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Deep Plasma Proteome Profiling of Systemic Lupus Erythematosus Patients for Biomarker Discovery

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Introduction

Systemic Lupus Erythematosus (SLE) is a chronic, life-long autoimmune disease with heterogenous clinical presentation that affects vital organs. Patients with renal involvement are considered as worst prognosis, thus lupus nephritis (LN) is sometimes even considered as separate indication from extra-renal lupus (ERL). Due to a drought of mechanism-driven treatment options with favorable safety profiles, there is a need for exploiting new global technologies for development of better biomarkers as early predictors of patient classification for (i) determining suitable treatment paradigms and (ii) the identification of novel potential therapeutic targets.

Here, we present an optimized workflow for deep plasma proteome profiling that was applied on prospective collection of 62 longitudinal samples from ERL and LN patients, as well as healthy controls.

Methods

25 µL human plasma samples were depleted on an Agilent MARS Hu-14 column followed by reduction, alkylation, digestion and C18 clean-up. Biognosys' iRT-peptide mix was spiked into the peptide samples and subsequently injected to an in-house packed reversed phase column on a Thermo Scientific™ EASY-nLC™ 1200 nano-liquid chromatography system connected to a Thermo Scientific™ Orbitrap™ Exploris 480™ mass spectrometer operated in positive mode and equipped with a FAIMS Pro™ ion mobility device. Spectral libraries were generated with SpectroMine™ (Biognosys, version 1.8) and the HRM/DIA data was analyzed with Spectronaut™ (Biognosys, version 15.4).

Preliminary Data

The analyzed ERL and LN samples were gender- and age-matched to healthy controls. Within each group, the longitudinal samples were taken at clinically determined active disease phase ("flare") and at recent onset remission phase, respectively. In addition to healthy control

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samples, long-term remission samples were taken from independent ERL and LN patients to serve as an additional disease reference.

The samples were processed with an optimized semi-automated depletion workflow combined with a DIA-FAIMS acquisition method. We achieved a proteomics depth of 2’237 protein groups (19’231 peptides) across the 62acquisitions with a low number of missing values (~1’900 protein groups were quantified in 70% of the samples). More than 1’000 Protein Groups could be quantified with a coefficient of variation < 25% (workflow variability) which is much lower than the inter-individual variability (median CV > 50%).

We performed a differential expression analysis and found previously reported biomarkers that confirm validity of our approach, as well as some novel biomarkers. In general, many of the affected proteins in the SLE patients were found to be related to innate and adaptive immune response/regulation or host defense such as C3, C9, C4A, LGALS3BP or CD14. Many of those proteins are gradually changing from active phase, through recent onset remission, to well-treated long-term remission. However, even the long-term remission signatures still differ from healthy controls and could therefore be used for molecular disease diagnosis and disease activity assessment.

In addition, we found disease-type specific biomarkers in ERL and LN patients. We found downregulated of proteins related to bone growth and development (CHAD, TSKU) in LN patients whereas not affected in ERL patients. On the other hand, ERL active patients showed a specific upregulation of proteins involved in antimicrobial defense mechanisms such as LTF, PGLYRP1 or PXDN.

Novel aspect

1) We present a deep and scalable plasma proteomics workflow. 2) We identified SLE biomarkers for classification and activity assessment.