expression and drug-resistance gene ontology (GO) in A549-hABCB1 cells. In addition, to quantitatively compare the NK killing ability of the two cells, cytotoxicity was measured by carboxyfluorescein succinimidyl ester (CFSE) and 7-AAD staining using flow cytometry. As a result, it was confirmed that hABCB1-A549 cells with anticancer drug resistance showed resistance to NK therapy. Our proteomics and cellular analysis demonstrated that hABCB1-mediated anticancer drug resistance in A549 cells is positively correlated with resistance to immune cell therapy.

PP05.39: Investigation of Potential Chemoresistant Breast Cancer Targets by Proteomics and Phosphoproteomics Approaches

Praneeta Bhavsar, India

Background: Acquired drug resistance during chemotherapy leads to tumor recurrence in breast cancer patients and is a significant cause of mortality. Chemotherapy induces enhanced DNA damage and DNA damage response pathways promoting resistance and creating a barrier for further improvement of patient survival. The resistance mechanism has been associated with altered proteins regulating several cellular functions and signaling networks. Identifying chemoresistant specific proteins and their aberrant phosphorylation-mediated signaling network is indeed important in determining the chemotherapy regimen for a patient.

Methods: To identify proteins involved in chemoresistance, doxorubicin (DOX) resistant MDA-MB-231 cells were generated with an increase in concentration from 0.5 µM to 5 µM (MDA-MB-231/R). Label-free quantitative proteomics and phosphoproteomics were conducted on MDA-MB-231/R (resistant) and sensitive cells using Orbitrap Fusion mass-spectrometer coupled to EASY-nLC system.

Results: 951 differentially expressed proteins were identified, 246 showed upregulation with a Fold Change, (FC) ≥ 2, and 705 were found to be downregulated with a FC ≤ 0.5. The majority of differentially expressed proteins including LACTB, FN1, FDXR, F11R, and ABCC1 have been implicated in various cellular processes, particularly in cell proliferation and drug efflux mechanisms. Similarly, the phosphoproteomics of the resistant and sensitive cells analyzed 570 differentially regulated phosphoproteins, and 359 showed upregulation (FC ≥ 2) indicating a significant increase in phosphorylation levels. Conversely, 13 were found to be downregulated (FC ≤ 0.5) with decreased phosphorylation. IPA analysis of the differentially expressed phosphoproteins revealed altered canonical pathways like EIF2 signaling, ERK/MAPK signaling, mTOR signaling, and caveolar-mediated endocytosis signaling. Subsequently, the functional characterization of the altered targets is under progress to understand their significance in crucial resistance pathways.

Conclusions: Overall, we identified potential therapeutic targets that would provide valuable insights into the mechanisms underlying drug resistance in breast cancer.

PROGRAM

PP05.40: A Novel S-Capped Cysteine and its Utilization for Targeted Proteomics

Kwon Hee Bok, Republic of Korea

The selection and synthesis of target peptides of signature proteins is very important in targeted proteomic analysis which provides sensitive and accurate quantitative information of target peptides. One of the crucial steps in targeted proteomics employing stable isotope-labeled (SIL) peptides is the selection and preparation of the standard peptide library serving as surrogates of the biomarker candidate proteins. Due to the chemical reactivity and properties of specific side chain functional groups, several rules were proposed to exclude specific amino acids in this step, cysteine being as an example.
Cysteine is one of the unstable amino acids because sulfhydryl group is easily oxidized. As a method to eliminate intra- or inter-disulfide bond formation of peptides during proteomic profiling, it is a common practice to reduce with DTT and then alkylate with iodoacetamide. Therefore, in order to synthesize target peptide that contains cysteine, alkylation of cysteine is necessary. However, during cysteine alkylation, other functional group such as N-terminal amino group or ε-amino group of lysine can also be alkylated, leading to overalkylation. The overalkylation prevent cysteinyl peptides for use as target peptides. In this study, we introduce the design and synthesis of a novel protected cysteine analogue, Fmoc-Cys(TrtCam)-OH. This compound is readily applicable as a building block in standard solid phase peptide synthesis (SPPS) campaigns, directly producing S-carbamidomethyl cysteine containing peptide without overalkylation, facilitating the use of cysteine-containing stable isotope-labeled (SIL) peptides as target peptides.

PP05.41: The Effect of gp120 in Glioblastoma: Targeting UPR Survival Proteomics Signatures

Nawal Boukli, Puerto Rico

INTRODUCTION: Human immunodeficiency virus (HIV-1) infected patients are more prone to developing cancers, including glioblastomas (GBM). New treatment methods that take into consideration the functional role of HIV on tumor progression and chemotherapeutic resistance are needed. The goal of this study is to identify the biological mechanisms of HIV infection influenced on GBM progression and chemotherapy resistance. Previous data from our lab showed that exposure to HIV-1 exterior envelope glycoprotein (gp120) caused an increased proliferation and resistance to temozolomide in established (U87, A172) glioma cell lines and increase in the endoplasmic reticulum (ER) stress. Based on these findings, we hypothesize that gp120 activates an unfolded protein response (UPR) induced pathways that enhances glioma cell proliferation. METHODS: Viability and flow cytometry analysis of cell cycle, western blot, qRT-PCR and quantitative proteomic approaches were used to verify if exposure to gp120 stimulates proliferation, survival and ER stress in glioma cells. RESULTS: Our data indicates a gp120 induced migration, proliferative and protective responses granted by the expression of GRP78, master regulator of the UPR. Quantitative proteomics studies have identified the activation of the ER stress/ UPR pathways in HIV-gp120-exposed glioma cells. GRP78, ATF6, PERK and IRE1α ER stress markers are currently being validated by western immunoblot and qRT-PCR. CONCLUSION: In this study, we utilized glioma cells and cell biology approaches coupled with proteomics screening to dissect intracellular mechanisms associated with gp120-activated glioma growth. The investigated signaling mechanisms will provide a platform for developing therapeutic strategies for HIV positive glioma patients.

PP05:42: Data-independent Acquisitions of Human Breast Cancer Tissue Subtypes Explore Molecular Profiles to Identify Potential Targets for Stratification and Stromal Reprogramming

Jordan Burton, United States

Breast cancer is a heterogenous disease with multiple subtypes that influence patient outcomes and treatment strategies. We identified proteins from five different human breast cancer subtypes to highlight potential therapeutic targets that may be involved in cancer progression of all subtypes and in individual subtypes. We quantified ~6,000 proteins (each with 2 quantifiable peptides) extracted from Formalin-Fixed, Paraffin-Embedded (FFPE) tissues and over 26% were changed in each differential comparison (cancer subtype vs. control) using stringent statistical filters, $|log2(FC)|≥0.58$ and q-value≤0.001, which allowed us to deeply mine changes that are conserved and unique in the different human breast cancer subtypes. We observed 576 proteins that were significantly altered in all breast cancer subtypes. Conserved changes in the abundance of laminins, heat shock protein 90-alpha, nidogen-1, profilin-1, and myosin-11 for all breast cancer subtypes were previously observed in tissue from chronic inflammation-associated cancers of the lung, esophagus, stomach, and colon. We focused this work on changes in extracellular matrix (ECM) and senescence-associated secretory phenotype (SASP) proteins as potential therapeutic targets for stromal reprogramming. Almost half of the identified ECM proteins were significantly altered in each of the subtypes compared to healthy tissue. The majority of small leucine-rich, desmosome and basement membrane proteins that contribute to the stiffness of the ECM microenvironment were down-regulated, including HSPG2, desmoplakin, lumican and many collagens with the exception of Col12A1 which was upregulated. Additionally, we found that over 74 quantified SASP factors were significantly altered when compared to healthy tissue. Importantly, SerpinH1 and periostin are up-regulated in all breast cancer subtypes, and SerpinH1, for example, was previously validated as a major marker in lung cancer by our group. Our findings demonstrate that changes in the ECM and SASP were observed in breast cancer progression and point to the potential of targeting these pathways for therapeutic strategies.

PP05.43: Proteomics Profiling of Tumor Tissues Reveals a Promoting Role of ENAH in Progression of Oral Cancers

Xiu-ya Chan, Taiwan

Oral cavity squamous cell carcinoma (OSCC) is one of common cancers worldwide. The high incidence of recurrence and metastasis of OSCC leads to a poor prognosis of the disease, indicating that it is necessary to understand molecular mechanism of OSCC progression. To identify molecules involved in OSCC progression, integrated analyses of proteome and transcriptome have been performed with the tissues from OSCC patients and patient-derived xenograft (PDX) mouse models. Protein enabled homolog (ENAH), one of upregulated proteins in OSCC tissues, was selected to determine as an OSCC progression-associated protein. Immunohistochemical analysis revealed that OSCC patients with higher expression of ENAH had worse survival rates compared to those with lower ENAH expression. Knockdown of ENAH resulted in the inhibition of proliferation and migration in OSCC cells, suggesting that ENAH may involve in OSCC progression. Deciphering promoting roles of ENAH in growth and migration of OSCC cell may advance in our knowledge of OSCC prognosis. We found increased level of ENAH proteins in OSCC cells upon EGF treatment. Inhibition (PI3K/Akt) and overexpression (β-catenin) of EGFR downstream signaling molecules resulted in down- and up-regulation of ENAH in OSCC cells, respectively. Furthermore, we demonstrated that depletion of ENAH led to reduced levels of integrin β5 (ITGB5). And the ITGB5 overexpression can partially restore ENAH knockdown-induced reduction of growth and migration capabilities in OSCC cells. Collectively, ENAH is up-regulated by the EGFR signaling pathway and enhances growth and migration ability via ITGB5 regulation in OSCC cells. The information may be useful for development of ENAH-based treatment approaches in oral cancers.

See you in Busan!
BEXCO, Busan, Korea
2023.hupo.org
#HUPO2023

Together Through Proteomics

PROGRAM

PP05.44: Targeting Drug Resistance in Colorectal Cancer Through the Lens of Multiomics Analysis

Hsin-Yi Chang, Taiwan

5-Fluorouracil (5-FU), oxaliplatin, and irinotecan are commonly used as the first-line standard chemotherapies for metastatic colorectal cancer (mCRC). All these three agents target DNA synthesis and repair in different aspects: 5-FU acts as an antimetabolite to disrupt the synthesis and repair of DNA and RNA by inhibiting thymidylate synthase; the platinum-based anticancer drug oxaliplatin causes DNA damage; irinotecan inhibits topoisomerase I by preventing the reunion of DNA breaks. Nevertheless, only a small proportion of patients benefit from conventional chemotherapy due to the development of drug resistance. Drug response heterogeneity in CRC patients correlates to the genetic background and intracellular context. We apply next-generation sequencing-based transcriptome analysis and mass spectrometry-based proteomics to understand cancer progression and improve patient diagnosis and elucidate the disease mechanism at the molecular levels. To obtain robust biomarkers for colorectal patients from blood exosomes, we isolated the extracellular vesicles (EVs) and applied the tandem mass tag-based quantitative method coupled with high-resolution mass spectrometry to identify about 500 EV proteins across 103 samples. We obtained a set of immune response-activating proteins that predominantly co-expressed in the blood exosomes from CRC patients by utilizing weighted gene co-expression network analysis. To dissect the drug resistance mechanisms from the viewpoint of extracellular signals to metabolism rewiring, we integrated the multi-layered proteome analysis and metabolome analysis to understand the molecular regulation in drug-resistant cells. Accordingly, we discovered dysregulated glutamine metabolism as a targetable signature for advanced CRC cancer. Our work shows that robust and high-throughput proteome profiling pipelines allow us to accelerate precision medicine development.

PP05.45: Histopathologically Contextualized Proteomic Analysis of Prostate Cancer Whole-Mount Patient Specimens Leveraging Ion Mobility Mass Spectrometry

Matthew Chang, United States

Background: Prostate cancer diagnosis and prognosis traditionally have relied on PSA blood testing and prostate biopsy with imaging modalities such as MRI increasingly employed. In this proof-of concept study, we used spatially contextualized, discovery-mode proteomics leveraging high-sensitivity, ion mobility mass spectrometry (MS) to profile targeted resections from formalin-fixed paraffin-embedded (FFPE) prostate tumor whole-mount specimens. In addition to facilitating discovery of new prostate cancer-specific proteomic marker candidates, this platform will enable investigation of cellular and molecular factors underlying variability in MRI detection.

Methods: FFPE whole-mount prostate cancer tumor specimens were prepared using standard clinical workflows following surgical prostatectomy. Specimen sections were annotated by a clinical pathologist to define histopathologic areas. Targeted tumor and matched contralateral normal specimen resections were harvested by macrodissection and subjected to optimized preparation workflows in advance of shotgun mass spectrometry. Proteomic interrogation leveraged dia-PASEF mode acquisition on a Bruker timsTOF Pro ion mobility MS platform, and a supporting spectral library was generated using DDA-PASEF analysis of fractionated tumor and non-tumor resection peptides pools. Raw data was processed using DIA-NN.

Results: Analysis revealed identification of ~5100 protein groups per sample and ~6000 proteins across the entire cohort. We observed <31% median CV distributed over protein measurements and across study arms, demonstrating low variability and high reproducibility for this preparative approach. Statistical analysis yielded 82 significant, differentially expressed proteins in the tumor versus adjacent normal sections (FDR < 0.05, log2FC > ±1). Specimen annotation on clinical measurements such as MRI detectability and tumor volume were used for additional contextualization of these results.

Conclusion: Differential detection analysis reveals many known and new candidate markers of prostate cancer disease and suggests potential proteomic mechanisms underlying tumor MRI detectability for further future exploration in expanded whole-mount specimen cohorts.

PP05.46: Next Generation Proteomics Reveals Signature for Metastasic Cancer Years Prior to Diagnosis Enabled by a Longitudinal Wellness Cohort

Ray Chen, United States

Understanding the dynamics of the human proteome is crucial for identifying biomarkers to be used as measurable indicators for disease severity and progression, patient stratification, and drug development. We highlight the use of a broad-scale proteomics approach, including coverage of the low abundant plasma proteome, to reveal preliminary insights of early markers of disease transition in a longitudinal wellness cohort where samples were collected at approximately 6 month intervals over 4 years. In this preliminary study, 10 individuals with a minimum of 3 biobanked plasma samples prior to diagnosis were longitudinally compared to controls (n=69). Protein measurements were collected revealing not only a potential tissue agnostic signature for metastases, but also cancer specific signatures in multiple cancers. We will compare the signatures found in Magis et al 2021 to those in the Pan Cancer Blood Proteome (Uhlen et al 2023).

References:
Wik et al 2021 https://www.mcponline.org/article/S1535-9476(21)00140-7/fulltext
Uhlen et al 2023 https://www.researchsquare.com/article/rs-2025767/v1
Magis et al https://www.nature.com/articles/s41598-020-73451-z

PP05.47: Activity-Based Protein Profiling and Global Proteome Analysis Reveal MASTL-NEDD4-1 Axis Promotes Gastric Cancer Tumorigenesis

Kyoung-Min Choi, Republic of Korea

Background: Surgery and chemoradiation are traditional techniques for the treatment of patient with GC, however treatment options for metastatic GC has not been established. Thus, we aim to discover novel therapeutic targets to improve targeted therapy of metastatic GC.
Methods: HSP90 client kinases were profiled by mass spectrometry-based activity-based protein profiling (ABPP) using desthiobiotin-ATP probe. Global proteome alteration in GC cells transfected with RNAi targeting MASTL were profiled by mass spectrometry. Bioinformatic analyses were performed via GEPIA based on TCGA database.
Results: We identified 5 kinases (MASTL, CDK1, MET, CHEK1, STK11), which could be potential drug targets for GC treatment. Among them, MASTL (microtubule-associated serine/threonine kinase-like) is upregulated in GC as well as associated with poor prognosis of GC patients. We found that MASTL knockdown decreases migration, invasion and proliferation of GC cell lines. To investigate underlying mechanism of tumor promoting function of MASTL, we profiled global proteome alteration in SNU-484 transfected with RNAi targeting MASTL. We identified NEDD4-1, an E3 ligase reportedly upregulated in GC as a novel downstream effector of MASTL. Bioinformatic analyses revealed that NEDD4-1 was associated with poor prognosis of GC patients as well as the expression levels of MASTL and NEDD4-1 were positively correlated in GC patient tissues (P<0.01; R=0.61). Silencing NEDD4-1 also significantly decreased migration, invasion and proliferation of GC cells. We are currently investigating the detailed molecular mechanism by which MASTL-NEDD4-1 promotes GC.
Conclusions: Multi-proteomics analysis revealed MASTL-NEDD4-1 axis promotes gastric cancer tumorigenesis. Updated works will be presented.

PP05.48: Molecular Phenotyping of 3D Cultured Triple-Negative Breast Cancer Cells Reveals Pathways Influenced by Culture System

Luis Coy, United Kingdom

Breast cancer has the highest incidence and mortality rate of female cancers globally, with 10-15% of cases classified as triple-negative breast cancer (TNBC), which has a worse prognosis and fewer treatment options. 3D culture systems as an in vitro platform for cancer cell modelling are considered more clinically representative and have the potential for ultra-high-throughput therapeutic research. Here we employed proteomic analysis to further the understanding of the biological pathways involved in 3D TNBC spheroid models.

MDA-MB-231 and HCC1143 cell lines were cultured with complete medium for 4-days using standard monolayer or scaffold-free ultra-low adherent culture. Protein extracts were reduced, alkylated and digested using trypsin. Proteomic profiling was performed using a Dionex nano-LC system coupled to an Orbitrap Fusion mass spectrometer with raw data processed and searched against the human SwissProt database. Data were further analysed by Pathway analysis conducted using the R package clusterProfiler. Cell viability was assessed with Celltiter-glo 3D after exposure to the chemotherapeutic agents doxorubicin or paclitaxel for 48 hours.

In scaffold-free culture, both cell lines displayed a distinct proteomic signature compared to traditional monolayer culture. In comparison to MDA-MB-231, HCC1143 exhibited more significant differences with 2098 DEPs out of a total of 4309 proteins, while MDA-MB-231 had 1041 DEPs out of a total of 4498. This may reflect their contrasting morphologies with HCC1143 exhibiting spheroid formation, while MDA-MB-231 formed loose aggregates. Pathway analysis highlighted several pathways that were enriched in scaffold-free culture including DNA repair, cell-cell junctions and metabolic reprogramming. The evaluation of cell viability post-treatment indicated that scaffold-free culture displayed greater resilience to doxorubicin and paclitaxel compared to monolayer culture.

This study identified key cancer pathways enriched in 3D TNBC spheroid models compared to 2D monolayer models and highlights the importance of 3D spheroid models in advancing our mechanistic insight of therapeutic response.

PP05.49: New Proteomics Insights in the Characterization of Pancreatic Cancer Extracellular Vesicles as Lymphocyte Immune Activators

Federica Di Marco, Italy

Background: Pancreatic cancer (PC) is unresponsive to immunotherapy with checkpoint inhibitors and its microenvironment is highly immunosuppressive. Tumor-derived extracellular vesicles (EVs) play may activate or inhibit immune responses. However, their role in immunomodulatory regulation is still unknown. In this complex scenario, our study aimed to investigate the potential immunomodulatory effects of PC-derived EVs on circulating lymphocytes by proteomics approach.

Methods: Capan-2 PC and non-tumoral-derived EVs were isolated by ultracentrifugation. Fresh PBMCs from healthy donors were treated with EVs and then, CD3+ lymphocytes were isolated by FACS in order to evaluate their protein cargoes. The EVs and CD3+ lymphocytes EVs-treated or untreated proteomes were evaluated by nanoLC-Orbitrap-Fusion-Tribrid Mass Spectrometer. Quantitative proteomics data obtained from MaxQuant were used for functional analysis through Ingenuity Pathway Analysis (IPA).

Results: EVs derived from Capan-2 were characterized by proteomics approach by identifying 95 proteins that were connected in a single functional network involved in "extracellular exosomes". Intriguingly, Capan-2 EV proteins were involved in "immune-mediated inflammatory disease" according to the identification of mesothelin, HSP90 and HSP70. Then, we compared the protein outfit of CD3+ Capan-2 EVs treated with untreated ones quantifying 1065 and 1118 proteins, respectively. Moreover, CD3+ treated with non-tumoral EVs protein cargo was compared to untreated CD3+ by quantifying 875 and 952 proteins, respectively. According to IPA Comparison Analysis "cell proliferation and migration of T lymphocytes" were the most activated downstream effects in CD3+ lymphocytes treated with Capan-2 EVs. Meanwhile, Interferon-γ (IFNG) was one of the most activated upstream regulators in CD3+ treated with Capan-2 EVs. This data was confirmed also with the evaluation of INFG in supernatants of EV-treated PBMCs by ELISA assay. On the other hand, these biofunctions and predictions were unmodulated in the CD3+ treated with non-tumoral EVs. Our data support the potential role of EVs as antitumor immunity stimulators in PC.

See you in Busan!
BEXCO, Busan, Korea
2023.hupo.org
#HUPO2023

Together Through Proteomics

PROGRAM

PP05.50: ANKRD1 Promotes Breast Cancer Metastasis via NF-κB-MAGE-A6

Penchatr Diskul Na Ayudthaya, Thailand

In the year 2023, the survival rate for breast cancer is relatively high. However, patients with metastatic disease have much worse prognosis and decreased patient survival rate. In this study, we did microarray compared between low and high metastatic cell lines. We found gene called ANKRD1 was significantly increased in high metastatic cells. We did overexpressed ANKRD1 in low metastatic cells and knockdown ANKRD1 in high metastatic cells. Then cell proliferation, wound healing, cell migration and invasion were performed. The knockdown cells were both subcutaneous and tail vain injection in mice to observe cancer metastasis. Both in vitro and in vivo functional studies demonstrated that ANKRD1 is essential for cancer cell migration and invasion. Immunohistochemistry of human tissue array shown that the advanced stage tumors increased levels of ANKRD1 compared to the early stage. The over expressed and knockdown cells were sent for RNA-SEQ to explore the downstream pathway of ANKRD1, the results showed that NF-κB and MAGE-A6 were increased and decreased, respectively. Furthermore, label-free MS/MS of knockdown cells were performed, same results with RNA-SEQ were observed. The treatment of MAGE-A6 recombinant protein and si-MAGE-A6 shown induced and reduced in cell migration and invasion, respectively. Knockdown of NF-κB was performed to confirm whether it acts as upstream of MAGE-A6 or not. The results showed that after knockdown NF-κB, MAGE-A6 was significantly reduced. This showed that MAGE-A6 is a downstream of NF-κB. Our studies show that ANKRD1 promotes metastasis in breast cancer by activating NF-κB-MAGE-A6 signaling. These findings suggest that ANKRD1 could facilitate the development of novel targeted therapeutics and diagnostic strategies for breast cancer metastasis.

PP05.51: Mitochondrial Proteome and Acetylome in Glioblastoma Cells with Contrasting Metabolic Phenotypes

Sergio Encarnación-Guevara, Mexico

Introduction: Glioblastoma is a central nervous system cancer type with a poor prognosis. One of its main features is the constant change of metabolism phenotype to support its development and progression. Mitochondria play a critical role in cellular metabolism. Acetylation in the lysines of proteins influences many cellular processes. It has attracted researchers' attention since its consequences in cell biology, metabolism, interactions between proteins, subcellular localization of proteins, and their involvement in some pathologies like cancer. This PTM modification negatively affects the mitochondrial proteome and is counteracted by the enzyme sirtuin 3 (SIRT3). Our study focused on finding the different proteins regulated by SIRT3 in two glioblastoma cell lines with different energy metabolism.
Methods: An oxidative (T98G) and a glycolytic (U87MG), glioblastoma cell lines were used in addition to mass spectrometry-based proteomics strategy to map acetylation sites. SIRT3 was inhibited, and total protein and mitochondrial extract were obtained and chemically acetylated with a reagent that added a heavier acetyl group. The acetylation stoichiometry of all proteins was obtained by mass spectrometry due to the distinction in the spectrum of endogenously acetylated proteins and those chemically acetylated with the heaviest reagent.
Results: Our results indicate that SIRT3 inhibition promotes the overexpression of mitochondrial proteins, regardless of the cell's metabolic phenotype. Furthermore, we found that SIRT3 targets key enzymes involved in glycolytic metabolism, such as HK2, ACO2, SDHA, and GOT2, in the glycolytic cell line. We also identified new SIRT3 targets that have not been previously associated with specific functions, highlighting their potential as future targets for further study.
Conclusion: This study shed light on the role of SIRT3 in the mitochondria and its implication to cellular metabolism. These findings enhance our understanding of the complex regulation of energy metabolism in glioblastoma and provide potential targets for future therapies.

PP05.52: Plasma Proteomic Signature of Ovarian Cancer: Precise Biomarkers for Early Detection

Stefan Enroth, Sweden

Background

Ovarian cancer is the 8th most common cancer among women and has a 5-year survival of only 30-50%. The survival is close to 90% for stage I tumours but only 20% for stage IV. Presently available biomarkers have insufficient sensitivity and specificity and there is an urgent need to identify novel biomarkers.

Methods

The proximity extension assay (PEA Explore) was used to characterize 3072 plasma proteins in 610 samples from two separate clinical cohorts with women surgically diagnosed with benign or malign tumours based on suspicion of ovarian cancer. In addition to univariate case/control-analysis, supervised learning was used to build a predictive model for separating benign conditions from malign tumours using the first cohort (discovery) requiring at least 95% sensitivity. The performance of the model was then evaluated in the second cohort (validation).

Results

77 biomarkers were significantly different between cases and controls in the discovery cohort out of which 96% (74) replicated in the validation cohort using strict adjustment for multiple hypothesis testing. A multivariate model consisting of three proteins (WFDC2, KRT19 and RBFOX3) was built based on the discovery cohort achieving 96% sensitivity at a specificity of 67%. Notably, although available in the feature selection step, MUCIN-16 (CA-125) was not included. In the validation cohort, the model achieved a sensitivity of 93% at a specificity of 77% with an overall AUC of 0.92. The performance in the validation cohort was not statistically different (p > 0.45) from the performance in the discovery cohort.

Conclusions

Our results demonstrate the ability of using high-throughput precision proteomics for identification of novel accurate plasma protein biomarker for ovarian cancer. Coupling such technologies with machine learning approaches to detect combinations of biomarkers with robust predictive power is a powerful approach to break new ground and go beyond the current knowledge.

PP05.53: Pan-Cancer N-Glycoproteomic Atlas of Patient Derived Xenografts for Therapeutic Target and Serum Biomarker Discovery

Meinusha Govindarajan, Canada

Proteins exposed to the extracellular space (i.e., secreted and plasma membrane proteins) are an essential class of proteins due to their biological importance as the gatekeepers between a cell and its environment, and their clinical significance as favourable liquid biomarkers and therapeutic targets. Cell surface proteins, however, are under-represented in standard mass spectrometry-based proteomics analyses due to their low abundance and biochemical characteristics. We have recently devised a mass spectrometry-based strategy for detecting ovarian cancer (OV) associated surface proteins, in which N-glycosylated peptides were enriched from OV patient-derived xenograft (PDX) sera and tumors (Sinha et al., Cell Systems, 2019). Since >80% of the cell surface proteome is predicted to be N-glycosylated, N-glycoproteomics is a valuable approach to interrogate this clinically relevant sub-proteome. PDXs are models in which surgically resected patient tumors are grown in immunocompromised mice. In contrast to directly interrogating patient samples, the use of PDXs enables the unique advantage of bioinformatically distinguishing peptides of cancer (human) and microenvironment (mouse) origin. Applicability of PDX N-glycoproteome analysis for detecting OV serum biomarkers was demonstrated through validation of candidates in longitudinal sera from patients. Here, we have applied our previously established workflow on matched sera and tumors collected from 73 unique PDX models spanning six cancer types (LUSC, LUAD, GBM, COAD, PAAD and OV). We detected 7149 N-glycopeptides mapping to 3049 N-glycoproteins, of which > 90% of proteins have a predicted cell surface localization. We have identified 419 cancer-derived serum N-glycopeptides that may serve as non-invasive markers of tumor burden and show that cancer secretion is largely cancer-type specific. We also developed a target prioritization pipeline to identify cancer-specific therapeutic target candidates from PDX tumor N-glycoproteomes. Integration with our in-house proteomic database of over 300 primary and recurrent GBM patient tumors has prioritized five putative targets for functional interrogation using CRISPR.

PP05.54: Mouse Stromal Cells Confound Proteomic Characterization and Quantification of Xenograft Models

Piliang Hao, China

Introduction: Xenografts are essential models for studying cancer biology and developing oncology drugs, and are more informative with omics data. Most reported xenograft proteomics projects directly profiled tumors comprising human cancer cells and mouse stromal cells, followed by computational algorithms for assigning peptides to human and mouse proteins. The high sequence similarity between mouse and human is a great challenge for protein identification and quantification from xenografts.
Methods: We evaluated the performance of three main algorithms by carrying out benchmark studies on a series of human and mouse cell line mixtures and a set of liver patient-derived xenograft (PDX) models.
Results: Our study showed that approximately half of the characterized peptides are common between human and mouse proteins, and their allocations to human or mouse proteins cannot be satisfactorily achieved by any algorithm. As a result, many human proteins are erroneously labeled as differentially expressed proteins (DEP) between samples from the same human cell line mixed with different percentages of mouse cells, and the number of such false DEPs increases superquadratically with the mouse cell percentage. When mouse stromal cells are not removed from PDX tumors, about 30%–40% of DEPs from pairwise comparisons of PDX models are false positives, and about 20% of real DEPs cannot be identified irrespective of the threshold for calling differential expression.
Conclusions: Our study demonstrated that it is advisable to separate human and mouse cells in xenograft tumors before proteomic profiling to obtain more accurate measurement of species-specific protein expression. This study advocates the separate-then-run over the run-then-separate approach as a better strategy for more reliable proteomic profiling of xenografts.

PP05.55: Silencing DTX3L Inhibits the Progression of Cervical Carcinoma by Regulating PI3K/AKT/mTOR Signaling Pathway

Wei Hu, China

Cervical carcinoma (CC) is the second most prevalent gynecologic cancer in females across the world. To obtain a better understanding of the mechanisms underlying the development of CC, high-resolution label-free mass spectrometry was performed on CC and adjacent normal tissues from eight patients. A total of 2631 proteins were identified, and 46 significant differently expressed proteins (DEPs) were found between CC and normal tissues (p < 0.01, fold change >10 or <0.1). Ingenuity pathway analysis revealed that the majority of the proteins were involved in the regulation of eIF4 and p70S6K signaling and mTOR signaling. Among 46 DEPs, Integrinβ6 (ITGB6), PPP1CB, TMPO, PTGES3 (P23) and DTX3L were significantly upregulated, while Desmin (DES) was significantly downregulated in CC tissues compared with the adjacent normal tissues. In in vivo and in vitro experiments, DTX3L knockdown suppressed CC cell proliferation, migration, invasion and xenograft tumorigenesis, and enhanced cell apoptosis. Combination of silencing DTX3L and cisplatin treatment induced higher apoptosis percentage compared to cisplatin treatment alone. Moreover, DTX3L silencing inhibited the PI3K/AKT/mTOR signal pathway. Thus, our results suggested DTX3L could regulate CC progression through the PI3K/AKT/mTOR signal pathway and is potentially a novel biomarker and therapeutic target for CC.

PP05.56: Targeting Cholesterol Esters Synthesis Combats Colorectal Cancer

Tsui-chin Huang, Taiwan

Abnormal lipid metabolism, particularly the accumulation of cholesterol esters (CEs) in lipid droplets (LDs), is a recognized characteristic of various cancers and has been associated with tumor development. Sterol O-acyltransferase (SOAT), an enzyme that catalyzes the formation of cholesterol esters, is investigated in this study for its role in colorectal cancer (CRC) cells. Our previous research has shown that SOAT has higher expression in CRC tissues and is associated with prognosis. In this study, we further explore the function of SOAT in CRC cells by using siRNA to knock down SOAT1 expression and treating HCT116 CRC cells with the SOAT inhibitor avasimibe. Both approaches significantly inhibited cell growth and colony formation. Avasimibe treatment downregulated the expression of downstream genes of sterol regulatory element-binding proteins (SREBPs) involved in fatty acid synthesis, cholesterol uptake, and biosynthesis. We also observed increased expression of Bip and phosphorylated eIF2α proteins, markers of unfolded protein response, suggesting that SOAT inhibition induces endoplasmic reticulum (ER) stress in CRC cells. Furthermore, quantitative proteomics analysis revealed significant alterations in 3676 identified proteins, including 230 upregulated and 313 downregulated proteins, between avasimibe-treated and untreated groups. Functional enrichment analysis highlighted notable changes in biological processes, particularly a significant decrease in cell membrane signaling pathways, including SLIT/ROBO and FGFR2 singaling. This study provides new insights into the mechanisms of progression in CRC related to SOAT and LDs, contributing to the development of novel biomarkers and targeted therapies based on tumor-specific lipid metabolism. Our findings emphasize the potential of SOAT as a promising metabolic target in CRC therapeutics.

PP05.57: Integrative Multi-omics Analysis Prioritizes Potential Therapeutic Targets in TNBC Stem Cells

Sunghyun Huh, Republic of Korea

Introduction: Triple-negative breast cancer (TNBC) represents ~15% of global breast cancer diagnosis. TNBC is notorious for poor survival rate and aggressiveness. Cancer stem cells (CSCs) are thought to underlie the malignancy of the TNBC subtype. However, lack of targetable molecules in TNBC CSCs hinders development of an effective therapy. Recently, CAR-NK is being explored as an attractive therapeutic strategy against cancers. Similar to CAR-T, CAR-NK cells present receptors against tumor-associated antigens and can redirect immune system for anti-tumorigenic activity but with minimal side effects.
Methods: We performed an integrative analysis of multi-omics dataset to identify potential therapeutic targets of TNBC CSCs. For in-house data, we performed proteomic analysis of whole cell lysate as well as enriched plasma membrane for a normal breast cell line, TNBC non-CSCs (i.e., low CSC %), and TNBC CSCs (i.e., high CSC %). Additionally, through literature mining, we systematically collected public cancer cell line, tissue, and scRNAseq data.
Results: Analysis of the in-house proteomics data revealed signaling pathways dysregulated in TNBC CSCs such as cell cycle, TGF-β signaling, and Wnt/β-catenin signaling. Surfaceome analysis identified membrane proteins up-regulated specifically in TNBC CSCs, including well-known CSC markers. In addition, analysis of two public cancer cell line data identified proteins up-regulated specifically in TNBC CSCs compared to non-cancerous cells and TNBC non-CSCs. Combining results from the in-house and public data, several membrane proteins were shown to be consistently up-regulated in TNBC CSCs. We showed that expression patterns of these proteins are significantly associated with TNBC patient survival outcomes. We further narrowed down our potential targets by confirming their TNBC CSC-specific expressions in TNBC tissues using a public scRNAseq data.
Conclusions: By integrating in-house and public multi-omics dataset, we identified potential therapeutic targets for CAR-NK therapy in TNBC. We are currently undertaking a series of experiments to validate our findings.

PP05.58: Proteomic and Proteogenomic Characterization of Triple Negative Breast Cancer

Henrik Johansson, Sweden

Triple negative breast cancer (TNBC) has the worst prognosis in breast cancer with limited therapy options. Here we investigate the phenotypic proteome level of TNBC using mass spectrometry (MS) based proteomics methods, with the aim to foster a better biological understanding of TNBC, identify subtypes and link them to therapy response for proteome precision medicine.

The multi-omics population-based discovery cohort consists of 225 TNBC samples from SCANB in Sweden with WGS, RNA-seq, data independent acquisition (DIA) and TMT based quantitative MS based proteomics data. The TMT based proteomics data is generated using peptide fractionation using immobilized pH gradient -isoelectric focusing (IPG-IEF) before nanoLC-MS/MS, which enable searching a 6-reading frame translation of the human genome to identify novel protein coding regions and potential neoantigens.

In the discovery cohort we have identified subtypes with different biological characteristics and clinical outcomes. For validation of the subtypes and linking them to drug response we are developing proteome-based classifiers based on the DIA data and are acquiring DIA data from clinical trials with chemo- and immunotherapy.

PP05.59: A Novel Therapeutic Strategy: Overcoming AKT inhibitor-induced Cell Migration via β-catenin Targeting in CRC

Yonghyo Kim, Republic of Korea

In advanced colorectal cancer (CRC), a substantial majority of cases (approximately 90%) are characterized by mutations in the APC gene. This genetic alteration triggers the simultaneous activation of the Wnt/β-catenin and AKT signaling pathways, presenting significant challenges to the prevailing therapeutic strategies that predominantly utilize AKT inhibitors. These challenges stem from the induction of epithelial-mesenchymal transition (EMT) and the evasion of apoptosis facilitated by nuclear β-catenin. This study introduces a novel combinatorial therapeutic strategy that employs an AKT inhibitor in conjunction with KY1022, a β-catenin destabilizer. This approach effectively addresses these challenges by reducing nuclear β-catenin levels. The findings of this research highlight the potential of a therapeutic strategy that concurrently suppresses both Wnt/β-catenin and AKT signaling pathways. This dual suppression strategy offers a promising avenue for mitigating AKT-inhibitor-induced metastasis and augmenting the therapeutic efficacy of AKT inhibitors in CRC treatment.

PP05.60: The Regulatory Roles of Mitochondrial Metabolism Dynamics and Mitochondria Calcium Uniporter (MCU) in Bevacizumab Resistance of GBM

Yejin Kim, Republic of Korea

Adapted OXPHOS and TCA cycle activations are essential tumor microenvironments for abnormal energy consumption to acquire malignancy and drug resistance during cancer development and progression. To elucidate the molecular mechanism related to the mitochondrial metabolic dynamics and drug resistance in GBM, we established a longitudinal GBM orthotopic mouse model with acquired resistance to bevacizumab. The proteomic analysis results showed that OXPHOS, TCA, and calcium signaling gene sets were enriched in the bevacizumab pro-resistance phase. To investigate the metabolic dynamics changes that occurred in mitochondria induced by bevacizumab treatment in GBM, we used the APEX system to biotinylate proteins in the mitochondria matrix and analyzed the proteins of the brain tumors. The proteomic analysis showed that the pore-forming subunits of the mitochondrial calcium uniporter protein (MCU) are essential for acquiring bevacizumab resistance. Additionally, a combination effect of hypoxia and the MCU-specific inhibitor DS16570511 in vitro showed that cell growth and proliferation were reduced via inhibition of NF-κB and CEBP/β signaling pathways. In conclusion, the hypoxic tumor microenvironment induced by bevacizumab treatment affects mitochondrial metabolic dynamics, and targeting MCU is a promising therapeutic option in combination with bevacizumab in GBM.

PROGRAM

PP05.61: Functional Study of Nucleus-localized Proteins in Temozolomide-resistant Glioblastoma

Chanil Kim, Republic of Korea

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.

PP05.62: Proteomic Analysis of Non-Canonical Proteins in Non-Small Cell Lung Cancer.

Dae Ho Kim, Republic of Korea

Proteins are the result of translation. Most proteins are made by being translated from the translation initial site (TIS) of a commonly known open reading frame (ORF). However, advances in experimental techniques have allowed us to identify proteins that are translated at sites other than those known as canonical translation sites, and proteins translated at these non-canonical sites are called non-canonical proteins. So far, non-canonical proteins have been shown to be associated with a variety of diseases and have been implicated in protein-protein interactions but have not made much progress in non-small cell lung cancer (NSCLC). Non-canonical proteins are proteins translated from alternative ORFs or non-canonical TISs, and because of this feature, they require a different database than those commonly used in general proteomics. Therefore, we generated the database by 3-frame translation (stop codon to stop codon) of all RNAs including UTRs and ORFs in the RNA database downloaded from UCSC Genome Browser. Then, to validate the database by mass spectrometry (MS)-based proteomics, we analyzed the expression of non-canonical proteins in real NSCLC patients. We found that non-canonical proteins also differed between tumor and normal tissue adjacent to tumor (NAT), especially those expressed from non-coding RNA. By comparing the results with existing lung cancer data, we were able to identify differences in the expression of non-canonical and canonical proteins, as well as proteins expressed from long non-coding RNAs (lncRNAs), which are known to regulate many important biological processes. Thus, our results seem to correlate with the results of non-canonical protein canonical protein and RNA analyses, and we have identified non-canonical peptides specific to each patient. The results allowed us to confirm the association of non-canonical proteins with lung cancer and identify candidates with potential therapeutic effects.

PP05.63: Differential Expression of Deubiquitinating Enzymes in Cisplatin-treated Lung Cancer Cells

Tae-Woo Kim, Republic of Korea

Lung cancer is a highly lethal disease characterized by a significant mortality rate. Cisplatin, a common drug used for lung cancer treatment, frequently develops resistance over time. Overcoming cisplatin resistance is crucial in the effective management of lung cancer. The ubiquitin-proteasome system serves as a vital regulatory mechanism for maintaining protein homeostasis within cells. Recent studies have shown that manipulating deubiquitinating enzymes (DUBs) can overcome cisplatin resistance, and certain DUB inhibitors can sensitize cancer cells to cisplatin. This study aims to investigate the expression levels of DUB genes under cisplatin treatment. Multiplex RT-PCR analysis was performed to identify potential biomarkers by comparing expression patterns and levels of DUB genes. The results of the multiplex RT-PCR analysis showed differences in the mRNA expression levels of several DUB genes, including USPA, USPB, and USPC in A549 lung cancer cells under cisplatin treatment. In addition, qRT-PCR analysis revealed that decreased expression of USPA and USPB, while USPC exhibited increased expression under cisplatin treatment. These findings strongly suggest that DUBs may play a crucial role in overcoming cisplatin resistance and improving the treatment efficacy for lung cancer.

PP05.64: Discovery and Validation of Immuno-oncology Therapeutic Targets in Human Immune Cells by Mass Spectrometry Based Proteomics

amaury lachaud, Switzerland

Immuno-oncology (IO) has substantially improved the survival of cancer patients over the past several years encouraging the discovery of novel IO targets which are typically proteins expressed on the surface of immune cells. Sensitive quantification of proteins in complex biological samples is routinely achieved by immunoassays that use antibodies specific to target proteins. Such approaches can be a limitation in IO drug discovery and development as de novo development of antibodies is associated with long lead times, high costs, and high failure rates.
Protein quantification using mass spectrometry (MS) is agnostic to species and matrices and removes the barriers of availability or specificity of antibody-based assay. Further, MS proteomics workflows can support both large scale discovery studies but also represent an attractive alternative to targeted quantitative studies.

The main purpose of this work is to assess the performance of MS-proteomics platforms for the deep proteome profiling of human immune cells compared to flow cytometry solutions. We assess the number of quantified proteins and specifically the coverage of immune cell marker proteins in primary human immune cells across cell count groups from 2 million down to 2500 immune cells. In addition, we compared the quantification of a multiplexed surface antigens panel using PRM-MS and QIFI® flow cytometry platforms.

We found that the applied MS-based proteomics workflows achieve high sensitivity and robustness in detection and quantification of immune cell markers down to 2500 primary immune cells. Additionally, we observed a strong correlation of the quantitative data derived from our MS-based proteomics workflows with flow cytometry supporting the substitution of immunoassays by MS-based proteomics workflows in target discovery and validation.

PP05.65: Novel Insights into the Mechanisms of Tumorigenesis Through Proteome and Glycoproteome Analysis of Secretome from EGFR TKI-Resistant Lung Cancer Cells

Ju Yeon Lee, Republic of Korea

Drug resistance to targeted anticancer reagent is a major hurdle in cancer therapy. Tumors are composed of heterogeneous cells that differently respond to drugs and communicate between heterogeneous cell populations in tumors are associated with drug resistance. In cell-cell communications in the microenviroment, glycoproteins secreted by cells play essential roles in the regulation of extracellular activities and are often reflective of cellular status, and can serve as excellent biomarkers for disease detection. Secretome from cultured cells extensively employed as models in the research fields of biology and biomedicine were analyzed with LC-MS/MS after labeling TMT reagent and fractionation to 10 with the combined TMT labeled samples to investigate deep insights into mechanism study of drug resistance between parent EGFR mutant non-small cell lung cancer cells (PC9 and HCC827) and the corresponding cells (PC9ER and HCC827ER) with acquired resistance to EGFR tyrosine kinase inhibitor, erlotinib. The proteins which commonly up and down expressed in drug resistance cell were analyzed the enriched pathway in PC9ER/PC9 and HCC827ER/HCC82. The extracellular matrix organization and cell-cell adhesion pathways which regulate cell proliferation, movement and invasion, were enriched and specially MUC18 protein was up regulated in proteome and glycoproteome analysis of drug resistance sample. The abundance of MUC18 was validated by immunoprecipitation approach. The combining proteome and glycoproteome analysis may provide novel and deep insights into mechanism of drug resistance as well as mechanism by which resistance cells regulate tumor microenvironment.

See you in Busan!
BEXCO, Busan, Korea
2023.hupo.org
#HUPO2023

Together Through Proteomics

PROGRAM

PP05.66: Repurposing Neuroactive Drugs for Brain Cancer Drug Discovery

Sohyon Lee, Switzerland

Glioblastoma is the most common yet deadliest primary brain cancer, with a median survival of 15 months. The alkylating agent Temozolomide (TMZ) remains the only approved first-line chemotherapy while targeted therapies have been largely unsuccessful. Therapeutic roadblocks in glioblastoma treatment include the blood-brain barrier (BBB) limiting tumor accessibility, the presence of treatment-resistant glioblastoma stem cells (GSCs), and the lack of clinically predictive models. Here, we systematically address these therapeutic roadblocks by developing a clinically predictive drug-screening platform in primary GBM surgery material to screen for repurposable BBB-permeable drugs with potent anti-glioblastoma activity. Using this image-based drug-screening platform across 27 surgically resected glioblastoma patient samples, we identify the antidepressant Vortioxetine as the most promising therapeutic candidate among 132 repurposable drugs. Vortioxetine shows high BBB-permeability, selectively targets GSCs associated with poor patient prognosis, and displays potent anti-glioblastoma efficacy across all tested human and murine model systems. By genome-wide deep learning of drug-target protein networks we uncover AP-1-driven glioblastoma suppression as a tumor-intrinsic vulnerability targeted by Vortioxetine. This AP-1 connectivity signature is validated by in silico drug screening across >1 million compounds, as well as by multi-omic profiling of Vortioxetine-treated glioblastoma cells. Following drug treatment, integration of different proteotyping methods including time-resolved phosphoproteomics and structural chemoproteomics with gene-expression profiling elucidates the temporal sequence and direct targets involved in Vortioxetine-mediated glioblastoma suppression. The mode-of-action of Vortioxetine is epitomized by a rapid Ca2+ response and cytoskeletal reorganization that triggers induction of the canonical AP-1 transcription factor JUN, resulting in a cellular stress response and apoptosis. In summary, this study demonstrates how phenotypic drug discovery can synergize with machine learning of drug-target protein networks and chemoproteomic methods to identify a clinically compelling repurposable candidate for brain cancer treatment.

PP05.67: HER2 Expression in Breast Cancer by Proteomics Analysis

Gi Yeon Lee, Republic of Korea

Purpose Breast cancer is one of the most commonly diagnosed cancer in women worldwide, and HER2-positive breast cancer is a particular subtype. Over the past few decades, the development of HER2-targeted therapies has significantly improved patient outcomes. However, there is emerging need to determine low HER2 expression in context of novel HER2 targeted therapy. Here, we measured HER2 expression by proteomics analysis and compared the results with immunohistochemistry.

Methods Total of 245 breast cancer patients with median age 38.4(25-59). The breast tumor samples were cryopulverized, tryptic-digested, and 804 stable-isotope standard peptides containing HER2 were added for absolute quantification. All samples were analyzed using Orbitrap Eclipse Tribrid mass spectrometer equipped with an Ultimate 3000 RSLCnano system. Raw data was analyzed and quantified using SpectroDive (Biognosys). Immunohistochemistry was done by C-erbB2(4B5, Ventana or polyclonal, DAKO) antibody, scored scale of 0-3.

Results The quantitative values for ERBB2 and ERBB2/GAPDH/Tumor purity by proteomics analysis were ranged from 0.06-13.78 fmol/ug and 0.029-48.258%. The correlation between IHC score and ERBB2 or ERBB2/GAPDH/Tumor purity were significant (r=0.5469 and r=0.4493, respectably) The values of immunopathological result were significant in each Positive (3.588±0.476 fmol/ug, 7.106±10.076%), Negative (0.311±0.017 fmol/ug, 0.780±0.978%). However, ERBB2 and ERBB2/GAPDH/Tumor purity represented wide values as 0.06-13.78 fmol/ug and 0.131-48.258% in IHC score 3.

Conclusions A discrepancy between ERRB2 protein analysis quantitative results and IHC scores was confirmed in this study. We would further analyze the clinical features to elucidate the meaning of this finding. (This work was supported by National Cancer Center (No.NCC-2110181))

PP05.68: Secretome Analysis Reveals Potential Driver of Tumorigenesis Driven by EGFR-TKI Resistant Lung Cancer

Su-Min Lee, Republic of Korea

Background : Resistance to targeted anticancer drugs is one of the most important issues in lung cancer therapy. Previous studies reported various resistant mechanisms, however mechanism of how resistant cells regulate neighboring cells in tumor microenvironment (TME) remains elusive. Here, we aim to profile differential secretome between parent and EGFR tyrosine kinase inhibitor (TKI) resistant lung cancer cell in order to gain further insights into mechanism by which TME is regulated by EGFR-TKI resistant lung cancer cells.

Methods : EGFR-TKI resistant cells were established by exposing EGFR mutant lung cancer cell lines (HCC827 and PC9) to erlotinib. For secretome analysis, conditioned media (CM) collected from parent and resistant lung cancer cells were filter-concentrated and trypsin digested. The resulting peptides were analyzed by 6-plex TMT-based quantitative proteomics.

Results : CM from resistant cells promoted migration of parent cells as well as AKT, ERK activities. We also found resistant cell CM could promote AKT and ERK activities in lung fibroblasts as well. The pathway analysis revealed that extracellular matrix organization, cell-cell adhesion and regulation of cell adhesion pathways were enriched from proteins decreased in resistance cells, while heterocycle biosynthetic process, cellular amino acid metabolic process and small molecule biosynthetic process were enriched from increased proteins. Further, we identified proteins upregulated in TKI resistant cells, which are associated with increased cellular motility and drug resistance.

Conclusions : CM from EGFR-TKI resistant lung cancer cells could activate multiple signaling pathways in parental cells as well as lung fibroblasts. The quantitative secretome analysis revealed potential drivers of tumorigenesis driven by EGFR-TKI resistant cells.

PP05.69: Proteome Profiling of Peripheral Blood Mononuclear Cells in Dog with Mammary Gland Tumor

Jeong Woon Lee, Republic of Korea

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. Here, we profiled the proteomic dynamics of PBMCs related to canine mammary gland tumor, and compared with those in human breast cancer using machine learning. Through this profiling study, we found that the PBMCs in dogs bearing benign tumor were quite more different from normal than those in cancer patients. Furthermore, gene ontology(GO) and protein-protein interaction(PPI) analysis with ML-validated DEPs showed the features which differentiate normal and tumor group, or benign and cancer group. In particular, several proteins related to pentose phosphate pathway were down-regulated in tumor group compared to normal group, while those related to mitochondrial translation were down-regulated in cancer group compared to benign group. This study shows how tumor could be affect the proteomic dynamics, especially related to metabolism, of circulating immune cells, which was cross-validated by machine learning technique.

PP05.70: Sonication-assisted Protein Extraction from Tumor Tissues Improves Proteomic Detection of DNA- and Plasma Membrane-associated Proteins

Qing Kay Li, United States

Background: Studies of cellular protein changes can be biologically significant and clinically important, especially in diseases and cancers. It is well-known that proteins play critical roles in cellular functions and can be prognostic markers and/or therapeutic targets. However, the current tissue protein extracting protocol may not be effective for isolating all classes of proteins, limiting downstream detection and analysis of certain proteins. In this study, we investigated the impact of sonication-assisted protein extraction on tissue samples and characterized the proteome changes using TMT-labeling and mass spectrometry.

Methods: Urea lysis buffer was used to extract proteins from patient-derived xenograft (PDX) breast tumors of luminal and basal-like subtypes. A portion of samples from each subtype was further treated with sonication. Both sonicated and non-sonicated tissue samples were labeled by TMT-10plex. Global- and phospho-proteomic data were generated by liquid chromatography tandem mass spectrometry; searched against the RefSeq database containing both human and mouse proteins, and quantified by MS-PyCloud software (https://bitbucket.org/mschnau1/ms-pycloud/src/main/).

Results: In global proteomic data, more DNA- and membrane-bound proteins were identified in tissue samples extracted with sonication than that without sonication. Similarly, in phosphoproteomic data, more phosphorylated proteins were identified in sonicated tissue samples than that of non-sonicated samples. These identified phosphoproteins are ones with critical roles in the regulation of tumor progression, such as OSBPL8, PGRMC1 and HACD3. Furthermore, some of them are also considered to be potential therapeutic targets for cancers, such as NOLC1 and SEC62.

Conclusions: Our study is the first comprehensive and systematical investigation of the impact of sonication on protein extraction from tissue samples. Our results showed that sonication improved the identification of DNA- and membrane-associated proteins as well as phosphorylated proteins, which could have important biological, prognostic, and therapeutic significance.

PP05.71: Laser Capture Microdissection-based Trace Proteomics Accurately Defines the Characteristics of Esophageal Squamous Cell Carcinoma Progression

Xumiao Li, China

Esophageal squamous cell carcinoma (ESCC) is one of the most common malignant cancers with high incidence and poor prognosis, especially in East Asia. ESCC develops from normal esophageal epithelium to dysplasia and eventually to aggressive carcinoma. However, the molecular mechanism by which these precancerous lesions develop into ESCC remains unclear.

We collected normal esophageal epithelium, esophagitis, mild dysplasia, moderate dysplasia, severe dysplasia and ESCC biopsy specimens' formalin-fixed paraffin-embedded (FFPE) tissues, demographic characteristics, and follow-up data to construct a comprehensive sample bank at Yanting, China, one of the Cancer Incidence in Five Continents data acquisition area. For each tissue, trace epithelial lesion (L) and non-lesion (N) were obtained precisely by laser capture microdissection (LCM) for refined proteomic analysis separately.

Applying our LCM-Magnetic Trace Analysis (LCM-MTA, bioRxiv-2022-504675), we quantified a total of 4,565 protein groups, of which an average of 2,135 were quantified in L tissue and 1,422 in N tissue. Principal component analysis clearly distinguished L and N tissues, highlighting the advantages of LCM-based proteomics. By Fuzzy C-means Clustering, we found that some protein clusters changed significantly during the transition from normal esophagus, esophagitis, dysplasia to ESCC, for example, proteins related to RNA metabolism are progressively up-regulated and proteins related to the formation of the cornified envelope and amide metabolic process are progressively down-regulated.

To our knowledge, this is the first large-scale LCM-based proteomic study of trace FFPE tissues across the entire cancerous process. Trace L tissues provided a refined protein profile of ESCC precancerous lesions and avoid tissue heterogeneity. The significant differences between L and N tissue and changes in protein expression during ESCC precancerous lesions illustrated the advantages and reliability of LCM-based proteomics. In conclusion, our in-depth and refined proteomic data and comprehensive sample bank provided valuable resources for subsequent analysis such as biomarker and biological mechanism exploration.

PROGRAM

PP05.72: Decoding the Immune Microenvironment of High-Grade Serous Ovarian Cancer

Loren Méar, Sweden

High-grade serous ovarian cancer (HGSOC) is a highly aggressive gynecological malignancy with a poor patient prognosis. Diagnosis and treatment pose significant challenges as HGSOC are extremely heterogeneous tumors with a unique microenvironment, including cancer-associated fibroblasts (mCAF) and immune cells. We aimed to set up a pipeline for systematic mapping of the immune microenvironment of HGSOC tumors, which is crucial for developing personalized immunotherapy strategies.

To address this challenge, we utilized a multi-omics approach, by integrating publicly available single-cell RNA sequencing (scRNA) datasets, antibody-based proteomics data from the Human Protein Atlas (HPA), and relevant literature. This integration enabled us to identify genes enriched in specific cell types within HGSOC tumors, particularly in mCAF and immune cells. We then developed a 9-plex immunofluorescence workflow using the Opal™ system, allowing us to quantitate key immune and cancer cell markers (e.g., PD-L1, CD8A, FoxP3, CD163, KRT7, PDGFRB, and CD79A) in tissue sections from primary HGSOC tumors. By reanalyzing the scRNA data, we discovered novel protein candidates relevant for the immune microenvironment (n=160) to investigate alongside with our multiplex panel for determining their spatial localization. Two markers (SLAMF7 and GZMK) were selected for further investigation in a pilot study. We also implemented an automated image analysis pipeline to facilitate analysis.

In summary, we established a stringent pipeline for mapping the immune microenvironment of HGSOC tumors, which will expand to include a broader range of tissue samples with metadata and clinical parameters. The validated pipeline can be used for exploring novel protein candidates identified by other methods, and our study has the potential to revolutionize the diagnosis and treatment of HGSOC. Lastly, by deciphering cell type-specific markers within the microenvironment, we can refine immunophenotype stratification, leading to tailored immunotherapy approaches that might significantly improve patient outcomes.

PP05.73: A Comparison of Tribrid Mass Spectrometer Architectures for Deep Blood Plasma Proteomics

Rafael Melani, United States

Early cancer detection saves lives, and plasma proteins can potentially allow earlier less invasive cancer detection as they reflect host-tumor interactions. Here we compare the performance of two Tribrid mass spectrometers (Orbitrap Eclipse and Orbitrap Ascend) in mapping the human plasma proteome in unlabeled and TMTpro-labeled samples. Human plasma samples were analyzed both unlabeled and labeled with the TMTpro multiplexing reagent on the Orbitrap Eclipse and Ascend instruments equipped with FAIMS. TMTpro labeled sample pools were analyzed using a real-time database search (RTS) based SPS MS²/MS³ method. The Orbitrap Ascend has an additional ion routing multipole (IRM) in front of the C-Trap, allowing ion injection and accumulation to occur in parallel. A 120 min LC-MS method on the Ascend gave similar numbers to a 180 min run on the Eclipse (unique peptides ~2,000, quantified peptides ~ 800, and proteins ~300). An increase of 30% in all 3 categories was achieved for a 180 min method on the Ascend for unfractionated and TMT-labeled samples. Furthermore, we analyzed a fractionated TMT-labeled plasma sample using both instruments. Analyzing 12 fractions using 120 min gradients on the Ascend produced more quantified peptides (5,134) and proteins (2,490) than analyzing each fraction with 180 min gradients on the Eclipse (3,479 peptides and 2,308 proteins). In 80 minutes per sample, we quantified 2,500 plasma proteins (approximately 70 % of the plasma proteome). Moreover, we evaluated an AI-driven algorithm for assigning chimeric MS² data (CHIMERYS PD 3.0). Unfractionated and unlabeled plasma samples were analyzed using 90 min gradients on the Ascend with either 0.7 or 4 Da MS2 isolation windows. An increase of 26% was achieved for identified proteins with the combination of CHYMERIS and 4 Th isolation window (1,176 vs. 1,487).

PP05.74: Multi-omics Profiling of Metastatic Spread in a Large Breast Cancer Patient Cohort

Sergio Mosquim Junior, Sweden

In breast cancer (BC), as with all cancers, the presence of metastases is directly associated with morbidity and mortality. They take place primarily via lymphatic vessels, where the presence of tumor cells is a key prognostic indicator [1]. A combination of histological and molecular classification provides useful prognostic and diagnostic information. However, late recurrences still occur, particularly in estrogen receptor (ER) positive disease [2, 3]. Taken together with unnecessary complete Axillary Lymph Node Dissection (cALND) and incomplete patient stratification, patients may be overtreated or undertreated [4]. Therefore, better tools are needed in BC precision medicine.

Here, we adopt a multiomic approach to investigate and predict metastases and lymph node involvement in ER-positive breast cancer. We analyzed the flowthroughs from 182 BC samples belonging to the Sweden Cancerome Analysis Network – Breast (SCAN-B) [3] cohort. A semi-automated protocol previously described in [5] was further optimized in house to allow for parallel acquisition of full proteome and phosphoproteome data, and subsequent integration with matched transcriptome data. Data were acquired in Data Independent Acquisition (DIA) mode on a Q-Exactive HF-X (Thermo Scientific) coupled to an Evosep One (Evosep) liquid chromatography system. Preliminary results show that over 10500 protein groups were identified in total, with over 4300 found in all samples. Exploratory analyses show similarities and differences in the different omic layers when comparing patient groups. We discuss how these results can be combined to elucidate and resolve these molecular processes in invasive breast cancer, contributing to the field of personalized medicine.

1. Liu, X., et al. Interdiscip Sci, 2023.
2. Onkar, S.S., et al. Cancer Discov, 2023. 13(1): p. 23-40.
3. Saal, L.H., et al. Genome Med, 2015. 7(1): p. 20.
4. Narbe, U., et al. Br J Surg, 2021. 108(12): p. 1465-1473.
5. Mosquim Junior, S., et al. Cancers (Basel), 2022. 14(23).

PP05.74: Proteomic Analysis of the Function of IMPDH2 in Osteosarcoma Metastasis

Sutpirat Moonmuang, Thailand

Metastasis is the major cause of death in osteosarcoma patients, and the treatment has not been effective in improving the survival rate of this group of patients for decades. Better understanding the molecular mechanism of osteosarcoma metastasis will provide insight into osteosarcoma biology and identify novel therapeutic approaches for metastatic osteosarcoma. Inosine-5′-monophosphate dehydrogenase (IMPDH) is the rate-limiting enzyme for the de novo synthesis of guanine nucleotides. Targeting IMPDH has been shown to possess anti-tumor activity in various cancers, including osteosarcoma. Here, we investigated levels of IMPDH isoform 2 (IMPDH2) in 20 post-chemotherapy tissues of high-grade osteosarcoma patients using immunohistochemical (IHC) analysis. We showed that high IMPDH2 expression was significantly associated with shorter metastasis-free survival and could be used as an independent postoperative prognostic marker in osteosarcoma patients. We further investigated the role of IMPDH2 in osteosarcoma metastasis by employing the CRISPR-Cas9 system to knockout the IMPDH2 gene in an osteosarcoma cell line. The results showed that in vitro migration and anoikis resistance effects were drastically reduced in IMPDH2-KO 143B cells. To investigate the change in protein profile as a consequence of IMPDH2 knockout, a quantitative proteome analysis using SWATH-MS was carried out in a pair of IMPDH2 knockout and wild-type cells. We identified 223 proteins whose expression levels significantly changed between IMPDH2 knockout and wild type cells, which were involved in multiple molecular functions based upon gene ontology enrichment analysis, particularly cadherin binding. Notably, we demonstrated that the cytoskeletal components were among the significant dysregulated proteins in IMPDH2 knockout cells. Taken together, these results reveal that the IMPDH might somehow axis the cadherin binding involved in cell-cell adhesion for osteosarcoma metastasis. Inhibition of IMPDH2 might be a potential target for clinical intervention in osteosarcoma metastasis.

PP05.76: S100A10 is Associated with Ovarian Cancer Progression and Chemotherapy Resistance

Tannith Noye, Australia

Ovarian cancer is the most lethal of gynaecological malignancies; with a 5-year survival rate of 46%. Most being diagnosed as high-grade (HGSOC), these carcinomas have a high chemosensitivity to first line platinum-based therapies. However, >80% develop resistance, following a relapse. Effective molecularly targeted therapies to improve survival are urgently required. Mass spectrometry and Data Independent Acquisition (DIA) was employed to identify proteins that had dysregulated expression in patients that relapsed early (< 24 months) compared to patients that relapsed after 60 months. One of the proteins elevated in patients that relapsed within 24 months was S100A10. S100A10 is a calcium-binding phospholipid protein and through interactions with annexin A2 can increase cancer invasion. To date limited studies have examined the role S100A10 in ovarian cancer. We investigated the effect of S100A10 inhibitors (siRNA and neutralizing antibodies) on ovarian cancer motility and invasion in vitro and in vivo using the chick chorioallantoic membrane assay (CAM) assay. Expression of S100A10 protein was assessed in tissue cohorts of advanced-stage serous cancer: chemonaïve (n=24), post-chemotherapy (n=16), a matching cohort at diagnosis and relapse (n=4) and a tissue microarray cohort (n=140). S100A10 knockdown significantly decreased the motility and invasion of OVCAR5 and OV90 cancer cell lines. S100A10 neutralising antibody significantly reduced the motility and invasion of OV90, OVCAR5 and CAOV3 cancer cells in vitro and invasion of CAOV3 in vivo in CAM assays. S100A10 expression was also found to associated chemotherapy resistance and increased in CAOV3 cells resistant to carboplatin. S100A10 protein levels were increased in HGSOC following chemotherapy treatment and relapse. High S100A10 protein levels were associated with reduced overall survival in patients with HGSOC.
We conclude that S100A10 plays an important role in HGSOC progression and chemotherapy resistance and strategies to inhibit S100A10 have promising potential as a novel therapy for HGSOC.

PP05.77: Proteomic Analysis to Identify the Molecular Differences between Dermatofibrosarcoma Protuberans and Fibrosarcomatous Dermatofibrosarcoma Protuberans

Takuya Ono, Japan

Dermatofibrosarcoma protuberans (DFSP) is a locally advanced tumor characterized by a fusion gene. However, 5% of DFSP cases transform into fibrosarcomatous DFSP (FS-DFSP), which is associated with more aggressive clinical features, such as local recurrence and distant metastasis. While imatinib has shown efficacy in treating DFSP, it fails to elicit a response in FS-DFSP. The underlying cause for this discrepancy in clinical behavior and response to imatinib remains unclear. Abnormal kinase activity has been implicated in the progression of cancer, making it an important therapeutic target. This study aimed to identify potential targets for therapy and gain insights into imatinib resistance by investigating abnormal kinase activity in DFSP and FS-DFSP.

Although cell line is useful in exploring mechanisms of differential malignancy and drug responsiveness between DFSP and FS-DFSP, cell lines of DFSP and FS-DFSP are limited.

Therefore, we first attempted to establish cell lines from surgically resected tumor tissues of 15 DFSP and FS-DFSP patients. We then examined the proliferative, invasive potential and the presence of typical fusion genes. We also examined whether the kinase activity of the established DFSP and FS-DFSP cell lines changed before and after treatment with the kinase inhibitor imatinib using the PamStation (peptide array). Mass spectrometry was used to examine protein expression levels.

We successfully established five cell lines (two DFSP and three FS-DFSP) that contained the typical fusion gene. In vitro assays demonstrated differences in proliferative and invasive capacities among these cell lines, but no significant distinctions were observed between DFSP and FS-DFSP cell lines. However, comprehensive analysis using the PamStation revealed differences in kinase activity profiles and protein expression levels between DFSP and FS-DFSP, before and after imatinib treatment. These findings indicated the potential of proteomic analysis and the use of patient-derived cell lines in advancing research to identify therapeutic agents for FS-DFSP.

PP5.078: Development of Treatments for CIC-rearranged Sarcomas: Multiplex Kinase Activity Analysis and Drug screening Using Patient-derived Cancer Models

Julia Osaki, Japan

[Backgrounds] CIC-rearranged sarcomas are extremely rare mesenchymal malignancies, characterized by the unique fusion genes. They exhibit unfavorable prognosis because of their aggressive biological features and resistance to conventional chemoradiotherapies. Even after curative resection, the patients often experience local and distant metastasis, and the novel therapy has long been required. To discover seeds for the treatments, for the first time in the world, we had established patient derived cell lines using tumor tissues of CIC-DUX4 sarcomas, which occupied the majority of CIC-rearranged sarcomas.
 [Methods] Using our original cell lines, we employed two approaches with different principles for innovative treatments. Firstly, with special concerns for the small number of patients with CIC-DUX4 sarcomas, we aimed the expanded indication of major molecular targeted drugs such as kinase inhibitors. Their growth suppressive effects were screened in multiple CIC-DUX4 sarcoma cell lines, and in parallel, the kinase activity in the cells was comprehensively examined before and after the treatments. Secondly, we focused on the unique molecular backgrounds of CIC-DUX4 sarcomas, such as the fusion genes, to develop the anticancer drugs with less adverse effects. We try to identify therapeutic targets by investigating the protein complex of the fusion gene products.
 [Results] We identified several kinase inhibitors as candidates for the treatments of CIC-DUX4 sarcomas based on the screening and comprehensive kinase activity assays. Growth of CIC-DUX4 cells was significantly inhibited by the identified kinase inhibitors. The aberrantly high activity of kinases was detected by the membrane peptide array. We also detect the proteins binding to the CIC-DUX4 gene products in the cells by mass spectrometry.
 [Conclusion] Our multifaceted approach will lead to the innovative seeds for novel therapy to CIC-rearranged sarcomas. Considering the clinical diversity of disease, we need to establish more patient-derived cancer models, and use them for pre-clinical research.

PP05.80: Oxidative PTM by Exogenous ROS at Lysosome as a Chemical Leverage to Regulate Autophagy

Mingyu Park, Republic of Korea

Significance: Inhibition of autophagy is an emerging strategy to overcome drug resistance of cancer. However, its photodynamic control has not been clarified since reactive oxygen species (ROS) can either induce or inhibit autophagy. Thus, a rational strategy to perturb autophagy without the activation is required.
Approach: To inhibit autophagy, we developed Ir(III) complex B2 that can target and spatiotemporally oxidize lysosome. To study the mechanism of action, we conducted label-free proteomics followed by in vitro experiments for its verification.
Results: To selectively inhibit autophagy using ROS, we hypothesized that localization of a photodynamic agent must be restricted to lysosome, the major organelle of autophagic process, so that can minimize undesired activation of autophagy. For this aim, we substituted morpholine, a pH-sensitive moiety, for targeting lysosome to Ir(III) complex for generating ROS. The product named B2 was then analyzed in vitro including proteomics. Oxidized proteome suggested lysosomal proteins that are related with lipogenesis and membrane fusion were significantly oxidized. Following in vitro experiments showed that the cellular processes that were identified to be oxidized were damaged. Lysosomal membrane permeabilization was observed using confocal microscopy and fusion-malfunction was shown by dysfunction of SNARE complex assembly. Western blot analysis demonstrated accumulation of P62 and decrease of LC3, which imply inhibition of autophagy. Overall, the lysosome localized photosensitizer B2 induced autophagy-inhibitory cell death that is coupled with lysosomal protein oxidation
Conclusions: This study shows that chemically controlled ROS accumulated at lysosome can successfully inhibit autophagy.

PP05.81: Establishing Proteome Divergence Between Chemo Naïve High-grade Serous Ovarian Tumors and Patient-derived Xenograft Experimental Models

Jesenia Perez, United States

Introduction: Patient-derived xenograft (PDX) models of ovarian cancer recapitulate histologic features and genomic aberrations found in original tumors. However, data from published studies have demonstrated significant transcriptomic differences between PDXs and primary tumors, suggesting that these models exhibit phenotypic divergence. Here, we utilize a mass spectrometry-based proteomic approach to establish similarities and differences between the proteomes of patient-derived high-grade serous ovarian carcinoma (HGSOC) tumors and their serially-passaged PDX models. Method: Tumor tissue was obtained from a peritoneal nodule or the ovary of two chemo naïve HGSOC patients in addition to PDX models into which these tumors were serially passaged. Protein was extracted using heat and sonication prior to trypsin digestion in a RapiGestTM-containing buffer. Peptides were chemically labeled with 10-plex TMT tags and subjected to offline reversed-phase fractionation followed by LC-MS/MS analysis on an Orbitrap Q Exactive Plus using a Top 10 DDA method. Protein identification and pathway analysis was performed using MaxQuant (v.2.1.4.0), Perseus (v.2.0.6.0), Cytoscape (v.3.9.1), and ClueGO (v.2.5.9). Results: A total of 7,384 quantified proteins (2,860 human proteins) were analyzed across all samples. Analysis of Pearson Correlation coefficients highlighted significantly strong and positive correlations (>0.90) between protein abundances in patient tumors and their PDX tumor counterpart. Interestingly, PDXs retained HGSOC-specific phenotypes only from the patient-derived peritoneal nodule HGSOC tumor across two serial passages. In addition, proteins associated with DNA replication were significantly more abundant (p < 0.005) in the passaged PDXs compared to their corresponding primary tumor. Analysis of the mouse proteome in each PDX-model revealed proteins associated with angiogenesis and cell migration to be significantly increased (p < 0.05) from passage 1, indicating the presence of mouse cell infiltration within implanted tumors. Conclusion: We identified the protein signaling pathways that account for the unique proteomic divergence between human HGSOC tumors and PDX mouse models.

PP05.82: Integrative Proteomic Characterization of Epithelial Ovarian Cancer

Liujia Qian, China

As the second most fatal cancer in gynecology, epithelial ovarian cancer (EOC) still lacks biomarkers for early detection, recurrence prediction as well as therapeutic targets. To systematically characterize the molecular pathology of EOC, we profiled global proteome of 1041 surgically resected ovarian tissue samples and 180 plasma samples collected exact before surgeries from 813 patients with diverse histological types and therapeutic regimens, covering the expression of 10,715 proteins. Eight proteins with diagnostic potential firstly discovered to be ascending along with increased malignancy in ovarian tissues then verified to be significantly upregulated in the plasma samples from EOC patients when compared with non-carcinomas. In addition, we identified 759 histotype-specific differentially expressed proteins, revealing pathological changes underlying five histotypes and potential molecular mechanism of clinical outcomes. Remarkably, we compared prognostic proteins among three cohorts and found opposing effects of inositol compound metabolism on recurrence between primary and relapsing patients. To predict one-year recurrence, we firstly verified prognostic proteins by multiple reaction monitoring assays and built two machine learning models based on tissue and plasma proteomic data, respectively. The two groups of the independent validation cohort predicted by both two proteome-based models showed significant differences in recurrence-free survivals (Log-rank test, p value = 0.0094 and 0.012), outperforming the model based on five clinical factors (p value = 0.079). Using the integrative analysis of 295-gene sequencing and proteome data, we found that gene mutations in homologous recombination repair (HRR) showed few associations with chemoresistance, but proteomic regulation in DNA damage plays an opposite role in chemoresistance when compared with the primary cohort, indicating complementary insights provided by proteomics. In summary, this study characterizes a multi-histotype proteomic landscape of ovarian cancer, providing clues for underlying molecular pathology of EOC and potential biomarkers.

PP05.83: Olink Insight and Human Disease Blood Atlas to Uncover Human Disease Proteome and Accelerate Adoption of Proteomics

Marijana Rucevic, Sweden

Despite decades of outstanding research and the impact of genomics, cancer remains an overwhelming burden on global healthcare resources and challenge for the scientific community. Next generation proteomics technologies are increasingly emerging to better understand cancer biology and identify early diagnostic, prognostic, and therapeutic response biomarkers essential to drive development of new, more effective therapies. To progress cancer research more rapidly, data sharing and free access to generated data remains an essential step forward. Here, comprehensive proteome profiling, using Olink Explore platform, was used to measure 1,463 high and low-abundant proteins in plasma, collected at the time of diagnosis and before treatment, from more than 1,500 patients representing 15 common cancer types. The obtained results were used as a foundation for establishment of the Olink Insight platform, an open-access digital knowledge data resource to accelerate adoption of proteomics in the research community. Olink Insight comprises key features such as pathway browser, panel and biomarker selection, and proteomics publication explorer. In Olink Insight, we are creating a collection of proteomic profiles for some of our most important diseases, starting here with cancer. Using a multi-step statistical approach, a panel of 83 proteins was identified that can discriminate 12 different types of human cancer with extremely high accuracy. The plasma profiles for all measured proteins across 12 types of cancers are available in the Human Protein Atlas (HPA). Results available in Olink Insight complementing those of HPA can be interactively explored through a variety of analyses and visualizations, including differential expression analysis, pathway enrichment, pathway annotation with hexmaps, and predictive protein groups identified by machine learning. Olink Insight and the Human Disease Blood Atlas represent a significant step towards uncovering the human disease proteome and will be a valuable resource for researchers in many areas of medicine and biology.

PP05.84: Proteogenomic Landscape of Non-small Cell Lung Cancer

Adrianna Seredynska, Germany

Introduction:
Non-small cell lung carcinoma (NSCLC) is the most common type of lung cancer, with adenocarcinoma (LUAD) and squamous cell carcinoma (LUSC) being the most prevailing histological subtypes with distinct biological signatures. Correct classification and accurate molecular profiling are essential for effective therapeutic guidance. Therefore, a correct distinction between LUAD and LUSC and deeper understanding of the molecular alterations on the genetic and protein level are required in order to detect biologically relevant biomarkers.

Aims:
In this study, we aim to perform an in-depth mass spectrometry (MS)-based proteogenomic study of a large patient cohort representing the two aforementioned subtypes of NSCLC. The MS-based profiling of the LUAD and LUSC and their primary types revealed the proteogenomic alterations that might determine their clinical behavior. This approach along with high-coverage proteome analysis should also unravel the mechanisms of immune evasion in NSCLC. Moreover, a follow up immunopeptidomics study of the patient's frozen tissue should reveal the tumor-associated antigens, which are pivotal for the development of epitope-specific cancer immunotherapies.

Methods:
Patient-derived samples were processed with optimized, semi-automated single-pot, solid phase enhanced sample preparation (SP3-beads) workflow utilizing a pipetting BRAVO robot. Thereby, extracted proteins were digested and the resulting peptides were analyzed via liquid-chromatography tandem-mass spectrometry (LC-MS/MS).

Results:
The MS-analysis of the patient cohort (consisting of n = 153 tumor and n = 138 normal tissue) resulted in high proteome coverage of a total of 10 164 proteins. Initial statistical analysis shows significant differences between LUAD and LUSC and suggest a possible distinction of proteome profiles based on patient's gender.

PP05.85: Proteomics-based Identification of S100 Protein Members in Pancreatic Tumor Secretome and its Potential Role in Collective Invasion of Cancer Cells

Dongwoo Son, Republic of Korea

Pancreatic ductal adenocarcinoma (PDAC) is highly invasive and treatment refractory cancer with poor prognosis. The tumor is usually stromal rich, with activated stellate cells and extracellular matrix that generate a barrier for therapeutic agents. To identify targetable protein candidates in the PDAC stroma, we performed secretome analysis of patient-derived xenograft of PDAC. Out of 1642 proteins identified, we found three S100 family members upregulated in PDAC secretome. As the PDAC cells are known to migrate collectively and S100P plays a positive role in this process, we proceeded to evaluate the role of S100 protein members in the collective invasion. To this end, we developed a co-cultured tumorsphere with activated stellate cells to mimic PDAC stromal condition. Our preliminary results indicate the stable knockdown of S100A4 and S100A10 in a matrix-embedded Panc1 tumorsphere inhibits cellular outgrowth from the sphere, suggesting a positive role of S100A4 and S100A10 in collective invasion. Currently, we are studying the molecular mechanism of this finding using a live cell imaging system and additional multi-omics analysis of multiple cancer cell lines and primary PDAC cells. The potential therapeutic application of this study will be discussed further.

PP05.86: Mutual phosphorylation of FAK and SRC is Involved in Osimertinib Resistance in Non-small Cell Lung Cancer

Takehiro Tozuka, Japan

Introduction: Osimertinib, a third-generation EGFR tyrosine kinase inhibitor, is a standard treatment for patients with EGFR mutant non-small cell lung cancer (NSCLC). However, almost 50% of resistant mechanisms for osimertinib are not identified yet. The aim of this study was to identify mechanisms of osimertinib resistance not involving genetic alteration.
Methods: We established two sets of osimertinib-resistant cells and their parent cells from two independent NSCLC cell lines (PC-9 and HCC827) with EGFR mutation using a stepwise method. We compared the phosphoproteomics profile between osimertinib-resistant and parent cells using mass spectrometry. We inferred upstream kinases using Kinase Enrichment Analysis version 3 (KEA3). KEA3 is a web application that infers upstream kinases whose substrates are overrepresented in a list of phosphorylated proteins.
Results: Phosphoproteomic analysis isolated 80 phosphoproteins mutually upregulated in the two different osimertinib-resistant cell lines. Estimation of upstream kinases by computational methods using KEA3 showed that FAK and SRC were upstream kinases of up-regulated phosphoproteins in the two osimertinib-resistant cell lines. Knockdown of FAK using siRNA methods reduced SRC phosphorylation in the two osimertinib-resistant cell lines, while knockdown of SRC using siRNA methods reduced FAK phosphorylation in both the osimertinib-resistant cell lines. These results suggest that FAK and SRC were mutually phosphorylated. Furthermore, FAK-specific siRNA treatment and SRC-specific siRNA treatment restored phosphorylation of EGFR in the two osimertinib-resistant cell lines. In vitro, combination of FAK inhibitors and SRC inhibitors resulted in inhibition of cell proliferation in osimertinib-resistant cells. In a xenograft mice model, combination of FAK and SRC inhibitors led to inhibition of tumor growth.
Conclusions: Phosphoproteomic analysis might be useful for elucidating the mechanisms of resistance to molecular-targeted therapies in lung cancer. Mutual phosphorylation of FAK and SRC is involved in osimertinib resistance. Hence, inhibition of FAK and SRC might be a novel treatment for osimertinib-resistant NSCLC.

PP05.87: Sources of Technical Variability in Manual Proteomic Sample Preparation Workflows Incorporating Multiplexed Isobaric Labeling and LC-MS/MS Analysis

Carly Twigg, United States

Introduction:
Technical variability is a critical parameter to assess during LC-MS/MS sample preparation and analysis. Identifying the steps associated with the largest technical variability provides an opportunity to enhance the rigor of experimental designs of proteomics experiments, strengthening the biological significance of the results.

Methods:
Variability of enzymatic protein digestion, peptide de-salting, intra-labeling and inter-labeling with tandem mass tags (TMTpro), and LC-MS/MS analysis was determined using protein extracted from three ovarian cancer cell lines (CAOV3, COV362, PEO1) and three patient-derived ovarian tumor tissues. Peptides were analyzed on a Q Exactive Plus Mass Spectrometer using a top-10 ddMS2 method. Data were searched using Proteome Discoverer 2.5 with Sequest HT and Percolator. Filtered abundances were log2 transformed and median-normalized.

Preliminary data:
The reproducibility at each of the five sample processing steps was determined by the average correlation (r) of the replicate protein abundances and the CVs. The correlations of protein abundances from the tissue and cell samples were the highest following LC-MS/MS analysis (n=3 injections/sample): 0.990 and 0.996, respectively, whereas the desalting step accounted for the lowest reproducibility: r=0.950 and 0.757. Only 58.91% and 14.70% of the proteins from the tissue and cell samples had a CV < 30% following de-salting (n=3). At the protein level, LC/MS-MS analysis had the highest reproducibility, whereas the upstream sample processing steps (digestion, desalting, TMT labeling) had decreased reproducibility and increased sample variability between replicates. We determined the following hierarchy of contributions to technical variability at the protein level: De-salting >> Inter-TMT labeling > Intra-TMT labeling > Digestion ≈ LC-MS/MS analysis. Ongoing extended analyses are assessing the technical variability contributed by offline peptide fractionation.

Novel aspect:
Determination of technical variability associated with manual sample preparation steps of proteomic workflows to facilitate experimental designs yielding biologically significant results.

PP05.88: Unique Protein Patterns Identified from Multilevel Proteomic Characterization of Intraductal Papillary Mucinous Neoplasms of the Pancreas

Yuefan Wang, United States

Intraductal papillary mucinous neoplasms (IPMNs) of the pancreas are precancerous lesions that grow in the pancreatic ducts. Over time, some will progress to invasive cancer, which has a 5-year survival rate of only 10%. Therefore, IPMNs present an opportunity to treat pancreatic neoplasia before an invasive cancer develops. The challenge is that many IPMNs never progress, and overtreatment is a real risk. There is therefore an urgent need to understand the molecular features of these noninvasive precursor lesions. Multilevel proteomic approaches that integrate global proteomics and post-translational modification (PTM) analyses have the potential to provide a more comprehensive view of IPMNs.

Herein, we performed a multilevel proteomic characterization of 76 IPMNs and 79 macrodissected normal ducts (34 paired normal pancreatic ducts, and 45 normal pancreatic ducts from pancreatic resections for other pancreatic diseases) obtained from fresh surgical specimens. Label free data independent acquisition (DIA) based proteomics, glycoproteomics and phosphoproteomics analyses were applied to these samples. We identified a total of 10629 protein groups with median of 8307 protein groups identified among all samples. There was high measurement reproducibility in the quality-control samples. We found 231 proteins with >2-fold increase in abundance in IPMNs relative to normal ducts, and 98 of these proteins were identified in more than half of the samples. Gastrointestinal epithelium maintenance and structure proteins, and glycosylation proteins were enriched and upregulated in the IPMNs. Furthermore, integrated clustering identified 3 subtypes of IPMNs, which may help us to understand the biology of IPMNs. Overall, this multilevel proteomic characterization of IPMNs will help the community to understand IPMNs at proteome level and pave the way for informed clinical management of patients with one of these precancerous neoplasms.

PP05.89: Label-free Quantitative Proteomics of Nicotine-treated A549 Cells Reveal a Potential Therapeutic Target for Metastatic Lung Cancer

Churat Weeraphan, Thailand

Adenocarcinoma of lung is strongly related to cigarette smoking. It is well established that cigarette smoke is the initiators and promoters of lung cancer development. Empirical studies indicate that nicotine, the major alkaloid in tobacco, can enhance the growth and metastasis of lung cancer. Here, the effect of nicotine exposure on human lung adenocarcinoma A549 cells was investigated to gain insight into the critical role of proteins in cancer cell invasion using label-free quantitative proteomics. An invasive subpopulation of nicotine-treated A549 cells was selected using Boyden chamber assays. Mass spectrometry-based proteomic analysis was then applied to compare the differentially expressed proteins in the invasive subpopulation of nicotine-treated A549 cells and untreated A549 cells using trapped ion mobility spectrometry coupled with quadrupole time-of-flight mass spectrometry. One hundred and thirty-six proteins were identified with a significant change in protein expression levels using PEAKS Studio. The candidate proteins were confirmed using immunodetection techniques. Notably, most of proteins involve in signaling receptor binding, Wnt/β-catenin and Notch/Snail signaling pathway. Therefore, these proteins might serve as a potential target for treatment in lung cancer patient with cigarette smoking status.

PP05.90: The Effect of Allelic Bias in Cancer Cell Line Proteomes

James Wright, United Kingdom

Genomic variants and mutations are widespread in individuals, however only a small number of these have significant impact on the proteome and cellular phenotype. Somatic mutations are one of the foremost driving forces behind cancer development and understanding them is important for discovery of neoantigens, cancer therapies and biomarkers. Mutant peptides and proteoforms caused by non-synonymous mutations are notoriously difficult to detect in mass spectrometry-based proteomics experiments, confounding efforts to profile their expression in cancers and directly link mutations to changes in protein abundance and function.

There is a plethora of technical and biological factors at play that impact the detectability and quantitation of mutant peptides, including but not limited to peptide "flyability", ambiguity, protein abundance and allelic bias. In this research we have explored these factors in a large set of 375 cancer cell lines previously profiled as part of CCLE (Cancer Cell Line Encyclopedia). Mapping known non-synonymous cancer mutations from the COSMIC database we have searched TMT labelled and multiplexed experiments using a bespoke proteogenomics pipeline against cancer cell line specific protein sequences. Although many mutant peptides (1336) are observed, they only represent a tiny fraction (~1%) of those available and curated in COSMIC. Based on the detectability of a mutant peptide, its paired reference peptide, and parent protein abundance we have categorized observed and absent mutations. Most of these are heterozygous, hence using the relative abundance of non-mutant peptides, and protein abundances we have calculated allelic bias for 31,219 COSMIC mutations (~25% of mapped mutations). This analysis shows that the non-mutant proteoform is often dominant in the proteome, even when there is less allelic bias in the transcriptome, suggesting that allelic bias is moderated pre and post translationally. This data is being made available via the PRIDE database and as genomic 'TrackHubs' in the Ensembl genome browser.

PROGRAM

PP05.91: The Small Open Reading Frame-encoded Peptides: Method Development and Functional Studies

Qian Zhao, Hong Kong

Small open reading frames (sORFs) are novel coding DNA sequences that are shorter than 100 codons. They used to be considered non-coding or even junk. In recent years, accumulating evidence suggests that sORFs can encode microproteins or peptides with important functions. Given 98% of the human genome is defined as non-coding regions, sORFs-encoded peptides (SEPs) remain an underexplored territory and gold mine. However, large-scale identification and functional study of SEPs have been technically challenging. We have established a systematic approach to discover, quantify, and characterize novel SEPs. First, we analyzed samples with ribosome profiling to predict thousands of sORFs hidden in 5'UTR, 3'UTR and lncRNA, which showed high temporal and spatial specificity. Next, we detected SEPs by mass spectrometry with the following optimizations to improve the identification number and data reproducibility: 1) Peptide enrichment; 2) Tailored data acquisition and searching; 3) Customized sORF database. With our approach, thousands of novel SEPs were identified and quantified from cells and tissues. We investigated representative SEPs that play important functions in cancer progression and drug resistance. The SEPs regulated cancer cell growth, migration, colony formation and apoptosis. Our work not only provided a mass spectrometry platform to identify SEPs, but also, reported novel peptides with important biological functions.

PP05.92: Integrating Multiplex Staining and Multiplex-DIA: Profiling the Tumor Microenvironment Proteome for Precision Cancer Research

Xiang Zheng, Denmark

Background: Multiplex-staining allows simultaneous visualization of molecular markers, deepening our understanding of tumor-stroma interactions. However, it is still limited to pre-selected markers. Our group recently developed a robust dimethyl-based multiplex-DIA workflow, enhancing proteome depth in the Deep Visual Proteomics (DVP) technology. We now seek to integrate multiplex-staining with multiplex-DIA to profile the tumor microenvironment proteome, advancing our understanding of the intricate cancer ecosystem and enhancing personalized oncology.

Methods: We conducted multiplex immunofluorescent staining on human cancers (colorectal/ tonsil cancer and skin lymphoma) using the MACSima platform. AI-based single-cell phenotyping and automated single-cell laser microdissection were employed as in DVP. We loaded dimethyl-labelled 3-plex tryptic peptides onto Evotips and performed LC-MS analysis using the Evosep One coupled to timsTOF SCP. Peptide identification was accomplished through DIA-NN against an AlphaPeptDeep-generated library, with data preprocessing and visualization using AlphaPeptStats.

Results: Proteome analysis conducted pre- and post-22-plex staining confirmed no significant protein loss, validating our staining procedure effectiveness in microdissection and excellent protein recovery. Three key findings have emerged: 1) colorectal tumor cells were selectively microdissected based on their proximity to immune-cell-enriched lamina propria. A protein-group depth of approximately 3022 was achieved from 700 contours, equivalent of around 150 cells. Highlighted cancer cell heterogeneity revealed 464 differentially expressed proteins between tumor cells near and far from immune-cell-enriched regions; 2) tonsil cancer exhibited tumor-infiltrating cytotoxic T cells with lost expression of CD45RA/RO, a phenomenon that remains incompletely characterized. We profiled the proteomes of this T cell subtype to characterize cytotoxic activity; 3) skin lymphoma showed malignant T cells homing to the epidermis, prompting us to profile the proteomes of malignant T cells and the surrounding immune cells to gain insights into homing mechanisms and uncover potential diagnostic markers. Our approach integrates multiplex-staining and multiplex-DIA, propelling advances in personalized diagnostics and potentially transforming therapeutic interventions.

PP05.93: Data-driven Exploration of the Inflammatory Proteome Associated with SARS-CoV-2 Infection and/or Vaccination from Home-sampled Blood Spots

Leo Dahl, Sweden

Background:
From the start of the administration of COVID-19 vaccines in 2021, estimates predict that more than 14 million deaths have been prevented. Today, many people are either infected, vaccinated, or both. Owing to the rich proteome response of SARS-CoV-2 infection, proteins have been identified to differentiate between states of immune response or between severities of the disease. However, the proteomic responses to infection, vaccination and combinations thereof in the general population are still poorly studied.

Methods:
Using quantitative home-sampling kits (Capitainer AB), we collected dry blood spots (DBS) from 440 random individuals in Sweden between May and September of 2021. Each participant also completed a questionnaire about possible events of infection and vaccinations and health-related aspects. Serological analyses used multi-analyte Luminex-based assays to characterize the immune responses against five SARS-CoV-2 proteins and 22 human interferons. The DBS proteomes of the donors were subsequently analysed with the Olink Explore assays targeting 384 inflammation-related proteins. A two-step machine learning approach of feature selection followed by a deep search within the selected features was used to identify interesting proteins.

Results:
We used questionnaire information and serological data to cluster the donors in a knowledge and data-driven manner. In both, we identified four groups based on infection and/or vaccination. There was a minor but noticeable mismatch between serological data and questionnaire answers, mainly owing to the timepoints of infection or vaccination. Using the two schemes to classify subjects, we used proteomic data to identify features associated with the states of the immune response.

Conclusion:
This study shows the possibilities of home-sampled DBS to determine antibodies and proteins associated with response phenotypes of SARS-COV-2 infections and vaccination.

PP05.94: Super-resolution Proximity Labeling Reveals Antiviral Protein Network and Its Structural Changes Against SARS-CoV-2 Viral Proteins

Yun-bin Lee, Republic of Korea

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) replicates in human cells by interacting with host factors after infection. To understand the virus and host interactome, proximity labeling methods (biotin ligase or APEX) have been utilized. However, conventional proximity labeling workflow often provides rather ambiguous results likely due to the indirect identification of biotinylated proteins. Herein, we developed a super-resolution proximity labeling (SR-PL) method with "plug and playable" PL enzyme, TurboID-GFP binding protein (GBP) and we applied it for interactome mapping of GFP-tagged SARS-CoV-2 ORF3a and M proteins, which generated highly perturbed ER structures. Through SR-PL analysis of the biotinylated interactome of ORF3a and M, 224 and 272 peptides were robustly determined as ORF3a and M interactomes, respectively. Within the ORF3a interactome, RNF5 co-localized with ORF3a and generated ubiquitin modifications of ORF3a related to protein degradation. We also observed that SARS-CoV-2 infection rate was efficiently reduced by the overexpression of wild-type RNF5 in the host cells compared to cells overexpressing the nonfunctional mutant C42SRNF5. Overall, we introduced a new virus–host interactome mapping workflow using SR-PL and we could identify novel anti-viral ubiquitin ligase (RNF5) interfering with SARS-CoV-2 infection in human cells. This interactome data obtained using this SR-PL method was presented as a web-based platform (https://sarscov2.spatiomics.org) for readers to make it more accessible. This method contributes to revealing virus–host interactomes of other viruses in an efficient way in the future.

PP05.95: Antiviral Activity of a Nanoscale Phospholipid Dosage Form Containing an Antiviral Component against SARS-COV-2

Yuliya Romashova, Russian Federation

The coronavirus pandemic has prompted researchers throughout the world to search for new drugs that provide a high degree of antiviral action. Umifenovir (Umi) an indole derivative is used for the treatment and prevention of infections caused by influenza virus A and B. Umifenovir is insoluble in water, what has a significant effect on its bioavailability. To improve that, a nanoscale phospholipid dosage form containing Umi (UmiNP) was developed using the microfluidization method. The aim of this research - investigation of antiviral activity of UmiNP against coronavirus. We used two methods – analyzing the cytopathic effect of the virus (CPE) using the MTT assay and the ELISA method using monoclonal antibodies to the nucleocapsid protein SARS-CoV-2 (500 ng/ml, HyTest, RF) on the Vero (CCL81) cell line.

Cells were cultured until a complete monolayer was reached. The investigational compounds were added at concentrations (5; 10; 20 and 30 µg/mL). The cells were incubated (37°C, 5% CO_2) for 2 hours. Then, the virus was added at a dose of 20 or 100 MOI and incubated in the same conditions for 5 days until a clear CPE appears in the viral control cells for the MTT assay and 24 h for the ELISA method.

The results confirmed the UmiNP antiviral activity against the coronavirus. The estimated 50% effective concentrations (EC50) were similar for UmiNP and Umi at low dose of virus (20MOI). At high doses (100MOI), EC50 for UmiNP was twice lower than for Umi. The 50% cytotoxicity concentration (CC50) was more than 80 µg/mL for UmiNP, and 31.5 µg/mL for Umi. Activity of UmiNP is similar to that of Umi, but the selectivity index (CC50 to IC50 ratio) is significantly higher (7,4 for UmiNP vs 3,1 for Umi) which is an important advantage as an antiviral drug compared to the free form.

The study was performed employing "Avogadro" large-scale research facilities, and was financially supported by the Ministry of Education and Science of the Russian Federation, Agreement No. 075-15-2021-933, unique project ID: RF00121X0004.

PP05.95A: Uncovering a Neurological Protein Signature for Severe COVID-19

Houari Abdesselem, Qatar

Uncovering a Neurological Protein Signature for Severe COVID-19
Omar El-Agnaf1, Ilham Bensmail1, Maryam Al-Nesf2, James Flynn3, Mark Taylor4, Nour Majbour1, Ilham Abdi1, Nishant Vaikath1, Abdulaziz Farooq5, Mohammed Al-Maadheed2,5, Vidya Mohamed-Ali2,5, Praveen Vemulapalli6, Frank Schmidt6, Khalid Ouararhni1, Heba Al-Siddiqi1, Patrick Wijten1, Abdelilah Arredouani1, Julie Decock1, and Houari Abdesselem1
1Hamad Bin Khalifa University, Qatar. 2University College London, UK. 3Illumina Corporation, USA. 4Bioinfo Solutions LLC, USA. 5Anti-Doping Laboratory-Qatar. 6Weill Cornell Medicine-Qatar.

Introduction: Coronavirus disease of 2019 (COVID-19) has sparked a global pandemic with severe complications and high morbidity rate. Neurological symptoms in COVID-19 patients, and neurological sequelae post COVID-19 recovery have been extensively reported. Yet, neurological molecular signature of severe COVID-19 remain still unknown and need to be identified. Plasma samples from severe COVID-19 patients were subjected to Olink proteomics analysis. By using a multi-approach bioinformatics analysis, we identified a 34-neurological protein signature for COVID-19 severity.

Methods: Using Olink proteomics technology, we profiled plasma samples of 49 patients with severe COVID-19 disease, 50 patients with mild- COVID-19 disease, and 40 healthy controls. 184 CNS and neurodegenerative disease proteins were used in our study.

Results: 34 diffrentially expressed proteins in severe COVID-19 displaying co-expresssion and protein-protein interactions profile were identified as neurological protein signature for severe COVID-19, and then validated in different independent cohorts using blood and postmortem brain samples at both protein and RNA levels.

Conclusion: Unveiled neurological protein signature and disrupted neurological pathways for COVID-19 severity. The new protein signature can potentially contribute to the development of prognostic and diagnostic tools for neurological complications in post COVID-19 convalescent patients.

1.   Al-Nesf MAY., et al. 2022 Nat Commun 13:946.
2.   Varatharaj A., et al. 2020 Lancet Psychiatry 7:875-882.
3.   Virhammar J., et al. 2021 Eur J Neurol 28:3324-3331.

PP05.96: Systematic Analysis of Specific Antibody Response to SARS-CoV-2

Sheng-ce Tao, China

COVID-19 (SARS-CoV-2) has resulted in over 760 million global infections and a reported death toll of nearly 6.9 million. Specific antibodies against the virus are crucial in the infection and recovery process of COVID-19. Understanding the production and variation patterns of these antibodies at a systemic level has practical implications. It can deepen our knowledge of the virus's infection mechanisms and guide the development of therapeutic antibodies and vaccines. To address the demand for systematic analysis of specific B cell immunity to SARS-CoV-2, we have:

Constructed a SARS-CoV-2 protein microarray, analyzing serum samples from over 3,000 COVID-19 patients and 600 controls. This revealed specific immunological patterns at the protein level, identifying highly immunogenic spike (S) and nucleocapsid (N) proteins, as well as other proteins with strong immunogenicity. The immunogenicity of these proteins is significantly correlated with age, sex, lactate dehydrogenase (LDH), and lymphocyte percentage (Ly%).

Developed a comprehensive S protein peptide microarray, analyzing the same set of samples. Multiple B cell immune hotspot regions were identified, including the CTD region and a section covering the FP and S2' protease cleavage site. Surprisingly, no significant linear epitopes were found in the RBD. Based on these findings, 19 significant peptides/epitopes were determined, with three non-RBD region linear epitopes demonstrating neutralizing effects. Certain peptides showed discriminative effects on COVID-19 infection.

Developed a high-throughput antibody recognition epitope analysis technology (AbMap) to identify key amino acid residues in the recognition epitopes of specific antibodies against SARS-CoV-2 in recovered patients.

In summary, using protein microarray, peptide microarray, and AbMap technologies, we analyzed COVID-19-related sera, constructing a specific antibody response atlas at the protein, peptide, and amino acid residue levels. The identified highly immunogenic SARS-CoV-2 proteins and significant epitopes can be used for specific diagnosis and guide the development of therapeutic antibodies and vaccines.

PP05.97: Characterization of Lymphocytes Protein Cargo in Covid-19: Unveiling the Impaired Coagulation

Silvia Valentinuzzi, Italy

Background. SARS-CoV-2 is a highly transmissible and pathogenic beta-coronavirus that caused pandemic of pneumonia named Covid-19 since 2019. The striking variability of clinical manifestations, relying on reduction in lymphocyte subset counts, cytokine storm, neurodegeneration and hypercoagulopathy, highlighted the urgence to characterize the molecular networks involved. In this context, peripheral lymphocytes bring the cellular basis of adaptative immune responses, playing a considerable role as predictors of Covid-19 outcomes. Methods. A "SORTomics" approach through fluorescence activated cell sorting (FACS) combined with mass spectrometry (HR-MS) was employed to purify and analyze 100,000 CD3+ T and 100,000 CD19+ B cells from pooled plasma of hospitalized patients during infection (I), recovered (R) and healthy subjects (H). Peptides from tryptic digestion were analyzed by Orbitrap-Fusion-Tribrid-MS analysis. Data were processed through MaxQuant and Perseus software; quantified proteins underwent Ingenuity Pathway Analysis (IPA) for functional enrichment analysis of the interacting networks. Validation of putative markers was performed by FACS and ELISA on plasma specimens from expanded cohorts of I and H. Results. 221 (I), 165 (R) and 234 (H) proteins were quantified in T cells, whereas 205 (I), 118 (R) and 161 (H) proteins in B cells. I pool was devoid of effective lung healing markers and expressed markers of cytoskeleton remodeling, inflammation, proteasomal activity, virus entry, coagulation, and metabolic switch. IPA suggested the interplay between inflammation, viral infection, and coagulation cascade by confirming the acute phase response signalling, vascular dysfunction, and the infection by RNA-viruses. Interestingly, we demonstrated that iron homeostasis is affected in patients as shown by upregulated levels of transferrin (TF). Its role in coagulation is mostly supported by the overexpressed levels of the platelet factor 4 (PF4) in patients, that have been confirmed in the validation set. These data provide useful tools in the assessment of response to Covid-19 and to predict the outcomes.

PP05.98: Viable Single Cardiomyocytes Applications in Proteomics

Aleksandra Binek, United States

Introduction
The cardiomyocyte is a specialized heart cell that is responsible for heart tissue contraction and is easily damaged. These large rod shape cells (with wide size range of 35-180 um) are challenging to isolate in large numbers using conventional cell sorting. Our aim is to develop a method to monitor proteome changes of single cells in disease and control state and evaluate their heterogeneity.
Methods
After sacrifice, hearts were digested and perfused with enzyme mix containing collagenase. Human induced pluripotent stem cells (hiPSC) derived from healthy individual with no known cardiovascular risk were differentiated at iPSC core into cardiomyocytes by adapting an established monolayer differentiation protocol, after which cells were enzymatically dissociated into a cell solution. Live/dead stained cell suspensions were sorted on CellenONE instrument to isolate single cardiomyocytes into a 384-well PCR plate. Trypsin digested peptides were analyzed using SCP-TIMS-TOF (Bruker). The data was analyzed in FragPipe 17.1 and DIA-NN 18.1.1 using the match between runs label-free quantitation workflow.
Results
Benchmarking of the proteomics analysis was performed using the DDM-lysed mouse cardiac cells resulted in detection of 15654 peptides (1374 proteins) with an average identification of 920 proteins per mouse cardiomyocyte. Hierarchical clustering analysis of the whole proteome segregated the cardiomyocyte population into 2 main clusters grouping them by cellular diameter into 50-65μm and 76-112 μm groups. Cardiomyocytes with a 66-75μm cellular diameter size were equally segregated between the 2 main populations. Single cell proteomics applications developed in cardiomyocytes are now being tested on and translated to human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs). Our first preliminary analyses of those clinically relevant single iPSC-CMs samples rendered an average of 2358 precursors and 629 proteins detected per cell.
Conclusions
We established isolation of cardiomyocytes using label free methodology requiring high sensitivity applications.

PP05.99: Exploring the Human Aging Blood Proteome

Dohun Lee, Republic of Korea

Introduction: Aging is a major risk factor for most chronic diseases including cancer, cardiovascular diseases, and neurodegenerative diseases. Recently, the blood proteome has emerged as a promising candidate to explore the aging process given that circulating proteins are good predictors of chronological/biological age, markers for age-associated pathologies, and even direct regulators of aging. Despite its significance, systematic investigation on aging blood proteome has been limited. Here, we describe our integrative analysis of the human blood proteome as a dynamic system that manifests multi-faceted processes of aging.
Methods: We systematically collected human blood proteomics data from in-house projects and public databases. The resultant dataset consisted of MS data from >1,500 individuals with different sex, age (20~90), and health conditions (healthy/diseased). Additionally, through literature mining, we built a compendium of aging-associated molecular features including aging-associated genes, SASPs, and centenarian-associated proteins.
Results: In our integrated human blood proteome dataset, a total of >1,400 circulating proteins were identified. Linear modelling revealed proteins correlated with age have known associations with risk of specific diseases. Combination of the circulating proteins accurately predicted chronological age, validating previous notions of blood proteome as an 'aging clock.' Clustering analysis revealed major expression patterns of blood proteome across lifespan, which recapitulated known aging processes in cells/tissues. Interestingly, aging trajectories differentiated among different groups of individuals, mainly by health conditions. This led us to distinguish biomarkers of aging and those of aging-associated diseases. Lastly, an integrative analysis involving clustering, correlation analysis, and functional annotation predicted potential aging regulatory proteins. Our list of 'aging regulators' included previously validated aging factors such as B2M and CDH13, corroborating our analytical framework.
Conclusions: Our analysis revealed that the blood proteome closely mirrors the aging process and contains potential biomarkers and therapeutic targets for aging. These results will be a rich resource for future aging studies.

PP05.100: Tandem Mass Tag-based Quantitative Proteomic Profiling Identifies Candidate Biomarkers of Aging-induced Hematopoietic Stem Cells

VAN DUC PHAM, Republic of Korea

All immune cells are differentiated from hematopoietic stem cells (HSCs). HSCs are characterized by the self-renewal and regenerative potential that will be lost gradually during aging, subsequently leading to the altered production of blood cells that may induce aging-related blood diseases. In addition, growing evidence shows that HSCs in old mice may exhibit altered functions that are different from that of young mice. Thus, understanding underlying mechanisms contributing to HSC phenotype in aging is crucial to maintain a regular healthy blood production system. However, it remains to explore the critical factors associated with pivotal cellular processes induced during aging that could be discovered through the proteomic analysis of aging HSCs. To do so, we carried out a TMT-based quantitative proteomic analysis of HSCs purified from bone marrows of young (~ 2 months) or old (~18 months) mice. Since the number of HSCs in a mouse is very limited, we pooled them from multiple mice (n=4, triplicate HSC samples from young or old mice). First, we established the HSC enrichment method by utilizing the antibody-based isolation protocol (Lin-, Sca+, cKit+, CD34-, CD15+, Flt3-). A total of ~104 HSCs pooled from four young or old mice were prepared to extract proteomes equivalent to ~0.5 ug peptides. Six-plex TMT was employed to perform quantitative proteomic analysis. A total of >3,000 proteins were identified with differentially expressed proteins associated with cellular activities related to transcription and translation. Further bioinformatic analysis predicted key transcription factors activated in HSCs in old mice.
Collectively, our work overcomes the challenge of studying a small sample amount and discovering a set of prospective proteins expected to be HSC drivers in aging. Targeting these transcriptional and translational regulatory proteins is promising for maintaining HSC function during aging.

PP05.101: Differential Modulation of the Phosphoproteome by the MAP Kinases Isoforms p38α and p38β

Arie Admon, Israel

Introduction:
The stress-activated p38 mitogen activated protein kinases (p38-MAPK) mediate rapid responses to different stresses, such as osmotic shock, radiation, immune stimuli, inflammatory cues, and many other stresses. The MAPKs are activated rapidly, within seconds of stress induction, but respond differently to continuous activation, such as during inflammations and cancer. This study aimed to follow the specific signaling cascades induced by the p38-MAPK p38α and p38β, after activation by strong and rapid stress, relative to continuous activation.
Method:
The analysis was based on large-scale proteomics and phosphoproteomics analyses using stable isotope labeling of mouse embryonic fibroblasts (MEF) deficient in each of these p38 kinases, and expressing constitutively wild-type p38α or p38β, or their intrinsically active variants. These effects on the phosphorylation patterns were compared to the effects of strong and rapid stress response induced by anisomycin in wild-type MEF or MEF knocked-out for these p38α or p38β MAPKs.
Results and Discussion:
The constitutive activation induced adaptations of the cells expressing the intrinsically active p38 mutants resembling chronic and specific stress responses in each of the p38s. The analyses reveal feedback loops that inactivated other p38s when one p38 is continuously active. Furthermore, several interesting phosphorylation sites on proteins relevant to stress responses and cancer were noticed. These responses were different from the rapid changes in the phosphoproteome induced by the anisomycin treatment.
Conclusions:
In conclusion, this study shed new light on the differences between chronic and transient p38 signaling and the associated stress responses induced by them, which resemble different disease states.

PP05.102: Characterizing Serine ADP-ribosylation in Breast Cancer Cell Lines After DNA Damage Stimulation Using Mass Spectrometry

Holda Anagho, Denmark

Since it was discovered that PARP inhibitors (PARPi) selectively target BRCA1/2 mutant cells, PARP inhibitors have been used to treat breast cancer patients with BRCA mutations. Because PARP1-mediated ADP-ribosylation (ADPr) targets a variety of proteins after DNA damage stimulation, we wanted to figure out whether differences in DNA damage-stimulated ADP-ribosylation correlate with PARP inhibitor sensitivity in breast cancer cell lines. To do this, we profiled the proteome and ADP-ribosylome in six wild type or BRCA1/2 mutant cell lines with different sensitivities to PARP inhibitors. Specifically, we used mass spectrometry to characterize protein expression levels, and we used Af1521-based enrichment to profile ADPr sites in these cell lines after H_2O_2-induced DNA damage. We identified 968 ADPr sites on 492 proteins across the cell lines, with over 90% of ADPr events occurring on Serine residues. The ADPr sites and proteins identified in this study match those previously identified in our lab in HeLa and U2OS cells. In untreated and H_2O_2-treated cells, most of the ADPr signal was localized to Histone H2B, Histone H3, and PARP1. In response to H_2O_2-induced DNA damage, the majority of ADP-ribosylation sites were identified in all six cell lines, and these protein targets are involved in various DNA damage response pathways. This suggests that ADPr signaling after DNA damage induction is robust and conserved in cells regardless of BRCA mutation status or PARP inhibitor sensitivity. Nevertheless, we detected site-specific differences in ADPr intensities in the PARP-inhibitor-sensitive BRCA mutants, which suggests that sensitivity may lie in the differential modulation of these pathways. These sites are interesting targets for follow-up studies to elucidate specific mechanisms of how ADPr signalling contributes to PARP inhibitor sensitivity.

PP05.103: How Does the Conventional Cell Culturing at Atmospheric Oxygen Influence the Response to Oxidative Stress and the Thiol-based Proteome Remodeling?

Sandra Anjo, Portugal

Conventional cell culture conditions using atmospheric or hyperoxic levels of oxygen (~18-21% O_2) do not replicate the physiological in vivo environment[1]. This discrepancy can lead to differential cellular behavior in vitro compared to in vivo conditions, particularly when studying oxygen-related mechanisms such as redox processes. Cells cultured under atmospheric oxygen conditions are under constitutive high levels of oxidative stress and exhibit reduced energy production, thereby impacting their response to external agents. Controlling this artifact is crucial to obtain more relevant results in in vitro studies and improve the translation of findings to disease pathology and the development of successful therapeutics. While a few studies have considered physiological oxygen levels, none have focused on studying redox alterations.
In this study, we aimed to evaluate the impact of culturing different neuronal cell lines under hyperoxia (conventional conditions) and physioxia (3% oxygen mimicking brain cell conditions) in the presence or absence of two oxidant agents (auranofin and carmustine). As expected, cells cultured under hyperoxia demonstrated increased vulnerability to the oxidants, indicating a diminished capacity to respond to toxic stimuli. To uncover the underlying mechanisms responsible for these differences, we conducted oxSWATH[2] analysis on the cells and their secretomes, enabling simultaneous evaluation of alterations in both the redox and total proteome. Therefore, our experiment is groundbreaking as it is the first investigation to examine the effects of oxygen on proteome redox remodeling. This research can provide valuable insights into mechanisms that may have been overlooked in previous studies focusing on redox-related phenomena.

[1]Pinho, S.; Anjo, SI. and Cunha-Oliveira, T, 2023 Antioxidants 12(5), 1072; https://doi.org/10.3390/antiox12051072
[2]Anjo, SI et al. 2019 Redox biology 22: 101130. doi:10.1016/j.redox.2019.101130

PP05.104: Targeted Cancer Treatment Using Cell-Penetrating Peptide-Conjugated vDUB

Kwang-Hyun Baek, Republic of Korea

Synthetic therapeutic peptides have emerged as a highly promising strategy with significant potential for advancing cancer research and treatment by enabling cellular transduction. This study focuses on exploring the potential of deubiquitinating (DUB) enzymes for targeted cancer treatment. Specifically, we have developed a recombinant peptide-based anticancer composition using the vDUB enzyme, one of DUB enzymes, as the active ingredient. To enhance the selective and effective cell penetration ability of vDUB, protein-transduction domain (PTD) sequences were incorporated. Through this modification, we achieved improved cellular penetration and effectively suppressed cancer growth in various cancer cell lines, including HeLa, HEK293T, MCF7, JEG3, OVCAR5, and A549 both in vitro and in vivo. Our recombinant peptide-based anticancer composition was evaluated using CCK-8 and LDH assays. The CCK-8 assay confirmed the ability vDUB to inhibit the proliferation and migration of various cancer cell lines in vitro. Moreover, the LDH test demonstrated minimal cytotoxicity, suggesting its potential as a safe and effective therapeutic option. Our findings demonstrate that the cell-penetrating peptide-conjugated vDUB composition effectively regulates the proliferation of specific cancer cells by activating the apoptotic pathway. Moreover, by examining the correlation between the cell penetration ability of protein therapeutic compositions and the reactivity of different cancer cell lines, we gain valuable insights into the selective targeting of specific cancer types. These findings establish the potential clinical applications of our approach. Overall, through an in-depth investigation of cellular transduction mechanisms of synthetic proteins, our study aims to unlock new avenues for improved cancer therapies, enhance our understanding of cancer biology, and ultimately contribute to the development of more targeted and effective treatments for cancer patients.

PP05.105: Exploring the Role of Post-translational Modifications in Tardigrade Cryptobiosis

Samantha Balboa, United States

Tardigrades are aquatic microorganisms known for their incredible ability to survive extreme conditions. Upon exposure to severe environments, tardigrades enter a state called cryptobiosis where metabolic activity is decreased to a near standstill. The underlying biological mechanisms to initiate, continue, and exit cryptobiosis is vastly understudied. Recent work in our lab emphasizes the importance of reactive oxygen species (ROS) to initiate cryptobiosis formation. Additionally, we have delineated the dynamic interaction between cysteine and ROS in tardigrades by showing that cysteine oxidation is crucial for cryptobiosis formation. However, the specific proteins that are oxidized and involved in cryptobiosis have not been identified. To investigate the proteins regulating cryptobiosis formation and the role of cysteine oxidation, we leveraged a cysteine specific fluorescent probe to label reversibly oxidized cysteines between hydrated and cryptobiotic tardigrades and visualized via 2D gel electrophoresis. We hypothesize that by first alkylating free sulfylhydryl groups, then reducing oxidized thiols, we can label the resulting free thiols with a fluorescent probe. Then, we can visualize an increase in fluorescence signal in the cryptobiotic state compared to the hydrated state via analysis by 2D gel electrophoresis. These differential spots will be excised and analyzed via LC-MS/MS to reveal potential protein contributors to tardigrade survival.

PP05.106: Combining Time-resolved Phosphoproteomics and Causal Network Models Elucidates Deregulated Signaling Mechanisms in Cancer

Mira Lea Burtscher, Germany

Background

Cellular signaling deregulation is a hallmark of cancer. Malignant melanomas often carry a phosphomimetic mutation of the BRAF kinase resulting in its hyperactivity and altered downstream signaling. Quantitative phosphoproteomics is a powerful method to study such deregulated signaling processes. However, low phosphoproteome coverage, as well as the need for sophisticated computational methods often constrain the biological insights that can be obtained.

Methods

We applied a phosphoproteomics method which combines high-sensitivity detection and precise multiplexed quantification to profile the steady-state phosphoproteome of different human cell lines and the time-dependent response of BRAF-mutant melanoma cells to the BRAF inhibitor Dabrafenib. We used computational methods such as neural gas clustering, kinase activity prediction with DecoupleR and the KinaseLibrary as well the causal reasoning method PHONEMEs to extract mechanistic insights from these datasets.

Results

We first compared the steady-state profile of human malignant melanoma cells (A2058) to other cell lines and extracted BRAF mutation related signaling characteristics. Multiplexing of six different Dabrafenib treatment time points resulted in a quantitative time-resolved profile for more than 80 000 phosphopeptides. Kinase activity prediction analyses identified known key players of BRAF signaling and related pathways. Clustering analyses revealed distinct time-dependent patterns of phosphorylation reflecting processes which are up- or downregulated over time. Combined with prior knowledge, this enabled us to derive mechanistic network models which suggest a variety of biological processes that are potentially affected by drug treatment, from various signaling cascades to RNA processing pathways.

Conclusions

Understanding the effects of kinase perturbations is critical for drug development, but is constrained by the complexity and plasticity of signaling networks. These findings highlight the potential of deep phosphoproteome profiling combined with computational methods for the analysis and interpretation of drug effects on deregulated signaling networks in cancer.

PP05.107: Investigating Proteome-wide Effect of PARP Inhibitor in Human Cancer Cells Using Quantitative Mass-spectrometry

Hayoung Cho, Denmark

ADP-ribosylation (ADPr) is a type of reversible post-translational modification (PTM) where ADP-ribose is transferred from NAD+ to the target protein by ADP-ribosyltransferases (ARTs). Like other PTMs, ADPr regulates pivotal cellular processes including DNA damage repair, metabolism, cell proliferation, apoptosis, and immune responses. ADPr modification is carried out by a family of proteins called poly (ADP-ribose) polymerases (PARPs). Recent insights into the cellular roles of PARP have led to the development of PARP inhibitors (PARPi), such as Olaparib, that are actively used for cancer treatments. In this study, we performed deep proteome analysis of HeLa cells after Olaparib treatment using a quantitative mass-spectrometry approach. Proteins were digested with Lys-C and trypsin, after which peptides were reversed-phase fractionated at high pH into 48 pre-fractions, which were concatenated into 12 final fractions. All samples were analyzed by LC-MS/MS using an Orbitrap Exploris 480 mass spectrometer. Across four biological replicates and three treatment conditions, the mean of Pearson correlation coefficient was 0.971, indicating that the proteome profiling was highly robust. In total, we identified 104,629 unique peptides mapping to 8,991 proteins, of which 6,199 (~69%) were label-free quantifiable at n=4/4. Olaparib treatment significantly regulated 139 proteins in HeLa cells (83 up and 56 down), and 24 proteins among the upregulated proteins were involved in mitosis. Our findings suggest that ADPr is a critical modification process that regulates cell cycle in human cancer cells, which can affect signaling pathways that lead to many disorders.
Keywords: ADP-ribosylation; PTM; PARP; Olaparib; quantitative mass spectrometry

PP05.108: Pro-apoptosis Protein Bax is Regulated by Deubiquitinating Enzymes

Hae-seul Choi, Republic of Korea

The Bcl-2 family of proteins plays a critical role in regulating apoptosis, and among them, Bax is a well-known pro-apoptotic protein that induces mitochondrial apoptosis. Bax is located in the cytosol in an inactive form, but upon activation, it translocates to the outer membrane of mitochondria. There, it forms an oligomerization with Bax or Bak, which leads to increased mitochondrial outer membrane permeabilization (MOMP) and the release of cytochrome c from mitochondria. This, in turn, activates caspases and initiates apoptosis. In this study, we aimed to investigate the regulation of Bax by the ubiquitin-proteasome system. We conducted a yeast two-hybrid (Y2H) screening to identify deubiquitinating enzymes (DUBs) associated with Bax, and found that two ubiquitin-specific proteases (USP12 and DUB-Bax1) directly interact with Bax. Our immunoprecipitation and GST pull-down assays confirmed this interaction, which these DUBs function as a deubiquitinating enzyme and regulate ubiquitination on Bax. Enzyme assays revealed that they have deubiquitinating activity by detaching ubiquitins from Lys63-linked chains. This suggests that USP12 and DUB-Bax1 affect the cellular functions of Bax, including the regulation of stress signals such as UV damage, hypoxia, oxidative stress, and DNA damage, but they are not related to proteasomal degradation. In addition, we determined that the half-life of Bax protein by performing site-directed mutagenesis of putative deubiquitination sites. We found that two lysine sites in Bax showed less ubiquitination, resulting in a longer half-life compared to wild-type Bax. Overall, our study provides insight into the regulatory mechanisms of Bax and the role of DUBs in modulating Bax-mediated apoptosis.

PP05.109: Proteoform Detection in Deep Plasma Proteomics Using Peptide Expression Correlation

Jennifer D'Angelo, United States

Biological complexity exceeds what can be explained by protein-coding genes alone. Post-translational modifications contribute significantly to this complexity. In particular, blood proteins often undergo proteolytic cleavage, generating multiple proteoforms. Here, we highlight the utility of a novel and automated proteomics workflow, Proteograph™, which leverages physicochemically distinct nanoparticles for deep and unbiased proteomics at scale to reproducibly quantify thousands of plasma proteins across large cohorts, enabling downstream proteoform inference.

To systematically detect proteoforms, we analyzed the peptide quantitative profiles. First, we employed Correlation-based functional ProteoForm (COPF) to identify clusters of peptides with distinct quantitative patterns in disease cohort data, resulting in hundreds of proteoform candidates. Next, we used the Wilcoxson rank-sum test on the peptide positions to filter for candidates with peptide clusters demonstrating a clear N-/C - terminal cleavage. Finally, biological relevance was assessed by mapping the peptide clusters to known UniProt splice/cleavage sites and by interrogating whether their quantitative profiles displayed differential expression for the disease. We identified numerous proteoform biomarkers in four different disease cohorts, including two cancers, type 2 diabetes and Alzheimer's Disease.

To further illustrate our ability to detect proteoforms, we compared data collected using a 23-protein ELISA panel to Proteograph™ workflow data in a dataset comprising of 15 normal and 15 lung cancer patients. We found an anti-correlative signal in the N-terminus peptides of the von Willebrand Factor protein between the two data modalities. When applying the proteoform inference above, we noticed that the anti-correlative peptides correspond to a known N-terminal chain of the protein that is cleaved post-translationally, suggesting ELISA measures protein quantity by targeting only one chain of the protein, whereas the Proteograph™ workflow can capture peptides from both chains.

In summary, the Proteograph™ workflow can quantify thousands of proteins in a deep and unbiased manner, enabling high-resolution data to detect biologically relevant proteoforms.

PP05.110: Label Free and Isobaric Labeling Mass Spectrometry to Monitor Ubiquitination Dynamics Upon Modulation by Small Molecule Inhibitors

Jeroen Demmers, Netherlands

Proteins are tagged with the small protein ubiquitin to target them for degradation by the proteasome. Malfunctioning of the ubiquitin–proteasome system leads to proteome imbalance and, thus, to cancer and neurodegenerative disorders. Using quantitative isobaric labeling and label free data independent acquisition (DIA) mass spectrometry in combination with ubiquitination enrichment technologies, we can monitor the dynamics of the ubiquitinome upon proteasome perturbation or the inactivation of deubiquitinating enzymes (DUBs) or ubiquitin ligases in great detail.

We have developed an improved workflow for the enrichment and detection of diGly peptides. Using a combination of crude peptide fractionation and an efficient peptide fragmentation regime, we were able to routinely identify >23,000 diGly peptides from a single sample. Next, we perturbed proteasome activity by either selective depletion of subunits or by small molecule inhibitors. Malfunctioning of the proteasome resulted in a largely affected proteome, characteristic for changes in stress response, cell cycle regulation, apoptosis and the UPS. The effects were even more pronounced for the ubiquitinome, which was dramatically remodeled upon proteasome modulation. Next, we selectively depleted the three proteasome associated DUBs (USP14, RPN11 and UCH37) and observed remarkable differences in proteome and ubiquitinome remodeling, suggesting unique targeting specificities. Several small molecule DUB inhibitors such as Bortezomib and b-AP15 were used to target the proteasome in a specific manner and we used both SILAC and isobaric TMT quantitation to monitor the deep ubiquitinome and to characterize ubiquitination profiles over time. To take these ubiquitinome assays to the next level, we have compared the above methods to DIA-MS label free mass spectrometry in order to assess the quantitative accuracy. In cells lacking the RING1A/1B ligase we identified and quantified several known and yet unknown affected ubiquitination target sites.

PP05.111: Investigating the Impact of Lysine Acetylation on Short-Chain Fatty Acid Production in the Human Gut Microbiome

Haonan Duan, Canada

Post-translational modification (PTM) holds significant importance in modulating protein functionality. Extensive research has been conducted on PTM in mammalian systems, whereas investigations pertaining to microorganisms have remained limited. Our previous studies have uncovered the widespread presence of lysine acetylation within the human gut microbiome. Furthermore, we have observed a substantial distribution of lysine acetylation proteins along the metabolic pathway responsible for short-chain fatty acid (SCFA) biosynthesis, with altered expression patterns in individuals diagnosed with Crohn's disease. The regulation of lysine acetylation is mediated by enzymes known as lysine acetyltransferases and deacetylases. Consequently, we postulate that the production of SCFAs may be subject to regulation by lysine acetylation levels. In this study, we have cultured the human gut microbiome in vitro using inhibitors targeting lysine acetyltransferases and deacetylases with an optimized culture medium. Notably, these inhibitors have exhibited a significant influence on the protein-level composition of the microbiome, as well as the production of SCFAs. Future investigations will focus on elucidating the intricate relationship between lysine acetylation levels and SCFA production.

PP05.112: A New Simple Glyco-check with Lectin/antibody Dotcoding Using a Fully Automated System

Sayaka Fuseya, Japan

Protein glycosylation is an important post-translational modification for protein function. Monitoring and evaluating glycosylation are essential for biopharmaceutical production because glycosylation affects their stability, potency, and immunogenicity and depends on the type of production cells, cell culture conditions, and product purification methods. Lectin microarrays, which are arrays of various lectins that recognize glycans, have been recognized as a complementary technique to mass spectrometry. This can find structural features of glycoproteins, especially differences in glycan structures, even in trace amounts of clinical specimens. However, it is necessary to simplify the system as a versatile automated instrument for this system to be widely used in industry. To enhance this versatility, we developed a fully automated glycan profiling instrument that applies a unique technical concept called BIST (Bead array In a Single Tip). Various millimeter-sized lectin beads are stored in a plastic nozzle. A simple robotic operation for 30 min performs automatic reaction, measurement, and data acquisition for glycan profiling. In this study, to make a standard GlycoBIST tip, we examined simultaneous signal reproducibility tests using 90 lectin-coated beads randomly picked from 1,000 lectin-coated beads. The results showed that about 30 of the lectins were robust with a CV of less than 10%. Fifteen lectins were verified to show different binding specificities by conducting binding tests using polyacrylamide (PAA)-glycans and glycoproteins and were implemented in the standard GlycoBIST chip. Current attempting suggested the possible combinational use of anti-sugar antibodies, such as anti-NeuGC and anti-alpha Gal antibodies in this system. Furthermore, the additional bead immobilizing an antibody against a target protein was efficiently used for normalization by protein amount. This hybrid platform becomes lectin/antibody dotcoding, enabling more specific evaluation and broadening the scope of analysis. This may realize a simple glyco-check in biopharmaceutical and glycobiomarker development.

PP05.113: Proteomics Analysis of Pathological Alpha synuclein in Synucleinopathies in Search for New Biomarkers and Therapeutic Targets

Belén García Sintes, Denmark

Synucleinopathies are among the most common groups of neurodegenerative diseases, including Parkinson's disease, multiple system atrophy (MSA) and dementia with lewy bodies. They are characterized by a pathological aggregation of alpha-Synuclein (α-Syn) protein in neurons. The intracellular α-Syn fibrillary aggregates are widely linked to neurotoxic pathways such as mitochondrial impairment, autophagic- and synaptic dysfunction, and are thought to be eventually released and spread to neighboring cells, causing aggregation in different brain areas. Changes of post-translational modifications (PTMs) of α-Syn have been observed in α-Syn inclusions and may contribute to pathology development, regulating aggregation, clearance, uptake and secretion of α Syn. However, a systematic analysis of α Syn modifications across synucleinopathies and mechanistic links to the pathology development are lacking.

In collaboration with the Center for Neuroscience & Stereology at Bispebjerg hospital, we have performed a quantitative analysis by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) of post-mortem human brain tissue from patients with α-Syn pathology. The primary focus of our investigation is on the identification of the disease-associated PTMs of α-Syn, including ubiquitination, acetylation, phosphorylation and truncated species of α-Syn. In addition, we have conducted a PTMomics analysis utilizing an in vivo α-Syn seeding model to investigate changes associated with α-Syn pathology. The analysis of human disease material will be compared to the in vivo α-Syn seeding model, expanding the investigation to include the examination of cerebrospinal fluid (CSF) and interstitial fluid (ISF) samples. The aim of this study is to generate a comprehensive map of α-Syn PTMs in disease. Ultimately, the observed α-Syn modifications will be used in the identification of novel disease-relevant biomarkers of synucleinopathies, and in the search of new intracellular therapeutic entry points.

PP05.114: Ubiquitome Profiling in Dozens of Cells Without Enrichment Using a Boosting Channel

Minsang Hwang, Republic of Korea

Ubiquitinylation is essential post-translational modification (PTM) for numerous cellular activities such as degradation of misfolded or damaged proteins. The enrichment process with ubiquitin remnants(K-ε-GG) specific antibodies and multiplexing with isobaric labeling tags are necessary for deep-profiling ubiquitome changes, due to the low stoichiometry of ubiquitinylated proteins. Recent studies have showed significant quantitative results with fractionation of over-milligram amounts of peptides. However, since at least 500ug of peptide is necessary for enrichment process, the conventional method is limited by the amount of sample. Owing to large batch effect during the enrichment processes, sample variations between channels are also inevitable. To address this issue, we presented a highly sensitive, and accurate multiplexed strategy by adding boosting channel instead of an enrichment step per channel, termed UbiBoost. For this method, we collected a larger amount of enriched ubiquitinylated peptides in common cell lines as a boosting channel. By exploiting UbiBoost, we were able to perform quantification especially in nano-scale samples, corresponding to dozens of cells. Here, our results showed that >2,400 ubiquitinylated proteins were quantified from 35ng of tryptic peptides from HeLa lysates. In addition, those also indicated that UbiBoost produced comparable quantification results with less amounts of samples and higher accuracy, compared to the conventional methods. Therefore, the newly proposed method is expected to be able to quantify changes in ubiquitomes of primary cells or rare tissue samples that were previously impossible to monitor ubiquitinylation in the conventional method.

PP05.115: Global, in Vivo, and Site-specific Profiling of Protein Lipidation

Koshi Imami, Japan

Proteins can be modified by lipids in various ways, for example by myristoylation, palmitoylation, farnesylation, and geranylgeranylation—these processes are collectively referred to as lipidation. Current chemical proteomics using alkyne lipids has enabled the identification of lipidated protein candidates but does not identify endogenous lipidation sites and is not readily applicable to in vivo systems. Here, we introduce a proteomic methodology for global analyses of endogenous lipidation sites that combines liquid-liquid extraction of hydrophobic lipidated peptides with liquid chromatography-tandem mass spectrometry using a gradient program of acetonitrile in the high concentration range. We applied this method to explore lipidation sites in HeLa cells, and identified a total of 90 lipidation sites, including 75 protein N-terminal myristoylation sites, which is more than the number of high-confidence lipidated proteins identified by myristic acid analog-based chemical proteomics. Isolation of lipidated peptides from digests prepared with different proteases enabled the identification of different lipidated sites, extending the coverage. Moreover, our peptide-centric approach successfully identified dually modified peptides having myristoylation and palmitoylation. Finally, we analyzed in vivo myristoylation sites in mouse tissues and found that the lipidation profile is tissue-specific. This simple method (not requiring chemical labeling or affinity purification) should be a promising tool for global profiling of various protein lipidations.

PP05.116: Investigation of Brain N-glycosylation Alteration in Acute Social Isolation Models using Mass Spectrometry Imaging (MSI)

Hyun Jun Jang, Republic of Korea

N-glycans regulate protein folding, stability, and signal transduction, and are involved in the release of neurotransmitters and the formation of new synapses in the CNS. Interestingly, several studies have reported changes in N-glycan profiles in serum from patients with neurological disorders or mental stress. The recent social isolation led by Covid-19 has caused social and psychological stress for many people. We assumed that acute social stress affects brain N-glycan levels and used a social isolation model in our experiments. For the social isolation model (SI), a mouse was housed alone in a cage with a partition. And for the indirect social communication model (PAR), two mice were housed with a partition between them in a cage. The group housing (GH) mice used as control were housed in a cage without partition. To investigate brain N-glycan changes caused by acute social isolation, elevated plus maze behavioral test and mass spectrometry imaging (MSI) were performed 24 hours later. In behavioral tests, SI mice spent less time in the open arm than GH and PAR mice. These results show that anxiety-like behavior is induced by acute social isolation. And the MSI results showed a decrease in the relative amount of N-glycan in the six brain regions of the SI model. In this study, we showed dynamic changes in N-glycan in different brain regions of an acute social isolation model with anxiety-like behavior.

PP05.117: Global Oxidative Modifications and Molecular Alterations in the Heart after Ischemia-reperfusion Injury

Inmaculada Jorge, Spain

Cardiovascular diseases are the leading cause of death worldwide. After an episode of coronary artery occlusion, reperfusion alters the healing process in the heart. Although many relevant proteome changes take place in the cardiac tissue following the ischemia-reperfusion process, no systematic studies have tackled the protein posttranslational modifications (PTMs) that take place in the post-reperfused myocardium. We have performed an unbiased, open-search analysis of PTMs using Comet-PTM along with redox proteomics using the FASILOX technique to unveil the dynamic map of oxidative PTMs in pig heart tissue along the first week after ischemia-reperfusion.

After ischemia, blood flow restoration generates an initial oxidative damage in the mitochondria. This first intracellular oxidative wave, detected as early as 20 minutes after reperfusion, consisted on irreversible oxidations and affected intracellular proteins essential for cardiac function. Later, immune cells are recruited to the lesion site as part of the cardiac inflammatory process concomitant with a second oxidative event at 2 h, peaking at 24 h after reperfusion, which impacted irreversible and reversible cysteine oxidations in mitochondrial, sarcomere and inflammation-related proteins.

Results provide the first systematic, detailed map of the molecular alterations that take place in the heart during the first week after the ischemia-reperfusion event, encompassing a deep study of the posttranslationally modified peptidome, the redoxome and the proteome in a highly translational pig model. We anticipate that this detailed map will help define new therapeutic targets and diagnostic methods for improving early post-myocardial infarction remodeling.

PP05.118: Fe-IMAC Phosphopeptide Enrichment of Plasma Phosphoproteome for Diagnosis of AD

Seunghyeon Jung, Republic of Korea

Plasma is one of the most accessible biofluids for various tests and proteome analysis, but its complexity and dynamic makes difficult to analyze minor compositions of plasma for diagnosis. Meanwhile, tau protein has been highlighted due to tauopathy including Alzheimer's disease. Among various tau isoforms, phosphorylated 2N4R tau proteins including p-tau 181, p-tau 217 and p-tau 231 are recommended as AD biomarkers. However, many studies that utilized p-tau biomarkers for cohort study often use SIMOA instead of LC-MS analysis. Antibody based analysis have its own strengths, but it requires antibody and variable results due to the differences between antibodies. Thus, in this study, we optimized phosphopeptide enrichment method to produce phosphoproteome samples from plasma samples for LC-MS analysis.
We utilized commercial Fe-NTA phosphopeptide enrichment kit as enrich material and in house buffer solution for washing, binding, and elution. We proceed skeptical in-sol digestion/desalting procedure and apply plasma protein digest to enrichment step. After phosphopeptide enrichment, we apply parallel reaction monitoring (PRM) method for LC-MS analysis with AD phosphopeptide biomarker list from previous studies. As the result, we could optimize phosphopeptide enrichment process for plasma, and produces reproducible results from PRM analysis. Also, we used isotope labelled standard peptides for absolute quantification, we could calculate enrichment efficiency and estimate concentrations of biomarkers in plasma. Now, we are dealing with actual AD patients samples, to determine significant increases or decreases in biomarker panel concentrations.

PP05.119: ReCom: A Semi-Supervised Approach to Ultra-Tolerant Database Search for Improved Identification of Modified Peptides

ANDREA LAGUILLO GOMEZ, España

Open-search methods allow unbiased, high-throughput identification of post-translational modifications (PTMs) in proteins at an unprecedented scale. However, the performance of current open-search algorithms is diminished by experimental errors in the determination of the precursor peptide mass. In this work we propose a semi-supervised open-search approach, called ReCom, that minimizes this effect by taking advantage of a priori known information from a reference database of PTMs, such as Unimod or a database provided by the user. We present a proof-of-concept study using Comet-ReCom, an improved version of Comet-PTM [1]. Comet-ReCom achieved a 68% increase in identification performance over Comet-PTM. This increased performance of Comet-ReCom in scoring the MS/MS spectrum comes in parallel with a significantly better assignation of the monoisotopic peak of the precursor peptide in the MS spectrum, even in cases of peptide coelution. Our data demonstrate that open searches using ultra-tolerant mass windows can benefit from a semi-supervised approach that takes advantage of previous knowledge on the nature of protein modifications. The ReCom concept is also fully compatible with other open-search algorithms such as MSFragger [2]. We foresee that the use of semi-supervised approaches will improve statistical algorithms for the high-throughput analysis of PTMs by mass spectrometry.

[1] N. Bagwan, et al. Comprehensive Quantification of the Modified Proteome Reveals Oxidative Heart Damage in Mitochondrial Heteroplasmy, Cell Rep. 23 (2018) 3685-3697.e4
[2] A.T. Kong, et al. MSFragger: ultrafast and comprehensive peptide identification in mass spectrometry–based proteomics, Nat. Methods 2017 145. 14 (2017) 513–520.

PP05.120: Analysis of Oxidized Proteomes in Two Cellular Organelles Exhibiting Synergistic Cell Death

Chae Gyu Lee, Republic of Korea

Iridium complexes are potent anticancer photosensitizers with high singlet oxygen generation efficiency, although they are limited to being applied in clinical systems due to their severe cytotoxicity. Therefore, determining the most efficient target within the cancer cells, such as specific organelles or signalling pathways, could minimize the required dose of cytotoxic photosensitizer, followed by reduced physical burden on the patient. Here, we control the target cell organelles of iridium photosensitizers by modulating their charges, hydrophobicities, and pKa. Based on the confirmation of different localization patterns and photodynamic efficacy of three iridium complexes, we further investigate the combination that makes synergism in breast, lung, cervix, stomach, colon, and pancreatic cancer cells. To elucidate the plausible mechanism of the synergistic effects exhibited by this combination of two photosensitizers, proteomics analysis was conducted. Proteins were extracted from whole cells containing oxidized proteins after photodynamic therapy and conducted proteomic analysis. We identified a group of proteins showing high oxidation signals specifically related to methionine oxidation, and investigated their association with synergistic effects. Finally, the synergy between two iridium complexes could reduce the required amount of cytotoxic photosensitizers for a similar therapeutic effect, successfully reducing the in vivo toxicity.

PP05.121: Evaluating Data Analysis Pipelines for Large-Scale Citrullination Proteomics

Chien-Yun Lee, Germany

Introduction: Post-translational modifications (PTMs) regulate protein functions. Citrullination, a relatively understudied PTM, correlates with autoimmunity and inflammation. Despite its recognized pathological significance, identifying citrullination sites is challenging due to limited enrichment tools. Deep proteomics profiling has provided insights into direct identification of citrullination sites, but faces difficulties such as shared mass increases with deamidation of Gln and Asn, potentially leading to errors. Manual inspection is often required, this hampers throughput in large-scale proteomics studies. Here, we evaluate search algorithms and post-processing approaches to propose optimal pipelines for large-scale citrullination proteomics.

Methods: An evaluation data set was generated by spiking in different ratios of synthetic citrullinated peptide pool into cell lysates to assess citrullination identification precision. Public proteomics data sets of human brain and Arabidopsis root tissues were obtained from PRIDE. Andromeda (MaxQuant 2.2.0.0) and MSFragger (FragPipe 19.0) search algorithms were used for citrullinated peptide identification and quantification. Evaluation included Prosit PTM and IonFinder post-processing approaches for automated neutral loss (HNCO) detection.

Results: Citrullination site identification was evaluated using different approaches in the evaluation dataset. MSFragger performs better precision without post-processing than MaxQuant, while Prosit PTM achieved the highest precision at lower citrullinated peptide spike-in samples. Filtering identifications by neutral loss increased precision but reduced total identifications. We applied our pipeline to reanalyse human brain proteomes from multiple sclerosis and Arabidopsis tissue proteomes, revealing increased citrullinated peptide and protein identifications. Notably, we confirmed non-vertebrate citrullination in Arabidopsis, highlighting its wider importance. These findings provide novel insights into citrullination by re-searching existing proteomics data with evaluated post-processing.

Conclusions: We proposed an optimal data analysis pipeline with increased accuracy and throughput of large-scale citrullination identification, facilitating the systematic understanding of citrullination.

PP05.122: Unveiling Organ-Specific Glycan Profiles in Porcine Xenograft Model: Insights into Non-Human Glycan Antigens

Daum Lee, Republic of Korea

Porcine models have emerged as crucial tools for studying human diseases due to their physiological similarities and organ structures. Xenotransplantation utilizing porcine organs offers a promising solution to the organ shortage crisis, potentially saving countless lives. However, successful clinical trials face challenges posed by the differing glycosylation patterns between humans and pigs. Non-human glycan structures, such as galactose-α1,3-galactose (α-gal), Neu5Gc, and Sda antigens, act as xenoantigens triggering hyperacute rejection in porcine-to-human xenotransplantation. Despite the pivotal role of glycans in the immune system, comprehensive research on glycosylation in porcine organs, which serve as representative models for xenotransplantation, is lacking. In this study, we conducted a comprehensive qualitative and quantitative analysis of glycan profiles in porcine organs, including the heart, pancreas, and kidney. Utilizing state-of-the-art LC-MS/MS techniques, we successfully identified various glycan isomers and examined their heterogeneity characteristics. Additionally, we employed LC-MRM/MS to quantitatively determine the absolute content of Neu5Gc in porcine organs. Our findings revealed that the majority of glycans in these organs were Neu5Ac-sialylated, while approximately 30% exhibited non-human glycan structures. Through MS/MS spectra analysis, we successfully identified non-human glycans expressing the Neu5Gc, Sda antigen, or α-gal epitope in each organ. Furthermore, we accurately quantified trace amounts of Neu5Gc in each porcine organ. The comprehensive qualitative and quantitative glycan profiling presented in this study serves as a valuable resource for future research endeavors in glycobiology and immunology. The identified glycan isomers and their heterogeneity characteristics provide a solid foundation for investigating the interactions between glycans and immune receptors, ultimately facilitating the development of strategies to overcome barriers in porcine-to-human xenotransplantation and enhance the success rates of these life-saving procedures.

See you in Busan!
BEXCO, Busan, Korea
2023.hupo.org
#HUPO2023

Together Through Proteomics

PROGRAM

PP05.123: Proteome-wide Analysis of Post translational Modification Reveals Dynamic Alterations in Protein Function During Lipopolysaccharide-induced Neuroinflammation in Microglia.

GEEEUN LEE, Republic of Korea

Introduction: The inflammatory response is a tightly regulated process involving complex signaling networks and post-translational modifications (PTMs) of proteins. PTMs are critical regulatory mechanisms that modulate protein function and cellular responses, playing a crucial role in the context of inflammation. Lipopolysaccharides (LPS) are well-known inducers of neuroinflammation in central nervous system, associated with neurodegeneration. In this study, we focused on investigating alterations in protein expressions and PTMs in LPS-induced neuroinflammation in microglia.
Methods: Microglia cell (BV2) was cultured and treated with LPS (1ug/mL) for a specific duration. To inhibit the proteasome activity, BV2 cell was treated with 5uM MG132 for 4 hours. The collected cells were lysed directly using 2% Sodium deoxycholate, followed by in-solution digestion methods. For global proteome analysis, 10ug peptides were desalted using SDB-RPS StageTips and analyzed by Q Exactive HF-X mass spectrometer with data-independent acquisition method. For PTM analysis, phosphor and ubiquitinylated peptides were enriched using TiO2 and antibody, respectively. The enriched PTM samples were analyzed by data-dependent acquisition. Mass raw data files were processed using Spectronaut, and all statistical analyses of mass data were performed using Perseus software.
Results: By comparing the proteome and PTM profiles between control and LPS conditions, we identified a total of 8000 proteins in the proteome. Additionally, we identified about 1000 unique phosphorylation and ubiquitinylation sites that exhibited significant alterations in inflammatory response. Of the differentially expressed PTM events occurring at specific sites, we classified the patterns of PTM and identified key proteins related to immune signaling.
Conclusions: Our proteomic study demonstrates the power of quantitative PTM analysis in unraveling the dynamic changes in the inflammatory response at the molecular level. This finding contributes to the current knowledge of how PTMs mediated processes modulate inflammation in microglia and provides valuable insights into potential targets for therapeutic intervention in neuroinflammatory diseases.

PP05.124: Optimization of Peptide-to-bead Ratios for In-depth Phosphoproteomic Analysis

Jung-hyun Lee, Republic of Korea

Protein phosphorylation, a type of post-translational modification, plays a critical role in cell signal transduction and cellular processes. The dysregulation of cell signaling is the underlying mechanism of numerous pathological conditions. It triggers a cascade of aberrant responses that significantly alter the functions of cells and tissues. Thus, the orchestration of kinase and phosphatase activities upon endogenous or exogenous stimuli can be inferred by quantitative measurement of phosphorylation level on substrates to better the understanding of cellular regulations.
 Various methods have been developed to enrich phosphopeptides for mass spectrometry-based phosphoproteomic analyses. Among many methods established, immobilized metal affinity chromatography (IMAC) or titanium dioxide (TiO2) beads are the most widely used in the field. However, there are several analytical variables to be considered for reproducible and in-depth results, including time, pH, temperature and acidifying chemicals(e.g. formic acid, phosphoric acid, acetic acid, or trifluoroacetic acids). In addition, optimal peptide-to-beads ratio is another factor that can vary depending on the sample origin, as the phosphorylation profiles differ. By using diverse biological samples, such as cell lines and tissues, together with an appropriate peptide-to-bead ratio, we could provide an overview of sample characteristics through phosphoproteomic outcomes.
 In conclusion, we have collected wide-scale phosphoproteomic data sets to pinpoint an optimal condition for in-depth phosphoproteomic analysis of cell lines or tissues. This study will provide insights towards the utilization of phosphoproteomics in biomedical and clinical applications, addressing the prevalent challenge of limited sample quantities.

See you in Busan!
BEXCO, Busan, Korea
2023.hupo.org
#HUPO2023

Together Through Proteomics

PROGRAM

PP05.125: A Tip-Based N-terminal Proteome Enrichment Method and Its Applications

Seonjeong Lee, Republic of Korea

Protein N-termini contain valuable information such as protein half-life, but studying the N-terminome faces challenges due to the requirement of large sample sizes. This can be problematic when analyzing limited samples, such as low number of cells or clinical biopsies. To overcome this, we developed a tip-based N-terminal Proteome Enrichment Method, named tipNrich. The entire procedure is carried out in a single pipette tip in order to minimize sample loss. With tipNrich, we could analyze the femtomole-scale of a single protein at minimum even using a low-resolution mass spectrometer. Also, we analyzed different single proteins purified from different organisms and discovered that even purified single protein could have various N-termini caused by cleavages or modifications. The simplicity of tipNrich, requiring only a gel loading tip and a PCR tube cap as a reactor, would allow for easy adoption in other laboratories, including clinical fields. In addition, we present tipNrich platform coupled with matrix-assisted laser desorption/ionization (MALDI) strategy to shorten the total analysis time. Our method with MALDI would suit the needs for quality check of protein products such as paratope of antibody in a fast way. Lastly, our method can be utilized as a diagnostic tool, especially for diseases related to N-terminal proteoforms because our method is easy-to-use and enables rapid sample processing. In this regard, we applied our method to plasma of patients with wild-type transthyretin amyloidosis and detected disease-related N-terminal sites. Published [Seonjeong Lee et al., Analytical Chemistry, 2021].

PP05.126: Identification of Histone Lysine Acetoacetylation as a Dynamic Post-translational Modification Regulated by HBO1

Sangkyu Lee, Republic of Korea

Ketone bodies has been long known as a group of lipid-derived alternative energy source in situations of glucose shortage. Nevertheless, the molecular mechanisms underlying their non-metabolic functions remain largely elusive. Here, we identified acetoacetate as the precursor for lysine acetoacetylation (Kacac), a new and evolutionarily conserved histone post-translational modification (PTM). We comprehensively validated this protein modification using chemical and biochemical approaches, including HPLC co-elution and MS/MS analysis using synthetic peptides, Western blot, as well as isotopic labeling. Histone Kacac can be dynamically regulated by acetoacetate concentration, likely via acetoacetyl-CoA. Biochemical study showed that HBO1, traditionally known as an acetyltransferase, can serve as an acetoacetyl-transferase. In addition, we identified 33 Kacac sites on mammalian histones, depicting the landscape of histone Kacac marks across species and organs. Our study thus discovers a physiologically relevant and enzymatically regulated histone mark that sheds light on the non-metabolic functions of ketone bodies.

PP5.127: Global Characterization of Lysine Acetylation and Lactylation in Kupffer Cells

Sangkyu Lee, Republic of Korea

Of the various cell types that make up the liver, Kupffer cells (KCs) remove gut-derived foreign products in this organ. Protein lysine acetylation (Kac) and lactylation (Kla) are dynamic and reversible post-translational modifications, and various global acylome studies have been conducted in the liver and liver-derived cells. However, no studies have been undertaken on KCs. In this study, we identified 2,198 Kac sites in 925 acetylated proteins and 289 Kla sites in 181 lactylated proteins in mouse immortalized KCs using global acylome technology. The subcellular distribution of proteins with Kac and Kla site modifications was shown to differ. Similarly, the specific sequence motifs surrounding the acetylated or lactylated lysine residues also showed differences. Gene Ontology and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were also performed to obtain a better understanding of the differentially expressed proteins with Kac and Kla. In the novel identified Kla, we found K82 lactylation in high mobility group box-1 protein in the neutrophil extracellular trap formation category in KEGG enrichment analyses. Herein, we report the first proteomic survey of Kac and Kla in KCs.

PP05.128: An Improved Workflow for Robust and Comprehensive Lysine Acetylome Analysis

Tao Liu, United States

Introduction
Protein lysine acetylation plays key regulatory roles in various cellular physiological and pathological processes. However, developing a robust and effective approach for lysine acetylome analysis remains difficult, primarily due to copurification of lysine carbamylated peptides during immunoprecipitation (IP), LC issues caused by non-covalent antibody, and wrong precursor selection during database searching.

Methods
Instead of urea, SDS buffer was used to avoid generation of artificial lysine carbamylated peptides. The S-Trap method was then used for protein digestion and SDS removal. After TMT labeling, the peptides were fractionation by high-pH RPLC and concatenated, followed by automated acetyl peptide enrichment using magnetic bead-conjugated antibody (PTMScan HS) and KingFisher. Optimized search parameter in FragPipe was used to reduce the false-positive in acetylation peptide identification.

Results
Compared to urea, SDS greatly reduces the proportion of carbamylation peptides from 29% to 13%. When operated in manual mode, the magnetic-bead-conjugated antibody provided a 45% increase in acetyl peptide identification and higher specificity (n=3659, 65%) compared to the agarose beads (n=2518, 43%), using 500 ug TMT-labeled peptides. Up to 5306 and 6579 of acetyl peptides were identified from 500 ug and 1 mg peptides, respectively, by processing the samples using KingFisher; the specificity was further improved to over 85%. The magnetic-bead-conjugated antibody also enabled robust LC-MS/MS analysis by eliminating "leaking" antibody from the IP process that was causing LC issues. Finally, the 6-fraction method provided a 33% increase in acetyl peptide identifications (n=17,280) compared to the 4-fraction method. The false-positives in acetyl peptide identifications could be effectively reduced by optimization of the precursor mass tolerance and selection of variable modification in FragPipe.

Conclusions
This optimized acetylome workflow allows for robust and comprehensive lysine acetylome analysis, and can be readily implemented for integrated analysis of other PTMs such as ubiquitination, phosphorylation and glycosylation.

PP05.129: Exposing the Molecular Heterogeneity of Glycosylated Biotherapeutics

Rafael Melani, United States

Current biotherapeutic analysis methods measure compound fragments or rely on glycan digestion to reduce sample complexity. We created a new platform for the direct compositional analysis of highly glycosylated biotherapeutics using the Orbitrap Tribrid platform. The method involves the glycoform fingerprinting of biotherapeutics using a broadly applicable data-independent method for analyzing intact glycoproteins by proton-transfer charge-reduction tandem mass spectrometry (DIA-PTCR). When integrated with a bioinformatic data analysis scheme, the approach enabled the elucidation of a biotherapeutic's glycoform-resolved molecular profile. Notably, we obtained putative assignments of hundreds of glycoforms for the eight-times glycosylated fusion drug IL22-Fc as a function of sialic-acid content. Glycan annotation for IL22-Fc was challenging, given its eight glycosylation sites and the millions of potential glycoforms that could explain the detected intact masses. Assigning the detected masses to combinations of monosaccharides using only DIA-PTCR data resulted in broad distributions of matching compositions due to hundreds of possible monosaccharide combinations matching each molecular weight. Hence, we integrated the DIA-PTCR data with glycoproteomic data, which constrained the search space of glycan structures and allowed the filtering of the potential assignments to only the most likely ones. The resultant 'glycan fingerprint' confirmed the expected increases in sialic acid content, displayed constant levels of GlcNAc, and revealed an increase in hexose content concomitant with sialic acid. The 'glycan barcode' for IL22-Fc provided a site-resolved map of IL22-Fc glycosylation and probability-based assignments for each glycoform mass. This information was validated by orthogonal glycomic analysis and was in turn used to correlate high-level cellular effects, namely the potency of the biotherapeutic, to specific IL22-Fc glycoforms, defining a general path toward precision proteomics and the structure-function annotation of glycoforms.

PP05.130: Real-time Instrument Methods to Resolve Chimeric Spectra and Post-translational Modifications in Multiplexed Proteomics

Rafael Melani, United States

Real-time instrument control improves instrument acquisition efficiency and quantitative accuracy in sample multiplexed experiments. Here we sought to develop methods to resolve highly complex fragmentation spectra resulting from the co-isolation of multiple precursors (chimeric spectra) and post-translational modifications (PTMs).

By integrating new, optimized scoring algorithms on the Orbitrap Ascend for real-time searching (RTS) and real-time library searching (RTLS), we can effectively discriminate multiple peptides identified within a chimeric spectrum and trigger individual quantitative scans. The primary sample we analyzed was a two-proteome (human and yeast) standard labeled with TMTpro. For RTS, databases were pulled from UniProt. For RTLS, libraries were predicted using Prosit-TMT or pulled from existing data using SpectraST and preprocessed with DBKey.

We used the CHIMERYS algorithm to try to identify the proportion of confident identifications derived from chimeric spectra. Depending on MS² isolation width and the use of FAIMS, CHIMERYS identified anywhere from 4.5% to 63.2% of chimeric spectra. We then used a modified RTLS to attempt to trigger quantitative MS³ scans for multiple precursors identified in a single chimeric MS² spectrum. We were able to validate chimeric spectral triggering based on the differential human and yeast HYpro profiles. Triggering multiple quantitative measurements from a single MS² scan opens the door for more efficient instrument acquisition using RTLS and rescoring-based spectral matching. Similar to chimeric spectra, the identification/quantification of PTMs represents an enticing analytical challenge related to the exponential increase in library sizes. The larger database results in more candidate spectra and often lower delta scores, reducing the accuracy of peptide/PTM matches. We developed a phospho-RTLS pipeline and scoring method that enables discrimination of phosphorylated peptides using ~1ms per spectrum to search, match, and score phosphopeptides. These improvements enabled real-time discrimination of target and decoy phosphopeptides with similar sensitivity and selectivity to unmodified RTLS methods.

PP05.131: Flavone-induced S-Nitrosylated Proteins Facilitate Schwann Regeneration

Shirley Thy Nguyen, Republic of Korea

Compared to the central nervous system, peripheral nerves have higher intrinsic regeneration capacity after damage. This difference relies largely on Schwann cell's plasticity to undergo dedifferentiation into a reparative phenotype primed for regeneration. However, substantial defects from traumatic injuries or pathological complications might interrupt this process and lead to peripheral neuropathy.

Utilizing TMT-based proteomics, we characterized the in vivo dynamics of the sciatic nerve after injury in a time-dependent manner. We discovered that its proteome was altered significantly starting from day 2 of injury. Interestingly, the regeneration process could be aided by a natural compound that was previously reported to have antioxidant and anticancer effects. Moreover, we observed an elevated level of neuronal nitric oxide synthase in the mouse's sciatic nerves upon degeneration yet suppressed by the treatment with this compound. We subsequently investigated the S-nitrosylated landscape of Schwann's proteome and its orchestration during degeneration and treatment-induced regeneration. From this multi-level proteomic analysis, vital cellular pathways that contribute to myelination and axonal regrowth were noted. We further assessed the regulating role of S-nitrosylated protein on other post-translational modifications, notably phosphorylation, to unveil the underlying mechanisms of this compound to counteract peripheral neuropathy. In conclusion, time-course proteomics provided a holistic understanding of Schwann cell properties and neuronal repair. We also proposed a therapeutic agent from natural sources that could expedite the regeneration process. Of note, by linking the crosstalk between post-translational modifications and related pathways, our findings might lay a foundation for future research endeavors into S-nitrosylation and its overlooked roles in pathological conditions.

PP05.132: Disentangling the Tubulin Code: Characterization of Tubulin Polyglutamylation in the Flagellum of Trypanosoma Brucei

Marija Nisavic, France

Dimers of α- and β-tubulin represent microtubule (MT) building blocks. The tubulins can exist as several isotypes and can have different posttranslational modifications (PTMs) such as acetylation, polyglycylation, polyglutamylation or tyrosination. This variety is referred to as the tubulin code and it controls the properties and function of MTs. Polyglutamylation entails the addition of lateral glutamate chains of variable lengths to C-terminal tubulin tails, catalyzed by tubulin tyrosine ligase-like (TTLL) enzymes. Defective polyglutamylation affects MTs and is associated with different diseases such as ciliopathies and neurodegeneration. A model system to study this PTM is Trypanosoma brucei, a protist parasite that causes sleeping sickness in humans. Here, α- and β-tubulins are encoded by a single gene each and polyglycylation of the C-terminals is absent, which simplifies precise characterization of polyglutamylation. Literature on tubulin polyglutamylation in T. brucei is scarce and the exact role of TTLL enzymes is still to be determined. Here, we employ thermolysin digestion and strong anion exchange enrichment followed by nano-liquid chromatography coupled to tandem mass spectrometry (MS/MS) to identify and quantify polyglutamylated peptide variants in flagella purified from wild type (WT) cells as well as from TTLL4 and TTLL9 enzyme knock out cell lines. Our data reveals the existence of numerous unique polyglutamylation patterns, commonly involving two specific glutamylation sites. In WT T. brucei, C-terminal peptides of α- were detected with up to eleven and those of ß-tubulin with up to five supplementary glutamates. Long chain polyglutamylations were detected exclusively on detyrosinated tubulin. Apart from previously described glutamylation sites, we discovered completely novel ones and could determine the precise role of TTLL4 and TTLL9 enzymes in the polyglutamylation mechanism. Up to date, our data provides the most comprehensive study of tubulin polyglutamylation and gives novel insight into TTLL activity in T. brucei.

PP05.133: Unraveling Nitration Signaling in Early Onset Gastric Cancer: Insights from Nitroproteomics Analysis

JaeWon Oh, Republic of Korea

Nitration is one of the post-translational modifications (PTMs) occurring under the condition of oxidative stress. The generation of 3-nitrotyrosine through peroxynitrite formation involving nitric oxide synthase (NOS) and reactive oxygen species (ROS) has been well-documented. Once, the peroxynitrite is generated, MPO (Myeloperoxidase) catalyzes the nitration process, leading to the formation of 3-nitrotyrosine and 3-nitrosotyrosine. Previous nitroproteomics studies revealed that nitrated proteins have roles in inflammation, altered enzymatic activity, reduced cellular signaling, interruption in phosphorylation pathways, and protein degradation. Despite its importance, studying nitroproteomics and comprehending their intricate signaling mechanisms remains challenging due to the rarity of nitration compared to other PTMs such as phosphorylation and glycosylation. Advancements in mass spectrometry and the integration of diverse data from various projects have significantly expanded the nitroproteome coverage. Leveraging these developments, we conducted an in-depth investigation into nitration using the Clinical Proteomic Tumor Analysis Consortium (CPTAC) data. By re-analyzing global proteogenomics and phosphoproteomics data from the Early Onset Gastric Cancer (EOGC) project in CPTAC, we identified a robust association between nitration and crucial biological processes, including migration, inflammatory response, and actin polymerization in EOGC patients. In summary, our study demonstrates the power of integrating advanced mass spectrometry techniques and large-scale proteomic datasets to uncover the role of nitration in cancer biology. The identified association between nitration, MPO expression, and critical biological processes in EOCG enhances our understanding of nitro-signaling pathways and paves the way for potential therapeutic interventions targeting this PTM.

PP05.134: Proteomic and Phosphoproteomic Profiling of Transglutaminase 2 Inhibition in Human Kidney Tubular Epithelial Cells

Hong-Beom Park, Republic of Korea

Introduction: Transglutaminase 2 (TG2) is a multi-functional Ca2+-dependent enzyme that catalyzes protein crosslinks and post-translational modification such as transamination, serotonylation, and phosphorylation. In kidney fibrosis, excessively secreted TG2 proteins from kidney tubular epithelial cells (TECs) promotes fibrosis to induce the expression of TGF-β, increases the accumulation of extra cellular matrix (ECM), and impairs kidney function to induce apoptosis of TECs. But, functionality of TG2 as a protein kinase and phosphoproteins in kidney fibrosis are not fully understood.
Methods: Primary cultured human TECs were treated with TGF-β (2 ng/mL) to promote fibrosis for 24 h or 48 h and Cysteamine (2 mM) for TG2 inhibition. Proteins extracted by TECs were digested by Trypsin/LysC and we performed global proteome and phosphoproteome analysis based on tandem mass tag 10-plex and phosphopeptide enrichment using TiO2. Peptides and phosphopeptides were analyzed by Orbitrap Exploris 480 with data-dependent acquisition mode. MS raw data was analyzed by Protein Discovery, and proteome and phosphoproteome data were analyzed by Perseus software and gene ontology enrichment analysis.
Results: In-depth proteome and phosphoproteome analysis led to the quantification of about 8,000 proteins. A parallel phosphoproteome analysis identified about 10,000 unique phosphopeptides. Integrated proteomic and phosphoproteomic analyses showed significant differential expressed proteins (DEPs) and phosphoproteins related to the ECM component, inflammatory response, and apoptosis. Also, inhibition of TG2 decreases the expression of kidney fibrosis markers such as α-smooth muscle actin, fibronectin, and TGF-β. Interestingly, phosphorylated proteins related with various pathway were dysregulated by TG2. These pathways are significantly related with progression of kidney fibrosis.
Conclusions: We newly discovered overall TG2-induced alteration of proteins and phosphoproteins and showed that pathological significant of TG2 in the phosphorylation of various proteins, and is closely related to the pathogenesis of kidney fibrosis. Therefore, DEPs and phosphoproteins can be putative therapeutic strategies for kidney fibrosis.

PP05.135: Label-free Quantitative Phosphoproteomics of Isogenic Cholangiocarcinoma Cell Lines Reveal the Crucial Protein Phosphorylation as Potential Therapeutic Targets for Metastatic Cancer

Metarsit Patchimaekapol, Thailand

Cholangiocarcinoma (CCA) is a highly metastatic tumor with poor prognosis and globally increasing incidence. Growing evidence indicates that protein phosphorylation level is predominantly altered in metastatic cancer cells to promote the onset and development of cancer. Here, an in vitro metastatic model of isogenic CCA cells was used to gain insight into the crucial role of protein phosphorylation in enhancing the invasive ability of cancer cells using label-free quantitative proteomics. One of metastatic subpopulation from the RMCCA-1 cell line was selected by using Boyden chamber assays. Mass spectrometry-based proteomic analysis was then performed to identify the different level of phosphorylated proteins in relation to the invasive ability using the combination of immobilized metal affinity chromatography (IMAC) and trapped ion mobility spectrometry coupled with quadrupole time-of-flight mass spectrometry (timsTOF Pro2). Approximately 9,500 proteins were identified and 6,400 proteins were detected as phosphorylated proteins using PEAKS studio. Three hundred and fifty-four phosphorylation sites on 322 proteins were identified with a significant change in phosphorylation level. The candidate proteins were validated using immunoblotting technique. Interestingly, most differential phosphorylated proteins are involved in angiogenesis, Wnt/β-catenin, PDGF and Ras signaling pathway. Therefore, the aberrant phosphorylation of proteins might be served as a novel and potential therapeutic target for treatment in CCA patients.

PP05.136: MSFragger-Labile: Improving Analysis of Post-translationally Modified Peptides

Daniel Polasky, United States

Identifying protein post-translational modifications (PTMs) by mass spectrometry (MS) is a key task complicated by the fact that many such modifications are labile, dissociating from peptides during tandem MS. Database search algorithms often struggle to identify peptides following PTM loss as the observed fragment ions do not match the expected fragments with intact modification(s). We have recently developed a flexible framework for labile PTM searches in the MSFragger search engine, termed MSFragger-Labile. In labile searches, PTM-specific diagnostic ions are used to filter which spectra are searched for modifications, and peptide- and fragment-remainder ions can be used to improve search scores for peptides bearing labile modifications. Fragment remainder ions can also be used to localize a modification, even after partial dissociation. Using these features, we are able to identify more modified peptides and modification sites in wide variety of PTM searches, including of phosphopeptides, ADP-Ribosylated peptides, RNA-crosslinked peptides, and more. The degree of improvement from labile searches depends on the how labile a modification is, ranging from more than doubling the number of highly labile RNA-crosslinked spectra identified to more modest gains of 10-30% in phosphopeptides. Labile search can also be combined with conventional search for partially labile modifications, like phosphorylation, providing a flexible framework to maximize search performance across modifications and fragmentation conditions. MSFragger-Labile mode is fully supported in the FragPipe search environment, where it can be combined with our recently described diagnostic ion mining module in PTM-Shepherd to automatically identify abundant fragment ions to use in labile mode search. Labeled and label-free quantitation of peptides bearing labile modifications is incorporated into TMT-Integrator and IonQuant, respectively, and visualization of labile fragment ions is also supported in the accompanying FP-PDV results viewer.

PP05.137: Scop3PTM: a Knowledgebase on Human Post-translational Modifications of Proteins Built by Reprocessing and Reusing Public Proteomics Experiments

Pathmanaban Ramasamy, Belgium

A common mechanism for protein regulation is through post-translational modifications (PTMs), which can affect the activity, subcellular localization, and stability of proteins. The most frequently used tool to study proteome-wide protein modification is mass spectrometry (MS)-based proteomics experiments. With the increasing availability of public data submitted from such high throughput proteomics experiments, several databases related to one or more PTMs in one or more model organisms have been established. Most of the available databases on protein PTMs collect and integrate PTM information from different resources, but they provide very little or no information on the reliability of the identified PTM sites, tissue-specificity, pathological status and their functional relevance. Moreover, the modified peptides measured from different experiments are typically identified using different search engines with different false discovery rate thresholds, which may artificially increase the heterogeneity of the PTM sites when these are integrated into a database that has no information on the significance of the reported PTM sites. In order to remove the hidden layer on the proteomics experiments using different versions of sequence databases with different search settings and FDR controls, and to potentially look for additional modifications that could explain the identified spectra from the original experiments, we created Scop3PTM. Scop3PTM is an interactive knowledge-base on human PTMs built by reprocessing all available human proteomics experiments from PRIDE with the uniform and updated sequence database, search settings and FDR control. Furthermore, PTM sites are put into sequence, structural and biophysical context by annotating every protein with per-residue structural propensity, solvent accessibility, disordered probability, backbone and sidechain dynamics, and early folding information. Scop3PTM acts as a much-needed bridge between the fields of proteomics, structural bioinformatics and intrinsically disordered proteins (IDPs), and presents a unique resource for visualization, and to understand the impact of PTM-sites on structure-function relationship of proteins.

PP05.138. Digging Deeper into Phosphoproteomes Through AI-Driven Deconvolution of Chimeric Spectra

Florian Seefried, Germany

Background. The analysis of phoshopeptides requires accurate identification and correct localization of the post-translational modification within the peptide. Here, we extend the capabilities of our deep-learning framework to accurately predict physicochemical properties of phosphorylated peptides and demonstrate the usefulness of these predictions in our search algorithm enabling the analysis of such analytes.

Methods. We curated a large training dataset with >1.6 million spectra of phosphorylated peptides to develop an accurate model for predicting their physicochemical properties. Our CHIMERYS™ algorithm utilizes these predictions to score experimental MS2 spectra. All relevant peptides in each MS2 isolation window are considered simultaneously, aiming to explain as much experimental intensity as possible with as few peptides as possible and yielding fractional contributions of peptides to the experimental spectrum. FDR-control is performed using Mokapot. Post-processing is performed using Thermo Scientific™ Proteome Discoverer™ software.

Results. When analyzing single-shot DDA data from an IMAC-based phosphopeptide enrichment, we observe a 1.4-fold increase in the number of identifiable and localizable phosphorylation sites. Next, we benchmark our prediction-based localization strategy of scoring all possible positional phosphoisomers of a given peptide precursor against established localization tools like phosphoRS using synthetic peptide standards. Here, the precision-recall curve at different localization probability thresholds highlights that fragment ion intensities are a valuable source of information to improve the localization of the modification site. We demonstrate the compatibility of our algorithm with TMT™- and TMTpro™-labeled phosphopeptides, resulting in a similar increase in the number of identified phosphopeptides as for label free data. Finally, we benchmark the localization performance of our algorithm in convoluted DIA spectra from phosphopeptides and derive error rates for such workflows.

Conclusions. AI-driven, intensity-based identification and localization of phosphopeptides enables more sensitive and reliable data analysis, unlocking biological insight through added sensitivity.

PP05.139: Protein Arginylation: A New Key Player in SARS-CoV-2 Infection

Janaina Silva, Brazil

SARS-CoV-2 infection induces endoplasmic reticulum stress (ER-stress) and activation of the unfolded protein response (UPR) pathway. Maintaining proteostasis is an essential element for cell survival during biotic and abiotic stress. N-terminal protein arginylation is a mechanism activated during stress conditions and induces ubiquitination and proteasomal degradation through the N-degron pathway. Here, we explore the role of arginylation during SARS-CoV-2 infection. The functions of arginylation were elucidated in Vero CCL-81 and Calu-3 cells infected at different times (2, 6, 12, 24, 48 hpi) with SARS-CoV-2. A reanalysis of public omics in vivo and in vitro data, combined with immunoblotting, was performed to measure levels of arginyl-tRNA protein transferase (ATE1) and its substrates. Furthermore, proteomics and glycoproteomics of cells Calu-3 ATE1-silenced cells were explored. Transient transfection of HEK-293T cells was performed using a plasmid expressing full-length spike protein or plasmids encoding the Spike Protein Receptor Binding Domain (RBD) from classical (Wuhan), P1, gamma, and delta strains. We found that SARS-CoV-2 infection increases ATE1 expression in Calu-3 and Vero CCL-8 cells. We report that ATE1 inhibitors reduce viral release. This finding was confirmed in Calu-3 ATE1-silenced cells. Furthermore, we found that ATE1 induction was variant-dependent. We showed that the reduction of ATE1 levels implies a reduction in the activity of the oligosaccharyltransferase complex (OST complex) of the N-glycosylation machinery and in the activation of the UPR pathway, preventing normal viral replication. Treatment with ER-stress and glycosylation inhibitors/activators showed for the first time an arginylation-glycosylation-ER-stress axis that sheds light on events involving both PTMs. Moreover, we found that Calu-3 ATE1-silenced cells are more sensitive to stress-induced death. We demonstrate that ATE1 increases during SARS-CoV-2 infection and its inhibition has potential therapeutic value; in addition to regulating other important pathways, such as N-glycosylation and UPR, being an important mechanism in infectious and other diseases.

PP05.140: Interactome of Intact Chromatosome Variants with Site-specifically Ubiquitylated and Acetylated Linker Histone H1.2

Florian Stengel, Germany

Post-translational modifications (PTMs) of histones have fundamental effects on chromatin structure and function. While the impact of PTMs on the function of core histones are increasingly well understood, this is much less the case for modifications of linker histone H1, which is at least in part due to a lack of proper tools. In this work, we establish the assembly of intact chromatosomes containing site-specifically ubiquitylated and acetylated linker histone H1.2 variants obtained by a combination of chemical biology approaches. We then use these complexes in a tailored affinity enrichment mass spectrometry workflow (AE-MS) to identify and comprehensively characterize nucleosome and chromatosome-specific cellular interactomes and the impact of site-specific linker histone modifications on a proteome-wide scale. In particular, we find involvement of chromatin-bound H1.2 in the recruitment of proteins involved in DNA double-strand break repair and confirm this function in vitro. Taken together, our optimized workflow constitutes not only an important tool for future AE MS investigations of histone epigenetic modifications, but also supports the role of H1.2 as a regulatory protein with distinct functions beyond DNA compaction.

PP05.141: Structural Characterization of SARS-CoV-2 Dimeric ORF9b Reveals Potential Fold Switching Trigger Mechanism

Xue Sun, China

Background
Enhanced innate immune evasion via boosting viral antagonists' expression including the accessory protein ORF9b, contributes to the improved transmission of SARS-CoV-2 variants, suggesting we need to pay more attention to these viral proteins. The crystal structure of SARS-CoV ORF9b revealed a homodimer β-rich structure with a long hydrophobic lipid binding tunnel, suggesting its function on membrane binding and mature virion assembling.
Methods
The full-length gene of SARS-CoV-2 ORF9b protein was synthesized and codon-optimized in E.coil. We employed trapped ion mobility-time of flight mass spectrometry (timsTOF MS) to analyze the specific phosphorylation site of ORF9b. Besides, we identified the lipid-ligand molecules of ORF9b expressed in different expression systems using QE-HFX Orbitrap mass spectrometry. We proposed a model of multifunctional ORF9b with distinct structures, proving that both lipids and phosphorylation in ORF9b contributed to the fold-switching.
Results
Herein, we demonstrated that the dimeric structure of supernatant and refolding SARS-CoV-2 ORF9b with a long hydrophobic tunnel containing lipid molecules were crucial for the dimeric conformation, and determined that the specific lipid ligands in ORF9b expressed in different expression systems were consistent to the LC-MS/MS identification, indicating their critical role for viral life cycle. Of note, a long-intertwined loop accessible for host factor binding was observed in the structure. Eight phosphorylated sites in ORF9b were identified using timsTOF MS, and residues S50 and S53 were confirmed to be critical for dimeric ORF9b stabilization. Additionally, we proposed a model of multifunctional ORF9b with distinct structures, proving that both lipids and phosphorylation in ORF9b contributed to the fold-switching.
Conclusion
We demonstrated crystal structure of SARS-CoV-2 ORF9b with lipid binding tunnel, and phosphorylation site S50/S53 are critical for ORF9b stabilization. Our results inspire a better understanding of SARS-CoV-2 viral evolution and explore the crucial targets of treatment against COVID-19.

![HUPO BUSAN 2023 logo]

See you in Busan!
BEXCO, Busan, Korea

🌐 2023.hupo.org
🐦 f 📷 in #HUPO2023

Together Through Proteomics

PROGRAM

PP05.142: The Protein Methylation Network in Yeast: a Landmark in Completeness for a Eukaryotic Post-translational Modification

Marc Wilkins, Australia

Defining all sites for a post-translational modification (PTM) in a proteome, and identifying their cognate modifying enzymes, is essential for a complete understanding of a modification's function. This goal, however, has been extremely difficult to achieve for any modification type. Here we present the first near-complete example of a PTM network, involving all methylated proteins in S. cerevisiae and all relevant methyltransferases (1).

Following on from extensive proteomic analysis, and incorporating enzyme-substrate relationships, a formal process was used to define and quantify all potential sources of 'network incompleteness'. This was done for both the methylation sites in the proteome and also protein methyltransferases. From that we proved that this protein methylation network is essentially complete, containing 33 methylated proteins and 28 methyltransferases, comprising 44 enzyme-substrate relationships, and a predicted further 3 enzymes.

The completeness of this protein modification network is unprecedented. Interestingly, it allowed us to holistically explore the role and evolution of protein methylation in the eukaryotic cell. We show that while no single protein methylation event is essential in yeast, the vast majority of methylated proteins are themselves essential, being primarily involved in the core cellular processes of transcription, RNA processing and translation. This suggests that protein methylation in lower eukaryotes exists to fine-tune proteins whose sequences are evolutionarily constrained, providing an improvement in the efficiency of their cognate processes.

The approach described here, for the construction and completeness testing of post-translational modification networks and their constituent enzymes and substrates, defines a new formal process of utility for other post-translational modifications in the proteome.

(1) Hamey, J., Wilkins MR (2023) PNAS, in press.

PP05.143: Pushing the Boundaries for Robust and High-throughput Single Cell Analysis with Whisper Flow Technology Powered by dia-PASEF

Dorte Bekker-jensen, Denmark

Single cell proteomics is a field in rapid development and recent advancements deliver improved robustness and sensitivity in all parts of the workflow with an additional focus on sample preparation. This is essential to scale the number of cells for analysis, which is needed to gain biological insight from single cell proteomics. WhisperTM Flow technology combined with efficient capture and recovery of peptides from the Evotip Pure is critically important for the sensitivity needed for low sample amounts down to the single cell level. A prototype adaptor for single cell isolation and processing in the cellenONE has been developed for seamless integration and direct transfer of peptides into Evotips. Across 48 replicate injections, precursor identifications were more consistent using direct centrifugation compared to manual pipetting as would be expected.
96 single HeLa cells were isolated and digested in the Evotip adapter and transferred to Evotips via centrifugation and analyzed with the Whisper 40 SPD method on a timsTOF SCP Ultra in dia-PASEF mode. The single-cell proteome coverage was on average 3,500 protein groups per cell and close to 5,500 proteins across the dataset. Reassuringly, the performance remained stable throughout the 2.5 days required to measure the samples. During this period, the loaded Evotips were stored on the instrument at room temperature with no observed losses in proteome depth.
To demonstrate biological applicability, we isolated peripheral blood mononuclear cells (PBMCs) to perform unbiased cell type identification and assignment. This represents a highly relevant biological model system as PBMCs are an easily accessible source to understand immune system response. Overall, the data validates the technical robustness of the workflow and outlines a promising path for comprehensive proteome exploration at single-cell resolution.

PP05.144: Identification of Causal Genes for Nonalcoholic Fatty Liver Disease Using Multi-omics Based Single-cell Analysis

Sung Eun Hong, Republic of Korea

Background and aims
Nonalcoholic fatty liver disease (NAFLD) is an emerging liver disease associated with metabolic syndrome. Lack of effective treatment drugs urges the discovery of novel therapeutic targets. This study utilizes multi-omics-based single-cell analysis to discover biomarkers and therapeutic targets of NAFLD.

Methods
Liver biopsy samples obtained from 23 control individuals and 21 NAFLD patients were subjected to single nucleus RNA-sequencing (snRNA-seq). DNA samples obtained from the same participants were genotyped by low coverage whole genome sequencing. snRNA-seq profiles of the NAFLD liver were analyzed using various bioinformatics tools. Genotype and single-cell gene expression data were integrated to map single-cell expression quantitative trait loci (sc-eQTL).

Results
A total of 250K cells were detected, including hepatocytes and various non-parenchymal cells. Pseudotime analysis recovered the zonation information in hepatocytes, differentiation pattern in cholangiocytes, and activation process in stellate cells. Differentially expressed genes revealed cell type-specific changes in NAFLD. Multiple sc-eQTL signals in each cell type were detected.

Conclusions
We present transcriptomic profile of NAFLD in a single-cell resolution. sc-eQTL analysis identified NAFLD-associated genes and their regulatory variants in relevant cell types. The role of putative regulatory genes and variants will be subjected to functional validation.

PP05.145: Single-nucleus RNA-seq Reveals Key Contributors in Duchenne Muscular Dystrophy

Eun Young Jeon, Republic of Korea

Duchenne muscular dystrophy (DMD) is a devastating X-linked disorder caused by mutations in the dystrophin gene. Slowly deteriorating muscle forces patients to lose ambulation in their early teens and to die in their 20s, mostly due to respiratory or cardiac complications. Deflazacort, a glucocorticoid derivative, has been used to control inflammation and delay muscle weakness. Despite recent progress in understanding of the genetics and pathogenesis of the disease and attempts to treat it, DMD still remains a major pediatric muscle dystrophy without cure due to an incomplete characterization of the molecular and cellular interactions responsible for muscle maintenance and functions. Here, we present single-nucleus RNA-sequencing (snRNA-seq) results of muscles from DMD, milder-form Becker muscular dystrophy (BMD), and healthy controls, followed by spatial transcriptomics analysis of DMD and healthy muscles. In addition, to examine the effect of deflazacort, we administered the drug to D2-mdx mice and subjected their muscles to snRNA-seq. Integrated analysis of human and mouse muscles revealed pathogenic features in the patients and palliative effects of deflazacort. Meanwhile, it also highlighted the perturbation of proliferating satellite cells, leading to an increased signal transduction pathway involving EZH2, NR3C1, and cell cycle progressor proteins in the patient cells, which was confirmed by EZH2 ChIP-seq in satellite cells. We also demonstrate a therapeutic effect by perturbation of these pathways in D2-mdx mice through improved muscle phenotype. Our analysis of DMD patient muscles reveals pathogenic mechanisms that can be readily targeted by pre-existing therapeutic options.

PP05.147: Single Cell Proteome Analysis with Ultra-high sensitivity Using a timsTOF Mass Spectrometer

Christoph Krisp, Germany

For single cell proteome analysis, ultra-high sensitivity mass spectrometry is a key to reach proteome coverages necessary for understanding the cellular heterogeneity on a cell-by-cell level. Latest enhancements in ion transfer with a larger transfer capillary, an additional higher-pressure segment for more effective ion collection and two orthogonal deflections, to maintain robustness, and high-capacity trapped ion mobility spectrometry (TIMS) pushes the limits of detection to single cell level.

K562 cell digest (Promega) dilution series from 16 ng to 15 pg, in 2-fold dilution increments, was prepared. One, five, ten and twenty HeLa cells were isolated with a cellenONE into wells of a proteoCHIP Evo-96 prototype, lysed, digested, and transferred by centrifugation into a 96-well plate. Total volume was picked up and loaded onto an Aurora Elite column (IonOpticks) using a nanoElute2, separated with a 22 min active gradient, detected on a timsTOF mass spectrometer in dia-PASEF and analyzed using Spectronaut library-free and library-based approaches.

Here, we assessed the sensitivity of a timsTOF mass spectrometer using a dilution series of K562 cell digest showing excellent identification rates, reproducibility, and quantification accuracy per concentration replicates. Processing of the dia-PASEF data identified >1,000 protein groups out of 15 pg, and >7,000 protein groups out of 16 ng K562 peptides loaded on column. The quantitative accuracy improved inversely with loaded peptide amounts with 19% at 15 pg to 4% at loads of 4, 8 and 16 ng. Analysis of the isolated HeLa cells resulted in good identification rates and good reproducibility per individual cell count group with expected increase in protein abundance from the single cells to 20 cells.
The timsTOF mass spectrometer combined with automated single cell isolation and sample preparation using the cellenONE platform for protein-loss reduced preparation and transfer with the proteoCHIP format leads to deep proteome coverage and high reproducibility.

PP05.148: Increasing Proteomic Depth of Single-Cell Analysis by Feature Matching in diaPASEF data

Karl Kristian Krull, Germany

MS-based single-cell proteomics underwent a steep development in recent years, however, it still suffers from limitations in sensitivity and throughput. To overcome those, MS acquisition and data processing have received growing attention. In data-independent acquisition (DIA), collective precursor isolation allows accumulation of low abundant signals, but results in highly convoluted spectra. To cope with the resulting data complexity at generally low signal intensity, pre-generated libraries were successfully applied in the past, however, they disregard direct sample-to-sample comparison and thereby lose a valuable source of experimental information.
Here, we introduce DIA-ME, a novel data analysis strategy using matching enhancers (MEs). MEs are raw files derived from a higher-input samples that are analyzed in parallel with lower-input samples of the same type to increase proteome coverage. In this way, DIA-ME leverages the ability of existing DIA processing software to store peptide information in an internal library and augments inter-sample matching by providing a much greater information pool.
Using this concept, we increased identifications by 35% compared to conventional match-between-runs, reaching up to 4,800 protein groups from only 1 ng peptide input. We used a two-proteome model system and demonstrate that neither correct feature assignment nor quantification accuracy is disturbed. On the contrary, we found that error susceptibility of feature transfer was reduced as DIA-ME provides more experimental information to the matching algorithm. We applied our novel workflow to reproduce the known effect of IFN-γ on U-2 OS cells at peptide dilution and single-cell level and accomplished time-dependent differential expression analysis at unprecedented depth, highlighting gradual up-regulation of numerous IFN-γ-response proteins. These findings were in strong agreement with results from bulk experiments, thus validating the reliability of the DIA-ME analysis. As result, we expect it to overcome the requirement of pre-generating spectral libraries for low-input data by providing a simple and reliable solution.

PP05.150: Novel Developments in Single-Cell Proteomics: Clustering Analysis of Human PBMCs and High-Throughput LC-MS/MS Platform

Ziyi Li, China

Single-cell RNA sequencing has enabled comprehensively analysis of cell heterogeneity and systematic identification of cell populations in tissue by exploring gene expression profiles at single-cell resolution. However, the lack of amplification methods for proteins has hindered in depth single-cell proteomic (SCP) analysis. Recent developments of SCP technologies have overcome the limitation and realized such analysis. Nevertheless, there have been no reports on defining cell types in complex samples based on their SCP profiles.

In this study, we used the Ultra-sensitive and Easy-to-use multiplexed Single-Cell Proteomic workflow (US-SCP) platform, developed in our lab (bioRxiv, 2022), to achieve the proteomic analysis of 224 CD45+ peripheral blood mononuclear cells (PBMCs) sorted into a 384-well plate by CellenONE F1.4. This method allowed over 1200 quantified proteins per cell. By developing a clustering and cell assignment algorithm based on differentially expressed proteins, we successfully annotated the cell clusters of the 224 single-cells into 7 specific clusters. To further improve the throughput of the UE-SCP platform and make it possible for use in clinical applications, we combined the TMT6plex isobaric labeling method with the 30 sample per day (SPD) LC method of Evosep ONE liquid chromatography system. Subsequently, we were able to analyze 120 single cells per day under this high-throughput SCP platform, achieving an average of 1518 protein groups in each HEK-293T or Hela cells, and the two different cell lines could be unsupervised clustered based on their proteomes. The approach presented here demonstrates the potential for realizing clustering analysis of clinical samples with large-scale singe-cell proteomic profiles using the UE-SCP platform.

PP05.151: Single-cell and Low-input Proteomics Depicted an In-depth Landscape for Mouse Maternal-to-zygotic Transition

Chen Li, China

Single-cell and low-input multi-omics techniques have revolutionized the study of preimplantation embryo development. However, single-cell and low-input proteomics researches are relatively underdeveloped. To get enough depth proteome, hundreds of mouse embryos often need to be collected for each sample. Therefore, we developed a Comprehensive Solution of Ultrasensitive Proteome Technology (CS-UPT) for single-cell and low-input mouse oocytes and preimplantation embryos (J Pharm Anal, 2023, SI: Single-cell and ST Omics.). Both deep coverage and high-throughput routes significantly reduced the starting material and provided a full set of options for investigators' demands.

Maternal-to-zygotic transition (MZT) is a fundamental and conserved process, during which the maternal environment of the oocyte transitions to the zygotic genome driven expression program. Using the deep coverage route, we provided a large-scale snapshot of the very early stage of mouse MZT, including almost 5,500 protein groups from 20 mouse oocytes or zygotes for each sample. Moreover, significant protein regulatory networks centered on transcription factors and kinases between the MII oocyte and 1-cell embryo provided rich insights into minor zygotic genome activation.

N6-methyladenosine (m6A) is the most prevalent internal modification present in the mRNA across higher eukaryotes. However, the potential regulation role of m6A modification on RNA metabolism during MZT remains largely unknown. Here, we investigated m6A dynamics during mouse MZT through low-input multi-omics analysis. Our results showed that m6A could be maternally inherited or de novo gained after fertilization. By integrating SLIM-seq, LiRibo-seq, low-input proteomics and RIP-qRT-PCR data, we demonstrated that a group of maternal mRNAs with m6A mark were maintained throughout the MZT, and most of these genes were actively translated, indicating a role of m6A in safeguarding RNA stability for early embryo development (Genome Biol, 2023).

All these findings provided new insights and clues for the study of critical biological events during preimplantation embryo development in mice.

PROGRAM

PP05.152: Time-resolved Metabolite and Lipid Profiling Depicts Macrophage Continuum with Apoptotic and Ferroptotic Heterogeneity along Foam Cell Formation

Ling Lin, China

Abstract
Atherosclerosis is a chronic inflammatory disease driven by the maladaptive lipid metabolism and inflammatory response of macrophages. In the progression of atherosclerosis, macrophages work as a lipid scavenger and immune regulator by dynamic lipid and metabolic reprogramming in response to the microenvironment. Nevertheless, we are only starting to characterize the function of the heterogeneity in metabolite and lipid compositions. Herein, we developed a four-dimensional micro-manipulation platform for single-cell sample preparation, and observed the macrophage heterogeneity along foam cell formation via a single-cell time-resolved metabolite and lipid profiling. By integrating bulk metabolomic and lipidomic data with single-cell metabolomic and lipidomic data, we were surprised to uncover that macrophages have different fate outcomes during the late foam cell formation process. Some foam cells were more prone to apoptosis, while others are more susceptible to ferroptosis, suggesting heterogeneity in cell fate determination could have important implications for understanding atherosclerosis progression. Single-cell transcriptome sequencing was further performed on late-stage foam cells derived from both THP-1 and peripheral blood mononuclear cells, jointly confirming the divergent cell fates toward apoptosis or ferroptosis. Lastly, we validated the heterogeneity of late-stage foam cell differentiation through a series of in vitro experiments, including caspase activity assay and lipid peroxidation assay. In summary, single-cell multi-omics depict the molecular choreography that dictates the cell death in late atherosclerosis.

Key words: Single-cell analysis; Metabolomics; Lipidomics; Foam cell; Ferroptosis.

PP05.153: Water Droplet-in-oil Digestion Method with Enhanced Throughput for Single-cell Proteomics

Takeshi Masuda, Japan

Protein expression varies between cells, and observing this heterogeneity helps to understand biological phenomena. We have developed a water droplet-in-oil digestion (WinO) method for single-cell proteomics. The WinO method reduces adsorption losses of proteins and peptides during sample preparation and allows parallel sample preparation in multi-well plates. However, the original WinO method uses ethyl acetate, and requires a manual sample combining after digestion and drying steps, which are major obstacles to automating sample preparation. The purpose of this present study was to improve the throughput of the WinO method. In this study, we changed ethyl acetate to hexadecane, and a reversed-phase sulfonate column was used for desalting peptides instead of a reversed-phase column. The use of hexadecane, which is less volatile, eliminated the need for a refile step of organic solvent. In addition, the use of reversed-phase sulfonate column also eliminated the drying step and greatly simplified the process. Furthermore, the sample collection from the multi-wells was changed from manual operation to a liquid handling robot (24LX, BIOTEC). The 24LX dramatically reduced the sample collection time without decreasing the recovery rate. This modified WinO method enables automation of the sample preparation process and is expected to be applied to a large-scale single-cell proteomics.

PP05.154: Discovery of Post-translationally Modified and Variant Peptides in Single cell Proteomics Experiments

Dong-Gi Mun, United States

Recent advancement of mass spectrometry-based single cell proteomics enabled unbiased proteome profiling of thousands of proteins from single cell. The identification of proteins from single cells has been significantly improved through the implementation of an advanced sample preparation workflow and mass spectrometry with enhanced sensitivity. In particular, trapped ion mass spectrometry (TIMS) operated with parallel accumulation-serial fragmentation (PASEF) mode has provided significantly enhanced sensitivity in label-free approach. We optimized settings of TIMS for both data dependent acquisition (DDA) and data independent acquisition (DIA) modes for analyzing single cell samples. When applied for proteome profiling of single primary T cells, we demonstrated that the depth of proteome coverage was sufficient to delineate several essential metabolic pathways and T cell receptor signaling pathway, while also detecting several phosphorylated and acetylated peptides. After further optimization of DIA data interpretation strategy, we identified a total of 4,197 proteins from 200 single cell samples collected from three human cholangiocarcinoma cell lines and a normal cholangiocyte cell line. In addition to the identification of proteins, the improved depth of proteome coverage enabled the detection of various types of post-translational modifications of phosphorylation, methylation and acetylation at single cell level. Notably, for the first time, we report the identification of cancer cell line specific single nucleotide variants at peptide level in single cells of cancer cell lines including G12D variant of KRAS. We further tested whether we could quantitatively measure alterations in PTMs under perturbation. Increased phosphorylation was measured at the single cell level in calyculin A treated cells. Overall, we believe that this strategy will have direct impact on clinical applications to elucidate proteome and PTM changes at single cell level.

PP05.155: Evaluating Linear Ion Trap for MS3-based Multiplexed Single-cell Proteomics

Junho Park, Republic of Korea

The commonly used isobaric labeling-based multiplexed single-cell proteomics approach suffers from distorted protein quantification due to co-isolated interfering ions during MS/MS fragmentation, also known as ratio compression. We reasoned that the use of MS3-based quantification could mitigate ratio compression and provide better quantification. However, previous studies indicated reduced proteome coverages in the MS3 method, likely due to long duty cycle time and ion losses during multi-level ion selection and fragmentation. Herein we described an improved MS acquisition method for MS3-based single-cell proteomics by employing a linear ion trap to measure reporter ions. We demonstrated that linear ion trap can increase the proteome coverages for single-cell-level peptides with even higher gain obtained via the MS3 method. The optimized real-time search MS3 method was further applied to study the immune activation of single macrophages. Among a total of 126 single cells studied, over 1200 and 1000 proteins were quantifiable when at least 50% and 75% non-missing data were required, respectively. Our evaluation also revealed several limitations of the low-resolution ion trap detector for multiplexed single-cell proteomics and suggested experimental solutions to minimize their impacts on single-cell analysis.

PP05.156: Single-cell Proteomics Reveals the Difference on Early Tumor Evolution Between dMMR and pMMR Colorectal Cancer Organoids

Yan Ren, China

Introduction:
In Colorectal cancer (CRC), only mismatch repair deficiency (dMMR) patients positively respond to PD-1/PD-L1 blockade therapy. The difference in early tumor evolution between dMMR and mismatch repair-proficient (pMMR) would help to explain the situation and find good solutions for pMMR. CRC organoids reflect the spatial characteristics and genetic heterogeneity of their source samples, making them an innovative and promising model for nonclinical studies. Single-cell proteomics studies on them provide precision information on early tumor evolution at protein and function level.

Methods:
We obtained fresh tumor tissues from two CRC patients (one pMMR and one dMMR) and picked seven spheroids from their organoid cultures (4 pMMR, 3 dMMR). The spheroid cells were released by digestion and sorted by CellenOne. Mass-adaptive coating-assisted single-cell proteomics (Mad-CASP) method was adopted to quantify the protein changes in individual single cells.

Results:
Totally 132 cells were used for Mad-CASP analysis, and more than 5,000 proteins were identified. The variability on single cell protein expression among different spheroids was significantly higher than within the same spheroid. Hierarchical clustering analysis supported that spheroids performed distinct cellular activities, and represented the cellular subpopulations evolving to different stages during CRC development. Spheroids from dMMR are highly enriched in cellular respiration and cytokinesis pathways, while spheroids from pMMR prefer the function on lipid metabolic transport. It implies that in early tumor evolution, dMMR and pMMR tumor cells may adopt completely different metabolic pathways to accumulate substances and energy for their high-intensity protein synthesis and division activities.

Conclusion:
The single-cell proteome can provide more information on the characteristics of early tumor evolution, which could not be achieved in previous analyses on bulk samples, and can reveal the difference between dMMR and pMMR tumor evolution. This study provides pioneering evidence that Mad-CASP studies can well characterize key features of early CRC evolution.

PP05.157: Single-cell Proteomics Reveals Changes in Expression During Colorectal Cancer Organoids Development

Yan Ren, China

Introduction:
In Colorectal cancer (CRC), only mismatch repair deficiency (dMMR) patients positively respond to PD-1/PD-L1 blockade therapy. The difference in early tumor evolution between dMMR and mismatch repair-proficient (pMMR) would explain the situation and find good solutions for pMMR. CRC organoids reflect the spatial characteristics and genetic heterogeneity of their source samples, making them an innovative and promising model for nonclinical studies. Single-cell proteomics studies on them provide precision information on early tumor evolution at protein and function level.

Methods:
We obtained fresh tumor tissues from two CRC patients (one pMMR and one dMMR) and picked seven spheroids from their organoid cultures (4 pMMR, 3 dMMR). The spheroid cells were released by digestion and sorted by CellenOne. Mass-adaptive coating-assisted single-cell proteomics (Mad-CASP) method was adopted to quantify the protein changes in individual single cells.

Results:
Totally 132 cells were used for Mad-CASP analysis, and more than 5,000 proteins were identified. The variability of single-cell protein expression among different spheroids was significantly higher than within the same spheroid. Hierarchical clustering analysis supported that spheroids performed distinct cellular activities, and represented the cellular subpopulations evolving to different stages during CRC development. Spheroids from dMMR are highly enriched in cellular respiration and cytokinesis pathways, while spheroids from pMMR prefer the function on lipid metabolic transport. It implies that in early tumor evolution, dMMR and pMMR tumor cells may adopt completely different metabolic pathways to accumulate substances and energy for their high-intensity protein synthesis and division activities.

Conclusion:
The single-cell proteome can provide more information on the characteristics of early tumor evolution, which could not be achieved in previous analyses on bulk samples, and can reveal the difference between dMMR and pMMR tumor evolution. This study provides pioneering evidence that Mad-CASP studies can well characterize key features of early CRC evolution.

PP05.158: Single-cell Proteomics by Mass Spectrometry with Spatial Context in Mammalian Liver

Florian A Rosenberger, Germany

Introduction: Single-cell proteomics by mass spectrometry (MS)-based proteomics is an emerging and promising technology for the characterization of biological heterogeneity. It has so far been confined to cultured cells due to technological constraints. Extension of single-cell proteomics to the biologically relevant tissue context would greatly enhance our understanding of biological processes. Here we describe single-cell Deep Visual Proteomics (scDVP), a technology that integrates high-content imaging, laser microdissection and multiplexed MS.

Methods: We developed and benchmarked scDVP on mouse liver tissue. After staining and imaging, AI-guided segmentation outlined the hepatocytes. Single shapes were dissected with a laser microscope and collected individually in 384-well plates in an automated manner. We applied dimethyl labelling and the reference channel concept in multiplex-DIA to increase sensitivity. Peptides of 450 shapes were measured on a SCP timsTOF mass spectrometer in DIA-PASEF mode.

Results: Our scDVP workflow resolves the context-dependent, spatial proteome of murine hepatocytes at a current depth of 1,700 proteins per shape. Half of the proteome was differentially regulated in a spatial manner. This data enabled the reconstruction of metabolic pathways and their organization within intact tissue at single-cell resolution, including spatial partitioning of the urea cycle and degradation of very long chain fatty acids. Using a machine-learned model, we predicted the proteome of previously unseen cells solely from images. With this approach, we reconstructed whole-tissue spatial proteomes from only four staining markers. We are currently expanding scDVP to other cell types in human tissue and mapping high-resolution spatial proteomes, including the extracellular matrix in fibrotic liver.

Conclusions: Single-cell DVP delivers spatial proteomics of true single cells or cell shapes in intact tissue resulting in biologically relevant insights.

PP05.159: Accessible Low Input Proteomics Reveals Relationship Between Mitochondrial Potential, Lipid Metabolism, and Progenitor Associated Phenotype in the Basal Mammary Epithelium

Matthew Waas, Canada

Breast cancer, a heterogenous disease of multiple origins, is the most common cancer in women. Studies of the various subtypes of breast cancer have revealed similarities between the molecular profile of these subtypes to distinct populations of mammary gland epithelial cells. This evidence supports the hypothesis that the different epithelial cell populations serve as the cell-of-origin for the corresponding subtypes of cancer. Our hypothesis is that defining functional and molecular identities of distinct stem cell populations within the mammary gland epithelium will reveal potential markers and molecular vulnerabilities. Recently, we identified a metabolically defined subpopulation of mammary epithelial basal cells which harbor an increased capacity for colony formation representing a novel putative progenitor/stem population. We have adapted an innovative droplet-digestion sample preparation strategy relying on commercially available consumables and, in combination with systematic optimization of sample preparation, instrument acquisition, and data analysis methods, we reproducibly obtain >3,400 proteins on a Thermo Fusion mass spectrometer from 500 sorted cells. We have performed a repeatability study and observe median correlations between cell type replicates of 0.985 and 0.970 within and between batches, respectively. Finally, we have applied this platform to profile the proteomic phenotype of the metabolically defined putative epithelial stem/progenitor cells from six individual mice (~2000 cells/mouse). We identify over 3500 proteins per sample with high repeatability. From these data, we define the relationship between mitochondrial potential and the proteomic for both epithelial compartments. We identified and validated two surface markers which enrich for progenitor capacity. Future studies will compare the proteomic phenotype to stem cell populations sorted with other established markers and will investigate the functional capacity of this population. We anticipate the results of this research will harmonize our understanding of mammary stem cell biology and support breast cancer prevention, prognosis, and treatment efforts.

PROGRAM

PP05.160: Optimized Data-independent Acquisition Approach for Proteomic Analysis at Single-cell Level
Yuefan Wang, United States

Single-cell proteomic analysis provides valuable insights into cellular heterogeneity allowing the characterization of the cellular microenvironment which is difficult to accomplish in bulk proteomic analysis. Currently, single-cell proteomic studies utilize data-dependent acquisition (DDA) mass spectrometry (MS) coupled with a TMT labelled carrier channel. Due to the extremely imbalanced MS signals among the carrier channel and other TMT reporter ions, the quantification is compromised. Thus, data-independent acquisition (DIA)-MS should be considered as an alternative approach towards single-cell proteomic study since it generates reproducible quantitative data. However, there are limited reports on the optimal workflow for DIA-MS-based single-cell analysis.We report an optimized DIA workflow for single-cell proteomics using Orbitrap Lumos Tribrid instrument. We utilized a breast cancer cell line (MDA-MB-231) and induced drug resistant polyaneuploid cancer cells (PACCs) to evaluate our established workflow.We found that a short LC gradient was preferable for peptides extracted from single cell level with less than 2 ng sample amount. The total number of co-searching peptide precursors was also critical for protein and peptide identifications at nano- and sub-nano-gram levels. Post-translationally modified peptides could be identified from a nano-gram level of peptides. Using the optimized workflow, up to 1500 protein groups were identified from a single PACC corresponding to 0.2 ng of peptides. Furthermore, about 200 peptides with phosphorylation, acetylation, and ubiquitination were identified from global DIA analysis of 100 cisplatin resistant PACCs (20 ng). Finally, we used this optimized DIA approach to compare the whole proteome of MDA-MB-231 parental cells and induced PACCs at a single-cell level. We found the single-cell level comparison could reflect real protein expression changes and identify the protein copy number.Our results demonstrate that the optimized DIA pipeline can serve as a reliable quantitative tool for single-cell as well as sub-nano-gram proteomic analysis.

PP05.161: A High-sensitivity Low-nano -Flow LC-MS Configuration for High-throughput Sample-limited Proteomics
Runsheng Zheng, Germany

Recently, proteomics has seen a significant shift toward profiling smaller sample quantities down to individual cells. Obtaining high-quality data requires exquisite sensitivity, accuracy, and precision for all stages of the proteomics workflow, from sample collection to data analysis. As such, state-of-the-art liquid chromatography -mass spectrometry (LC-MS) technology plays a crucial role in investigating changes in protein expression.

Considering multiple factors for balancing the sensitivity and sample throughput in a low-nano flow LC-MS application, we established a novel configuration to run gradients at 100 nL/min on Thermo Scientific™ Vanquish™ Neo UHPLC system with an Acclaim™ PepMap™ 100 C18 50 µm ID x 15 cm column (2 um dp) in the direct injection and trap-and-elute workflows. Peptide ionization is carried out via a 10 µm ID glass emitter into a FAIMS Pro™ interface operated at a single compensation voltage to reduce background ion interference, followed by data acquisition in an Orbitrap Exploris™ 480 mass Spectrometer.
Ultimately, six high-performance methods were developed to permit fast sample loading, efficient column washing, and equilibration while maintaining a stable flow of 100 nL/min. For example, it enables up to 100 samples/day analysis with a 10-min peptide elution window (i.e., 70% MS utilization), offering extraordinary sensitivity for routine sample-limited proteomics analysis. Using this optimized method, we identified ~2,200 protein groups from 250 pg of standard protein digest using data-independent acquisition (DIA) in a library-free search. A proof of principle study indicated that >1,700 protein groups were identified from single-cell samples in a label-free quantification (LFQ) experiment with neglectable carryover in a 100 cells/day (CPD) throughput. Further employment of library-based search and a faster mass spectrometer significantly boost peptide and protein identification.
In conclusion, we demonstrate a high-sensitivity LC-MS/MS configuration that serves the needs for sample-limited analysis, permitting 100 CPD throughput for single-cell proteomics using LFQ-DIA.

PROGRAM

PP05.162: High Throughput XL-MS Analysis on a New High-resolution Accurate Mass Platform

Yi He, United States

Introduction
Cross-linking mass spectrometry (XL-MS) has grown dramatically into a routinely utilized strategy for elucidating protein higher-order structure and mapping protein-protein or protein-nucleic acid interaction networks on a proteome-wide scale. However, due to sample complexity and low abundance of the XL peptides, it remains a significant challenge for the sensitivity and speed of the mass spec instrument. In this work, we optimized XL-MS workflows on the novel high-resolution mass spectrometer and achieved three times throughput for XL-MS analysis.

Methods
The amine-reactive, enrichable crosslinker TBDSPP (tert-Butyl Disuccinimidyl Phenyl Phosphonate, tBu-PhoX)) was used to crosslink proteins and Ecoli cells. A novel HRAM mass spectrometer was coupled to Vanquish Neo nano-LC. Data were analyzed using Thermo Scientific™ Proteome Discoverer™3.0 software and XlinkX 3.0 nodes. The optimized operation condition was selected followed by testing MS2 acquisition speed, AGC control, collision energy and resolution.

Results
XL-MS has become a universal tool for studying protein structure and protein-protein interaction networks, however the challenge in this workflow is the low abundance of crosslinked species and sample complexity, especially for intra-cellular XL. Sample fractionation and long LC gradients are usually used to improve the identification of crosslinked peptides but lead to low throughput for this type of analysis. Compared to conventional mass spectrometry, the novel mass spectrometer increases analytical performance by combining high-resolution with fast-speed MS2 analysis up to 200 Hz. Using 60 min gradient we observed 35% increase in the identification of tBuPhoX crosslinks, 95 % increase in looplinks and 85% in monolinks peptides. To test the throughput of the instrument, we reduced the gradient to 20 min and identified comparable numbers of crosslinks to the 60 min gradient. More importantly, 75% of the identified crosslinks overlapped between two gradients. Taken together, we demonstrated a high throughput XL-MS workflow on the new mass spectrometer.

PP05.163: Differential N-Glycosylation Site Occupancy Depends on Distinct Amino Acid Sequence Features of Oligosaccharyltransferase and Acceptor Polypeptides

Marium Khaleque, Australia

Introduction:
Oligosaccharyltransferase (OST) is the key enzyme in the N-glycosylation process which catalyses the transfer of a pre-assembled glycan from a lipid-linked oligosaccharide (LLO) to select asparagine in Asn-Xaa-Thr/Ser (Xaa≠Pro) sequons of newly synthesized polypeptides in the lumen of the endoplasmic reticulum. Glycoproteins can contain more than one possible N-glycosylation site and 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. Here, we aimed to understand how control of LLO biosynthesis affects site-specific N-glycosylation occupancy and subsequently the interactions between the OST, N-glycan donor, and the peptide acceptor that contribute to site-specific N-glycosylation occupancy.

Method: We used 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 compared global site-specific glycosylation under two LLO stress conditions: structural and concentration stress. Then we performed a molecular dynamic simulation to investigate the possible amino acids in OST responsible for interacting with the donor glycan and/or acceptor peptide molecule. Finally, we tested these interactions through site-specific mutations and glycoproteomics to observe their contributions to determining site-specific N-glycosylation occupancy.

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. We also found that specific sites in OST were responsible for differential N-glycan occupancy in diverse N-glycosylation sites in substrate glycoproteins.

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 sequence features beyond the core N-glycosylation sequon by interactions with specific sites in OST.

PP05.164: Probing Protein Interactome Dynamics Using an Experimental Library of Protein Complex Interfaces

Cathy Marulli, Switzerland

Protein complexes are essential to cellular processes, and methods to systematically monitor their dynamics are much needed. Here we introduce FiLiP-MS, a structural proteomics workflow for global profiling of protein binding interfaces directly in cell lysates. We apply limited proteolysis and mass spectrometry (LiP-MS) to generate an atlas of peptides at protein complex-binding interfaces based on differences in protease accessibility between the protein complex-bound and monomeric forms. We then use this information to track changes in protein-protein interactions under conditions of interest. Applied to S. cerevisiae, FiLiP-MS yielded 6441 candidate interface markers from 1086 proteins at 5% FDR. After quality assessment, we used this FiLiP-MS protein binding interface library to probe interactome changes in yeast grown under DNA-replication stress, capturing known and novel protein complex rearrangements, and revealing that SAGA acetyltransferase activity is upstream of the rearrangement of SAGA and nucleosome complexes. Our data suggest that DNA replication stress in yeast is associated with the SAGA to SLIK transition and we uncover for the first time a link between Gcn5 activity and the regulation of P-bodies. Our library of binding interfaces is a stand-alone resource, and similar libraries could be generated for any organism of interest. FiLiP-MS enables protein complex dynamics to be monitored upon any perturbation on a proteome-wide scale, at high throughput and with peptide-level structural resolution, thus providing both global and molecular views of a system under study.

PP05.165: deMix_GUI Provides a Solution for Automatically Analyzing HDX-MS Data and Investigating Protein Conformational Changes

Seungjin Na, Republic of Korea

Hydrogen/deuterium exchange (HDX) with mass spectrometry (MS) is one of the most popular methods for examining protein conformational changes and dynamics. We previously developed a fully automated algorithm called deMix to analyze HDX-MS data in-depth. Here, we introduce a graphical user interface (GUI) version for deMix, which aims to facilitate the analysis and interrogation of data and results. deMix offers a peptide-level view and a protein-level view of analysis results. In the peptide centric view, deMix offers two windows for viewing spectrum data: one shows a natural isotope distribution (Dnat), and the other shows the corresponding deuterated isotope distribution (Ddeu) for the selected D2O labeling time. Users can see the input peptides and their HDX analysis results in the peptide & HDX results table. When the users select the specific peptide and D2O labeling time in the tables, deMix displays the experimental Dnat and Ddeu of the peptide with their theoretical Dnat and Ddeu in the spectral view window. For a better analysis of distributions, it contains zoom in and out of each scan's isotope distribution to allow examination of all isotope distributions over the elution time spans of peptides. With the deMix's deuteration rate plot across D2O labeling time, users can visually compare the deuterium uptake/rate between groups or estimate the saturation rate. In the protein centric view, deMix offers a sequence coverage map for HDX-MS data. For each peptide, the coverage map represents the HDX rate of each D2O labeling times using colors. Furthermore, deMix allows users to examine the protein's conformational change between control and experimental conditions through the included differential analysis tools. . These intuitive and interactive GUI or features helps users to effortlessly adapt to the deMix environment, allowing them to focus more on analysis. deMix is available at https://prix.hanyang.ac.kr.

PP05.166: Structural Premise of Selective Deubiquitinase USP30 Inhibition by Small-Molecule Benzosulfonamides; Implications for Mitophagy and Neurodegeneration

Darragh O'Brien, United Kingdom

Dampening functional levels of the mitochondrial deubiquitylating enzyme USP30 has been proposed as an effective therapeutic strategy against neurodegenerative disorders such as Parkinson's Disease. USP30 inhibition may counteract the deleterious effects of impaired turnover of damaged mitochondria which is inherent to both familial and sporadic forms of the disease. Small-molecule inhibitors targeting USP30 are currently in development, but little is known about their precise nature of binding to the protein. We have integrated biochemical and structural approaches to gain novel mechanistic insights into USP30 inhibition by a small-molecule benzosulfonamide containing compound, USP30inh. Activity-based protein profiling (ABPP) mass spectrometry confirmed target engagement of the inhibitor with endogenous USP30 in a neuroblastoma cell line, with a high selectivity and potency compared to 49 other deubiquitylating enzymes measured. In vitro characterization of USP30inh enzyme kinetics inferred slow and tight binding behavior, which is comparable with features of covalent modification of USP30. Finally, we blended hydrogen-deuterium exchange (HDX) mass spectrometry and computational docking to elucidate the molecular architecture and geometry of USP30 complex formation with USP30inh, identifying structural rearrangements at the cleft of the USP30 thumb and palm subdomains. Our results suggest that USP30inh binds to the thumb-palm cleft that guides the ubiquitin C-terminus into the active site, thereby preventing ubiquitin binding and isopeptide bond cleavage, and confirming the importance of the region to the inhibitory process. Our data will pave the way for the design and development of next-generation inhibitors targeting USP30 and associated deubiquitinylases.

PROGRAM

PP05.167: Identification of Novel Protein Metal-Binding Sites Using Limited Proteolysis-Coupled Mass Spectrometry (LiP-MS)

Jan-philipp Quast, Switzerland

Protein-metal interactions play critical enzymatic, structural and regulatory roles across all biological processes and kingdoms of life. Metal ions can be found in more than one third of enzymes where they serve as cofactors to enable catalysis. Moreover, they function as signaling molecules and stabilize proteins as structural elements. We still lack knowledge about the location of many metal-binding sites in proteins as well as their physiological relevance because system-wide characterization remains difficult. Studying protein-metal interactions at a proteome-wide level is therefore essential to achieve high-throughput kinetic as well as functional annotation of metal-binding sites.

We have developed a method that uses the structural proteomics approach limited proteolysis-coupled mass spectrometry (LiP-MS) in combination with titration of chelators in cell lysates to locate known and identify novel metal-binding sites in proteins. We validated our method with the well-annotated E. coli proteome for which we obtain enrichments of up to 60% metal-binding proteins among the hits based on UniProt annotations. If we exclude ribosomal proteins, which are well known to be sensitive to metal ions but rarely annotated as metal-interacting, we obtain enrichments of around 80%. For most of the remaining proteins, metal-site prediction tools often identify probable metal-binding sites. Furthermore, structural changes detected by LiP-MS are frequently in close proximity to annotated metal-binding sites, confirming that we can use our method to narrow down the location of metal-binding sites within proteins. Interestingly, we see that different chelators have different mechanisms of action. The chelator Deferasirox generally causes proteolytic resistance in metal-binding proteins as compared to EDTA, BAPTA and TPEN, which make them more susceptible to proteolysis. Finally, we can extract EC50 values from the fitted dose-response curves, which, for the first time, allow relative metal-affinity estimates for many proteins simultaneously.

PP05.168: Molecular Organization of Signaling at the Plasma Membrane in Mouse Brain Revealed by High-Resolution Complexome Profiling

Uwe Schulte, Germany

Signaling processes at and across the plasma membrane of eukaryotic cells are generally characterized by high functional specificity, spatial precision and speed. Targeted functional proteomic analyses of individual receptors and ion channels has demonstrated that these properties are achieved by defined biochemical association of the involved proteins into protein complexes and networks. A systemic assessment of the molecular organization of membrane signaling has however been lacking.

We applied our recently developed cryo-slicing Blue Native Gel-based high-resolution complexome profiling approach (csBN-MS, Müller et al., 2016) to study plasma membrane of mouse brain. Gently solubilized complexes were size-separated by Blue Native gel electrophoresis and the lane cut into 368 slices à 0.3 mm subsequently analyzed by LC-MS/MS. Proteins were identified using Mascot and quantitatively evaluated by MaxQuant and in-house developed software as described (Schulte et al., 2023). Selected examples of newly identified channel and receptor complexes were further characterized by biochemical and functional analyses.

The obtained complexome dataset provided detailed information on the quantitative size distribution (ranging from 80-5000 kDa) of more than 5000 proteins. Evaluation of these profiles showed a high degree of molecular complexity across all major classes of signaling proteins. In line with the results obtained recently from yeast mitochondria (Schulte et al., 2023), molecular appearance of proteins did not correlate with primary biochemical properties (such as membrane topology or size) but was distinct for functional classes. In addition, detailed cluster analysis provided a high-density map indicating thousands of distinct protein assemblies. We used examples from four different classes of proteins involved in excitatory synaptic glutamatergic signaling, namely AMPAR-type, Kainate-type, NMDA-type and metabotropic glutamate receptors (mGluRs) to demonstrate the broad applicability of this dataset.

The established mouse brain complexome represents a valuable resource for investigating molecular mechanisms, protein function, complex biogenesis and structure at the plasma membrane.

PROGRAM

PP05.169: How to Study Flexible Domains on Membrane Proteins - Combining Cryo-EM, Limited Proteolysis-coupled and Crosslinking Mass Spectrometry

Dina Schuster, Switzerland

Membrane proteins, due to their low expression levels and high hydrophobicity, are difficult to study and underrepresented in common bottom-up proteomics experiments. They often contain large stretches of flexible, partially disordered domains, which are not amenable to conventional structural biology techniques.

We use a combination of cryo-EM, limited proteolysis-coupled mass spectrometry (LiP-MS) and cross-linking mass spectrometry (XL-MS) to obtain a comprehensive understanding of how calmodulin interacts with the cyclic nucleotide-gated ion channel of retinal rods and thereby regulates visual signal transduction. We apply LiP-MS on native retinal membrane suspensions and XL-MS on purified protein to gain insight in structural features that cryo-EM could not resolve.

We additionally apply this combination to study membrane adenylyl cyclases (ACs), a class of proteins crucial for cellular signaling. We focus on AC8, an AC-isoform that is important for neuronal processes. Using LiP-MS, XL-MS and cryo-EM we manage to propose a structural model of how AC8 is regulated and demonstrate the role of its flexible domains.

For LiP-MS experiments, membrane suspensions were titrated with the respective interactor, followed by pulse proteolysis and a tryptic digest under denaturing conditions. Data was acquired in DIA mode on different Orbitrap mass spectrometers and analyzed using Spectronaut (Biognosys) and the R package protti.

Purified proteins were cross-linked using complementary chemistries (primary amine-primary amine, primary amine-acidic residues, acidic residues-acidic residues). Data was acquired on a Thermo Scientific Fusion Lumos Tribrid mass spectrometer and analyzed using xQuest.

For single-particle cryo-EM, detergent-purified proteins were blotted onto carbon grids, plunge-frozen and data was collected on a Thermo Scientific Krios electron microscope.

We benchmarked the application of LiP-MS to membrane suspensions, developed the R package protti to analyze the data comprehensively and apply the workflow to study two different membrane proteins involved in signal transduction, showing that their flexible domains are crucial for regulation.

PP05.170: Quantifying the Direct Human DNA Interactome in Response to Transcription-Modulating Drugs

Jakob Trendel, Germany

The human genome is embedded in proteins, which organize its structure, its replication and how the information it encodes is put into action. While many DNA-binding proteins and their DNA-binding domains are known and well-studied, so far no systematic inventory has been taken of the proteome that interacts with DNA directly. In this study we set out to record an atlas of DNA-interacting proteins from cultured cells, as well as quantify changes in this interactome when cells undergo a treatment. Therefore, we developed a novel high-powered photo-crosslinking system to covalently couple proteins to DNA in live cells. In combination with a new extraction strategy this allows us to purify protein crosslinked to the genome and quantify it by mass spectrometry (MS). Because crosslinking with the here applied photo-activatable nucleotide occurs at 'zero distance' the interactome gives a snapshot of proteins directly engaging with DNA at the moment of irradiation. Indeed, peptide-nucleotide crosslinks in our MS data document many of these direct interaction. We present a catalogue of more than 1300 high-confidence DNA interactors, many of which were previously unrecognized as DNA-binding. We show that DNA repair proteins are recruited to the genome and strongly increase their relative abundance in our MS quantification when DNA damage is induced by chemotherapeutic drugs. Moreover, we follow transcription factors engaging with the DNA in a dose-dependent fashion when cells are subjected to different concentrations of estrogen or cortisol. Along with the known interactions of the nuclear hormone receptors we find previously unreported dose-dependent protein-DNA interactions in response to hormone treatments, showcasing the methodology for the development of transcription-modulating drugs and more general the study of genomic regulation.
In summary, we present the human DNA-interacting proteome and a system to quantify changes in this interactome during cellular pertubations.

PROGRAM

See you in Busan!
BEXCO, Busan, Korea
2023.hupo.org
#HUPO2023

Together Through Proteomics

Session Date/Time: Wednesday, September 20, 2023 - 01:15 PM - 02:15 PM

ISS14: Seminar Supported by Nautilus Biotechnology

13:15	**Expanding Proteomic Horizons with Single**	
	Parag Mallick, United States	

13:45	**Molecule Analysis at Scale**	
	Birgit Schilling, United States	

Session Date/Time: Wednesday, September 20, 2023 - 02:30 PM - 03:35 PM

CS28: AI and Bioinformatics

Chair
Eunok Paek, Republic of Korea

Chair
Alexey Nesvizhskii, United States

14:31	**CS28.01: Keynote Speaker - Ending Brute Force Mass Spectrometry-Based Proteomics Data Acquisition Using Machine Learning**
	Mathieu Lavallée-Adam, Canada

14:56   **CS28.02: Completing the Human Functional Proteome Together!**

Lydie Lane, Switzerland

Background
Currently, about 8% of the human protein-coding genes have no function annotated. Yet, protein products have been confidently identified for many of these genes, and they might play important roles in human biology. The HUPO Human Proteome Grand Challenge was launched recently to fill this important knowledge gap. While protein structure prediction has become highly accurate, predicting protein functions remains very challenging. Therefore, finding a function for every protein will require the cooperation of experimental scientists, biocurators and computational scientists.
To stimulate such collaborations, neXtProt offers new community pages with manually reviewed functional predictions for uncharacterized proteins, ready for experimental testing.

Methods
Proteins lacking functional annotations and those that are solely annotated with broad Gene Ontology (GO) terms are considered uncharacterized. They can be retrieved here: https://www.nextprot.org/proteins/search?mode=advanced&queryId=NXQ_00022.
Functional hypotheses were either retrieved from the literature or manually generated by our own data mining protocol. They were standardized using GO molecular function (MF) or biological process (BP) terms and displayed in the Function prediction pages located in the Community section of the corresponding entries. For full traceability, the underlying data and method used are indicated (as ECO terms), as well as the organism in which the primary data was obtained, the reference of the publication and/or the ORCID of the submitter.

Results
Function predictions are provided for 239 uncharacterized proteins, on a total of 1521
(neXtProt release 2023-04-18). Out of these 239 proteins (https://www.nextprot.org/proteins/search?listId=3O1KQY7L), 227 have been validated by mass spectrometry.
Most of the predictions (90%) are GO BP terms. The data types that mostly contributed to the predictions were phenotypes, expression, subcellular location and interactions, in decreasing order.

Conclusions
We invite all scientists to test and refine these predictions and to submit new ones, obtained by manual or automated approaches. Let's complete the human functional proteome together!

15:09 **CS28.03: A Multi-Scale Map of Proteome Organization From Integration of Protein Interactions and Images**

Leah Schaffer, United States

Much of how the proteome is organized into subcellular components remains unknown. Towards this goal, we constructed a global Multi-Scale Integrated Cell (MuSIC) map of proteome organization in the U2OS cell line using a machine learning approach to systematically integrate large-scale protein imaging and interaction data. We integrated two major proteomic resources: immunofluorescence (IF) images from the Human Protein Atlas (HPA) and the BioPlex interaction networks derived from systematic affinity-purification mass spectrometry (AP-MS) experiments. The resulting U2OS MuSIC hierarchical map contains protein subsystems across biological scales, spanning from small protein complexes to large organelles.

Deep neural networks were used to embed each protein in each data modality separately. Embeddings from the two separate modalities were integrated with a recently developed unsupervised deep learning method for multi-modal structured embedding (MUSE) to create an integrated co-embedding for each protein that captures information from both original data modalities. Pan-resolution community detection was performed on the similarities between co-embeddings to construct a hierarchy of subcellular components. The pipeline for creating multi-scale integrated cell maps is freely available as part of a Python toolkit.

The U2OS multi-scale integrated cell map (MuSIC v2.0) places >5000 proteins into 270 distinct subcellular components, representing approximately half of proteins expressed in U2OS. The U2OS MuSIC map recovers known organelles and protein complexes as well as 152 putative novel components that have no significant overlap with a known Gene Ontology term. We used the U2OS cell map to interpret pediatric cancer patient mutational patterns, which are extremely rare across patients at the gene-level, by determining how different gene mutations significantly affect common multiprotein subcellular components in the map. Multi-scale integrated cell maps of proteome organization thus reveal novel biological systems and enable a mechanistic understanding of complex diseases.

15:22 **CS28.04: Deep Learning Based Mass Spectra Prediction Facilitates Proteomics Data Analysis**

Liang Qiao, China

During the past years, deep learning has enabled many practical applications and attracted extensive attention. Deep neural networks can learn different representations of objects and recognize complex patterns from large datasets. Efforts have been made using deep neural networks for MS/MS spectrum prediction, de novo peptide sequencing and RT prediction, indicating great potential of deep learning in the field of proteomics.
In 2020, we proposed DeepDIA [1], a deep learning-based approach to generate in silico spectral libraries for DIA analysis. We demonstrate that the quality of in silico libraries predicted by instrument-specific models using DeepDIA is comparable to that of experimental libraries, and outperforms libraries generated by global models. With peptide detectability prediction, in silico libraries can be built directly from protein sequence databases. We further illustrate that DeepDIA can break through the limitation of DDA on peptide/protein detection, and enhance DIA analysis on human serum samples compared to the state-of-the-art protocol using a DDA library.
In 2023, we further proposed DeepFLR [2], a deep learning-based framework for controlling the FLR in phosphoproteomics. DeepFLR includes a phosphopeptide tandem mass spectrum (MS/MS) prediction module based on deep learning, and an FLR assessment module based on a target-decoy approach. Compared to existing tools for MS/MS prediction of phosphopeptides, DeepFLR improves prediction accuracy. Furthermore, DeepFLR estimates FLR accurately according to evaluation using both synthetic and biological datasets, and localizes more phosphosites than probability-based methods. DeepFLR is capable of handling data from different types of instruments and organisms, and assisting in data analysis by both data-dependent acquisition (DDA) and data-independent acquisition (DIA) approaches.
Reference:
[1] Y. Yang, X. Liu, C. Shen, Y. Lin, P. Yang, L. Qiao, Nature Communications, 2020, 11, 146
[2] Y. Zong, Y. Wang, Y. Yang, D. Zhao, X. Wang, C. Shen, L. Qiao, Nature Communications, 2023, 14, 2269

Session Date/Time: Wednesday, September 20, 2023 - 02:30 PM - 03:35 PM

CS29: Covid and Long Covid

PROGRAM

Chair

Robyn Kaake, United States

Chair

Daesub Song, Republic of Korea

14:31 **CS29.01: Keynote Speaker - Multi Omics Analysis of SARS-CoV2 Infected Mouse**
Je Kyung Seong, Republic of Korea

14:56 **CS29.02: COVID-eQTL: Genetic Dissection of COVID-19 Susceptibility**
Jeongha Lee, Republic of Korea

A world-wide pandemic caused by SARS-CoV-2 virus provided an unprecedented opportunity to investigate the genetic susceptibility of virus infection and severity determination. For example, COVID-19 Host Genetics Initiative has released genome-wide associated variants from more than 100K infected individuals with COVID-19 severity. However, this study is not suited to analyze complex network of genomic variants and gene expression, which may eventually affect disease severity and prognosis. To address this, we took advantage of the multi-omics data of COVID-19 patients with mild or severe symptoms and performed expression quantitative trait loci (eQTL) analysis. The data set is composed of whole genome sequencing, blood-based single cell RNA-seq, cytokine profiling, TCR/BCR, and HLA data from 495 individuals with mild or severe COVID-19 symptoms over three time points. Our eQTL discovery pipeline uncovered a number of signals that are specific to severity and disease progression from various cell types and serum cytokine levels. This approach identified a number of variant-gene associations that are differentially regulated by disease status and their molecular upstream signals that eventually modulate disease progression in individuals of different genotypes.

15:09 **CS29.03: High-Throughput Mining of Proteomics Datasets for Evidence of Proteolysis during SARS-CoV-2 infection**
Peter Bell, Canada

Proteolytic processing is the most ubiquitous post-translational modification and regulator of protein function. There is a need to characterise proteolysis at the whole-proteome scale to better understand its role in biological processes, health and disease. To address this, various terminomics workflows (e.g. TAILS, COFRADIC, HUNTER) have been developed to enrich the proportion of protein termini in a sample. Subsequent proteomic analysis of these enriched samples then reveals evidence of cleavage sites within proteins. However, for an overwhelming majority of sample types and experimental conditions, terminomics methods have not yet been applied. Furthermore, non-enriched datasets – which comprise 99.5% of datasets in proteomics repositories – are seldom probed for evidence of proteolysis. As such, mining of non-enriched proteomics datasets for neo-termini presents an under-utilised opportunity to increase our understanding of proteolytic processing in diverse biological processes, sample types, conditions and stimuli.

In this study, we used FragPipe to perform a meta-analysis of >20 publicly available non-enriched proteomics datasets of SARS-CoV-2 infected cells. In doing so, we identified hundreds of neo-termini that were reproducibly generated upon infection, indicative of extensive proteolysis mediated by both host and viral proteases. Furthermore, our analysis revealed protein cleavages that were unique to subsets of cell lines, which may influence cell-tropism of SARS-CoV-2. These findings demonstrate that re-analysis of existing proteomics data is a valuable strategy for terminomics research, and increases our understanding of virus-host interaction in SARS-CoV-2 infection.

15:22   **CS29.04: Persistent and Prevalent New-Onset Autoantibodies in Mild to Severe COVID-19**

August Jernbom Falk, Sweden

Background
After the acute phase of COVID-19, many individuals experience persistent and greatly varying symptoms known as post-acute sequelae of COVID-19 (PASC). Although the underlying and likely diverse causes of PASC remain unknown, pre-existing and new-onset autoantibodies are major hypotheses. To elucidate the role of new-onset autoantibodies in COVID-19, we analyzed their prevalence, persistence, and PASC associations in 525 healthcare workers (HCW) and inpatients followed over 3-5 visits, spanning 8-16 months of the pandemic.

Methods
Using proteome-wide planar protein arrays, we performed an initial screening of the autoantibody repertoire of 32 HCW. Antigens corresponding to detected autoantibodies were coupled in a bead array which was used to characterize the longitudinal autoantibody profiles of all 525 individuals. Furthermore, two machine learning approaches were used to identify new-onset autoantibodies. Finally, we performed epitope mapping of 6 autoantibodies using peptide arrays.

Results
Among the 369 baseline seronegative individuals, 55% (n=204) developed new-onset autoantibodies at seroconversion. Of these, 60% remain elevated for 12 months. In total, we detect 22 new-onset autoantibodies that emerge in ≥1% of the cohort, with ICU patients having an increased autoantibody load. Three prevalent new-onset autoantibodies, anti-MYO16, anti-CALU, and anti-SNURF IgG, are associated to increased severity of neuropsychiatric symptoms following COVID-19. Furthermore, anti-PCYT1B and anti-SNURF IgG additionally increase at vaccination. Finally, we epitope-map 6 new-onset autoantibodies and validate them in an independent cohort with Neuro-COVID.

Conclusions
In this study, we show that diverse and prevalent new-onset autoantibodies develop with COVID-19. The autoantibodies persist for 12 months and are more common in severe than mild COVID-19. Furthermore, three autoantibodies are associated to neuropsychiatric PASC, and are found in independent cohorts with Neuro-COVID. In summary, these results illustrate the need for untargeted autoantibody profiling and strengthen the connection between new-onset autoantibodies and neuropsychiatric PASC.

Session Date/Time: Wednesday, September 20, 2023 - 02:30 PM - 03:35 PM

CS30: Epigenetic and Aging

Chair
Je Yoel Cho, Republic of Korea

Chair
Christina King, United States

14:31   **CS30.01: Keynote Speaker - The Aging Brain Proteome**

Alessandro Ori, Germany

See you in Busan!
BEXCO, Busan, Korea
2023.hupo.org
#HUPO2023

Together Through Proteomics

PROGRAM

14:56   **CS30.02: Proteomic and Functional Exploration of Anti-aging Molecules**

Haiteng Deng, China

Multiple chronic conditions are prevalent among elderly population over the world and warrant a great research effort to reduce diseases burden as World's elderly population grows at an unprecedented rate. The quest to slow ageing and to increase healthspan has become the frontier in biomedical research. It has been amply documented that intervention in biological systems can delay the onset and progression of chronic diseases. Herein, we examined the biological functions of two promising interventions: NAD+ enhancers and senolytics.

We recently used comparative proteomics analysis to profile age-associated changes in proteome in mouse organs. We found that nicotinamide mononucleotide (NMN) restores redox homeostasis via the Sirt3−Nrf2 axis and protects aged mice from oxidative stress-induced liver injury. Furthermore, NMN can restore peroxisome biogenesis in aged mouse kidney. We have showed for the first time that oxidative stress causes protein sulfation, which triggers protein degradation. Consequently, nicotinamide mononucleotide (NMN) stabilizes tumor suppressors, such as 15-PGDH, DHFR and STAT1, which prevent aging associated inflammation, liver fibrosis and tumors. On the other hand, we applied thermal proteome profiling to identify targets of known senolytics such as quercetin. We further revealed that Flavonoid 4,4'-dimethoxychalcone (DMC) induced ferroptosis to eliminate senescent cells by synergistically activating Keap1/Nrf2/HMOX1 pathway and inhibiting FECH.

In summary, the present study demonstrated that aging-associated oxidative stress degraded tumor suppressors via protein sulfation. NMN is an effective molecule for tumor chemoprevention. Our data provides a valuable resource for understanding the age-associated changes, and reaffirms that mass spectrometry is a powerful tool in aging studies.

15:09   **CS30.03: USP7 Regulates the ncPRC1 Polycomb Axis to Stimulate Genomic H2AK119ub1 Deposition Uncoupled from H3K27me3**

Jeroen Demmers, Netherlands

Ubiquitin-specific protease 7 (USP7) is a deubiquitylating enzyme that is involved in the regulation of multiple key cellular processes, including tumor suppression, transcription, epigenetics, the DNA damage response, and DNA replication. For debiquitinating enzymes, the regulatory mode of action is at the posttranslational level and proteomics tools are particularly suited to study this. Here, we took an unbiased multi-omics approach with a heavy targeted quantitative proteomics component to define the core USP7 network.

We performed interactomics and in-depth global proteomics to identify the USP7 interaction network. Targeted proteomics using parallel reaction monitoring (PRM) was done to accurately quantify all relevant players of the Polycomb system in a label free manner.

Integrating unbiased proteomics, genomic, and functional molecular approaches, we determined the core USP7 regulatory circuitry. This multi-angle analysis establishes USP7 as a regulatory hub in a multinodal network involved in tumor biology, protein (de)ubiquitylation, and genome regulation.

Here, we followed-up on the regulation of the Polycomb system by USP7. USP7 modulates the ncPRC1 axis at the posttranslational level through stabilization of ncPRC1.6 and ncPRC1.1. At the transcriptional level, USP7 silences AUTS2, the subunit that suppresses H2A ubiquitylation by ncPRC1.3/5. Collectively, these USP7 activities increase the genomic deposition of H2AK119ub1. Contradicting prevalent paradigms of Polycomb function, our findings reveal that changes in H2AK119ub1 are generally uncoupled from H3K27me3 and thus argue against a hierarchical relationship between these two repressive histone marks. Importantly, the connection of USP7 to the Polycomb system suggests that its role in cancer extends beyond regulation of p53. Our interactomics assay shows that USP7 has a remarkable range of interaction partners, of which only a portion appears to be stabilized by USP7.

Combined, our multi-omics results provide a resource for future studies on the role of USP7 in (neuro) development and cancer.

![HUPO BUSAN 2023 SEP 17-21 · 22nd Human Proteome Organization World Congress]

See you in Busan!
BEXCO, Busan, Korea
🌐 2023.hupo.org
🐦 f 📷 in #HUPO2023

Together Through Proteomics

PROGRAM

15:22   **CS30.04: Functional Phosphoproteomic Analysis of Insulin Signalling in Ageing Bone**

Mriga Dutt, Australia

Insulin signalling in bone plays a critical role in osteogenesis and the regulation of whole-body energy metabolism. However, a systems biology analysis to map in vivo signalling network has yet to be performed, and whether this signalling is rewired during ageing and insulin-resistance is still unknown. Furthermore, the bone tissue proteome is an underrepresented dataset within the Human Protein Atlas. Thus, here we present the first mouse ageing bone proteome and phosphoproteome of 8- and 73-week-old mice following acute in vivo insulin stimulation and identified >16,000 phosphorylation sites mapped to 4528 bone phosphoproteins, of which >4,600 sites are novel. Notably, >2100 phosphosites were differentially regulated between young and old bone revealing dramatic rewiring and defects in insulin signalling. Kinase : substrate prediction using machine learning coupled to phosphosite evolutionary conservation analyses and integration with human bone mineral density GWAS enabled us to prioritise novel phosphoproteins conceivable to play important roles in bone biology. We next developed a CRISPR/Cas9 loss-of-function screening pipeline in zebrafish to assess candidate protein function on bone development. This identified several novel insulin-regulated phosphoproteins as causal regulators of bone formation including protein AFF4, the core scaffold of the transcriptional Super Elongation Complex (SEC). Using targeted phosphoproteomics and affinity-purification coupled to mass spectrometry, we show that AFF4 is a novel substrate of nuclear-localised P70S6K, and the insulin-regulated phosphosite regulates SEC formation. Furthermore, ChIP-seq revealed the activity of the SEC is defective under insulin-resistant conditions and is associated with reduced phosphorylation of AFF4 and an inability to engage efficient transcription of Immediate Early Genes. Altogether, through combination of in vivo phosphoproteomics with functional genomics we have defined in vivo defective insulin-signalling events in old mouse bone and identified novel functional phosphorylation events.

Session Date/Time: Wednesday, September 20, 2023 - 02:30 PM - 03:35 PM

MS03: Mentoring Session 3 - Geographical Differences for Academic (Postdoc-PI) Job Applications (Asia, Europe, Africa, Americas)

Chair
Livia Rosa-fernandes, Australia

Chair
Andreas Hober, Sweden

MS03.01: Panelist
Nicolle Packer, Australia

MS03.02: Panelist
Uwe Völker, Germany

MS03.03: Panelist
Yu-Ju Chen, Taiwan

MS03.04: Panelist
Birgit Schilling, United States

Session Date/Time: Wednesday, September 20, 2023 - 04:30 PM - 06:30 PM

PROGRAM

PL07: HUPO Awards Ceremony and Closing Session

16:30   **PL07.01: Congress Summary**
Je Yoel Cho, Republic of Korea

16:30   **PL07.01: Congress Summary**
Youngsoo Kim, Republic of Korea

16:40   **PL07.02: ECR Summary and Announcement of ECR Award Winners**
Ruth Huttenhain, United States

16:40   **PL07.02: ECR Summary and Announcement of ECR Award Winners**
Mathieu Lavallée-Adam, Canada

17:05   **PL07.03: Rising Star Award - Presentation**
Nicholas Riley, United States

17:20   **PL07.04: Clinical & Translational Proteomics Sciences Award - Presentation**
Rebekah Gundry, United States

17:32   **PL07.05: Discovery in Proteomic Sciences Award - Presentation**
Hannes Röst, Canada

17:44   **PL07.06: Distinguished Achievement in Proteomic Sciences Award - Presentation**
Bernhard Küster, Germany

17:56   **PL07.07: Distinguished Service Award - Presentation**
Henning Hermjakob, United Kingdom

18:08   **PL07.08: Science & Technology Award - Presentation**
Alexey Chernobrovkin, Sweden

18:08   **PL07.08: Science & Technology Award - Presentation**
Tomas Friman,

18:18   **PL07.09: Closing Remarks**
Jennifer Van Eyk, United States

See you in Busan!
BEXCO, Busan, Korea
2023.hupo.org
#HUPO2023

Together Through Proteomics

PROGRAM

18:23   **PL07.10: HUPO 2024 Invitation**

Uwe Völker, Germany

HPP Day (September 21, 2023) – Emerging Strategies to Address Protein Functions

Registration Fee: 100 USD

The Human Proteome Project (HPP) is HUPO's flagship project. It is designed to map the entire human proteome in a systematic effort using currently available and emerging techniques. In 2021, the HPP launched the Grand Challenge, the objective of which is to identify a function for every human protein. During the HPP Day at HUPO 2023, different strategies to predict or address the functional properties of proteins in the context of cells will be presented and discussed.

Be sure to register for this outstanding program! All HUPO 2023 delegates are welcome to attend.

08:00 – 10:30 Session 1: Mission and Current Status

- 08:00 Welcome and Introduction – Charles Pineau, France & Cecilia Lindskog, Sweden
- 08:05 Overview of the Grand Challenge and Reflections from 2022 – Charles Pineau, France
- 08:15 2nd Update on the π-Hub Project – Fuchu He, China
- 08:45 2nd Update on the Chinese Westlake Pilot Project – Tiannan Guo, China
- 09:00 The HPP-ChemBioFrance Pilot Project – Charles Pineau, France
- 09:15 Proteins in Time and Space – Spatial Proteomics for Predicting Function – Cecilia Lindskog, Sweden
- 09:30 TBC – Gong Zhang, China
- 09:45 Involving Undergraduates in the HPP Grand Challenge: The Functionathon Experience at the University of Geneva – Lydie Lane, Switzerland

10:30 - 10:45 Coffee Break

10:45 – 12:30 Session 2: How Should We Get Organized to Contribute to the Grand Challenge?

- 10:45 Current HPP Structure and Suggestions of Future Directions – Charles Pineau, France
- 11:00 4x Breakout Sessions
- 11:30 Summaries from the Different Sessions and General Discussion

12:00 - 13:00 Lunch Break

13:00 – 15:30 Session 3: Moving Forward – Strategies and Initiatives to Predict Protein Function(s)

- 13:00 How to Propose Protein Function Based on Cellular Localization – Kathryn Lilley, UK
- 13:30 Proteomics and Protein Function in Clinical Precision Medicine and Pathology – Michael Roehrl, USA
- 14:00 Location matters! Position-specific Glycosylation Impacts Protein Function - Morten Thaysen-Andersen, Australia
- 14:30 Discussion on Future Directions - All
- 15:25 Wrap Up – Charles Pineau, France