Comparative Proteomics Of Abundant Protein-Depleted Plasma From Patients with Sickle Cell Disease-Related Pulmonary Hypertension

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Introduction:

Human plasma proteomics is becoming a powerful means to investigate the molecular etiology and pathogenesis of disease, as well as to identify disease biomarkers. Separation of plasma proteins by successive chromatofocusing and reversed-phase HPLC (2D-LC), particularly following abundant plasma protein depletion, greatly facilitates MS analyses and can provide differential protein expression maps that direct research into proteins displaying significant disease-related changes. Sickle cell disease (SCD), a common genetic hemoglobinopathy, is characterized by clinical heterogeneity suggesting a role for plasma post-translational protein modification in disease modulation. To explore this hypothesis, we utilized a comparative proteomics approach based on 2D-LC followed by MALDI-TOF MS and LC MS/MS analyses of abundant protein-depleted plasma from patients with sickle cell disease (SCD), with or without pulmonary hypertension (PH), a common cause of mortality in these patients.

Methods:

Platelet-poor plasma was prepared from peripheral blood samples obtained from age, sex and racially-matched patients in each of the following groups, SCD with PH, SCD without PH, PH unrelated to SCD, and normal volunteers without co-existent cardiopulmonary disease. Samples were albumin-depleted, or abundant plasma-protein depleted by off-line HPLC, separated by two-dimensional HPLC on a Beckman PF2D™. Differential 2D expression maps were generated with the Beckman software ProteoVue™ and DeltaVue™, and fractions showing appreciable differences in protein expression levels, pI, or reversed-phase retention time were targeted for MS analyses. Tryptic peptides were analyzed using a Bruker Reflex IV MALDI-TOF MS. Further analysis was performed on a Waters Cap-LC QTOF MS.

Results:

With coupling 2D-LC of abundant protein-depleted plasma to MS, we have conducted a comparative proteomic analysis of the plasma of SCD patients with or without PH, patients with isolated PH and normal volunteers. Differential 2D expression mapping has allowed us to target only those proteins in the plasma that changed significantly in abundance, pI, or retention time in correlation with disease. MALDI-TOF MS and LC QTOF MS/MS analyses have allowed us to identify several abundant and medium-abundance proteins that appear to vary with the disease process. For instance, we have identified two proteins from SCD patients with PH, transferrin and apolipoprotein A1, which exhibit pronounced changes in abundance and post-translational modifications. Modifications of transferrin, an important molecule in iron metabolism and transport, and apolipoprotein A1, a molecule required for HDL-mediated activation of endothelial nitric oxide synthase, could play a role in the pathogenesis of PH in SCD, or could serve as

important biomarkers of disease. Transthyretin, Hemopexin and Apolipoprotein A2 were also identified as potential biomarkers by exhibiting post-translational modifications such as oxidation and deamidation.

Conclusion:

Further MS analyses of these and other differentially expressed proteins will be instrumental in determining the role of these modifications in the pathogenesis of PH in SCD patients. Differential plasma proteomics such as this study may have a significant impact on the detection and treatment of SCD, PH and other human diseases.

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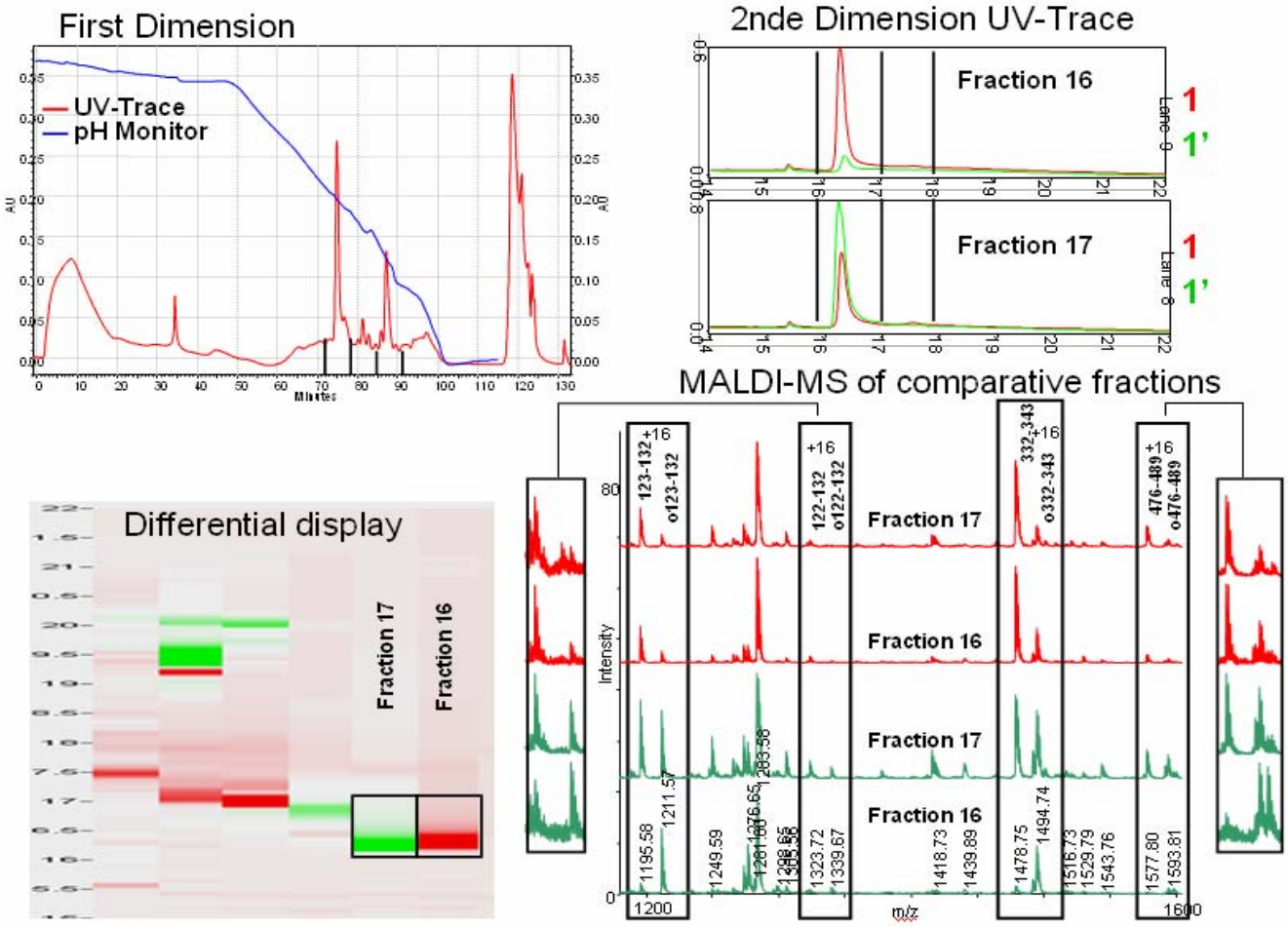

Figure1: Identification of Transferrin Post-Translational Modifications by MALDI-MS after separation by two-dimensional HPLC on a Beckman PF2D™.

Proteomes of two Albumin-depleted human serum samples were compared using the Beckman software ProteoVue™ and DeltaVue™ and targeted fractions were analyzed by MALDI-MS after trypsin digestion. MALDI data shows significant post-translational modifications.