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Mass spectrometry-based proteomics to evaluate the circulating proteome in idiopathic pulmonary fibrosis (IPF)

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Rationale: The circulating proteome in IPF may provide insights into its pathobiology and uncover candidate biomarkers. Recent proteomic analyses have employed protein extension analysis platforms that are limited to target primers. Here we report mass spectrometry (MS)-based proteomic analyses in participants with IPF compared to those without known lung disease of similar age and sex distribution.

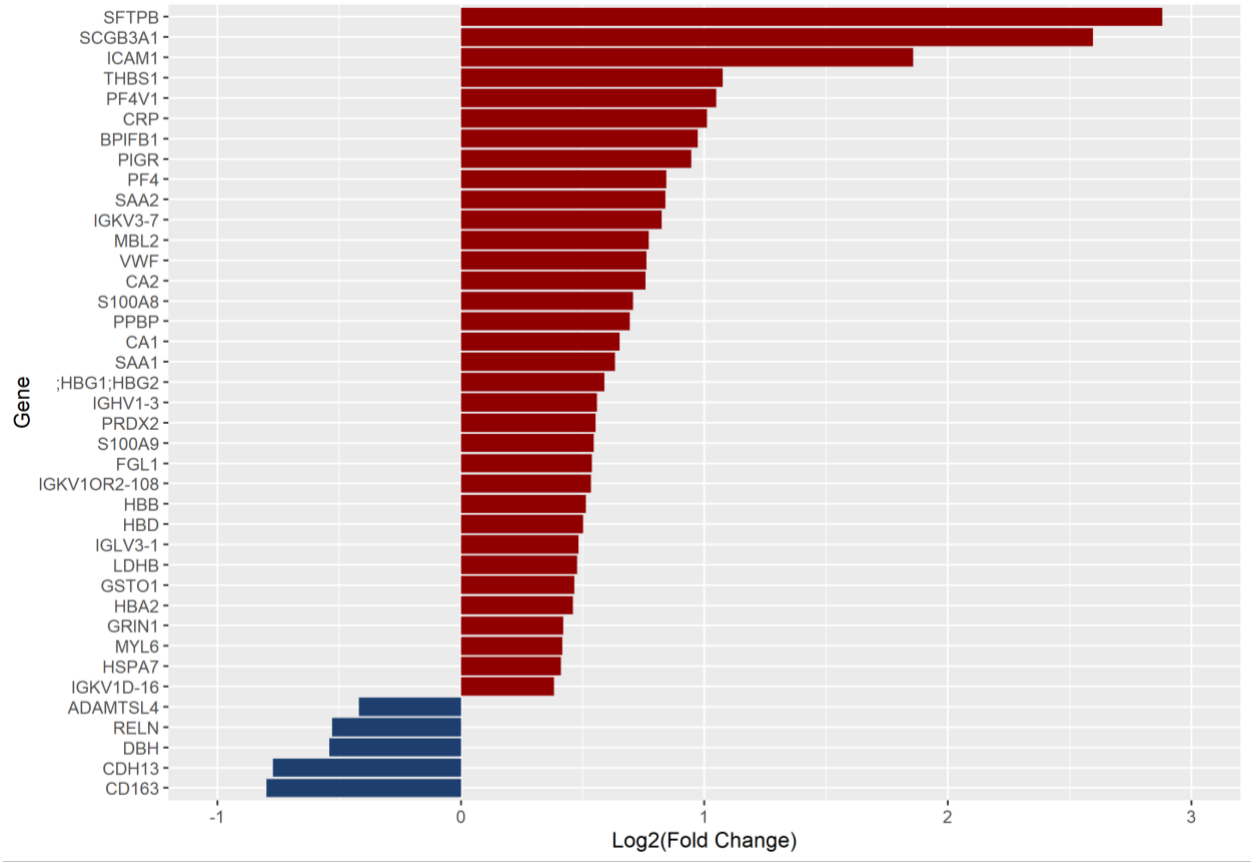
Methods: The IPF cohort came from the IPF-PRO Registry and included 299 subjects with IPF that was diagnosed or confirmed at the enrolling center in the prior 6 months. Controls came from a population-based registry and included 99 participants without known lung disease. Plasma taken at enrollment was processed with an automated liquid handling platform and measured using a Evosep One coupled to an Orbitrap Exploris instrument. Data analysis was performed with Spectronaut 14 with a spectral library to identify and quantify peptide sequences. Protein data were $\log_2$ transformed. Missing values were imputed, batch-corrected with pyCombat. Linear regression was used to compare abundances in the IPF vs. control participants. P-values were corrected for multiple comparisons using the Benjamini-Hochberg method to control the false discovery rate (FDR) at 5%. Pathway enrichment analyses on gene-level were performed with clusterProfiler using canonical pathways from Reactome.

Results: The IPF cohort was predominantly (75%) male with a median (interquartile range) age of 70 (65, 75) years. At enrollment, median % predicted FVC and DLCO were 69.7 (60.9, 80.2) and 40.5 (31.6, 49.4), respectively. The control cohort was 74% male with a median age of 66

(63, 71) years. 761 protein groups corresponding to 736 unique genes were detected in the plasma samples. 168 protein groups had significantly different abundances between the IPF and control groups ($p<0.05$), with 21 showing at least a 1.5-fold difference ($\log_2$[fold change]$\geq\pm0.58$) and 39 showing at least a 1.3-fold difference ($\log_2$[fold change]$\geq \pm0.38$) (Figure). Among the top differentially expressed proteins were the epithelial proteins surfactant protein B (SFTPB) and secretoglobin family 3A member 1 (SCGB3A1), intercellular adhesion molecule 1(ICAM1), thrombospondin 1(THBS1), and platelet factor 4(PF4v1). Pathways analyses showed enrichment in pathways associated with hemostasis, the complement cascade, and cell surface interactions at the vascular wall among others.

Conclusions: This MS-based proteomic analysis confirms proteins previously associated with IPF and reveals new candidate proteins for biomarker development. Future analyses will relate proteins to measures of IPF severity and quality of life and examine protein-outcomes associations, leveraging the rich longitudinal data collected in the IPF-PRO Registry.

Figure. Protein groups with different abundances between the IPF and control cohorts with an FDR-adjusted p-value threshold of <0.05 and at least a 1.3-fold difference ($\log_2$[fold change]$\geq \pm0.38$). Protein groups are labeled by corresponding gene name.



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