IPD Project Details

Project ID: IPD2535

Project Title: Label-free quantitative total membrane proteome analysis of Candida glabrata wild-type and eleven CgYapsins-deficient strain Cgyps1-11?.

Description: The project is aimed at characterizing the effect of loss of a family of eleven aspartyl proteases (Cg Yapsins) on the abundance of proteins in the total membrane factions of Candida glabrata.

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Sample Preparation: C. glabrata wild-type and Cgyps1-11? strains were grown in YPD medium till logarithmic-phase. Next, cells were harvested and washed twice using ice-cold water. The cells were normalized to 20 OD600 and suspended in buffer 1 [0.1 M Tris (pH 10.7), 5 mM EDTA, 2 mM dithiothreitol (DTT) and 1X protease inhibitor cocktail solution]. The 0.5 mm acid-washed glass beads were added to cells, and the cells were lysed using mechanical force. The cell homogenates were diluted using the buffer 2 [0.1 M Tris-HCl (pH 8.0), 0.33 M sucrose, 5 mM EDTA and 2 mM DTT], and centrifuged at 1000g for 3 min at 4°C. The supernatant was transferred into a new centrifuge tube, followed by centrifugation at 3000g for 5 min at 4°C to remove unbroken cells and cell debris, if any. Next, the supernatant was centrifuged at 19000g for 45 min at 4°C to obtain total membrane fraction pellets. The membrane pellets were washed with the buffer 2 [0.1 M Tris-HCl (pH 8.0), 0.33 M sucrose, 5 mM EDTA and 2 mM DTT] and suspended in membrane suspension buffer [6M Gn-HCL and 0.1M Tris (pH 8.8)]. Protein quantification was performed using the BCA protein assay kit. 100 µg TMF samples were sent to Valerian Chem Private Limited, New Delhi, India using the dry ice shipment. These samples, in duplicates, were sent on dry ice to Valerian Chem Pvt Ltd for label-free relative protein quantification, following LC-MS, using the Minora Feature Detector Node of the Proteome Discoverer 2.2 with default settings.

Peptide Separation: At Valerian Chem, protein samples were reduced with TCEP solution, followed by trypsin digestion, and resolution of the peptide mixture (1.0 ?g) on a 25 cm-long PicoFrit column (360 ?m outer diameter, 75 ?m inner diameter, 10 ?m tip) containing 1.8 ?m of C18 resin. The peptides were eluted with a 0-40% gradient of buffer

containing 95% acetonitrile and 0.1% formic acid at a flow rate of 300 nl/min for 100 min. The sample analysis was carried out using the EASY-nLC 1000 system (Thermo Scientific™) coupled to the QExactive mass spectrometer (Thermo Scientific™) equipped with nanoelectrospray ion source, and the data-dependent top10 method was used to acquire MS data. Sample with search name C1 refers to C. glabrata wild-type replicate 1, C2 refers to C. glabrata wild-type replicate 2, T1 refers to Cgyps1-11? replicate-1, and T2 refers to Cgyps1-11? replicate-2.

Protein Characterization: The raw data were analysed using the Uniprot C. glabrata reference database, with precursor and fragment mass tolerances set at 10 ppm and 0.5 Da, respectively, for Sequest search. The peptide spectrum match and the protein false discovery rate were set to 0.01, and the peptide spectrum matches with high and medium confidence were selected.

Experiment Type: Shotgun proteomics

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

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