dia-PASEF for targeted proteomics: development of large-scale assay for quantitation of more than 500 proteins in human plasma sample

Stephanie Kaspar-Schoenefeld[1], Sebastian Mueller[2], Tejas Gandhi[2], Sira Echevarria-Zomeno[2], Markus Lubeck[1], and Gary Kruppa[1]

[1]Bruker Daltonics GmbH & Co. KG, Bremen, Germany

[2]Biognosys, Schlieren, Switzerland

Introduction

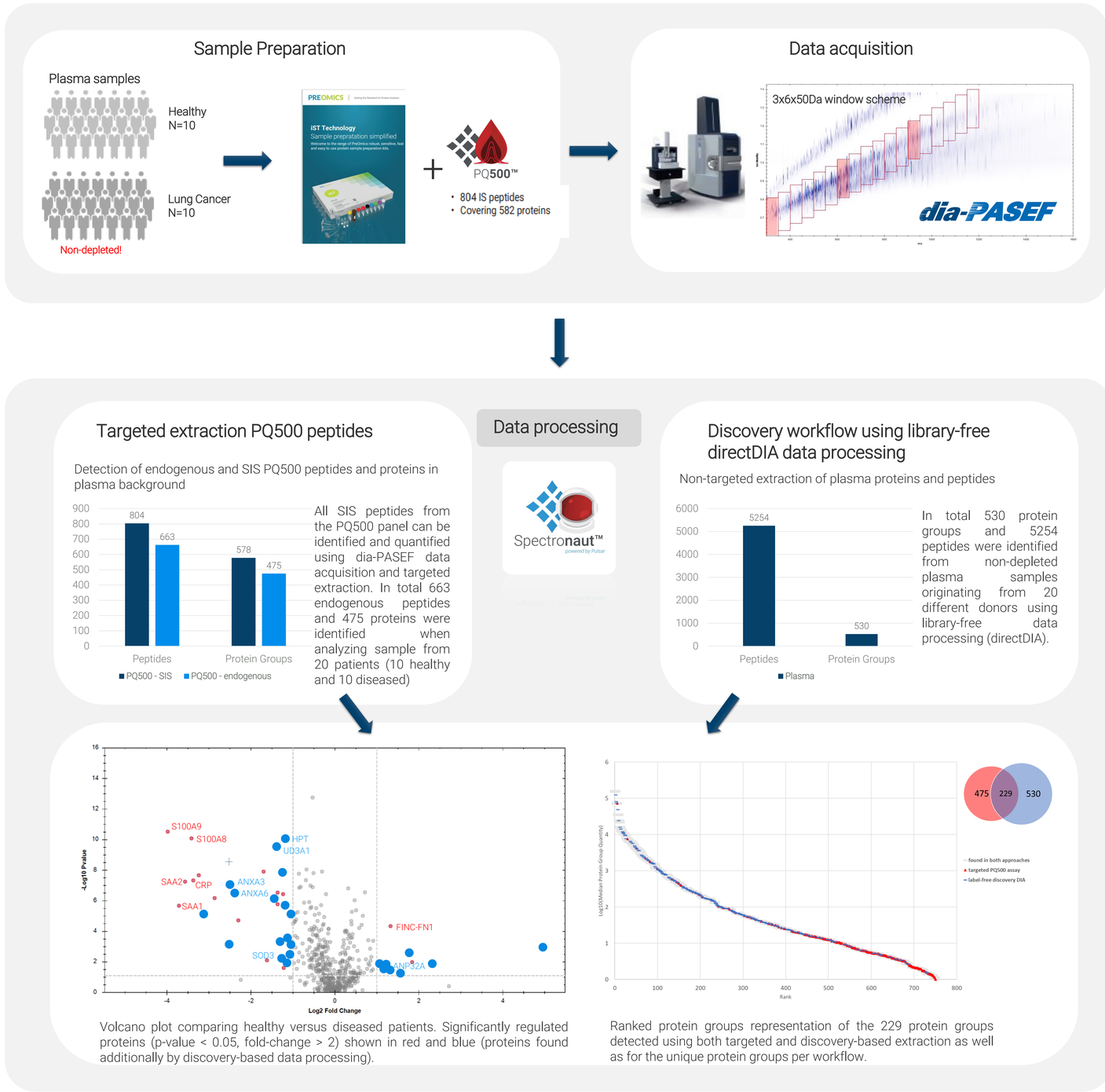
dia-PASEF merges the benefits of DIA with the advantages of ion mobility in proteomics experiments making it an advantageous method to be integrated in a platform for large-scale biomarker studies without the need for in-depth method optimization. Here, we use dia-PASEF in combination with the PQ500 kit to develop a targeted quantitation assay for peptides in human plasma sample.

Methods

Individual plasma samples were digested using the iST kit from PreOmics. The PQ500™ kit (Biognosys) was prepared according to the manufacturer's instructions and spiked into the prepared digests. Tryptic peptides were separated on a 25cm C18 column (75µm x 1.9µm, Aurora, IonOpticks) using a nanoElute coupled to a timsTOF HT mass spectrometer via a CaptiveSpray ionization source using a 30-min acetonitrile (ACN) gradient. For the dia-PASEF acquisition, a window placement scheme consisting of 6 TIMS ramps with 3 mass ranges per ramp spanning from 300–1200 m/z and from 0.6–1.40 1/K_0 with a cycle time of 0.7 seconds, including one MS1 frame, was utilized. Data was processed in Spectronaut (v16, Biognosys) using an ion mobility annotated PQ500 library for targeted data extraction. The library-free directDIA workflow was used for discovery-based proteomics.

Results

Here, we developed a targeted quantitation assay for human plasma proteins using dia-PASEF. The major advantage of the approach is that there is no need for tedious method development as is typically required for targeted approaches like SRM and MRM.
The assay was applied to a proof-of-concept study of non-depleted plasma samples from patients diagnosed with lung cancer. All 804 SIS peptides and 578 protein groups from the PQ500 panel could be detected. In total, 663 peptides and 463 protein groups were identified, covering around 80% of the PQ500 panel. Of those, 55 proteins were found to be significantly regulated (p-value < 0.05, fold change > 2). Three of the proteins (Fibronectin, Immunoglobulin lambda-like polypeptide 1, Immunoglobulin lambda-like 1 light chain) were detected to be higher abundant in healthy donors, whereas the remaining proteins showed significant upregulation in donors diagnosed with lung cancer. For example, elevated plasma levels of serum amyloid A (SAA) proteins (SAA1 and SAA2) have been detected in patients diagnosed with lung cancer. With dia-PASEF not only targeted peptides can be monitored, but quantitation information of all detectable peptides is preserved. Data processing using directDIA™ allows identification and quantitation without the need for library generation. In total 530 protein groups and 5254 peptides were identified during the experiment. Additional 26 protein groups were found to be significantly regulated, which were not part of the targeted quantitation assay. Among those, extracellular superoxide dismutase (SOD3) was found, which is known to be more highly expressed in tumor cells than in normal cells.



Volcano plot comparing healthy versus diseased patients. Significantly regulated proteins (p-value < 0.05, fold-change > 2) shown in red and blue (proteins found additionally by discovery-based data processing).

Ranked protein groups representation of the 229 protein groups detected using both targeted and discovery-based extraction as well as for the unique protein groups per workflow.

Fig. 1 Overview large-scale quantitation of more than 500 proteins in human plasma

Summary

Our results show that the applied multiplexed approach has the potential to identify disease biomarkers in non-depleted plasma samples without in-depth expert knowledge by using a standard proteomics workflow supported on the timsTOF platform.
In our study we further evaluated and optimized the dia-PASEF approach as it is less complex to set up and provides both targeted and non-targeted data extraction capabilities

Conclusion

- **Workflow for targeted quantitation in non-depleted human plasma using dia-PASEF was evaluated.**
- **The workflow eliminates tedious and time-consuming method development.**
- **By using a data-independent approach additional proteins not included in the target panel are measured and can be quantified resulting in a combination of targeted and discovery proteomics**

timsTOF HT