IPD Project Details

Project ID: IPD2865

Project Title: Assessment of host hemoglobin accumulation in P. berghei heme pathway ferrochelatase knockout parasite food vacuole by iTRAQ

Description: Two independent sets of fourplex iTRAQ labelling were carried out to assess the accumulation of host Hb in PbFCKO FVs. The results obtained have suggested that there is a ~3-4 -fold increase in Hb ï•¡ and ï•¢ chains in FCKO FVs as quantified by iTRAQ.

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Sample Preparation: Fourplex iTRAQ labelling was carried out as per the manufacturer’s protocol (iTRAQ kit, AbSciex, Cat No. 4381664). In brief, FVs were solubilized in 0.5 M triethylammonium bicarbonate (TEAB) containing 0.1% sodium dodecyl sulphate (SDS) and 5 mM reducing reagent tris-(2-carboxyethyl) phosphine (TCEP), and incubated at 60 °C for 1 h followed by treatment with methyl methane thiosulfonate (MMTS) for 10 min at room temperature.

Peptide Separation: 50 and 100 ?g total protein were used to perform trypsin digestion at 37ºC for 16 h. Two sets of four-plex reactions were carried out for three different preparations of WT and FCKO FVs along with two internal controls. The digested peptides of WT and FCKO FVs were labelled with either 115, 116 or 117 isobaric tag, and isobaric tag 114 was used exclusively to label the internal controls that had equivalent representation of all the samples for normalization. All the samples were spiked with bovine serum albumin (BSA) to ensure the loading accuracy. The samples labelled with different isobaric tags were pooled together as one single sample for individual reaction set and vacuum concentrated. After performing sample clean up with Waters Oasis SPE cartridge, the peptides were separated and the mass spectra and tandem mass spectra were recorded using Eksigent Ekspert Nano LC 425 system (SCIEX) coupled with a tandem quadrupole time-of-flight SCIEX TripleTOF 5600+ ESI-mass spectrometer. 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 100 to 1600.

Protein Characterization: Protein identification and quantification were performed using

Paragon algorithm (ProteinPilot Software Version 5.0.2, SCIEX) with a false discovery rate (FDR) < 1% against the reference proteome of Mus musculus (UP000000589, Taxonomy 10090) available at Uniprot (https://www.uniprot.org/). Proteins having peptides identified with 95% confidence or above were considered for further analysis. Proteins were quantified by summing the reporter ion intensities across the spectra matching to each protein and normalizing them with respect to the internal control labelled with isobaric tag 114. The normalized reporter ion intensities were used to calculate the fold changes of ? and ? chains of mouse hemoglobin in PbFCKO FVs with respect to PbWT FVs.

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: