BIOGNOSYS
NEXT GENERATION PROTEOMICS

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Cancer Biomarker Discovery by Proteome Profiling of the Human Blood Plasma Proteome at a Depth of over 2,700 Proteins

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Introduction

Blood is optimally suited to investigate health and disease states as it flows through the whole body and is readily accessible. Label-free discovery proteomics is a powerful method to study blood plasma. It enables identification and quantification of hundreds of proteins in large sample cohorts. Despite this progress, there is still a large fraction of the plasma proteome routinely not measured and therefore not accessible for biomarker discovery. Therefore, progress in coverage of the proteome remains highly relevant.

We present a deep data independent acquisition workflow based on automated plasma depletion and FAIMS suitable for biomarker discovery in plasma. We demonstrate the applicability of this workflow by profiling 180 human plasma samples from healthy individuals and cancer patients.

Methods

The healthy and cancer plasma samples were depleted in 96-well format using a MARS-14 depletion system (Agilent) automated with a UHPLC. Then the depleted samples were digested with trypsin and cleanup was performed using C18 material.

A FAIMS Pro device was connected to a Thermo Scientific Orbitrap Exploris 480. For chromatography, a Thermo Scientific EASY-nLC 1200 was used. Optimized, 2h FAIMS-DDA and FAIMS-DIA methods were generated. Libraries were generated using high pH reversed phase fractionation at a depth of 25 fractions. The DDA data were analyzed with SpectroMine 2 (Biognosys) and the DIA data with Spectronaut 14 (Biognosys).

Preliminary Data

To be able to profile human plasma at large scale and a maximal depth, we developed and optimized two main aspects. We automated the depletion of the top 14 most abundant proteins in human plasma to a throughput of 40 depletions per day per setup. Additionally, we further optimized FAIMS-DIA methods (presented at the last ASMS conference) for deep (plasma) proteome profiling. In combination we were able to identify and quantify 1,631 proteins in 1h

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gradients, 1,700 proteins in 2h and 2,035 proteins in 4h. The cumulative coefficient of variation for repeated depletions was 24.6% and for batches 26%.

To demonstrate the benefit in clinical samples, we used this new platform to process a study containing 180 plasma samples of healthy and cancer patients of early and late stage. There were 70 matched healthy donors and plasma from patients with cancer: 30 pancreatic, 30 breast, 30 prostate, colorectal and 30 NSCLC cancer. After sample preparation and acquisition, we were able to profile 2,741 proteins in the study and 1,849 proteins on average per plasma measurement using the 2h gradients. The missing values on protein level were at 33%.

Finally, we performed exploratory analyses, where we have compared protein identifications to the plasma proteome data coming from MS analysis and registered in the Human Protein Atlas. We reached 8 orders of magnitude of dynamic range given the concentrations in the database. Within this range we covered extensively tissue leakage proteome, interleukins and signalling proteins such as EGF, KLK3 (PSA), AKT1, CD89 and CD3. Using classification algorithms, we were able to identify a protein panels with a significant positive predictive value associated with individual cancer stages, reaching the best performance in pancreatic and colorectal cancer. Detailed, follow-up analysis of our findings to further understand the biology of cancer progression is planned.

Novelty aspect

Significant improvements in plasma proteome discovery analytics, applied to a study comprising multiple cancer types.