5135/18: "Characterization of a Data-independent Acquisition Mass Spectrometry-based Workflow in the Plasma of NSCLC Subjects Reveals Host Immune Response Mechanisms"

Kamil Sklodowski[1], **Nicholas Dupuis[1]**, **Linda Sensbach[1]**, **Sebastian Muller[1]**, **Lukas Reiter[1]**

[1]Biognosys AG, Schlieren, Switzerland

Background

Measurement of circulating biomarkers in cancer has proven utility for early detection, differential diagnosis, and predicting pre-treatment response to therapy. More recently, circulating proteomic biomarkers for pre-treatment prediction of therapeutic response have received additional attention due to the heterogeneous responses to immunotherapies. To develop a greater understanding of the circulating plasma proteome in subjects with cancer we have optimized a depleted plasma proteomic workflow, based on label-free data independent acquisition (DIA) mass spectrometry, and applied it to plasma from subjects with late stage NSCLC. This approach provides a deep and unbiased description of the plasma proteome and the dysregulated biological pathways associated with lung cancer.

Methods

Plasma samples from subjects with Stage III-IV non-small cell lung cancer (NSCLC, n = 15) and age matched healthy donors (n = 15) were depleted of 14 high abundance proteins using MARS Hu-14 spin columns (Agilent). The resulting flow through was prepared for mass spectrometry using a filter aided sample preparation method (FASP). All samples were analyzed using 2h gradients on a C18 column coupled to a Thermo Scientific Q Exactive mass spectrometer operated in DIA mode. Data was extracted using Spectronaut (Biognosys) with combined sample specific and resource spectral libraries and statistical analysis was conducted to identify disease associated biomarker candidates. Pathway analysis highlights dysregulated biological functions.

Results

An overview of assay optimization will be presented which resulted in the final workflow. In summary, a comprehensive protein spectral library was created containing 1,827 unique proteins and in DIA acquisition, 1,304 proteins were quantified across all samples. Univariate statistical testing identified 162 dysregulated proteins (125 up-regulated and 37 down-regulated;

Biognosys AG
Wagistrasse 21
8952 Schlieren (Zurich)
Switzerland

t: +41 (0) 44 738 20 40
f: +41 (0) 44 738 20 49
info@biognosys.com
www.biognosys.com

q-value > 0.05 and log2 fold change > 0.58). In addition to the acute phase proteins (e.g. CRP and SAA1) which were previously verified to be elevated in subjects with NSCLC, multivariate analysis identified additional proteins that are differentially expressed between the sample groups. Most relevant to immune function was CLC (Galectin-10), which has been identified as key component supporting the suppressive function of Tregs[1] and S100A9 which is a known therapeutic target associated myeloid-derived suppressor cells2. Furthermore, F13A1 was suppressed in the NSCLC samples which is known to be associated with macrophage activation.

Conclusions

Multiple plasma proteins were found to be dysregulated and associated with NSCLC reflecting the host immune response via acute phase response signaling and immunosuppressive mechanisms. Several of these markers have been linked to patient outcomes and represent known therapeutic targets.1. Kubach, J., et. al. Blood 2007 110:1550-1558 2. Shen, L., et. al. Cancer Immunol Res. 2015 3(2): 136-148.

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