IPD Project Details

Project ID: IPD8953

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

Description: With the increasing cases of SARS-CoV2 mediated COVID-19, which has taken the form of a savagely spreading pandemic, quick and effective detection and diagnosis is the need of the hour. While clinical testing is aimed at identifying the positive vs. negative cases, there are also enormous challenges that have emerged with the diagnosis of asymptomatic patients. We have here enumerated identification and detection of SARS-CoV-2 viral peptides and proteins using high throughput mass spectrometry from nasopharyngeal swabs, which can be used for measuring peptides from SARS-CoV-2. This study is aimed at constructing a detection kit and assay via which a simplified sample collection and testing can be performed using swab put in the tubes containing organic solvents (ethanol, acetone or isopropanol), that can then be sent for testing in laboratory using mass spectrometry. This would simplify the detection and increase the robustness of test. Further, the invention has scope for identifying the asymptomatic population using the enumerated proteomics procedure.

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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".The unique peptides were filtered using Human swissprot database in Skyline (Version 20.1). The statistical data analysis were done using Microsoft excel, R (Version 4.0.2), Python (Version 3.7.6), metaboanalyst, and Hierarchical Clustering Explorer (Version 3.0).

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

PubMed ID: