

IPD Project Details

Project ID: IPD4061

Project Title: Mass-spectrometry based plasma proteomics identifies biomarkers for the detection of COVID-19 progression

Description: COVID-19 manifests itself in an array of symptoms. While most patients experience very mild-to-moderate symptoms, around one in five patients develop pneumonia coupled with severe respiratory distress. These patients require treatment in the intensive care units (ICU), however, most of the times, it leads to multi-organ dysfunction and death. Mass spectrometry based proteomics can help us to identify the precise pathophysiological pathways that get perturbed during the course of the disease. Our study involved a comprehensive proteome-wide investigation of COVID negative (n=20), non-severe (n= 18) and severe (n= 36) COVID-19 patients plasma samples. We categorized patients into severe and non-severe groups and conducted LFQ based discovery proteomics for unraveling differentially expressed proteins followed by pathway enrichment studies and validation of targets by targeted studies on a batch of longitudinal samples.

Principal Investigator: Dr Sanjeeva Srivastava

PI Affiliation: Proteomics Lab, 304, BSBE Department, Indian Institute Technology Bombay, Powai, Mumbai, India-400076

Sample Preparation: Approximately 2ml of blood was collected from COVID RT-PCR confirmed and suspected patients in a sterile vacutainer by trained medical practitioner under aseptic condition maintaining proper biosafety protocol. After performing biochemical and serological tests, left over samples were centrifuged immediately at 3000 rpm for 10mins. Separated plasma was then incubated at 56°C for 30mins for viral inactivation and further stored at -80°C in cryovials. In order to improve the detectability of low abundance plasma protein, high depletion plasma samples were depleted. Depleted plasma sample was taken forward for quantification by Bradford assay taking BSA as standard. To 30µg of the depleted plasma sample, 6M of urea lysis buffer was added followed by 6 times dilution with ammonium bicarbonate. Prior to digestion of the protein, the plasma protein extract was reduced with TCEP (final concentration 20mM) at 37°C for 1hour and then alkylated with iodoacetamide (final concentration 40mM) for 15min under dark condition.

Peptide Separation: Protein was finally subjected to enzymatic digestion by Trypsin at an enzyme/substrate ratio of 1:30 for 16 hours at 37°C. The digested peptide was then vacuum dried and reconstituted in 1% (v/v) FA. To reduce the salt concentration in the digested peptide, it was cleaned up using C-18 column obeying the principle of reverse phase column chromatography. The cleaned peptide was further dried and dissolved in 1% (v/v) (FA). The concentration of peptide was calculated using scopes method from its O.D. value at 205nm and 280nm. For Liquid Chromatography-Tandem Mass Spectrometry, 1µg peptide of each sample was run in Orbitrap Fusion Tribrid Mass Spectrometer (Thermo Fischer Scientific) with easy nano LC 1200 system with a gradient of 80% ACN and 0.1% FA for 120 min with blanks after every sample. BSA was run at starting and end point of each set of run to check the instrument quality. All samples were loaded onto the LC column at a flow rate of 300nl/min. Mass spectrometric data acquisition was done in data dependent acquisition mode with a mass scan range of 375-1700 m/z and mass resolution of 60,000. A mass window of 10ppm was set with a dynamic exclusion of 40s. All MS/MS data was acquired by High energy Collision Dissociation method of fragmentation and data acquisition was done using Thermo Thermo Xcalibur software version 4.0.

Protein Characterization: Initial processing of raw files was done by MaxQuant (v1.6.6.0) and searched against databases, 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) and alkylation at N-terminals of protein. False-Discovery-Rate (FDR) was kept as 1% whereas 6AA 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: Data in species_details No Data

Tissue: Data in tissue_details No Data

Cell Type: Data in cell_details No Data

Disease: Data in disease_details No Data

Instrument Details: Data in instrument_details Data in instrument_details

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

PubMed ID: