IPD Project Details

Project ID: IPD5452

Project Title: Multiplexed Quantitative Proteomics Provides Mechanistic Cues for Malaria Severity and Complexity

Description: Management of severe malaria remains a critical global challenge. In this study, using a multiplexed quantitative proteomics pipeline we systematically investigated the plasma proteome alterations in non-severe and severe malaria patients. We identified a few parasite proteins in severe malaria patients, which could be promising from a diagnostic perspective. Further, from host proteome analysis we observed substantial modulations in many crucial physiological pathways, including lipid metabolism, cytokine signaling, complement, and coagulation cascades in severe malaria. We propose that severe manifestations of malaria are possibly underpinned by modulations of the host physiology and defense machinery, which is evidently reflected in the plasma proteome alterations. Importantly, we identified multiple blood markers that can effectively define different complications of severe falciparum malaria, including cerebral syndromes and severe anemia. The ability of our identified blood markers to distinguish different severe complications of malaria may aid in developing new clinical tests for monitoring malaria severity.

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Sample Preparation: Malaria and dengue samples were confirmed using different diagnostic techniques, and the positive cases were incorporated in the quantitative proteomic analysis. Protein quantification was performed using Bradford assay (Bio-Rad) following the manufacturer’s instructions. One hundred micrograms of depleted protein sample from each group (HC, CSA, CCB, SA, CB, SFM, and NSFM) were denatured using 15 µl of 6 M urea. The denatured proteins were reduced by adding tris (2-carboxyethyl) phosphine (TCEP) (Sigma Aldrich) to a final concentration of 20 mM and were incubated at 37 °C for 60 min. The reduced samples were alkylated by adding iodoacetamide [(IAA), Sigma Aldrich] to a final concentration of 40 mM and were incubated at room temperature in dark for 30 min.

Peptide Separation: The samples were then diluted eight times with 50 mM ammonium

bicarbonate buffer, and trypsin (Pierce Trypsin) was added to a 1:30 ratio (trypsin: protein) for performing in-solution digestion. Samples were incubated at 37 °C for 18 h for efficient digestion. The digestion was quenched by adding formic acid to a final concentration of 1%70. Subsequently, sample cleaning was performed using C18 Ziptip. Activation of the C18 ziptip was done by using 50% ACN in 0.1% FA, 40 µl, 1 min, 1500g thrice, followed by 99% ACN in 0.1% FA 40 µl, 1 min, 1500g thrice. Such multiplexing using stable isotope labeling provides increased throughput, higher precision, better reproducibility, reduced technical variations, and lower number of missing values. TMT-based multiplexed quantitative proteomics was used to map the plasma proteome (host) alterations, while we used a label-free quantitation approach for detection and quantification of the parasite (P. falciparum) proteins in host plasma. Quality control check (QC) of the data sets was performed by plotting the density plots for FM, VM, and DF using raw and normalized abundances at a proteome scale. The significantly ($p < 0.05$) altered proteins were considered for machine learning, and the elastic net regularized logistic regression method was applied to predict the best panel of proteins. Some selected targets were validated using Mass spectrometry (MS)-based Multiple Reaction Monitoring (MRM) assays. Eventually, we investigated the physiological pathways over-expressed in falciparum and vivax malaria.

Protein Characterization: TMT data analysis- TMTsixplex based quantitative proteomic analysis was carried out using individual samples of malaria patients. Raw instrument files were processed using Proteome Discoverer (PD) version 2.2 (Thermo Fisher Scientific). For each of the TMT experiments, raw files from 9 fractions were merged and searched with the Sequest HT and Mascot (v2.6.0) search engine against Homo sapiens database, which is downloaded (71,523 sequence entries, dated: 24/06/2018) from uniport. All searches were configured with dynamic modifications for the TMT reagents (+229.163 Da) on lysines and N-termini, oxidation of methionine residues (+15.9949 Da) and static modifications carbamidomethyl (+57.021 Da) on cysteines, monoisotopic masses, and trypsin cleavage (max 2 missed cleavages). The intensities of the TMT reporter - ions at m/z = 127 were used as the denominators for fold change calculation. The PSMs files have been exported from PD for FM, VM and DF. The protein level abundance summarized using MSstatsTMT.

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: iodoacetamide derivatized residue

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