

IPD Project Details

Project ID: IPD4298

Project Title: Food vacuole proteome of *P. berghei* heme pathway ferrochelatase knockout parasite by LC-MS/MS

Description: LC-MS/MS of in-solution trypsin digested FV protein extracts was carried out for the FVs isolated from PbWT and PbFCKO parasites. The FV preparations had signature FV proteins such as plasmepsin IV, berghepain, aminopeptidases, subunits of vacuolar-type H⁺ATPase (V-type H⁺ATPase), together with parasitophorous vacuolar (PV) proteins including exported protein 1 (Exp1), Exp2, early transcribed membrane protein, PV1, PV5 (lipocalin) etc., and Rab GTPases associated with cytosome-FV trafficking. A total number of 251 and 201 proteins could be identified for WT (P1) and FCKO (P2) FVs, respectively, and 175 proteins were common between them suggesting an overall consistency in the preparations. The results obtained have suggested that the FV proteome in FCKO parasites is compromised. None of the subunits of V-type H⁺ATPase - a proton pump maintaining the acidic pH of FV could be detected in FCKO FVs indicating the lower abundance of these proteins. In addition, berghepain-2 - a cysteine protease involved in Hb degradation could not be detected in FCKO FVs.

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Sample Preparation: To examine the protein content of the FVs from WT and FCKO parasites, proteins were extracted from three different preparations of WT and FCKO FVs using 25 mM ammonium bicarbonate containing 6M urea. The respective WT and FCKO FV protein extracts were pooled separately, followed by treatment with DTT and iodoacetamide. The urea concentration was then reduced to 0.6 M by performing dilution with 25 mM ammonium bicarbonate.

Peptide Separation: For LC-MS/MS, in-solution trypsin (SCIEX) digestion was carried out for 200 µg total protein, followed by sample clean up with Waters Oasis SPE cartridge. The samples were subjected to micro flow reverse-phase LC in Eksigent Eksport Nano LC 425 system (SCIEX) that was directly connected to a tandem quadrupole time-of-flight SCIEX TripleTOF 5600+ ESI-mass spectrometer. The samples were concentrated using a SCIEX Micro Trap Cartridge (Chrome XP; C18-CL, 5-µm, 120-Å pore size). The trap cartridge was washed with 0.1% (v/v) formic acid and 2%

(v/v) acetonitrile in water and the concentrated peptides were then separated using a SCIEX capillary reverse-phase column (ChromeXP, 3C18-CL-120, 3 μ m, 120 Å and 0.3 $\times$ 150 mm) at a flow rate of 5 μ l/min using the following solvents: solvent A - 98% water and 2% acetonitrile containing 0.1% formic acid (v/v), and solvent B - 98% acetonitrile and 2% water containing 0.1% formic acid (v/v). The gradient parameters were set at 2% to 50% of solvent B in 28 min, followed by 50% to 90% of solvent B in 1 min, sustaining 90% of solvent B for 3 min and then 90% to 5% B in 0.5 min with a final re-equilibration with 2% of solvent B for 2.5 min. Mass spectra and tandem mass spectra were recorded in positive-ion and high-sensitivity mode with a full scan resolution of 35,000 (full width at half maximum) and the ion source was operated with the following parameters: IonSpray Voltage Floating (ISVF) = 5500; Ion Source Gas 1 (GS1) = 25; Ion Source Gas 1 (GS2) = 22; Curtain Gas Flow (CUR) = 30. The precursor ions were fragmented in a collision cell containing nitrogen as a collision gas. The calibrations for TOF MS spectra and TOF MS/MS spectra were performed by injecting 100 fmol beta-galactosidase digest (SCIEX). The peptide spectra were recorded over a mass/charge (m/z) range of 350 to 1250, and MS/MS spectra were recorded over an m/z range of 150 to 1600 in data-dependent acquisition (DDA) mode.

Protein Characterization: Data acquisition was achieved using Analyst TF1.7.1. software and DDA was performed to obtain MS/MS spectra for the 15 most abundant parent ions following each survey MS1 scan (250-ms acquisition time per MS1 scan and 50-ms acquisition time per MS/MS). Dynamic exclusion features were set to an exclusion mass width of 50 mDa and an exclusion duration of 6 s. The acquired MS/MS data were annotated using Paragon algorithm (ProteinPilot Software Version 5.0.2, SCIEX) against the reference proteomes of *Plasmodium berghei* (UP000074855, Taxonomy 5823; UP000219974, Taxonomy 5821) available at Uniprot (<https://www.uniprot.org/>) with the following parameters: TripleTOF 5600 instrument; alkylation of cysteines by iodoacetamide; trypsin enzyme digestion; ID Focus on biological modifications and the detected protein threshold [Conf] set at >10%.

Experiment Type: Top-down

Species: Data in species_details No Data

Tissue: Data in tissue_details No Data

Cell Type: Data in cell_details No Data

Disease: Unknown No Data

Instrument Details: Data in instrument_details Data in instrument_details

Protein Modifications: iodoacetamide derivatized residue

PubMed ID: