IPD Project Details

Project ID: IPD3931

Project Title: Mining Meningioma Proteomics Alterations Using IP MS

Description: Herein we have used IP-MS based approach to identify potential interactors (PPI) that bind to Annexin A2. We have used Immunoaffinity based enrichment using antibody specific to ANXA2 followed by LC MS-MS of the Complex post running it in SDS-PAGE

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Sample Preparation: Patients with radiologically suspected meningiomas were enrolled after giving written informed consent. Surgically resected tissues were procured from patients diagnosed to be meningiomas by radiology.Global proteomic profiling/shotgun proteomics was performed across MG1 (n = 10), MG II (n = 11), MGIII (n = 2), Normal dura mater (n = 4), and Arachnoid mater (n = 4). Protein extraction was done using Urea buffer (8 M Urea, Tris–HCl buffer) with the addition of Phosphatase inhibitor cocktail (SigmaAldrich®, United States) as mentioned in the protocol by CPTAC Investigators (17). In brief, tumor tissue was washed with 1X PBS and cut (around 75 mg) tissue lysis was performed with sonication followed by bead milling at 90 s for 3 cycles.

Peptide Separation: The lysates were centrifuged at 12000 r.p.m at 4 degrees for 15 min to clear the debris, the supernatant was quantified using 2D-Quantification kit (Bio-Rad, United States) and 100 ?g of protein was digested using Trypsin (Pierce, Thermo Fisher Scientific, United States) for 16 h at 37°C Followed by vacuum drying the peptides and reconstitution with 0.1% Formic Acid. The peptides were quantified using Thermo plate reader using Scope’s method 1 ?g of the peptide was used for the LC-MS/MS run.

Protein Characterization: Data was analysed using Proteome Discoverer 2.2, all three replicates were run using the same gradient of 60 minutes in the LC MS-MS.

Experiment Type: Gel-based experiment

Species: Homo sapiens - 9606

Tissue: Brain (bto:0000142)

Cell Type: Epithelial cell (cl:0000066)

Disease: Brain cancer (doid:1319)

Instrument Details: Q Exactive (MS:1001911)

Protein Modifications: iodoacetamide derivatized residue

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