IPD Project Details

Project ID: IPD6641

Project Title: Deciphering the function of Angiomotin in trophoblast cells

Description: Trophectoderm-specific expression of Angiomotin (AMOT) in pre-implantation embryos followed by its unique expression in the post-implantation ectoplacental cone that harbors the trophoblast stem cell niche prompted our investigation on the function of AMOT in trophoblast cells. Using the in vitro trophoblast stem cell culture model, we established differentiation dependent up-regulation of AMOT expression in trophoblast cells. To understand the function of AMOT in trophoblast cells mass spectrometry-based proteomic analysis was employed to identify the AMOT interactome within the trophoblast proteome. This approach utilized immunoprecipitation of endogenous AMOT followed by fractionation on SDS-PAGE and subsequently subjecting the tryptic digested excised gel bands to mass spectrometry.

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Sample Preparation: The immunoprecipitates were resolved by 10% (w/v) SDS polyacrylamide gel. Staining was done using Coomassie staining solution (0.1% Coomassie Brilliant Blue R-250, 50% methanol, 10% glacial acetic acid).

Peptide Separation: The visible bands were excised out and in-gel digestion was performed. The mass spectrometric analysis was performed using TripleTOF 5600 (SCIEX).

Protein Characterization: All datasets (.wiff files) converted to .mgf file format. For NSAF values, datasets were analyzed using Andromeda in SearchGUI (version 3.3.3) with default engine-specific settings. The identification settings were as follows: enzyme: trypsin, specific, 2 missed cleavages; precursor charges +2 to +5; precursor mass tolerances 10 ppm, fragment mass tolerances 0.5 Da; fixed modifications: carbamidomethylation of C; variable modifications: Deamidation of N and Q, oxidation of M. The peptides and proteins were inferred from the spectrum identification results using Peptide Shaker (version 1.16.27). Peptide Spectrum Matches (PSMs), peptides, and proteins were validated at a 1% false discovery rate (FDR) estimated using the decoy hit distribution. The emPAIs was calculated from Mascot (version 2.3.02). The identification

settings were as follows: enzyme: trypsin, maximum missed cleavages 2; peptide mass tolerances 0.5 Da, fragment mass tolerances 0.2 Da; fixed modifications: carbamidomethylation of C; variable modifications: oxidation of M. The protein score and significance threshold for peptide matches were set to ? 30 and p? 0.05 respectively.

Experiment Type: Shotgun proteomics

Species: Mus musculus

Tissue: Trophoblast

Cell Type: Trophoblast

Disease: Disease free

Instrument Details: TripleTOF 5600 (MS:1000932)

Protein Modifications: monohydroxylated residue, iodoacetamide derivatized residue, deamidated residue

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