IPD Project Details

Project ID: IPD4667

Project Title: Proteomic Investigation Reveals Dominant Alterations of Neutrophil Degranulation and mRNA Translation Pathways in COVID-19 Patients

Description: The altered molecular proteins and pathways in response to COVID-19 infection are still unclear. Here, we performed a comprehensive proteomics-based investigation of of nasopharyngeal swab samples from COVID-19 patients to identify viral and host peptides by employing simple extraction strategies and also established a panel of host proteins using high-resolution mass spectrometry. The differentially expressed peptides/proteins identified from host and pathogen correlated with the viral load of the host which indicates that these proteins might be good prognostic biomarkers of severity prediction. A few host proteins such as Interleukin-6, L-lactate dehydrogenase, C-reactive protein, Ferritin and Aspartate aminotransferase was found to be upregulated in COVID-19 positive patients using targeted MRM study. Further, the proteins L-lactate dehydrogenase, Aspartate aminotransferase and Alanine aminotransferase was also validated in the clinical settings using immunological assays. We also identified neutrophil degranulation, platelet degranulation, interleukin-12 signaling pathways, mRNA translation of proteins and , co-factor metabolomic process protein metabolism, and stress responses to be key GO enriched pathways, thus altered in the COVID-19 infected patients.providing the detailed investigation of host response in COVID-19 infection , thus providing the landscape of COVID-19 pathophysiology. This study thus also revealed that mass spectrometry-based detected host proteins/peptides has a potential for clinical translation and a few proteins might be routinely monitored in clinics for the disease progression, peptide tests can be used by clinicians for diagnosis as well as identified pathways/ markers as the predictors of disease progression. Furthermore, the identified proteins and their drug binding studies might aid in COVID-19 therapeutic interventions.

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Sample Preparation: Nasopharyngeal swabs were collected from COVID-19 patients and viral inactivation procedure was performed by heat treatment of 65C for 45 min followed by precipitation using organic solvents. Samples were precipitated using three

solvents namely, Isopropanol, Ethanol and Acetone. The tubes were incubated at -20C for overnight to allow precipitation of the proteins followed by centrifugation. Pellets from the three tubes were heat treated, air dried and dissolved in buffer (8M Urea, Tris-HCl Buffer). Protein estimation was done using BCA assay and 25-50?g of protein was taken per sample for performing protein digestion.

Peptide Separation: The protein was digested using Trypsin (Pierce, Thermo Fisher Scientific, USA) for 16 hours at 37°C, followed by vacuum drying and reconstitution of the peptides with 0.1% Formic Acid. The peptides were quantified using Scope's method and 1 ug of peptide was used for the LC-MS/MS run. All patient samples were run in the Orbitrap Fusion Mass Spectrometer (Thermo Fisher Scientific, USA) using a gradient of 0.1% FA and Acetonitrile for 120 min with blanks after every sample.

Protein Characterization: Initial processing of raw files was done by MaxQuant (v1.6.6.0) and searched against databases, COVID-19 UniProtKB and Human Proteome Database of Uniprot (UP000005640). Orbitrap parameter was set to fusion mode. Trypsin missed cleavages were set to maximum of 2. Fixed chemical modification was set to Cysteine Carbamidomethylation (+57.021464 Da) and variable chemical modifications considered was Methionine oxidation (+15.994915 Da). False-Discovery-Rate (FDR) was kept as 1% whereas 7AA was kept as minimum amino acid length. Finally, decoy mode was set to "randomize", and the type of identified peptides was set to "unique+razor".

Experiment Type: Shotgun proteomics

Species: Homo sapiens - 9606

Tissue: Nasal lavage fluid (bto:0004977)

Cell Type:

Disease: Unknown

Instrument Details: Orbitrap Fusion (MS:1002416)

Protein Modifications: monohydroxylated residue, acetylated residue, iodoacetamide derivatized residue

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