Index number	Name	Title
33	Alvarez Penanes Pelayo	Exploring the potential of negative ion mode proteomics: an MS/MS free approach
15	Andersson Bennig Isabella	Structural and functional characterization of streptococcal SpnA-host interactions
25	Autret Nicolas	Reproducible and scalable one-day FFPE sample preparation method for LC-MS assays with Adaptive Focused Acoustics ® (AFA)
39	Benabdelkamel Hicham	plasma-Based Proteomic Profiling in Patients with Hyperplasia and Endometrial Cancer
18	Bendes Annika	Advanced protein profiling in dried blood spots
38	Blatnik Renata	Single cell proteome analysis with ultra-high sensitivity using a timsTOF mass spectrometer
65	Broman Axel	Characterization of acoustically isolated extracellular vesicles from pathogen activated platelets
53	Bromirski Maciej	AccelerOme automatic sample preparation enables highly reproducible quantitative proteomics analysis in data-independent acquisition (DIA) workflc
54	Bromirski Maciej	Unleashing the power of HT-DIA acquisition on Orbitrap Exploris 240 – Precise and accurate quantitation at 260 SPD
37	Camporesi Elena	Amyloid-beta peptide profiling in brain of Alzheimer's disease and Down syndrome patients using LC-MS and MALDI
29	Cannon Homaei Selina	The functional role of GADL1 in mouse brain and skeletal muscle tissues
44	Chen Ray	Therapeutic Target Discovery using proteome-wide analyses in large population health studies like the UK Biobank
59	Damoc Eugen	A benchmarking workflow for high-throughput DIA label-free quantification using a novel high-resolution accurate mass platform
62	Delanghe Bernard	Sensitive and robust high-throughput workflow for qualitative and quantitative single-cell/single cell like analysis
17	Emdadi Akram	Proteomic and proteogenomic characterization of triple negative breast cancer
24	Esmaeili Parisa	Ovarian cancer biomarker discovery using phosphoproteomics approaches
26	Farag Yehia Mokhtar	Prediction of spectrum identification rates based on spectrum features
22	Fernandes Gomes Bárbara	Novel strategy for quantification of phospho-Tau epitopes in cerebrospinal fluid by mass spectrometry
49	Gaetani Massimiliano	Chemical Proteomics
45	Ghosh Sudip	Proteomic deconvolution of response to IFNa stimulation in MLL-rearranged leukemia
36	Guedes Jéssica	The role of Tumor and Microenvironment in Early-Stage Primary Melanoma Recurrence: From Proteomics to Histopathological analysis
31	Hamm Prisca	The purposeful design of specific protease sensitive peptide linkers
66	Havers Megan	Accessing the exosome proteome in a minute blood plasma sample by rapid acoustic isolation of extracellular vesicles
60	Hermanson Daniel	High-throughput proteomics with the Orbitrap Astral mass spectrometer
41	Jha Durga	Multimodal Imaging Reveals Lipid and Peptide Dynamics in Familial and Sporadic Alzheimer's Disease
42	Jha Durga	Multimodal Imaging Reveals Lipid and Peptide Dynamics in Familial and Sporadic Alzheimer's Disease
56	Koch Heiner	Evaluation Of The Relative Quantitative Performance Using Tandem Mass Tags On A New High-Resolution Accurate Mass Platform
19	Kotol David	Unique concept for absolute quantification of proteins in blood
40	Koutarapu Srinivas	probing the molecular diversity and conformational polymorphism of tau pathology in alzheimer's disease
57	Koutarapu Srinivas	Delineating amyloid peptide signature patterns among heterogenous plaque population in Alzheimer's Disease
43	Kristensen Lara	Deeper plasma proteome coverage enables identification of novel biomarkers and classification of diseases
30	Krokengen Oda Caspara	Synergy and competition of myelin proteins in lipid membrane stacking and formation
28	Kuras Magdalena	Proteogenomics stratification of treatment naïve melanoma metastases associates the tumor microenvironment with disease progression and patient
35	Kuras Magdalena	Analysis of a paired primary and brain metastasis cohort from lung adenocarcinoma patients identifies novel proteomic subtypes correlating with progı
50	Lee Amanda	High-throughput XL-MS analysis on a new high-resolution accurate mass platform
58	Lee Amanda	Label-free DIA-based workflow for single-cell proteomic analysis on an Orbitrap Ascend mass spectrometer
48	Miliotis Tasso	Facilitating precision medicine through targeted proteomics analysis of dried plasma from fingerpricks
64	Mirgorodskaya Ekaterina	Analytical strategies for glycoproteomic studies
21	Montoliu-Gaya Laia	Optimal Blood Tau Species for the Detection of Alzheimer's Disease Neuropathology: An Immunoprecipitation Mass Spectrometry and Autopsy Study
32	Mosquim Junior Sergio	Multi-omics analysis of clinical breast cancer tissue
16	Nilsson Johanna	Novel LC-MS-MRM method development for simultaneous quantification in a synaptic and lysosomal protein panel
34	Onishchenko Evgeny	Age balance analysis of protein metabolism
61	Samra Stephanie	An in-depth plasma proteomics workflow powered by a Novel HRAM mass spectrometer
27	Sharma Nidhi	Navigating the melanoma brain metastasis landscape through spatial transcriptomics and multiregional tissue proteomics
55	Stewart Hamish	The Astral analyzer: a new class of sensitive high-resolution mass analyzer for deep and high-throughput proteomics.
63	Sultan Kristin	smart polymers for robust affinity-based capture of phosphorylated and methylated proteins
46	Szeitz Beata	A focused analysis on mutations of cancer-driving genes in the melanoma proteome
47	Watral Joanna	Exploring communication mechanisms in leukemic microenvironments: method development and analysis
20	Weiner Sophia	TMT proteomics reveals distinct cerebrospinal fluid signatures across genetic frontotemporal dementia subtypes
51	Xuan Yue	A novel intelligent data acquisition Hybrid-DIA mass spectrometry strategy: enabling data-driven and hypothesis-driven approaches in one go
52	Xuan Yue	Unlocking the potential of large-cohort proteomics studies with a novel high-resolution accurate mass platform
23	Zarrineh Mahshid	PhenoPCM: A Feasibility study of Proteomics and Multi-omics in Routine Clinical Samples

15

Structural and functional characterization of streptococcal SpnA-host interactions

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Background: Every major health organization has identified infectious diseases as serious global threats. We address these problems by studying the Streptococcus pyogenes bacterium; an important human pathogen, ranking globally among the top ten causes of mortality from infectious diseases with an estimated >150 000 annual deaths. During an infection, S. pyogenes encodes for a variety of virulence factors; many of these virulence factors have not been thoroughly studied. One of these virulence factors is the S. pyogenes nuclease A, SpnA, originally described as a cell-wall associated DNAse degrading host neutrophil extracellular traps (NETs) via its C-terminal endo/exonuclease domain.

Results & Material and Methods

We study the structure and function of SpnA by combining quantitative and structural proteomics mass spectrometry with integrative structural biology methods. Our results combining AlphaFold modeling, single-particle cryoEM and hydrogen-deuterium exchange mass spectrometry demonstrate that SpnA harbors disordered regions in its N-terminus. We have demonstrated by combining affinity-purification and bacterial surface adsorption mass spectrometry, that in human blood plasma SpnA binds the complement system membrane attack complex (MAC). By combining affinity-purification with crosslinking mass spectrometry we have been able to determine that SpnA specifically targets the MAC assembly intermediate C5b-C7. We hypothesize that SpnA acts like another streptococcal virulence factor SIC, streptococcal inhibitor of complement, which targets the C5b-C7 complex preventing its insertion into the streptococcal membranes, hence preventing bacterial lysis.

Conclusion

Our results indicate that in addition to degrading host DNA in NETs, SpnA mediates other central functions in host immune evasion in streptococcal pathogenesis.

16

Novel LC-MS-MRM method development for simultaneous quantification in a synaptic and lysosomal protein panel

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Introduction

Synapse loss is a key early event in Alzheimer's disease, along with endo-lysosomal system dysfunction, contributing to the disease's progression. Detecting biomarkers for these issues is crucial. Exploratory proteomic studies identified potential biomarkers where 60 proteins were chosen for validation. A multiplex MRM method was developed to quantify these proteins in cerebrospinal fluid (CSF) for a comprehensive panel assay.

Method

The developed method involves; alkylation, reduction, and trypsin digestion of 100 µL CSF samples after the addition of stable isotope standards, followed by solid-phase extraction. The method utilizes a micro-high-performance liquid chromatography-mass-spectrometry system (6495 Triple Quadrupole LC/MS system, Agilent Technologies) equipped with a Hypersil Gold reversed-phase column (dim. 100 × 2.1 mm, particle size 1.9 µm, Thermo Fisher Scientific), utilizing a 42-minute gradient.

Results

38 out of the 60 selected proteins were successfully quantified in CSF using the MRM assay, including neurogranin, complexins, VAMP-2, cathepsins, granins, LAMPs, VGF, contactins, glutamate receptors, neuronal pentraxins, synucleins, syntaxins, and 14-3-3 zeta/delta. Preliminary data have indicated that several of the proteins show different levels between controls and disease as well as between diseases. Interestingly not all proteins show the same changes. Neurogranin shows specifically increased levels in Alzheimer's disease while other proteins such as 14-3-3 zeta/delta are increased in Alzheimer's disease and other neurodegenerative diseases (such as Lewy body dementia). Similarly, but in the opposite direction, the neuronal pentraxins show decreased levels across diseases such as Alzheimer's disease and Parkinson's disease.

17

Proteomic and proteogenomic characterization of triple negative breast cancer

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The authors have chosen not to publish the abstract

18

Advanced protein profiling in dried blood spots

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Dried blood spots (DBS) offer a valuable alternative to traditional venous blood sampling in various clinical and research settings, including the benefit of at-home collection. However, newly emerging assay techniques are not optimized for the DBS sample type. Our goal was to demonstrate the utility of deep and accurate protein profiling in DBS samples.

Here, we present the utilization of multiplexed affinity proteomics techniques, such as Olink's PEA technology, to explore the protein content of DBS samples. We focused on developing a comprehensive workflow that integrates the advantages of highly accurate and quantitative DBS sampling with the specificity and sensitivity of affinity proteomics. We tested, optimized, and validated a series of steps in our protocols for extracting, enriching, and analyzing the proteins from DBS. This workflow includes a pipeline for DBS-tailored normalization for the subsequent data analysis.

We compared matched plasma and DBS samples from the same donors. We obtained a 99% detectability of cardiometabolic proteins in both sample matrices. Studying low abundant inflammatory proteins, we observed that an increased sample dilution of DBS during the elution process lowered the detectability compared to Plasma (64% vs 84%). Still, we found a strong concordance between the two sample types (rho = 0.84). When concurrently collecting multiple samples from the same study subject, we observed a coefficient of variation of 10.6, and average correlation of 0.99.

We demonstrate the utility of DBS sampling and advanced proteomics methods. Our workflow opens new avenues for microsampled dried blood and precision analyses.

19

Unique concept for absolute quantification of proteins in blood

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With mass spectrometry-based proteomics making a great move into precision medicine, there is an increasing pressure on data quality in studies of blood plasma profiles. Despite the immense endeavors to optimize and develop new data-analysis pipelines, the options for improving quantification results early on during the sample preparation are limited. ProteomEdge™ is a company from Stockholm, Sweden, with a mission to bring precision into absolute quantification of blood plasma proteins using heavy-labelled cleavable standards. Here we present a new strategy of our proprietary analysis platform that offers multiplex panels of heavy-isotope labelled Quantitative Recombinant Protein Standards – qRePS. qRePS are protein standards designed for targets of both clinical and research interests and always cover a longer amino acid stretch of a single endogenous protein. qRePS are enzymatically cleavable and ensure state-of-the-art accuracy in absolute protein quantification. They can be pooled into multiplex panels and utilized within standard mass spectrometry workflows and fully automated pipelines. ApoEdge™ and DiscoveryEdge™ offer both specialized panel for multiplex analysis of all human apolipoproteins and an exploratory panel covering over 150 protein targets. They are distributed vacuum dried in prealiquoted 96-well plate format which allows for addition-only protocols and high precision with low preanalytical variability. This setup outlines a new strategy for unbiased discoveries and confident results using thousands of experimentally verified proteotypic peptides.

20

TMT proteomics reveals distinct cerebrospinal fluid signatures across genetic frontotemporal dementia subtypes

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Frontotemporal dementia (FTD) encompasses a group of complex disorders characterized by progressive neurodegeneration in the brain's frontal and temporal lobes. The disease's multifaceted clinical and neuropathological nature has so far hampered diagnostic and therapeutic advances. This study thus aimed to explore proteomic differences in the cerebrospinal fluid (CSF) of genetic FTD cases, exhibiting a clear relationship between genetic mutation and underlying pathology, to discern FTD subtype-specific proteomic signatures.

We analysed 248 CSF samples from C9orf72 (n=78), GRN (n=56), and MAPT (n=38) mutation carriers as well as 76 controls using TMTpro 18-plex proteomics. TMT multiplex samples were fractionated into 24 concatenated fractions by reversed-phase chromatography at basic pH and analysed by LC-MS on an Orbitrap Lumos mass spectrometer. Differential protein abundance analysis, feature selection, and protein correlation network analysis were conducted to detect subtype-specific proteomic and pathophysiological alterations.

We found an overlap of 14 proteins including NEFL, YWHAG, and FABP3 that were significantly ($p<0.001$) changed in all symptomatic genetic forms compared to controls. Correlation network analysis revealed that commonly altered proteins were mostly related to neurodegeneration, the synapse or complement system (Figure 1). Furthermore, we identified several proteins which were only changed in abundance in specific FTD-subtypes, a notable example being lysosomal proteins such as PLBD2 and DNASE2 showing a selective decrease in MAPT mutation carriers.

We discovered shared and distinct proteomic signatures of specific genetic FTD subtypes that may have diagnostic value to detect FTD in patients and distinguish between disease subtypes.

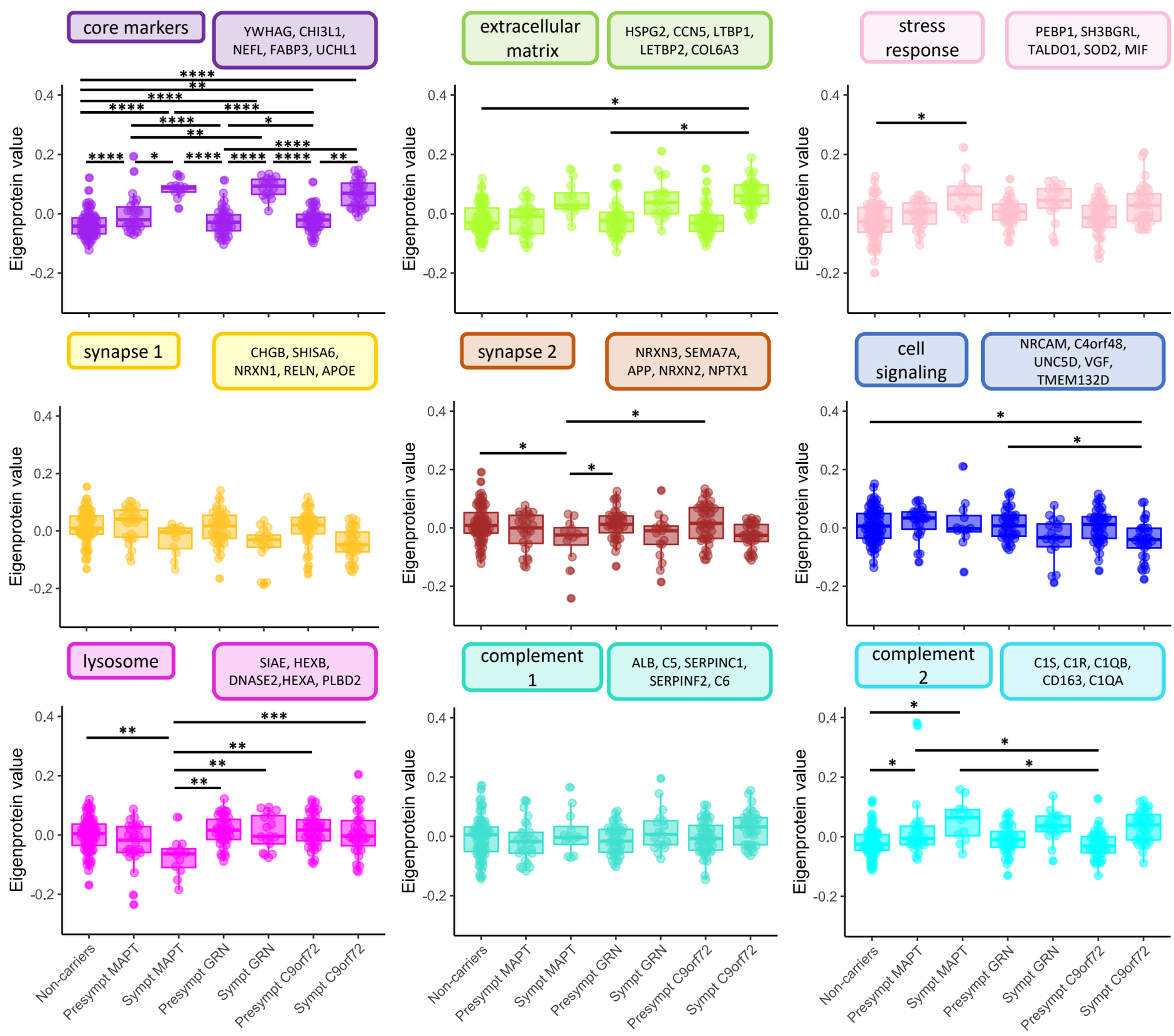

Figure 1. Network analysis revealing mutation specific patterns of proteomic changes.
This figure displays Eigenprotein values obtained through weighted gene co-expression network analysis (WGCNA), which identified 16 distinct modules of proteins, including one module (grey) which contained proteins that could not be assigned to a more specific module. In this figure, nine modules of special interest were selected, which include Eigenprotein values of (A) "core markers", (B) "extracellular matrix", (C) "stress response", (D) "synapse 1", (E) "synapse 2", (F) "cell signaling", (G) "lysosome", (H) "complement 1", and (I) "complement 2". Modules were named after the gene ontology (GO) terms assigned to them. The boxes contain the hub proteins of each module, as determined by having the highest module membership value (kME). *P*-values are derived from ANOVAs with post hoc Tukey's honestly significant difference (HSD) to adjust for multiplicity. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$.

21

Optimal Blood Tau Species for the Detection of Alzheimer's Disease Neuropathology: An Immunoprecipitation Mass Spectrometry and Autopsy Study

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Plasma-to-autopsy studies are essential for validation of blood biomarkers for Alzheimer's disease (AD). Few such studies have been done on phosphorylated tau (p-tau) and those that exist have made limited or no comparison of the different p-tau variants. This study is the first to use immunoprecipitation mass spectrometry (IP-MS) to compare the accuracy of eight different plasma tau species in predicting autopsy-confirmed AD. The sample included 123 participants (AD=69, non-AD=54) from the Boston University AD Research Center who had an available antemortem plasma sample and donated their brain. Plasma samples proximate to death were analyzed by targeted IP-MS for six different tryptic phosphorylated (ptau-181, 199, 202, 205, 217, 231), and two non-phosphorylated tau (195-205, 212-221) peptides. NIA-Reagan Institute criteria were used for the neuropathological diagnosis of AD. All tau species were increased in AD compared to non-AD, but p-tau217, p-tau205 and p-tau231 showed the highest fold-changes. Area under the receiver operating curve (AUC) analysis indicated that p-tau217 (AUC=89.8), p-tau231 (AUC=83.4), and p-tau205 (AUC=81.3) had excellent accuracy in discriminating AD from non-AD donors, even among those with CDR<1. Furthermore, p-tau217, p-tau205 and p-tau231 showed the highest Odds Ratio (OR) with both CERAD (ORp-tau217=15.29, ORp-tau205=5.05 and ORp-tau231=3.86) and Braak staging (ORp-tau217=14.29, ORp-tau205=5.27 and ORp-tau231=4.02) but presented increased levels at different stages of amyloid and tau pathologies. Our findings support plasma p-tau217 as the most promising p-tau for detecting AD brain pathology, and point to p-tau205 and p-tau231 to stage AD.

Figure 1. Box plots of the fold-changes of the tau peptide concentrations by Alzheimer's disease status. The non-AD group was used as a reference. National Institute on Aging-Reagan Institute criteria were used for the neuropathological diagnosis of Alzheimer's disease. Box plots include the median (bar) and interquartile range (whiskers) as well as the individual data points .

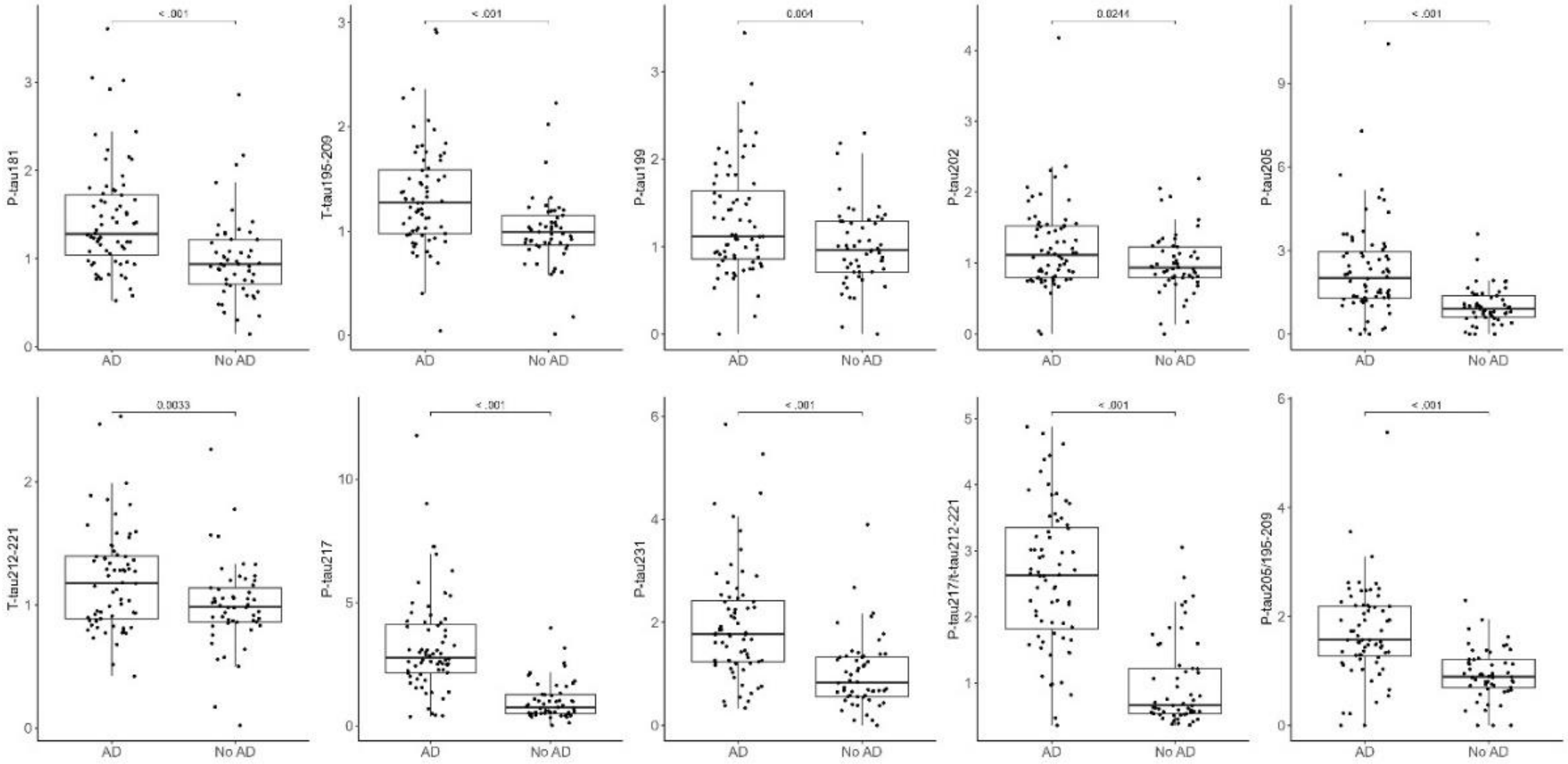


Figure 2. Box plots of the fold-changes of the tau peptide concentrations by CERAD neuritic amyloid plaque score. CERAD 0 was used as the reference group. Box plots include the median (bar) and interquartile range (whiskers) as well as the individual data points**.**

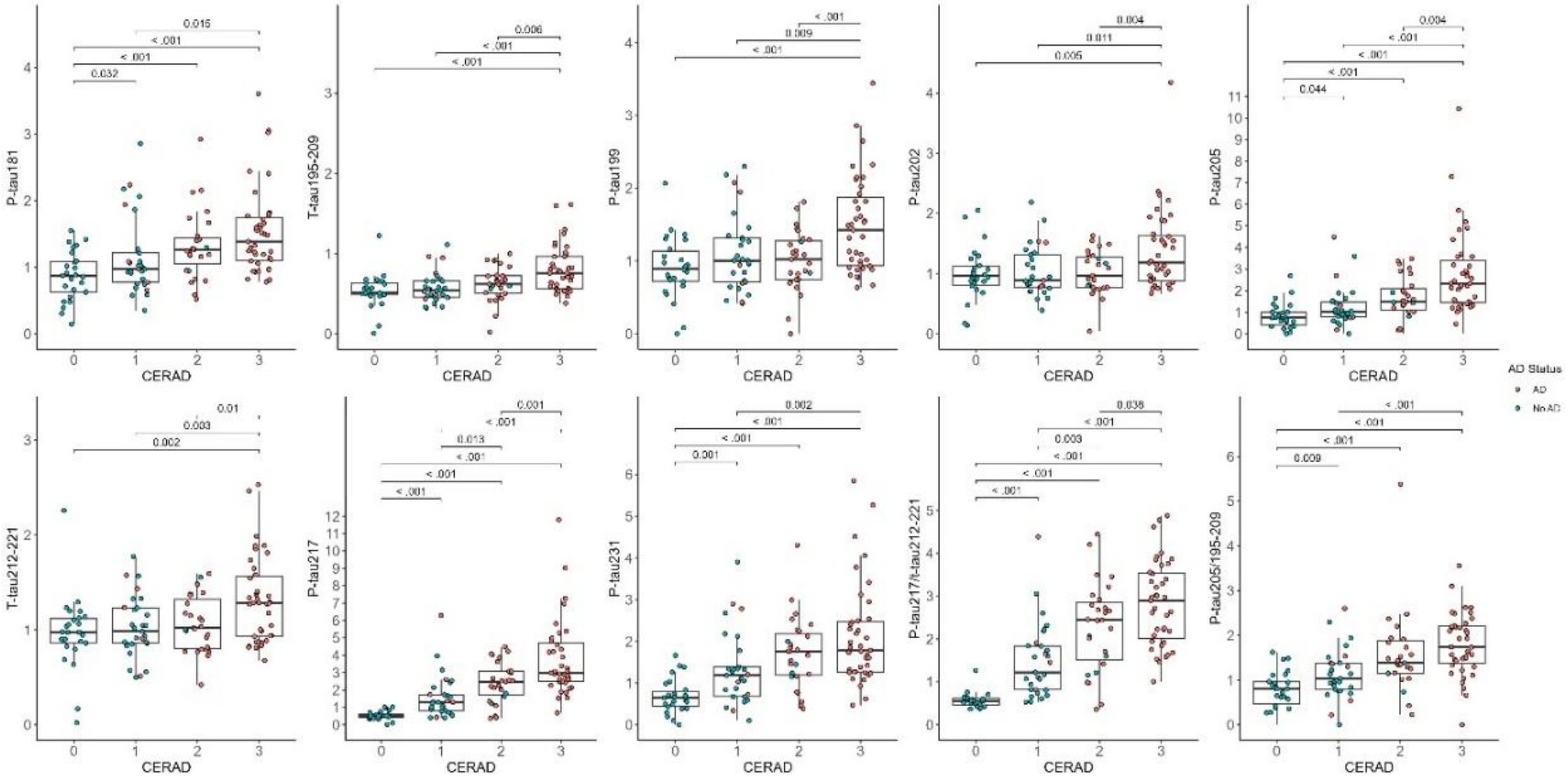
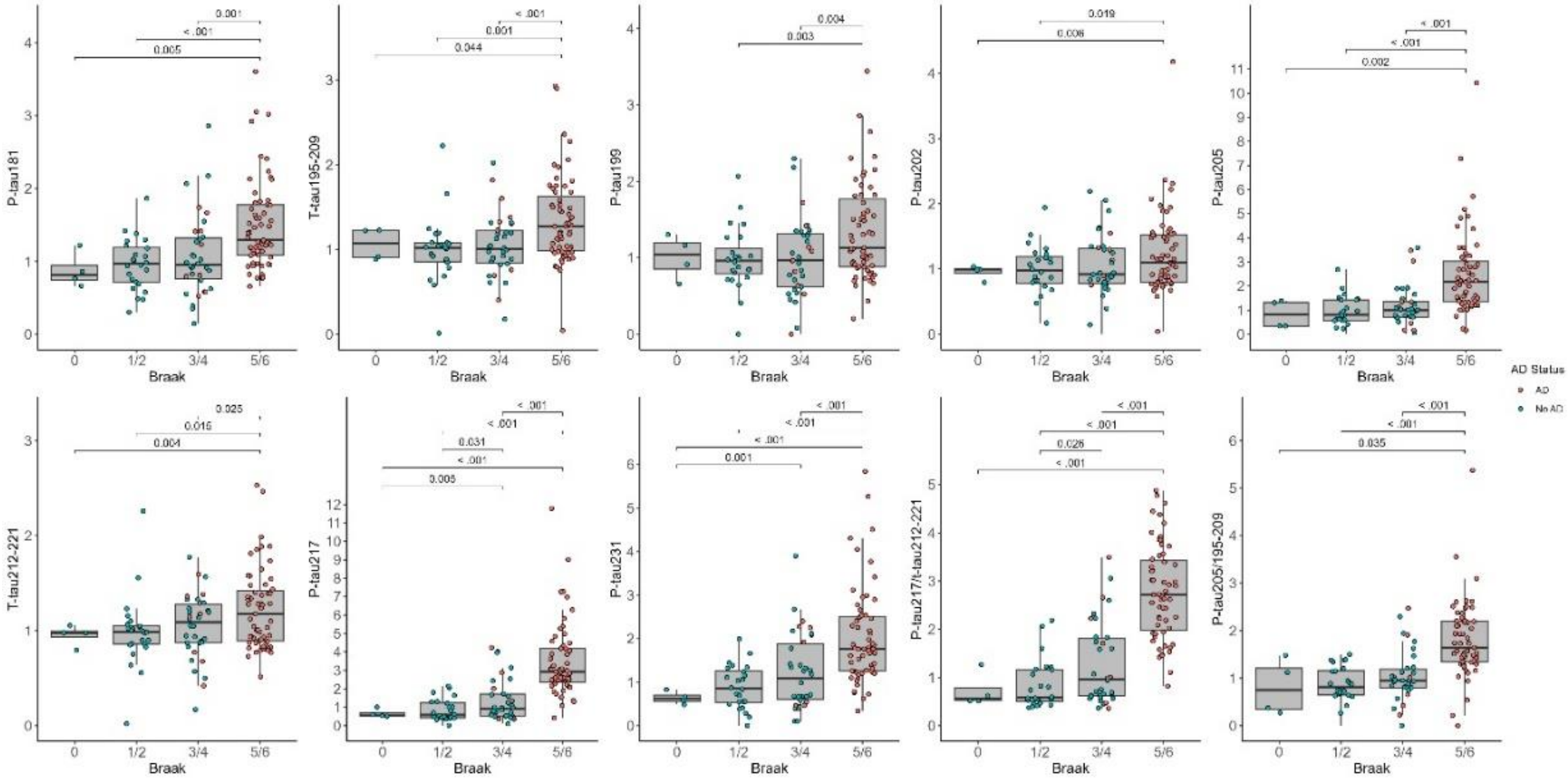


Figure 3. Box Plots of the fold-changes of the tau peptide concentrations by Braak staging for NFTs. Braak I-II was used as the refence group . Box plots include the median (bar) and interquartile range (whiskers) as well as the individual data points.



22

Novel strategy for quantification of phospho-Tau epitopes in cerebrospinal fluid by mass spectrometry

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Mass spectrometry is the standard for protein quantification, utilising tryptic peptides from the target protein relative to a stable isotope-labeled standard. Current approaches for quantifying total tau (tTau) and phosphorylated tau (pTau) in cerebrospinal fluid (CSF) rely on immunoaffinity enrichment or take advantage of tau's high solubility at 2.5% perchloric acid (PA), to precipitate abundant proteins. However, both strategies may introduce bias: immunoaffinity enrichment relies on antibody specificity, which may not be equal for all tau species, and PA may partially precipitate some tau forms. To address this, we developed an antibody- and PA-free method to measure tau/pTau epitopes in CSF by targeted mass spectrometry.

CSF samples (250 µl) from healthy individuals (HC) and Alzheimer's disease (AD) patients were spiked with stable isotope-labeled tau peptide standards containing the phospho-epitopes pTau181, pTau217, pTau205, and non-phosphorylated Tau212-221 and Tau282-290, and digested with trypsin. Desalted samples were fractionated by strong cation-exchange (SCX) solid-phase extraction. The fractions were analysed by parallel reaction monitoring on a nano-flow LC-MS on a Quadrupole-Orbitrap hybrid mass spectrometer.

Several tau peptides were detected and quantified with SCX, including pTau181, pTau217, pTau205, Tau212-221, and Tau282-290. Reproducibility of the assay was assessed, and results were compared with PA-based sample preparation in a pilot study of AD patients and controls.

This method allows for the detection of multiple tau/pTau peptides, while avoiding protein isolation methods like immunoaffinity enrichment or PA precipitation, thereby eliminating bias toward more/less soluble tau species.

23

PhenoPCM: A Feasibility study of Proteomics and Multi-omics in Routine Clinical Samples

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Background: Clinical proteomics has revolutionized the landscape of biomarker discovery and validation, drug screening in various cancer subtypes, and evaluation of treatment efficacy. Commonly, clinical samples are preserved using formalin-fixation and paraffin-embedding (FFPE), or optimal cutting temperature compound (OCT)-embedding followed by freezing. Adapting suitable sample preparation methods for Mass spectrometry (MS) analysis in combination with multi-omics analysis capabilities from these sample types has been an old challenge and needs to be optimized and evaluated in routine clinical samples.

Methods: In this study, we collected biopsy samples from 20 different patients with different types of cancer. Three different samples were collected from each patient in the form of FFPE, Fresh Frozen (FF), and OCT embedding. Subsequently, we processed the samples using two approaches, SDS lysing for all 3 sample types and All-prep kit preparation for FF and OCT samples, followed by optimized-SP3 magnetic bead tryptic peptide sample preparation and peptide clean up. The digested peptides were identified and quantified using label-free quantitative MS in Data-Independent Acquisition (DIA) mode, on a Tims-TOF HT instrument.

Results: We identified and quantified over 7000 proteins in FF and FFPE samples, and 6000 proteins in OCT samples. Using SDS lysis as extraction method yielded 30% more proteins and 5-10% more peptide identifications and quantifications compared to Allprep extraction.

24

Ovarian cancer biomarker discovery using phosphoproteomics approaches

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The authors have chosen not to publish the abstract

25

Reproducible and scalable one-day FFPE sample preparation method for LC-MS assays with Adaptive Focused Acoustics ® (AFA)

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[1] Covaris

Translational or clinical laboratories require automated, hands-off solutions for protein sample preparation to provide reproducibility, increased efficiency, higher quality results, and faster turnaround time. This communication highlights a simultaneous, one-day, multi sample processing from formalin-fixed and paraffin-embedded (FFPE) tissue for liquid chromatography-mass spectrometry-based (LC-MS) analysis, using focused ultrasonication (AFA).

AFA was used to efficiently extract proteins from FFPE and to allow a robust single pot sample preparation: this highly reproducible method works with short or long LC-MS gradients, and with every clean-up and digestion method. When working with bead-based protein aggregation capture, the workflow can include an accelerated trypsin digestion step (3 hours).

AFA makes FFPE tissue proteomics compatible with the clinical setting, through a non-toxic deparaffinization and rehydration approach. Short gradients (5 min) allow for ~2,500 protein IDs and longer gradients (60-90 min) for 6,000 to 9,000 IDs depending on the tissue type. Accelerated trypsin treatment yields similar quality compared to overnight digestion (below 10% miscleavages), in a more controlled way, and leads to 96 samples processed in 7 to 8 hours

AFA simplifies workflows by allowing protein extraction through digestion in a single well with an automation compatible high-throughput (HTP) approach, decreases turn-around-time, makes one-day sample prep also fully applicable to laser capture microdissections (LCM) and fresh/frozen tissue preparation.

26

Prediction of spectrum identification rates based on spectrum features

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Mass spectrometry is a major analytical technique for the identification and quantification of proteins. The generated data can however be challenging to interpret due to high variability in the individual spectra. To add to this, the spectrum identification rate can vary greatly between samples and a large portion of the spectra are often unidentified. In this study, machine learning was used to uncover underlying patterns within the spectra in order to assess the likelihood of a given spectrum to be identifiable, i.e. identified given optimal search parameters. Software to execute the spectrum classification and train a machine learning classifier was first developed and then tested on publicly available data sets from the PRIDE database. Several important spectrum features were thus detected which combined were able to confidently predict the spectrum identification rates for a wide set of test data sets.

27

Navigating the melanoma brain metastasis landscape through spatial transcriptomics and multiregional tissue proteomics

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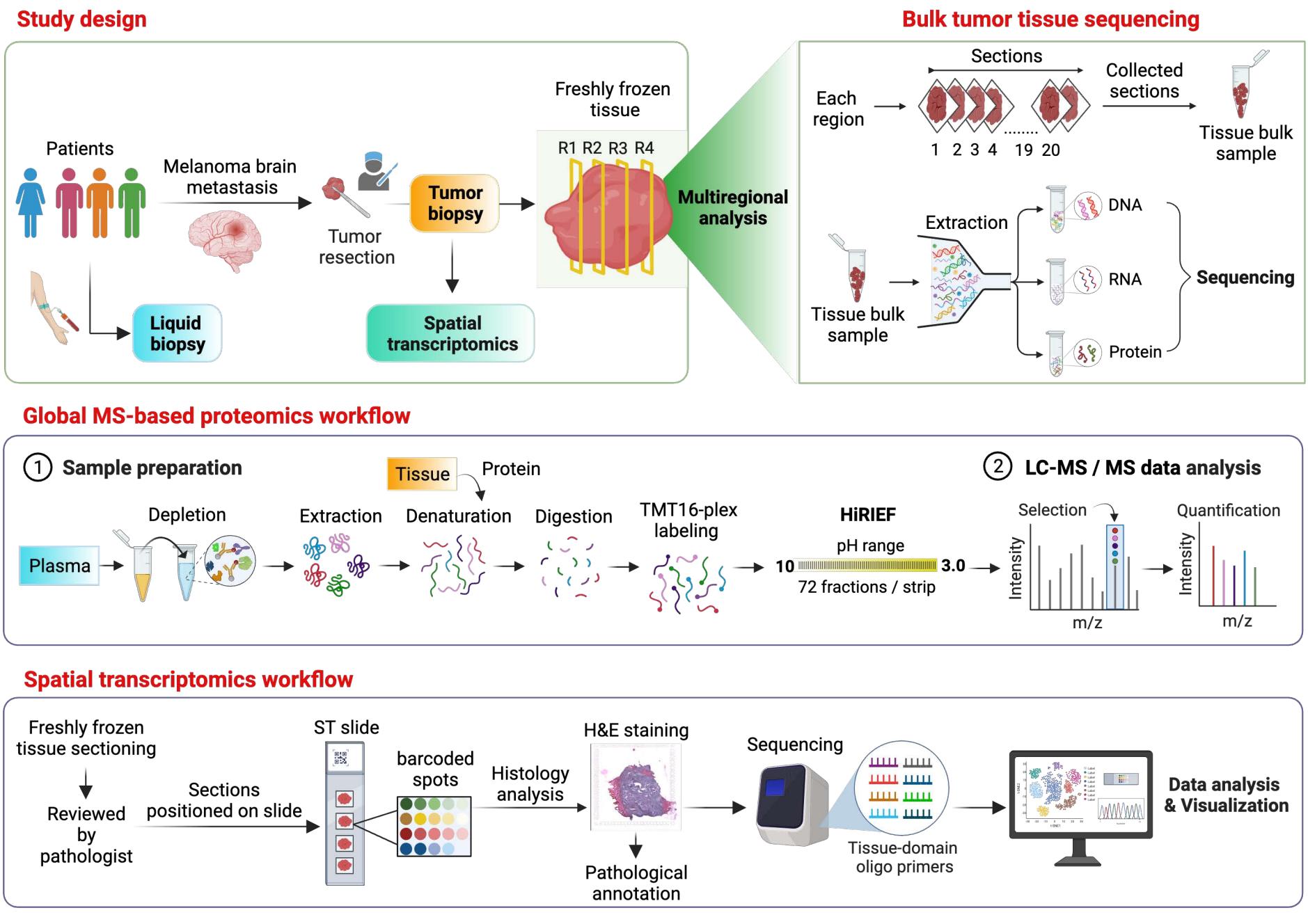
Background: Melanoma brain metastasis (MBM) presents a significant challenge for survival in advanced melanoma, requiring novel and enhanced therapeutic approaches. This amplifies the need for comprehensive tumor analysis and biomarker discovery to refine patient stratification and tailored treatment.

Materials and methods: We analyzed four MBM patient tumor biopsies (P1-P4) with diverse clinical traits and treatments. P1 underwent radiation therapy, P2 and P4 had systemic treatment, and P3 was treatment-naive. Inter-individual variability was identified using spatial transcriptomics (ST). To assess intratumor heterogeneity (ITH), we conducted multiregional bulk-sequencing (RNA-seq, DNA-seq, Proteomics) across multiple neighboring regions (R1-R4) as shown in Figure 1.

Results: Spatial transcriptomics revealed tumor organization and microenvironment deconvolution. Both ST and bulk tissue proteomics showed significant global protein enrichment associated with malignant cells, macrophages, and immune cells. Quantitative proteomics confirmed tumor-specific protein alterations related to melanoma and brain metastasis. Notably, key cancer pathways and T cell ligands, particularly T-cell-mediated adhesion signaling, were significantly enriched. Proteomics data clustering revealed distinct patient groups based on systemic treatment. ITH within multiple neighboring regions varied across the patient tumors. Differential expression analysis highlighted enriched protein clusters: innate immune proteins, macrophage activation, T- and B-cell signaling, and key cancer pathways (e.g., epithelial-mesenchymal transition, cell adhesion, notch signaling, oxidative phosphorylation and cell cycle checkpoints).

Conclusion: Spatially resolved multiregional tumor analyses provided us a holistic view of the ITH and inter-individual variations, identifying distinct enriched features in MBM. Novel biomarker detection could guide clinical decisions in MBM using advanced proteomics, sequencing and bioinformatics.


Study design
Patients
Melanoma brain metastasis
Tumor resection
Tumor biopsy
Freshly frozen tissue
R1 R2 R3 R4
Multiregional analysis
Liquid biopsy
Spatial transcriptomics
Bulk tumor tissue sequencing
Each region
Sections
1 2 3 4 ..... 19 20
Collected sections
Tissue bulk sample
Extraction
DNA
RNA
Protein
Sequencing
Tissue bulk sample
Global MS-based proteomics workflow
1 Sample preparation
Tissue
Protein
Depletion
Plasma
Extraction
Denaturation
Digestion
TMT16-plex labeling
HiRIEF
pH range
10
3.0
72 fractions / strip
2 LC-MS / MS data analysis
Selection
Intensity
m/z
Quantification
Intensity
m/z
Spatial transcriptomics workflow
Freshly frozen tissue sectioning
Reviewed by pathologist
Sections positioned on slide
ST slide
barcoded spots
Histology analysis
H&E staining
Pathological annotation
Sequencing
Tissue-domain oligo primers
Data analysis & Visualization

28

Proteogenomics stratification of treatment naïve melanoma metastases associates the tumor microenvironment with disease progression and patient outcome

Magdalena Kuras[1,2], Lazaro Betancourt[1], Leticia Szadai[3], Peter Horvatovich[4], Beata Szeits[5], Melinda Rezeli[2], Jeovanis Gil[1,2], Roger Appelqvist[2], Johan Malm[1], Elisabet Wieslander[1], David Fenyö[6,7], Istvan Balazs Nemeth[3], György Marko-Varga[2]

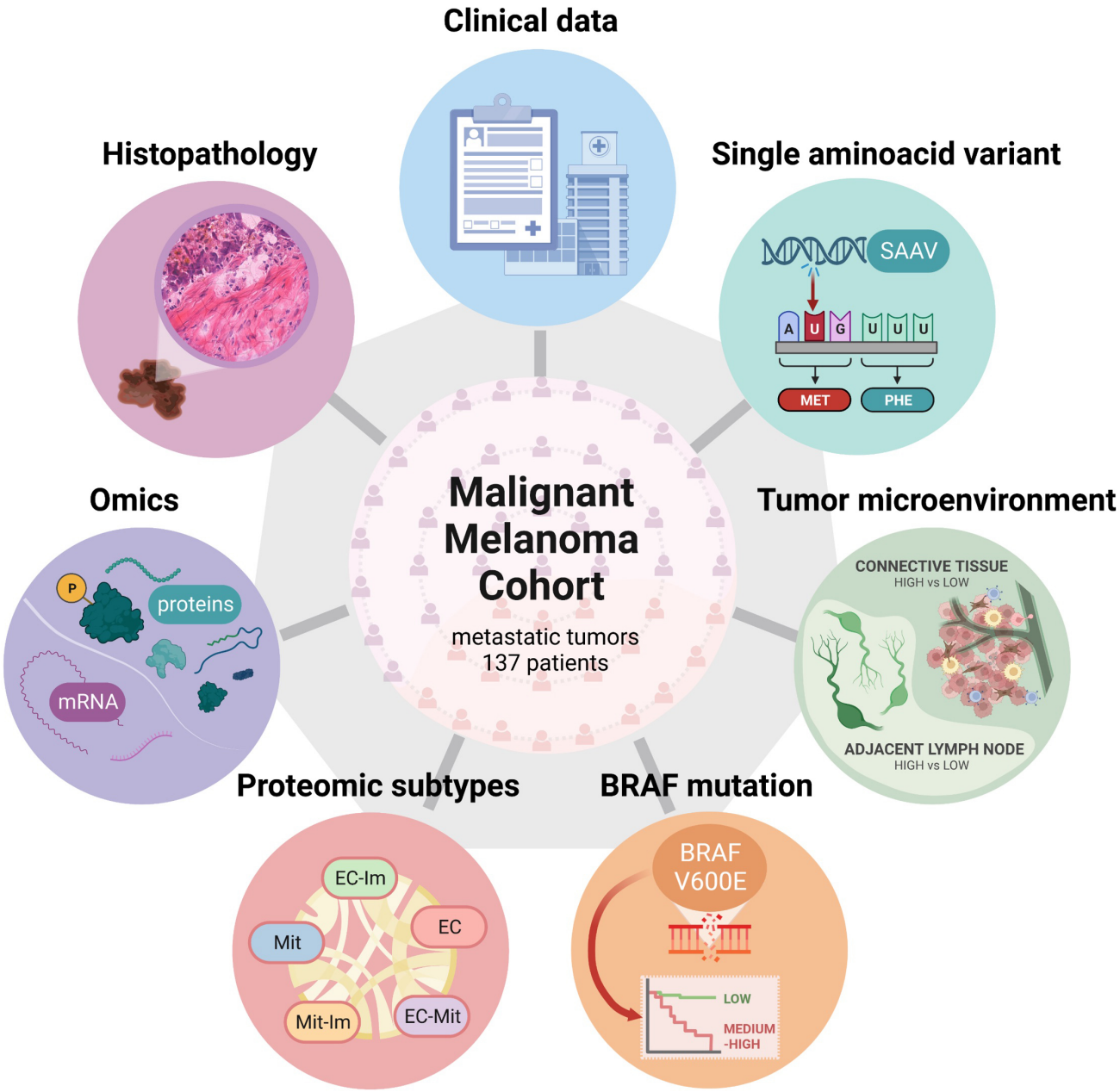
[1] Department of Translational Medicine, Lund University, [2] Department of Biomedical Engineering, Lund University, [3] Department of Dermatology and Allergology, University of Szeged, [4] Department of Analytical Biochemistry, Faculty of Science and Engineering, University of Groningen, [5] Division of Oncology, Department of Internal Medicine and Oncology, Semmelweis University, [6] Institute for Systems Genetics, NYU Grossman School of Medicine, [7] Department of Biochemistry and Molecular Pharmacology, NYU Grossman School of Medicine

Background: Although melanoma disease management has improved during the last decade, a significant fraction of patients relapse or do not respond to treatment. This can partly be explained by the complex signaling between the tumor, and its microenvironment. The aim of this study was to characterize treatment-naïve melanoma metastases using a proteogenomic approach.

Materials and Methods: Treatment-naïve frozen lymph node metastases from 142 patients with malignant melanoma were sectioned and histopathologically examined. Global proteomics was performed by isobaric labeling with TMT 11-plex. Phosphoproteomic analysis was performed using DIA-MS following Fe(III)-NTA enrichment. The peptides were analyzed by nanoLC-MS/MS using an Ultimate 3000 UPLC coupled to a QExactive HF-X.

Results: We identified five proteomic subtypes that integrate the immune and stroma microenvironment components, providing foundations for an in-depth molecular classification of melanoma. Our study showed that BRAF V600 mutated melanomas display heterogeneous biology, where the presence of an oncogene-induced senescence-like phenotype improves patient survival probability. Therefore, we propose a mortality-risk-based stratification, which may contribute to a more personalized approach to patient treatment. We also found a strong association between tumor microenvironment composition, disease progression, and patient outcome that point to straightforward histopathological connective tissue-to-tumor ratio assessment for better informed medical decisions. A melanoma-associated signature of single amino acid variants (SAAV) responsible for remodeling the extracellular matrix was uncovered with SAAV-derived neoantigen candidates as targets of anti-tumor immune responses.

Conclusions: This study provides insights into the complex biology of melanoma while highlighting the importance of the tumor microenvironment.


Clinical data
Histopathology
Single aminoacid variant
SAAV
A U G U U U
MET
PHE
Malignant Melanoma Cohort
metastatic tumors
137 patients
Omics
P
proteins
mRNA
Tumor microenvironment
CONNECTIVE TISSUE
HIGH vs LOW
ADJACENT LYMPH NODE
HIGH vs LOW
Proteomic subtypes
EC-Im
EC
Mit
Mit-Im
EC-Mit
BRAF mutation
BRAF V600E
LOW
MEDIUM -HIGH

29

The functional role of GADL1 in mouse brain and skeletal muscle tissues

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Glutamate Decarboxylase Like 1 (GADL1) is a pyridoxal 5'-phosphate (PLP)-dependent enzyme. We investigated the GADL1 mice model using multi-omics to characterize the functions of GADL1, including metabolomics (LC-MS, 1H-NMR) and transcriptomics (mRNA sequencing). GADL1 decarboxylates aspartate to beta-alanine, the rate-limiting precursor of the dipeptide carnosine (beta-alanyl-L-histidine). GADL1 deletion depletes levels of carnosine peptides in a tissue-specific manner, ranging from 11-80% reduction in various tissues (Mahootchi, et al. 2020, PMID: 32733999). In contrast, the levels of complex sphingolipids were increased, particularly in the olfactory bulb. In the olfactory bulb, the highest impacted pathway was beta-alanine metabolism (male mice, p=0.0070) and sphingolipid metabolism (female mice, p=0.0004). Moreover, PLS-DA analyses indicate a sex-specific difference in metabolites affected by GADL1 deletion. mRNA sequencing results showed that female mice have more differentially expressed genes than males (1194 vs 628 genes), specifically in the brain tissue sections (olfactory bulb and forebrain). Preliminary analysis of these data indicates that the gene transcript alterations vary with sex and tissue. Combined, these recent findings show that there is a sex-specific difference in metabolites and genes affected in the Gadl1 knockout mice. This may indicate that GADL1 plays a sex-specific role in the biosynthesis of beta-alanine. The observed effects could be caused by altered beta-alanine synthesis and compensatory mechanisms, or GADL1 could possibly have other, previously unknown substrates.

30

Synergy and competition of myelin proteins in lipid membrane stacking and formation

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Correct functioning of the myelin sheath is dependent on correct coordination and the combined effects of several important myelin proteins. Investigation of myelin protein synergy between myelin basic protein (MBP), the cytoplasmic tail of myelin protein zero (P0ct) and peripheral myelin protein 2 (P2), and their interaction with myelin membrane mimetics, was executed using several methods including small-angle X-ray diffraction (SAXD), differential scanning calorimetry (DSC) and microscopic techniques. Lipid phase transition temperatures reflecting the conformational freedom of the lipid hydrocarbon tails were studied using DSC. The proteins were screened against multilamellar vesicles, monitoring any protein-induced effects on the observed endothermic transition (Tm) of the lipids. DSC indicated changed lipid tail interactions dependent on the protein blend; the combination of MBP, P0ct and P2 decreased the Tm of the lipids. Epi-fluorescence patch experiments were utilized to explore the effects of the proteins on planar double-supported membrane patches. Preliminary results indicated that the three proteins stimulate bilayer formation, whereby P0ct and MBP promoted lipid transport. Membrane stacking was investigated by SAXD, and initial results revealed that the myelin proteins induced highly ordered structures, and lamellar d-spacing was accompanied by formation of lipid crystals. Research on myelin protein synergy is currently limited, even though these proteins function together in native myelin. Thus, research on how they affect each other's properties is crucial. Our results shed light on cooperative interactions between MBP, P0ct and P2, but more comprehensive studies are needed for untangling their full relationship.

31

The purposeful design of specific protease sensitive peptide linkers

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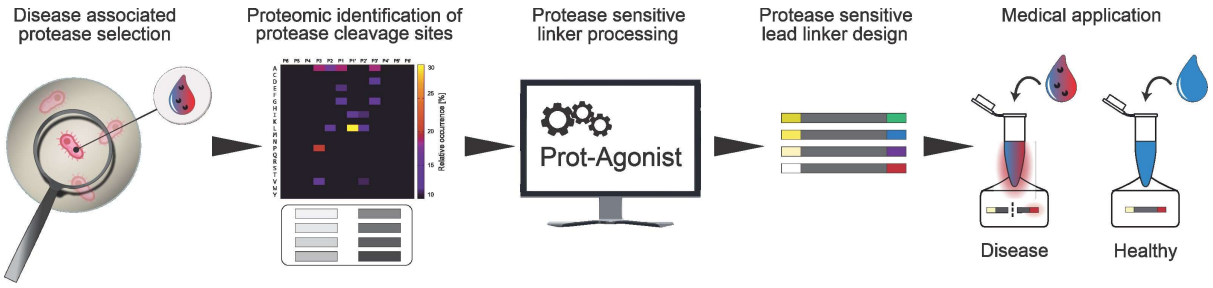
Proteases play a crucial role, not only in physiological, but also pathological processes, such as cancer, infection and inflammation (e.g. arthritis), to name but a few. Their ability to cleave peptides can be harnessed for a broad range of biotechnological purposes. As part of bioconjugates, cleavable linkers in general are used to reduce steric hindrance, enhance bioactivity and enable controlled drug release. Protease sensitive linkers (PSLs) have the ability to selectively respond to the local proteolytic environment at that. However, to do this efficiently, it is essential to find an amino acid (AA) sequence that meets the necessary requirements, including interdependent factors like specificity, selectivity, cleavage kinetics, synthetic accessibility, and others.

When PSL sequences can be derived from known cleavage sites of natural protease substrates, these often lack in several aspects, perhaps most commonly in specificity and selectivity. Hence, we describe a workflow for the systematic design of PSLs for specific target proteases. Guided by mass spectrometry based determination of the target protease cleavage site profile from a proteome-based peptide library, it includes not only a procedure for the identification of bespoke sequences, their optimization, synthesis and validation, but also introduces a program that can indicate potential off target cleavage sites by thousands of enzymes in any arbitrary amino acid sequence. Thereby altogether we provide a strategic approach to greatly accelerate the development and use of PSLs in diverse applications.


Disease associated protease selection
Proteomic identification of protease cleavage sites
Protease sensitive linker processing
Protease sensitive lead linker design
Medical application
Prot-Agonist
Disease
Healthy

32

Multi-omics analysis of clinical breast cancer tissue

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Background: Breast cancer (BC) was one of the first cancers to be tackled using precision medicine. However, late recurrences, undertreatment, and overtreatment are still a reality, especially for estrogen receptor positive disease [1-3]. Extended molecular characterization could potentially help to further stratify patients and guide treatment.

Materials and methods: A protocol previously developed in-house [4] was further optimized to include phosphoproteome data. 182 BC samples from the Sweden Cancerome Analysis Network – Breast (SCAN-B) cohort [2] were analyzed. They correspond to flowthroughs from freshly preserved biopsies processed via the Qiagen AllPrep method. Data were acquired in Data Independent Acquisition (DIA) mode on a Q-Exactive HF-X (Thermo Fisher Scientific) coupled to an Evosep One (Evosep) liquid chromatography system.

Results: We quantified over 15000 protein groups across 7000 unique genes in the cohort, a substantial coverage of the proteome. Furthermore, over 20000 phosphopeptides were quantified. Differential analysis between clinical sub-groups unveiled markers at the proteome and phosphoproteome levels which could potentially predict lymph node involvement and metastatic spread in patients.

Conclusion: We developed a semi-automated protocol which allows for the parallel acquisition of matching multi-omics data. We successfully applied it in the investigation of metastatic spread and lymph node involvement in BC.

1. Onkar, S.S., et al. Cancer Discov, 2023. 13(1): p. 23-40.
2. Saal, L.H., et al. Genome Med, 2015. 7(1): p. 20.
3. Narbe, U., et al. Br J Surg, 2021. 108(12): p. 1465-1473.
4. Mosquim Junior, S., et al. Cancers (Basel), 2022. 14(23)

33

Exploring the potential of negative ion mode proteomics: an MS/MS free approach

Pelayo Alvarez Penanes[1], Vladimir Gorshkov[1], Mark Ivanov[2], Mikhail Gorshkov[2], Frank Kjeldsen[1]
[1] University of Southern Denmark, [2] Russian Academy of Sciences

Background: Negative ion mode is highly underexplored compared to positive mode for proteomics; we aimed to develop a method for negative ion mode proteomics.

Materials and methods: Different alkaline compounds (piperidine and imidazole) and isopropanol as organic modifier were evaluated on a E. Coli digest and iRT peptide mixture. Best conditions for negative mode were evaluated on a HeLa standard using short gradients (5-15 minutes). After method validation, different enzymes (AspN, GluC, LysC and trypsin) were tested. Analyses were performed on a Orbitrap Eclipse. For protein identification, a deep-learning model (Biosaur2 and ms1searchpy) based on accurate mass and retention time has been applied.

Results: The best results in terms of spray stability and signal intensity were achieved with mobile buffers containing 2.5 mM imidazole and 3% isopropanol. Signal intensity was on the same order of magnitude as in positive mode. Method validation on a HeLa standard showed comparable performance between positive and negative mode, with more than 1,000 proteins in a single-shot 10-minute gradient in both modes (Figure1). 10% of the proteins and 50% of the PFMs (peptides) detected in negative mode were not present in positive mode. Data for the different enzymes revealed that trypsin and LysC performed best in terms of protein and PFM identification (Figure2). Combined search of 10-minute gradient data acquired in both polarities mode improved the number of identified proteins to 1,774 (Figure3).

Conclusion: A method for negative ion mode proteomics was developed, with the highest protein ratio identification to date in this polarity.

Exploring the potential of negative ion mode proteomics: an MS/MS free approach

Pelayo A. Penanes[1], Vladimir Gorshkov[1], Mark V. Ivanov[2], Mikhail V. Gorshkov[2], Frank Kjeldsen[1*]

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Figure 1. Number of identified proteins in positive and negative ion mode for a HeLa standard. Numbers represent the average of four replicates and error bars represent the standard deviation among all four replicates.

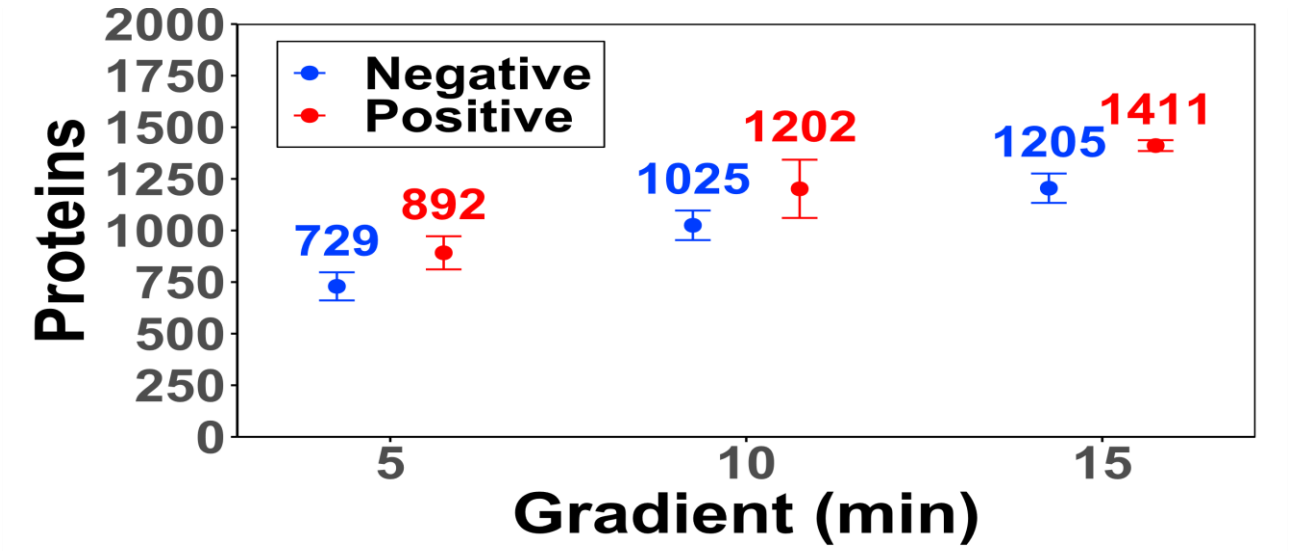


Figure 2. Number of identified proteins in positive and negative ion mode for the different enzymes evaluated. Numbers represent the average of four replicates and error bars represent the standard deviation among all four replicates.

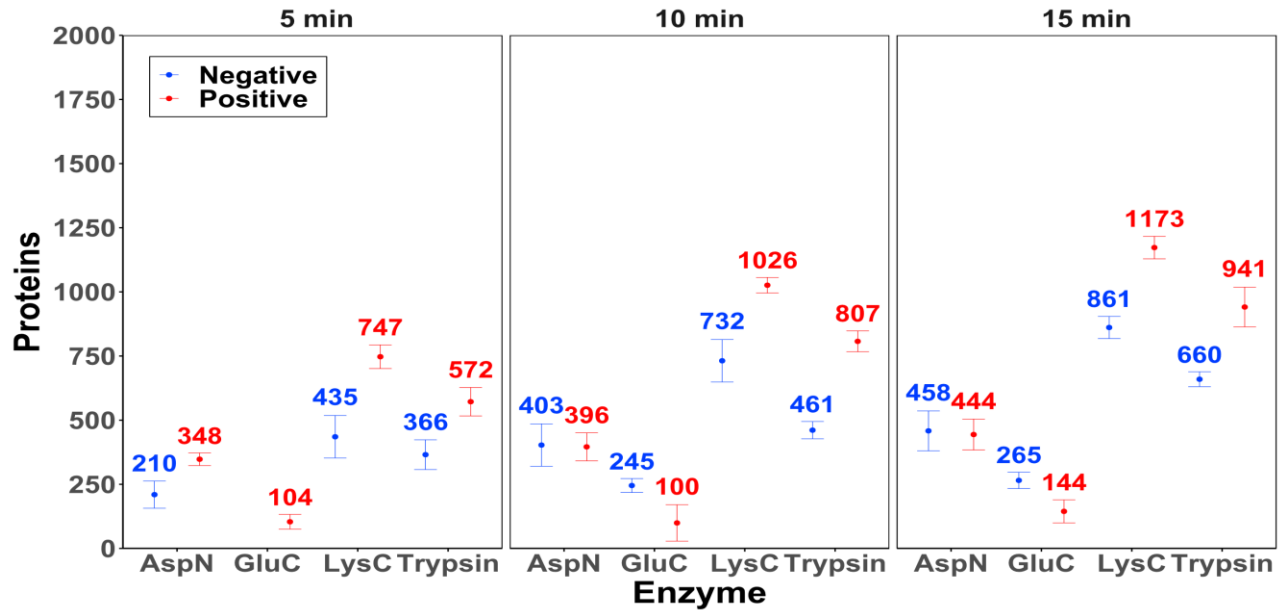
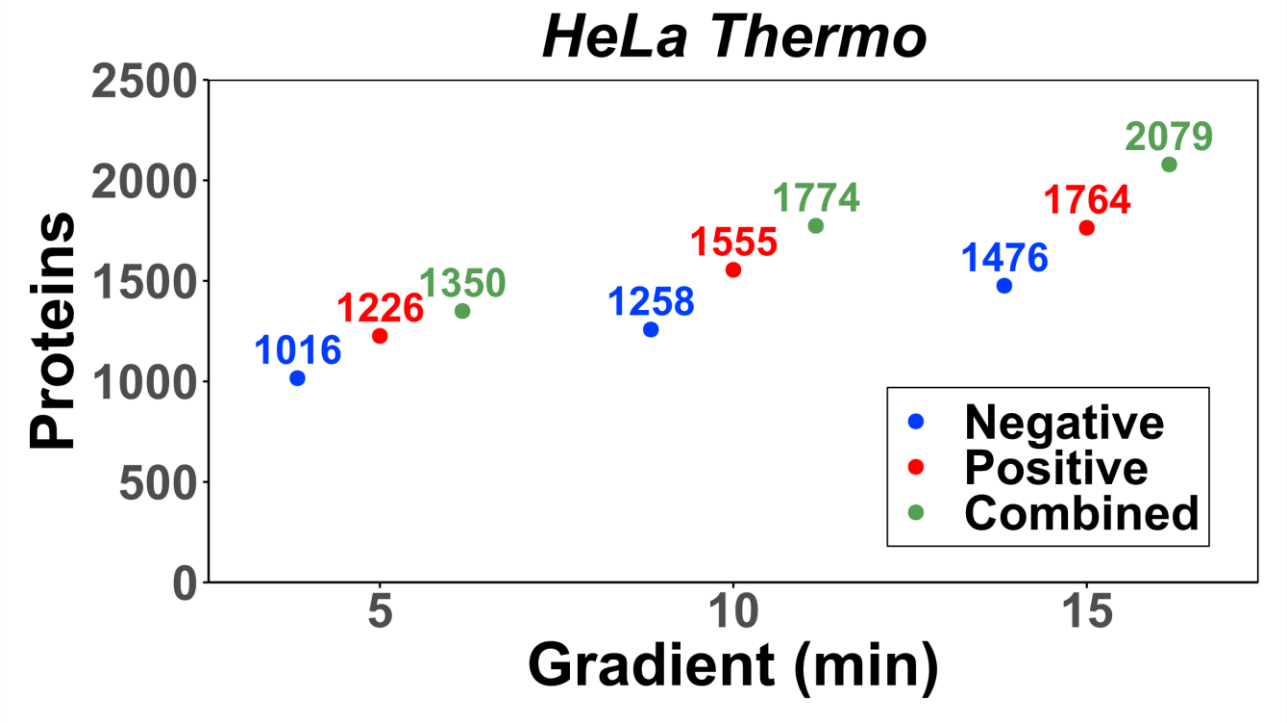


Figure 3. Number of identified proteins for the combined data for a HeLa standard.



34

Age balance analysis of protein metabolism

Evgeny Onishchenko[1], Kirill Jefimov[1], Elad Noor[2]
[1] University of Bergen, [2] Weizmann Institute of Science

Background: Pulsed stable isotope labelling by amino acids in cell culture and quantitative mass-spectrometry (pulse-SILAC-MS) is a powerful combination of tools to analyse protein metabolism as it happens in live cells. However, it is limited by the need of specific assumptions, for example that proteins degrade at a constant rate.

Materials and Methods: We theoretically analyzed general properties of labelling kinetic readouts in steady-state pulse-labelling experiments (example of which is log-phase cell cultures) using atom-mapping network model of cellular metabolism. Our theoretical findings were employed to investigate age dependency of protein-protein interactions and protein turnover in live yeast cells using a combination of pulse-SILAC, affinity pulldowns and DIA MS – based quantification of protein labelling kinetics.

Results: Our results demonstrate that kinetic parameters such as protein lifetime and age distribution can be inferred directly from the pulse-SILAC labelling readouts without further specific assumptions. We exemplify utility of these findings by determining Nuclear Pore Complex (NPC) age specificity for interactions with its assembly factor Brl1, with chromatin-remodelling factor Esc1, and, finally, by evidencing preferential degradation of young budding yeast proteins as a result of heat stress.

Conclusions: Taken together, our findings provide a convenient way to interpret results of metabolic labelling experiments, applicable both in proteomics and metabolomics.

35

Analysis of a paired primary and brain metastasis cohort from lung adenocarcinoma patients identifies novel proteomic subtypes correlating with progression-free survival

Magdalena Kuras[1], Nicole Woldmar[2], Judit Moldvay[3,4], Zsolt Megyesfalvi[5,6], György Marko-Varga[1], Balazs Döme[5,6], Beata Szeitz[7], Peter Horvatovich[8], Melinda Rezeli[1]

[1] Department of Biomedical Engineering, Lund University, [2] Laboratory of Molecular Biology and Proteomics of Blood/LADETEC, Institute of Chemistry, Federal University of Rio de Janeiro, [3] Department of Pathology and Experimental Cancer Research, Semmelweis University, Budapest, [4] Department of Bioinformatics, Semmelweis University, Budapest, [5] Department of Thoracic Surgery, Medical University Vienna, Vienna, Austria, [6] Department of Thoracic Surgery, National Institute of Oncology-Semmelweis University, Budapes, [7] Division of Oncology, Department of Internal Medicine and Oncology, Semmelweis University, Budapest, Hungary, [8] Department of Analytical Biochemistry, Groningen Research Institute of Pharmacy, University of Groningen

Background: Approximately 50% of all lung cancer patients have existing metastases at the time of diagnosis. In lung adenocarcinoma (LADC), brain metastases are the most common, and is related to poor outcomes. The aim of this study was to characterize primary tumors and the corresponding brain metastases from 58 patients with LADC using proteomics.

Methods: FFPE samples were processed as previously described (10.1021/acs.jproteome.0c00850). Proteomic analysis was then performed by isobaric labeling using TMTpro 16-plex. Peptides were analyzed by nanoLC-MS/MS using an Ultimate 3000 UPLC coupled to a QExactive HF-X following high pH fractionation.

Results: Protein-level stratification split the cohort into four primary and brain metastatic subtypes, respectively. These subtypes were distinctive in terms of histology, PD-L1 expression, and survival. Specific biological features were assigned to each subtype including nucleus and mitochondria, cellular response and vesicles, inflammatory response, and cell interaction and antigen presentation for primary tumors. While brain metastases were characterized by extracellular and oxidative stress, cellular response and vesicles, inflammatory response, and translation, mitochondria, and extracellular matrix. Additionally, the subtypes exhibit diverse stages of differentiation. Different immunological cell signatures could also be defined for each subtype. Interestingly, one of the primary subtypes was considered an independent prognostic factor associated with slower progression to brain metastasis and, consequently, better outcomes. It also presented a higher expression of immune-related markers, including upregulation of antigen processing and presentation.

Conclusion: Our findings provide insight into the biology of brain metastasis development in LADC through the identification of proteomic subtypes.

36

The role of Tumor and Microenvironment in Early-Stage Primary Melanoma Recurrence: From Proteomics to Histopathological analysis

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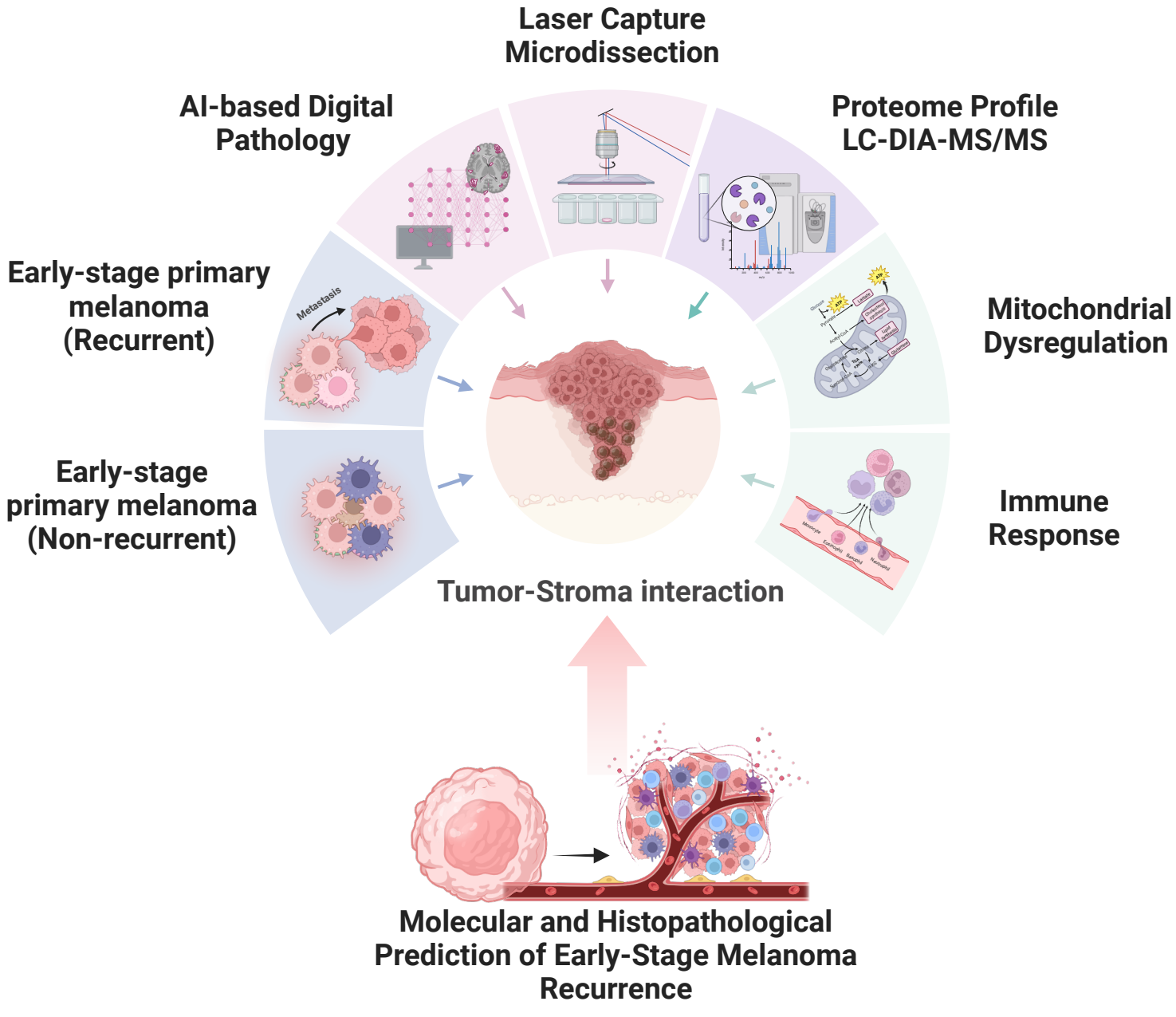
[1] Lund University, Lund, Sweden, [2] Federal University of Rio de Janeiro, Rio de Janeiro, Brazil, [3] University of Szeged, Szeged, Hungary, [4] Harvard Medical School, Boston, USA, [5] Biological Research Centre, Szeged, Hungary, [6] New York University Grossman School of Medicine, New York, USA, [7] Yonsei University, Seoul, Korea, [8] Tokyo Medical University, Tokyo, Japan

Background: Melanoma, the deadliest form of skin cancer, is the fifth most prevalent cancer in Sweden. Recurrent melanomas are closely connected to advanced stages and worse prognosis. However, current diagnostics cannot predict early-stage melanoma recurrence. Thus, this study aims to define early-stage melanoma changes by proteomics and histology to help in the recurrence prediction.

Materials and methods: Using an artificial intelligence approach, we identified tumor and stromal regions in 12 early-stage melanoma patients, with and without five-year recurrence. Laser capture microdissection isolated these areas. Quantitative proteomics and histopathology unveiled signatures in tumor cells and their surroundings.

Results: Our approach identified a proteomic signature in tumor cells and their microenvironments that reliably predicts a high risk of recurrence within five years. Dysregulated mitochondrial function and immune response-related pathways were the most significant drivers of recurrence. The tumor microenvironment of non-recurrent melanomas displayed more interleukin-related signaling pathways, extracellular matrix structure, and enriched complement, potentially preventing tumor recurrence. Furthermore, suppression of immune system response-related pathways in the tumor microenvironment of recurrent melanomas underscores the importance of immunotherapies to improve patient outcomes in this group of patients. Additionally, the histopathological analysis determined that the presence of microerosion, buddings, and melanocytic atypia, together with the absence of regression areas, are risk factors for melanoma recurrence.

Conclusion: Our findings determined the proteomic signatures for melanoma and its microenvironment, alongside distinct histopathological traits tied to recurrence. These insights may illuminate new early-stage melanoma prognostic factors.


Laser Capture Microdissection
AI-based Digital Pathology
Proteome Profile LC-DIA-MS/MS
Early-stage primary melanoma (Recurrent)
Metastasis
Mitochondrial Dysregulation
Early-stage primary melanoma (Non-recurrent)
Immune Response
Tumor-Stroma interaction
Molecular and Histopathological Prediction of Early-Stage Melanoma Recurrence

37

Amyloid-beta peptide profiling in brain of Alzheimer's disease and Down syndrome patients using LC-MS and MALDI

Elena Camporesi[1,2], Amal Kasri[3], Gunnar Brinkmalm[3], Eleni Gkanatsiou[1,4], Susana Boluda[3], Lev Stimmer[3], Yannick Vermeiren[5,6], Sarah E. Pape[7], Peter P. De Deyn[5,6], Henrik Zetterberg[1,2,8,9,10], Andre Strydom[7], Marie-Claude Potier[3]

[1] Institute of Neuroscience and Physiology, Department of Psychiatry and Neurochemistry, the Sahlgrenska Academy at University of, Gothenburg, Mölndal, Sweden, [2] Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden, [3] Paris Brain Institute, ICM, CNRS UMR7225 -INSERM U1127 –UPMC, Paris, France, [4] BioArctic, Stockholm, Sweden, [5] Department of Biomedical Sciences, Neurochemistry and Behavior, Institute Born-Bunge, University of Antwerp, Antwerp, Belgium, [6] Department of Biomedical Sciences, Neurochemistry and Behavior, Institute Born-Bunge, University of Antwerp, Antwerp, Belgium 7 Department of Neurology and Alzheimer Center, University of Groningen, University Medical Center Groningen (UMCG),, Groningen,Netherlands, [7] Institute of Psychology and Neuroscience, King's College London, 16 De Crespigny Park, London, United Kingdom, [8] Department of molecular Neuroscience, UCL Institute of Neurology, Queen Square, London, United Kingdom, [9] UK Dementia Research Institute at UCL, London, UK, [10] Hong Kong Center for Neurodegenerative Diseases, Hong Kong, China

Background

To investigate the amyloid-beta (Aβ) pathology present as plaques or cerebral amyloid angiopathy (CAA) and shared across Alzheimer's disease (AD), Down syndrome (DS) and cases carrying a duplication of the APP gene (APP-dup), we aimed at identifying qualitative and quantitative differences of the Aβ peptides profile.

Material and methods

Aβ peptides from brain tissues of sporadic AD (n=6), familial AD with APP duplication (APP-dup, n=4), DS (n=3), DS with dementia (n=8) and controls (n=15) were immunoprecipitated from soluble (TBS) and insoluble (70% formic acid) fractions, using a combination of 6E10 and 4G8 antibodies. Eluates were analysed using both MALDI and LC-MS.

Results

We identified more than 200 Aβ peptides, the majority of them being in the FA fraction. Low abundant species were more difficult to detect with MALDI. Despite both having an extra copy of the APP gene, the APP-dup group had the highest abundance in Aβ peptides in both fractions compared to the DS but also to all the other groups. The Aβ1-37/38/39/40 peptides were found to be the most abundant, while the Aβ1-42 peptide was lower. The high relative abundance of Aβ1-40 peptide in the APP-dup group is attributable to the high CAA present in this disease. Moreover, the APP-dup group showed higher relative abundance of Aβ2-X peptides, which might indicate preferential activation of different cleaving enzymes.

Conclusion

The different Aβ peptides profile of AD-related pathologies revealed by the MS analysis will be useful in the development of pathology specific biomarkers and might inform selection of therapeutic interventions.

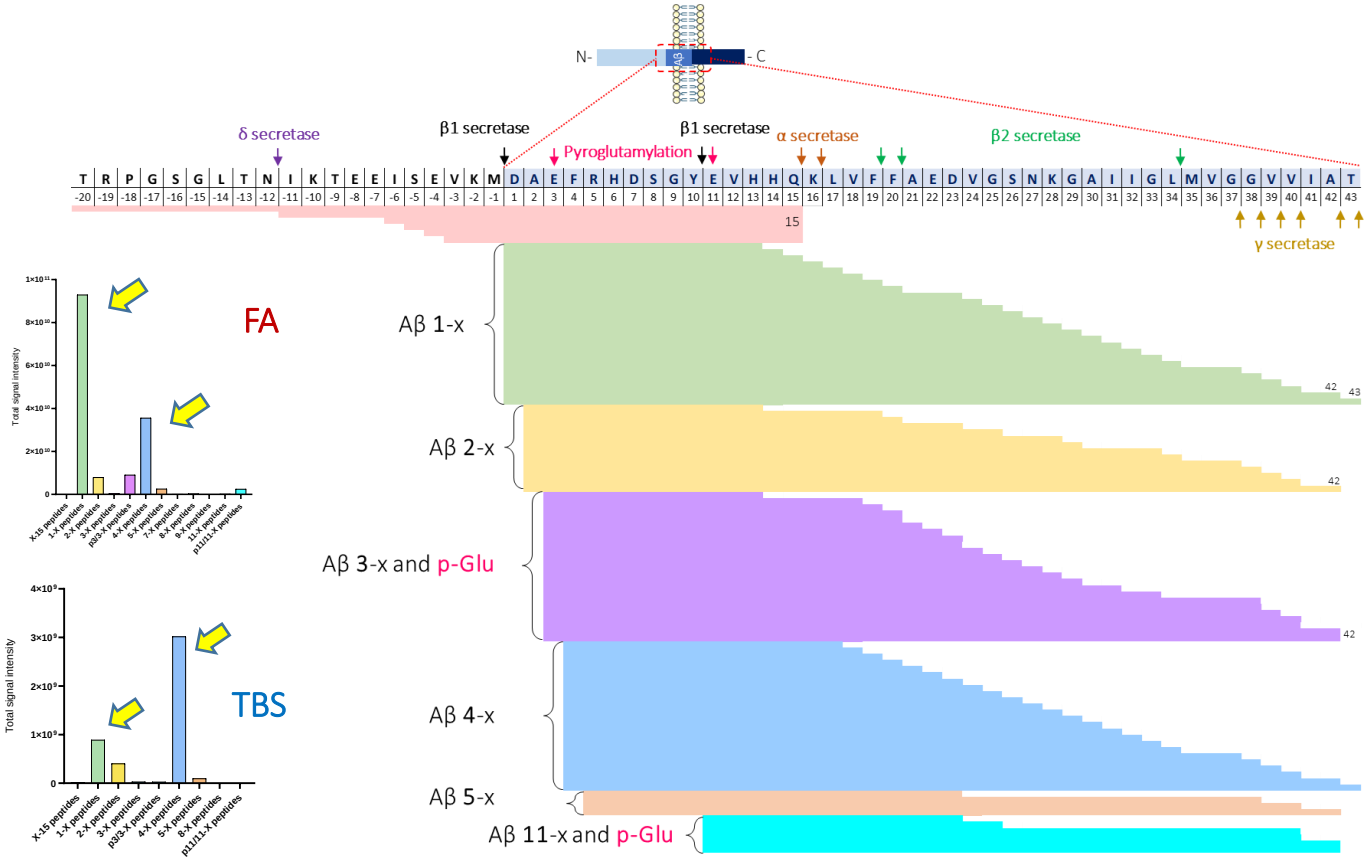


Fig. 1: Schematics of the Aβ peptide with indication of possible enzymatic cleavages (at the top) followed by a graphic representation of the main peptides detected by LC/MS-MS (below), and different abundance in the two fractions, tris-buffered saline (TBS) and formic acid (FA) (on the left). P-Glu= pyroglutamylated peptides.

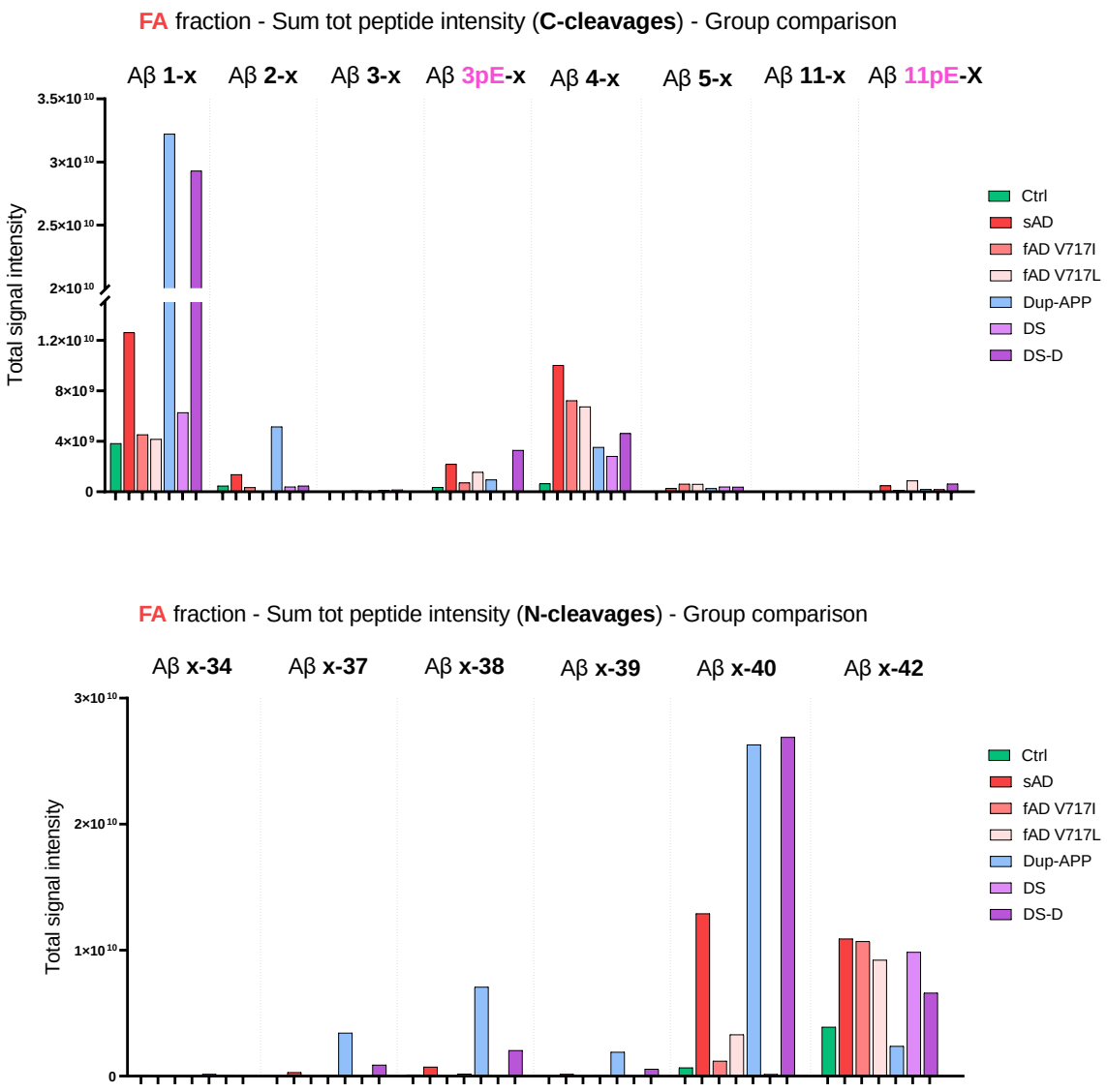


Fig. 2: Bar graphs representing the sum of the total intensity of different peptide-groups with the same N-terminal or C-terminal end and different cleavages. Ctrls= controls, sAD=sporadic AD, fAD=familial AD, Dup-APP=duplication of the *APP* gene, DS= Down syndrome, DS-D=Down syndrome dementia.

38

Single cell proteome analysis with ultra-high sensitivity using a timsTOF mass spectrometer

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[1] Bruker Nordic AB, Kista, Sweden, [2] Bruker Daltonics GmbH & Co. KG, Bremen, Germany, [3] Cellenion, Lyon, France, [4] Bruker s.r.o., Brno, Czech Republic

Background

For single cell proteome analysis, ultra-high sensitivity mass spectrometry is a key. 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) push the limits of detection to single cell level.

Materials and Methods

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 and prepared in a proteoCHIP. Tryptic peptides were loaded onto an Aurora Elite column (IonOpticks) using a nanoElute2, separated with a 22 min active gradient, detected on a timsTOF Ultra mass spectrometer in dia-PASEF and analyzed using Spectronaut.

Results

We assessed the sensitivity of a timsTOF Ultra mass spectrometer using a dilution series of K562 cell digest showing excellent identification rates, reproducibility, and quantification accuracy. Processing of the dia-PASEF data identified >1,000 or >7,000 protein groups in 15 pg or 16 ng K562, respectively. The quantitative accuracy improved inversely with loaded peptide amounts with 19% at 15 pg to 4% at 4, 8 and 16 ng loads. Analysis of the isolated HeLa cells resulted in good identification rates and reproducibility per individual cell count group with increase in protein abundance from 1 to 20 cells.

Conclusion:

The timsTOF Ultra combined with automated single cell isolation and sample preparation using the cellenONE® with the proteoCHIP leads to deep proteome coverage and high reproducibility.

39

plasma-Based Proteomic Profiling in Patients with Hyperplasia and Endometrial Cancer

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Background: Uterine cancers are the most prevalent gynecological malignancies; endometrial cancer (EC) is the most common in this group. This study used plasma-based proteomic profiling in patients with EC, hyperplasia, and control . Materials and methods: 2D differential in gel electrophoresis with mass spectrometry and bioinformatics, including a network pathway analysis identified differentially expressed proteins between the study groups. 36 patients (12 with endometrial cancer, 12 with hyperplasia (HY), and 12 controls (Ctrl)) were enrolled in this study. .

Results: The mean age of participants was 46–75 years. 87 proteins were significantly differentially expressed between the study groups, 53 were significantly differentially regulated (28 upregulated and 25 downregulated) in EC patients compared to the Ctrl, 26 proteins were significantly dysregulated (8 upregulated and 18 downregulated) in HY compared to Ctrl and 33 proteins (19 upregulated and 13 downregulated) in the EC group compared to the HY group. Proteins upregulated in the EC group compared to HY group included fructose bisphosphate aldolase A, alpha enolase, and keratin type 1, while desmin, peptidyl prolyl cis-trans isomerase A, and zinc finger protein were downregulated These proteins are known to regulate cellular processes (36%), followed by biological regulation (16%). Ingenuity pathway analysis found that differentially expressed proteins between EC and HY are linked to AKT, ACTA2, and other signaling pathways.

Conclusion: The panels of protein markers identified in this study could be used as potential biomarkers for distinguishing between EC and HY and early diagnosis nor progression of EC from hyperplasia

40

probing the molecular diversity and conformational polymorphism of tau pathology in alzheimer's disease

Srinivas Koutarapu, Junyue Ge, Wojciech Michno, Jörg Hanrieder, Henrik Zetterberg, Kaj Blennow

Aims

The primary goal of this project is to combine hyperspectral confocal imaging and SIMOA to probe the chemical and structural aspects of neurofibrillary tau pathology in Alzheimer's Disease.

Methods

We employed hyperspectral imaging of neurofibrillary tangles (NFTs) with co-staining of Luminescent Conjugated Oligothiophenes (LCOs) and pTau antibodies on AD brain sections. This is achieved using laser-scanning confocal microscopy that allows hyperspectral acquisition. Further, CSF and plasma samples from the same cohort can be further analyzed to examine the profiles of various phosphorylated antibodies like pTau-181 and pTau217: Microscopy data and SIMOA data are then correlated to understand the dynamic nature of tangle maturation correlates with that of the expression of p-Tau181 and p-Tau 217.

Results

In this study, we developed LCO-based imaging strategy to study the maturation stages of NFTs. For further analysis, this data would be analyzed in comparison with the pTau181 and pTau217 CSF/plasma profiles obtained from the SIMOA data of the same cohort.

Conclusions

The results obtained from this study aim to establish the chain of molecular events that underlie tau pathology with a particular focus on the role of different polymorphs of tau and the expression of different tau epitopes during the progression of the disease

41

Multimodal Imaging Reveals Lipid and Peptide Dynamics in Familial and Sporadic Alzheimer's Disease

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The authors have chosen not to publish the abstract

42

Multimodal Imaging Reveals Lipid and Peptide Dynamics in Familial and Sporadic Alzheimer's Disease

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The authors have chosen not to publish the abstract

43

Deeper plasma proteome coverage enables identification of novel biomarkers and classification of diseases

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Introduction

Blood plasma is one of the least invasive biopsies and a valuable specimen for clinical research. However, access to proteome information is limited by the highdynamic range in plasma, which spans approximately 10 orders of magnitude. 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

Starting from 20 µl plasma per sample, the described workflow achieves efficient dynamic range compression by capturing and thus enriching low abundant proteins on paramagnetic particles using dedicated buffer conditions (called ENRICH technology, PreOmics). The particles were separated from the residual plasma and briefly washed to remove residual low-affinity interactors. For subsequent LC-MS sample preparation, samples were processed with our iST-BCT protocol (PreOmics). Resulting peptides were analyzed by on-line nanoC18 separation on a nanoElute LC coupled to a TimsTOF HT mass spectrometer using a DIA-PASEF acquisition. Measured peptides are identified and quantified by Spectronaut 17 (Biognosys) against a plasma proteome database.

Summay

ENRICH-iST and biologically unbiased technology for dynamic range compression in biofluids by enriching low abundance proteins using only a small amount of starting material (10-20 µl). With ENRICH-iST, we observed between 2000 to 3500 proteins in small scale studies of lung cancer and colorectal cancer cohorts. We quantified more than 1500 protein groups reliably in both studies and could demonstrate that differences between groups are contained upon ENRICH-iST preparation. Moreover, we distinguished 2 cancer patient groups in the NSCLC study.

44

Therapeutic Target Discovery using proteome-wide analyses in large population health studies like the UK Biobank

Ray Chen[1], Cindy Lawley[1]
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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 ~5.400 proteins for up to 172 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.

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45

Proteomic deconvolution of response to IFNa stimulation in MLL-rearranged leukemia

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Background

Despite decades of research, MLL-rearranged (MLLr) acute leukemia remains a disease with few treatment options and a dismal prognosis in both infants and adults. Based on our previous findings, we hypothesize that interferon alpha (IFNa) has the capacity to induce a leukemia- and developmental-specific proteome and immunopeptidome in MLLr leukemia, and that such an approach can be utilized for the development of age-tailored immunotherapies of acute leukemia. Here, we aim to delineate the temporal changes in the cellular proteome of adult pre-leukemic MLLr cells upon IFNa stimulation.

Materials and methods

Hematopoietic progenitors were FACS-sorted from adult MLL-ENL-inducible mice, and cultured for 3 days with or without MLLr pre-leukemic induction, after which IFNa was added and the cells collected 17, 40 and 64 hours later. The cellular proteome was characterized by in-depth data-independent acquisition-based proteomics.

Results

We find that the level of induction of protein expression upon IFNa stimulation decreases rapidly in pre-leukemic cells over time, while the effect stays for a longer time in wild-type cells. We reveal that IFNa stimulation induces inflammatory response and translation-related processes while suppressing proliferation-associated proteins in pre-leukemic cells. MHC class I proteins and immunoproteasome are strongly induced in both wildtype and MLLr cells. Finally, we show a leukemic-specific protein signature upon IFNa stimulation.

Conclusion

Our study comprehensively describes the IFNa-regulated temporal changes in the proteome of healthy and MLLr hematopoietic progenitor cells, providing a rich resource in strategies that utilize IFNa for age-tailored treatment of MLLr leukemia.

46

A focused analysis on mutations of cancer-driving genes in the melanoma proteome

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Background: Melanoma is a cancer with exceptionally high mutation frequencies. In addition to the well-known driver mutations in BRAF, NRAS, or KIT, a wide array of proteins is functionally affected by mutations, which may carry potential prognostic and therapeutic relevance.

Materials and Methods: We reanalyzed shotgun MS-based proteomic data sets previously collected by the European Cancer Moonshot Lund Center (388 melanoma samples corresponding to 346 primary/metastatic tumors and 11 normal skin tissues from 286 patients) to conduct a focused search for mutations in genes from the COSMIC Cancer Gene Census. The reference and mutated protein sequences were concatenated for a closed database search in FragPipe. After a 1% FDR filter at PSM level, the MS/MS spectra matching to mutated peptides were subjected to a strict quality filter using PepQuery, SpectrumAI and MS2PIP tools.

Results: In total, 301 mutations with 2747 PSMs passed all quality filters. Only 128 mutations were identified in multiple tumor samples, highlighting the strong mutational heterogeneity within the cohort. Mutations were detected in 167 out of the 720 cancer-related genes, among which members of the WNT signaling- and cell cycle-associated pathways were overrepresented. 74, 4 and 12 mutations localized on protein domains, known binding sites and PTM sites respectively. In general, mutations in the HLA-A protein were more frequent in primary tumors than in metastases.

Conclusion: This study of mutations causing sequence changes to cancer-associated proteins offers new insights into dysregulated biological processes in melanoma.

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47

Exploring communication mechanisms in leukemic microenvironments: method development and analysis

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Background

Although much progress has been made in the treatment of some types of blood cancer (leukemia), others are still associated with dismal prognosis in both children and adults. New therapeutic options that are tailored to the age-specific biology of the disease are urgently needed. We and others have identified numerous intrinsic age-specific molecular features of leukemia-initiating cells, however, the understanding of the interactions between leukemic cells and their microenvironment, remains far from comprehensive. Therefore, the purpose of this study was to establish the methods for the exploration of communication mechanisms between leukemic cells and their microenvironment.

Materials and methods

The study involves proteomic analysis of the secretome of hematopoietic progenitor cells isolated from fetal liver and adult bone marrow of mice with inducible fusion oncogene MLL-ENL. The FACS-sorted progenitors were cultured in serum-free polyvinyl alcohol-based media for 6 days with and without leukemic initiation and the secreted proteins were analyzed through FAIMS Pro LC-MS/MS in DIA mode. The optimized protocol was extended to the study of secretome in co-cultured pre-leukemic cells and their niche cells.

Results

Our results highlight the suitability of the polyvinyl alcohol culture system for studying the secretome of fetal progenitor cells, while it has limitations for the adult counterparts. The analyses revealed distinct compositions in the secretome of fetal and adult pre-leukemic cells.

Conclusion

These findings underscore significant differences in the modulation of disease biology between infant and adult leukemia, offering valuable insights for developing age-tailored therapeutic approaches to enhance the outcomes for leukemia patients.

48

Facilitating precision medicine through targeted proteomics analysis of dried plasma from fingerpricks

Andreas Hober, Tasso Miliotis

Novel sampling approaches and their application in clinical trials have demonstrated the unique values of patient-centric sampling strategies that will be an invaluable component to the overall design of future clinical trials, focusing on the patient needs. Through longitudinal and cost-efficient monitoring of biomarkers, truly preventative medicine can become a reality. A crucial requirement for longitudinal monitoring 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 from a single fingerprick. An extraction protocol was optimized for targeted LC-MS/MS analysis using stable isotope labelled standards and selected reaction monitoring, which allows for reproducible and precise quantification of plasma proteins spanning a diverse concentration range. The workflow established shows 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%) while also showing excellent concordance with traditional plasma preparation. The results showcase the potential of microsampling devices for longitudinal monitoring of individuals for precision medicine purposes and has the potential to transform how clinical trials and health screenings are conducted as it retains precision and robustness while improving patient convenience.

49

Chemical Proteomics

Massimiliano Gaetani

Chemical Proteomics (ChemProt) is a unique, specialized national unit in BioMS and SciLifeLab infrastructures, dedicated to MS-based proteomics with the mission of target deconvolution and mechanism of action (MoA) elucidation for small molecules, compounds, drugs, or treatments interacting with and affecting biological systems. The strategy is adopting and developing the most efficient methods using cells or biological systems, similarly to other functional assays testing molecules.

ChemProt has fully equipped laboratories from cells to data analysis with a state-of-the-art nLC-MS/MS park and helps with project-tailored experimental design providing a complete workflow, from cultures and treatment of cells or lysates to MS data analysis and interpretation. Its methods achieve deep and multidimensional profiling of different cells, including cell lines, primary or iPSC-derived cells, organoids etc.

With drug development in mind, the focus is on sustainability, throughput, maximum proteome coverage, statistical power, data quality, cost-efficiency, reproducibility across a statistically relevant number of biological replicates (n≥3), and confidence in the results of the target and MoA discovery process.

The technology includes methods developed and optimized at ChemProt, as the PISA (Proteome Integral Solubility Alteration) assay – currently the only highly multiplexing and cost-efficient method for deep proteome profiling of the early (30-60 min) changes on proteins due to the treatment with a molecule – together with its integration with orthogonal methods (e.g., Expression and RedOx proteomics). Once the protein target is identified, ChemProt`s HDX-MS expertise and state-of-the-art platform can map the binding site and conformational changes on the protein sequence and structure.

50

High-throughput XL-MS analysis on a new high-resolution accurate mass platform

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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 leads 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. Amine-reactive crosslinkers were used to crosslink proteins and Ecoli cells. The Orbitrap Astral mass spectrometer with or without FAIMS was coupled to Vanquish Neo LC. Data were analyzed using Proteome Discoverer 3.0 software and XlinkX 3.0 nodes. MS2 acquisition speed, AGC target, and collision energy were tested for optimization. Using 60 min gradient we observed 35% increase in the identification of tbPHOX crosslinks, 95 % increase in looplinks and 85% in monolinks peptides compared to results generated previously on Orbitrap hybrid instruments. To test the throughput of the instrument, we reduced gradient to 20 min and identified comparable numbers of crosslinks and looplinks with 15% less monolinks compared to the 60 min gradient. More importantly, the identified crosslinks in 20 min gradient overlapped nicely (75%) with the results from the 60 min gradient. Taken together, we demonstrated a high throughput XL-MS workflow on the new mass spectrometer. High throughput XL-MS analysis on new high-resolution accurate mass platform- Orbitrap Astral mass spectrometer.

51

A novel intelligent data acquisition Hybrid-DIA mass spectrometry strategy: enabling data-driven and hypothesis-driven approaches in one go

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Translational scientists face the dilemma to choose between comprehensive profiling and sensitive targeted quantitation, especially with large cohorts. Proteomic profiling is commonly used to discover biomarkers, having a great potential for prognostic and predictive biomarkers; however, it still misses the sensitivity to quantify all the markers of interests. Therefore, targeted quantitation experiments of the potential markers are analyzed in the validation phase. This leads to high cost, time losses and more sample consumption. To address these challenges, we develop a novel intelligent data acquisition “Hybrid-DIA” MS strategy that enables comprehensive proteome profiling via high resolution data-independent-acquisition MS and on-the-fly intelligently switching the acquisition mode to parallel reaction monitoring (PRM) for sensitive quantification of the markers, substantially increasing throughput and reducing sample consumption.

The global profiling and targeted quantitation performance of Hybrid-DIA MS have been investigated and benchmarked against the standard DIA and the start-of-art targeted quantitation SureQuant methods, respectively. Comparable number of proteins/peptides are identified as standard DIA method, while Hybrid-DIA MS simultaneously achieves the similar quantitation performance of the low abundant biomarkers as SureQuant. We then applied Hybrid-DIA to analysis major signaling pathways in the cancer cell phosphoproteomes. With high precision and reproducibility, Hybrid-DIA MS can accurately quantify 100+ phosphorylation sites covering seven major signaling pathways in cancer cells (EGFR, RAS-MAPK, PI3K-AKT-mTOR, AMPK, apoptosis and stress response), while digitalizing their underlying proteotypes.

52

Unlocking the potential of large-cohort proteomics studies with a novel high-resolution accurate mass platform

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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 Orbitrap Astral MS 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 (SPD). Neat plasma digest was analyzed with >1000 injections on each LC-MS/MS setup. HeLa digest as QC were inserted every 12hours. 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 neat plasma digest. More than 80% of the proteins were reproducibly identified and quantified from all the runs on each LC-MS/MS setup. 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.

53

AccelerOme automatic sample preparation enables highly reproducible quantitative proteomics analysis in data-independent acquisition (DIA) workflow

Maciej Bromirski, Kevin Yang, Amirmansoor Hakimi, Amarjeet Flora, Julian Saba, Julia Kraegenbring, Santosh Renuse, Sally Webb

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. 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.

54

Unleashing the power of HT-DIA acquisition on Orbitrap Exploris 240 – Precise and accurate quantitation at 260 SPD

Maciej Bromirski, Dominic Hoch, Riccardo Stucchi, Jeff op de Beck, Julia Krägenbring, Eca Aydin, Robert van Ling

The ability to gain global proteome-wide information is key in the understanding of biological processes. To gain statistically significant quantitative information, study cohorts can comprise several hundreds of biological samples and replicates. A suitable workflow must accommodate for the need to process, measure, analyze a high number of samples with reproducible data and should maintain same performance over the duration of the whole study.

Here, we present a rugged workflow for LC-MS based high-throughput label-free quantitation using a Vanquish Neo LC system equipped with a 5.5 cm uPAC Neo High Throughput column, and an Orbitrap Exploris 240. We benchmark three different gradient lengths amounting to 100, 170 and 260 samples per day (SPD), showing high proteome coverage with reproducible quantitative results between replicates. We demonstrate the quantitative performance of these three methods by use of a three-species proteome mix, where we show accurate quantitation results for all three gradients. Additionally, we could show the robustness of the workflow with data from 1000 consecutive injections, as well as cross-site comparison of data collected at three different sites in Europe.

55

The Astral analyzer: a new class of sensitive high-resolution mass analyzer for deep and high-throughput proteomics.

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Background: Order of magnitude advances in mass spectrometer performance have enabled proteomics to expand into ever deeper, higher throughput methods and driven down the limits of sensitivity to single cell analysis. A novel multi-reflection mass analyzer has been developed that delivers a generational leap in sensitivity and speed. 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.

Materials and methods: Electrosprayed ions are concentrated in a high duty cycle dual-pressure linear trap, 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, and digested HeLa. Overall transmission was compared against an Orbitrap™ analyzer mounted upstream of the analyzer.

Results: 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. 200ng HeLa digest was analyzed to a depth of >7500 identified proteins at 300 samples-per-day, and 250pg single cell equivalent to >6,000 at 80.

Conclusion: The novel Astral analyzer represents a highly significant step above the state-of-the-art, with great potential application for the proteomics field.

56

Evaluation Of The Relative Quantitative Performance Using Tandem Mass Tags On A New High-Resolution Accurate Mass Platform

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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 at lower speeds. Despite using slower scan speeds compared to label-free samples, the improved instrument speed and sensitivity 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%.

57

Delineating amyloid peptide signature patterns among heterogenous plaque population in Alzheimer's Disease

Srinivas Koutarapu, Junyue Ge, Durga Jha, Wojciech Michno, Maciej Dulewicz, Henrik Zetterberg, Kaj Blennow, Jörg Hanrieder

Amyloid plaque deposition is recognized as the primary pathological hallmark of Alzheimer's disease that precedes other pathological events and cognitive symptoms. Plaque pathology represents itself with an immense polymorphic variety comprising plaques with different stages of amyloid fibrillization ranging from diffuse to fibrilized, mature plaques. The association of polymorphic Aβ plaque pathology with AD pathogenesis, clinical symptoms and disease progression remains unclear. Novel chemical imaging tools, mainly functional amyloid microscopy and mass spectrometry-based imaging (MSI), allow to delineate polymorphic plaque structures and identify the associated Aβ makeup. We here set out to make use of these tools to interrogate heterogenic plaque types and their associated biochemical architecture. We observed distinct Aβ signatures separating diffuse plaques from fibrilized plaques, which showed significantly higher content of Aβx-40. Moreover, among fibrilized plaques, we observed a sub-type of plaques, previously annotated as coarse-grain plaques. Both in sAD and fAD brain tissue, coarse grain plaques show significantly higher levels of Abx-40 and lower content of Aβx-42 deposition. We show that coarse grain plaques show higher levels of neuritic content including PHF and phosphor Tau positive neurites as well as dystrophic neurites and associated markers (RTN3). Finally, we demonstrate that coarse grain plaques show chemical similarities with vascular plaques, however show higher levels of Aβ 1-42 and increased levels of pyroglutamated Aβx-40 and Aβx-42 peptides.

58

Label-free DIA-based workflow for single-cell proteomic analysis on an Orbitrap Ascend mass spectrometer

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Recent advances in LC-MS have enabled label-free single cell proteome analysis revealing unexpected functional diversity of cells. However, there are still key challenges in this field application such as sensitivity, coverage, dynamic range, and throughput. To address some of these challenges, new method developments, as well optimization on existing LC-MS-based proteomics workflows are necessary. Here, we demonstrate the use of Orbitrap Ascend mass spectrometer, nano- UHPLC and solid silicon micro-pillar array column technology for high-throughput single cell applications.

Individual HeLa cells were isolated and Pierce™ HeLa digest was used for dilution series. Samples were analyzed using Orbitrap Ascend mass spectrometer FAIMS Pro™ interface coupled to a Vanquish Neo UHPLC system. Separation was performed on a 50 cm µPAC™ Neo low load column.

The performance of this ultra-sensitive LC-MS workflow was first optimized using a dilution series of HeLa digest. From 250 pg of HeLa digest load, we could identify on average > 3,015 protein groups by library-free approach whereas using DIA-library generated from 10 ng HeLa digest, we were able to identify >5,200 protein groups using a method with a throughput of 80 samples per day. Identifications from single-cell samples were on lower side due to different sample preparation method with associated losses and will also be presented.

End to end workflow for single-cell proteomics on a Orbitrap Ascend mass spectrometer with sensitive detections of proteins from single cell or low input samples.

59

A benchmarking workflow for high-throughput DIA label-free quantification using a novel high-resolution accurate mass platform

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Introduction

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.

Methods

Mixtures of three proteomes 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 15cm column in Trap&Elute mode, using a Vanquish™ Neo UHPLC system. Different gradient lengths were employed, ensuring throughput from 48 to 180 samples per day. MS data were acquired in DIA mode with 2Th window, and processed using Biognosys™ Spectronaut™ 17 software.

Results

The new benchmarking workflow enabled the quantification of >13,000 protein groups (7,600 Human, 4,100 Yeast and 1,400 E.coli) and >160,000 unique peptides, using a 20-min gradient and 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.

Conclusion

The Orbitrap Astral platform provides unsurpassed throughput while ensuring deep proteome coverage, thus opening new frontiers for life science applications.

60

High-throughput proteomics with the Orbitrap Astral mass spectrometer

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Single-shot LC-MS based proteomics has been an essential tool to help researchers unravel the proteome composition of complex biological samples. We combine the Orbitrap Astral mass spectrometer with a very short pillar array-based separation column to achieve both comprehensive as well as high throughput proteome analysis with a single LC-MS set-up. A set of robust high-to-medium throughput LC-MS methods with variable flow rates was developed by combining the extended flow capabilities and minimal delay volumes of the Vanquish Neo with the flow rate versatility of the µPAC Neo High Throughput column. Operating in trap-and-elute configuration, performing column re-equilibration in parallel to sample loading, and utilizing variable flow rate during the gradient formation achieved a significant increase in instrument productivity. LC-MS instrument productivity of 68, 79, and 87% could be achieved for respective 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 with DIA using a 2 Th isolation window. At a sample throughput of 180 samples per day (8 min method) we were able to identify 7900 protein groups on average from 5 technical replicates. Considerable increases in proteome depth could be achieved by extending the gradient length and reducing the active elution flow rate gradually. Within 14.4 and 24 min of total LC-MS time, we identified an average of 8579 and 9167 protein groups, respectively.

61

An in-depth plasma proteomics workflow powered by a Novel HRAM mass spectrometer

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Plasma is a rich source for protein biomarkers that can be used to understand disease biology, measure responses to therapeutic treatments or for diagnostic and prognostic purposes. Here we present a label-free plasma proteomics workflow on both Orbitrap™ Astral™ MS and Orbitrap Exploris™ 480™ MS as a robust analytical setup for in-depth analysis of plasma proteins.

Plasma samples were processed using 40 µL each mixed with five nanoparticle (NP) aliquots included in the Proteograph Assay Kit™. One-hour of incubation allowed for high-affinity proteins to displace high-abundance proteins, resulting in a reproducible protein corona on each NP surface. A series of washes removed non-specific and weakly bound proteins and resulted in a highly specific and reproducible protein corona that contained the high-affinity protein binding partners selected by the NPs. Protein coronas were reduced, alkylated, and digested with Trypsin/Lys-C to generate tryptic peptides for LC-MS analysis. All steps were performed in a one-pot reaction and in-solution digestion mixture was desalted, removed of detergents, and clean peptides were eluted in a high-organic buffer into a deep-well collection plate.

Thermo Scientific™ Orbitrap™ Astral mass spectrometer run in DIA mode was used for both high-throughput and in-depth analysis of plasma samples. Analysis of 500 ng of the neat plasma sample using the 15cm EasySpray column on a 5.5min active gradient resulted in ~600 protein groups. Increasing the gradient length for deeper coverage with a 75cm EasySpray column on a 60min active gradient improved the number of protein groups to ~1000.

62

Sensitive and robust high-throughput workflow for qualitative and quantitative single-cell/single cell like analysis

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Introduction

In recent years single cell analysis has profited from advances in LC-MS based proteomics approaches\tools. Nevertheless, there are still challenges in this field of application. Besides sample preparation, the key challenges in looking at individual single cell proteomes are sensitivity, coverage, dynamic range, and throughput. To address some of these challenges, new technological developments, as well as improvements on existing LC-MS-based proteomics workflows are a necessity. Here, we evaluated the performance of the Orbitrap Astral mass spectrometer for high-throughput single cell applications.

Methods

Individual HeLa cells were isolated using CellenONE® system from Cellenion, followed by reduction, alkylation and trypsin digestion as per manufacturer's instructions. For the dilution Pierce™ HeLa digest (10 ug) was reconstituted by adding 100 µL of 0.015% DDM solution, sonicated for 5 min, then dilution in 0.015% DDM solution to 5 ng/µL. Single cell digests and the diluted standard HeLa digest samples were analyzed using Orbitrap Astral mass spectrometer with Thermo Scientific™ FAIMS Pro™ interface coupled to a Thermo Scientific™ Vanquish™ Neo UHPLC system. Data was acquired in a DIA mode and processed with Spectronaut 18 software and Proteome Discoverer 3.1 software.

Preliminary Data or Plenary Speakers Abstract

. Using 80 SPD method and 250 pg Pierce HeLa digest we could identify >5,000 protein groups and >25,000 peptides using a library-free search. Median CV values at protein groups level were always <10%. The analysis of HeLa single cell samples resulted in ~5000 protein groups and 25,000 peptides using library-free search.

63

smart polymers for robust affinity-based capture of phosphorylated and methylated proteins

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Molecularly Imprinted Polymers (MIPs) offer an innovative platform for protein assays and sensors. These polymers are typically created using network polymerization, with a template that pre-organizes monomers and serves as a mold for shape-complementary binding sites. When the template is removed, MIPs exhibit antibody-like recognition behavior with strong affinities for the original template or similar targets (known as epitope imprinting). MIPs targeting protein post-translational modifications (PTMs), such as phosphorylation, glycosylation, and methylation, show promise as alternatives to traditional affinity reagents. We present high-affinity MIPs designed for phosphorylated and methylated proteins. Firstly, we focused on the Tyr-492 and Tyr-493 kinase regulatory motif of the SH2 domain in ZAP70, a critical player in T-cell receptor signaling. Phospho-tyrosine (pTyr) targeting MIPs are created to specifically recognize mono- or diphosphorylated tryptic peptides, binding with low μM Kd values in aqueous media and buffers. Secondly, we developed affinity reagents for histidine phosphorylation (pHis), enabling selective enrichment and detection of pHis peptides without the use of acidic solvents. Lastly, we explored methylation-specific affinity reagents using MIP technology, creating isomer-specific MIPs for methyl histidine (meHis) and high-affinity binders for methyl lysine (meLys). These MIP-based approaches offer robust techniques for enriching phosphopeptides and methylated peptides, showing great potential for clinical applications.

64

Analytical strategies for glycoproteomic studies

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Protein glycosylation is one of the most common and the most complex post-translational modification (PTM). The complexity of protein glycosylation results in the requirement of multiple analytical strategies tailored to the individual research projects. Due to large variations in recommended sample preparation, analysis and data processing workflows, the selection of correct and optimal methodologies for glycoproteomic studies can be challenging.

We regularly characterize N-glycosylation, mucin type O-glycosylation and glycosaminoglycans (GAGs). In general, proteomic samples are subjected to protease digestion and nanoLC-MS/MS is used to identify glycopeptides both with respect to glycan structure and peptide sequence to accomplish a site-specific glycan analysis. Depending on the project, purification protocols are often applied to enrich glycopeptides from the otherwise dominating ´naked´ peptides. Data analysis pipelines can effectively be used for automated identifications and quantifications. However, great care must be taken to verify the correctness of the data.

Here, we present our analytical workflows for (1) large-scale glycoproteome studies of complex biological samples, (2) deep glycoprotein profiling of recombinantly expressed glycoproteins, (3) in-depth analysis and verification of glycan structures based on analysis of diagnostic oxonium ions.

These analytical workflows were developed to address the increased interest and demands for protein glycosylation analysis at the Proteomics Core Facility, a node in SciLifeLab and BioMS.

65

Characterization of acoustically isolated extracellular vesicles from pathogen activated platelets

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Background

We have characterized and investigated differences in extracellular vesicles (EVs) from pathogen activated platelets, compared to EVs from Thrombin-activated or resting platelets.

Materials and methods

Platelet rich plasma was stimulated with either M1 protein from S. pyogenes, Thrombin or HEPES buffer, Figure 1. The platelets were removed by centrifugation, leaving the released EVs in plasma. The EVs were isolated and enriched by acoustic trapping and analyzed using mass spectrometry, immunoblotting, and immunogold labelling with transmission electron microscopy (TEM). Additionally, EVs were incubated with whole blood to investigate immunomodulatory effects.

Results

The mass spectrometry data showed clear distinction between isolated vesicles and plasma, figure 2A. There was also a clear distinction between EVs from activated platelets and resting platelets, figure 2B. Interestingly, M1 protein was enriched in EVs compared to plasma, figure 3A, suggesting that M1 protein binds to platelet EVs. This was supported by immunoblotting with clear bands around 54 kDa present in M1 exposed EV samples, figure 3B. Additionally, TEM images showed immunogold labelled EVs positive for both CD42b and M1 protein, figure 3C, confirming that M1 binds to platelet EVs.

The whole blood assay showed that platelet EVs stimulated platelet-neutrophil complex formation, figure 4A. Additionally, platelet EVs stimulated IL-8 cytokine release, figure 4B, suggesting functionally intact EVs.

Conclusion

We have acoustically isolated functionally intact EVs from plasma and characterized them with several downstream analyses. We found that M1 protein binds to and is transported with platelet EVs, a mechanism which could contribute to infectious progress in sepsis.

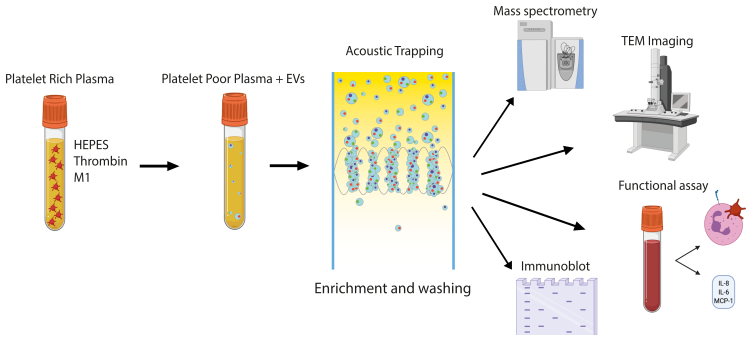

Figure 1: Schematic illustration of sample processing

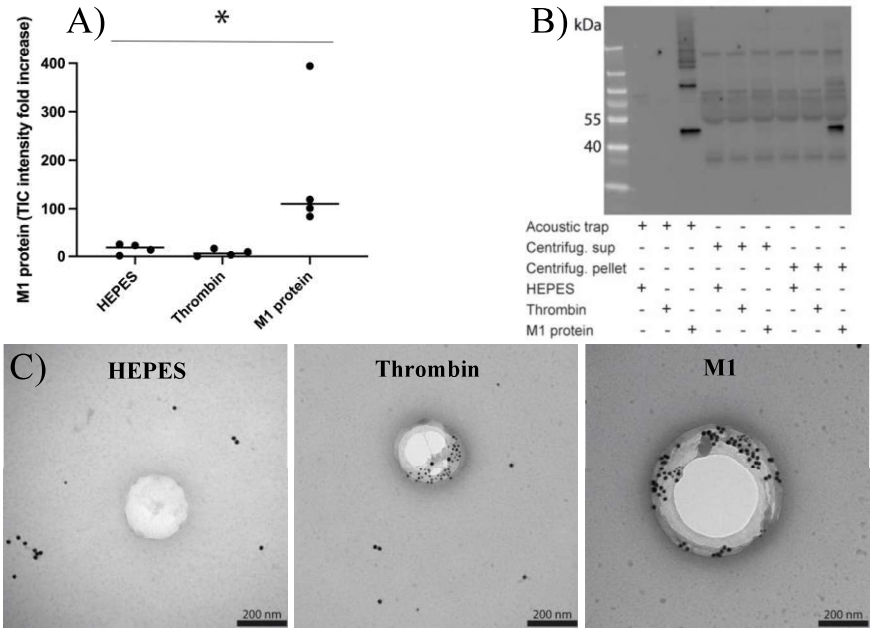

*Figure 3: Bacterial M1 protein associates with platelet vesicles. A) Fold increase of total ion current (TIC) normalized M1 protein signal when comparing isolated EVs to background plasma. Samples treated with M1 show a much greater signal, suggesting the M1 protein is enriched with the EVs. *=p<0.05 Mann-Whitney test B) Immunoblot against the M1 protein. M1 is found in EV samples that have been treated with M1 prior to isolation. C) TEM images of acoustically isolated EVs labelled against CD42b (10 nm gold) and M1 (15 nm gold).*

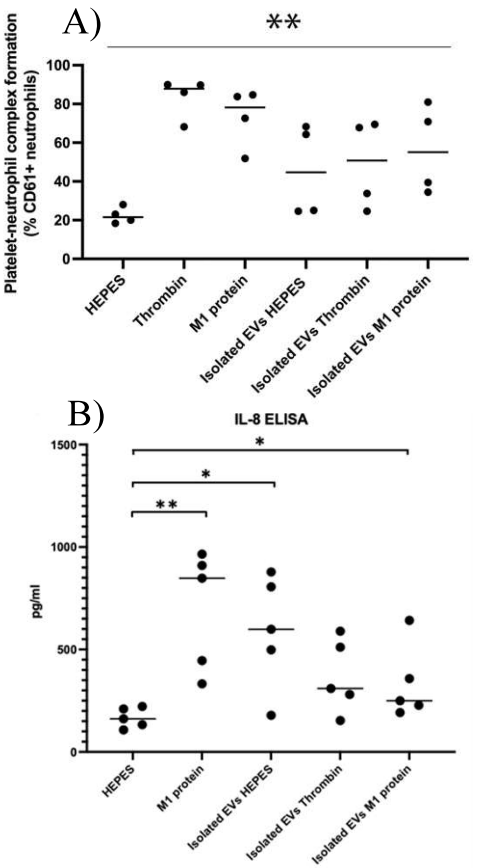

*Figure 4: Stimulation of whole blood with isolated EVs to investigate immunomodulatory effect of isolated EVs. A) Platelet EVs stimulated platelet-neutrophil complex formation. B) Platelet EVs mediate cytokine release from monocytes. *=p <0.05, **=p <0.01Mann-Whitney test.*

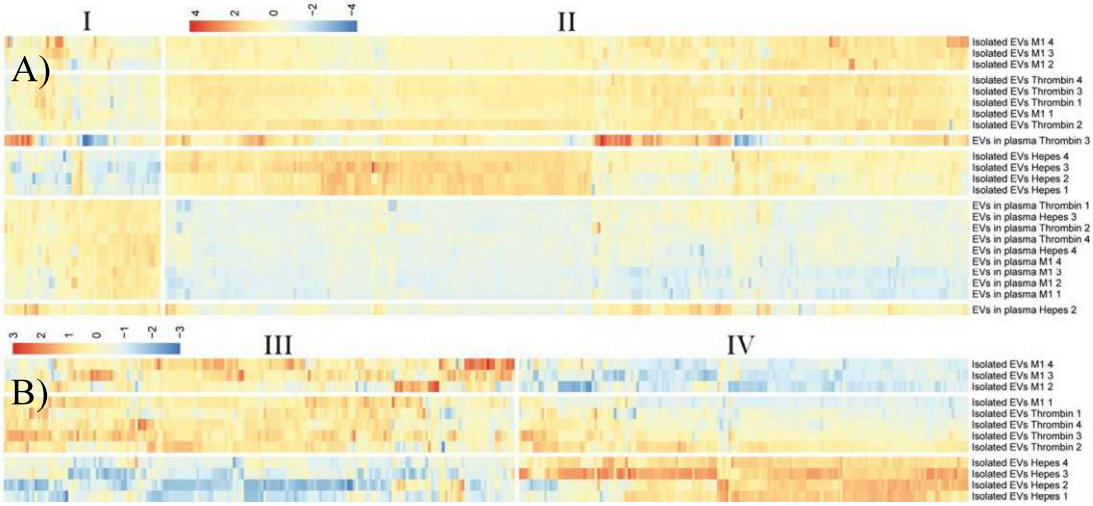

Figure 2: Heatmaps of normalized protein signal. The rows correspond to samples and columns to identified proteins. The legend gives the z-score. A) Plasma samples and isolated EVs. Cluster I shows weaker signal for EVs and contains mostly plasma proteins such as albumin and complement proteins. Cluster II shows stronger signal for EVs and contains predominantly proteins related to hemostasis. B) Isolated EVs from platelets treated with HEPES, Thrombin or M1 protein. Cluster III shows stronger signal for Thrombin and M1, which are known to activate platelets and contains proteins related to platelet degranulation and complement and coagulation cascades. Cluster IV shows stronger signal for HEPES and contains mainly proteins related to hemostasis.

66

Accessing the exosome proteome in a minute blood plasma sample by rapid acoustic isolation of extracellular vesicles

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The authors have chosen not to publish the abstract