IPD Project Details

Project ID: IPD5040

Project Title: A longitudinal study of COVID-19 patients using proteomics approach

Description: SARS-CoV-2, a highly contagious and infectious virus is responsible for causing this COVID-19 pandemic, which has a substantial impact on global health and economy. The spectrum of clinical manifestations of COVID-19 ranges from mild or non-severe state to severe life-threatening condition in some group of people. Many patients are likely to undergo non-severe to severe transition during their infection period. For this study, we have collected blood samples of different time points from patients showing both non-severe to severe and severe to recovered transition. The clinical information of the patient's condition is obtained from their medical records. We have investigated the proteome of different time points of the patient's sample to analyse their trend in prognosis of the disease

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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: All the raw datasets obtained from the instrument were processed with MaxQuant (v1.6.6.0) (Tyanova et al., 2016) and searched with the built-in Andromeda Search Engine against the Human Swiss-Prot database (downloaded on 09.07.2020) which contains total 20,353 proteins. The Label-Free-Quantification parameters are used for the processing of the raw files and used label-type setting as ‘standard’ with the multiplicity of 1. Fusion mode was set in the orbitrap and trypsin was used for the digestion with the maximum missed cleavage of 2. Carbamidomethylation of Cysteine (+57.021464 Da) was set as the static modification and oxidation of Methionine (+15.994915 Da) was set as variable modification and for identification of protein and peptide with high reliability False Discovery Rate was set as 1%. Decoy mode was set to ‘reverse’ and the type of identified peptides was set as ‘unique+razor’.

Experiment Type: Shotgun proteomics

Species: Homo sapiens -9606

Tissue: Blood plasma (bto:0000131)

Cell Type:

Disease: Unknown

Instrument Details: Orbitrap Fusion (MS:1002416)

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

PubMed ID: