

IPD Project Details

Project ID: IPD2453

Project Title: Placental proteomics identified elevated Aldo-keto Reductase 1-B1 level in spontaneous preterm birth

Description: The prevalence of preterm birth along with its associated mortality and lifelong morbidity warrant a better understanding of the underlying signaling events for better diagnosis and management of the condition. Placenta is an important transient organ that acts as a conduit between the fetus and mother. Any physiological and pathological changes taking place in the feto-maternal system are directly reflected in the placenta, making it a fitting choice to study the altered signaling mechanisms that are play. We undertook independent data acquisition of clinical placenta samples (n=40), obtained from Garbh-Ini cohort, to study their comparative protein profiles in spontaneous preterm vs term birth condition. When label-free quantitation (LFQ) was carried out, it yielded 23 differentially expressed proteins (DEPs) in the case-control comparison.

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Sample Preparation: 10 mm punches from lower-left quadrant (Q1) of human placenta were collected within 30 min of delivery. The tissues were then dissected (150 to 200 mg per cross-section), washed with ice-cold PBS and stored in 10% neutral buffered formalin, at -80°C until further use. For sample preparation 25 mg of stored tissue was weighed from each sample and cryo-ground using liquid nitrogen, transferred to microcentrifuge tubes containing RIPA lysis buffer followed by sonication thrice at amplitude 50, for 10 pulses of 0.5 seconds, using a probe sonicator. The samples were then centrifuged at 14,000 rpm for 45 min at 4 °C to remove insoluble tissue debris. Protein concentration in the homogenates was estimated by BCA assay.

Peptide Separation: 100 µg protein was aliquoted from each sample for further digestion. Aliquoted proteins from each placenta sample were then reduced with 10 mM DTT for 1 h at 55 °C and subsequently alkylated with 20 mM IAA in dark at room temperature for 1 h. Alkylated proteins were then digested with sequencing grade trypsin (1:20 w/w in 50 mM ammonium bicarbonate or ABC) and incubated at 37 °C overnight,

as per manufacturer's instructions. The digested protein samples were passed through the C18 tip to remove salts and buffer ions. The peptides were then sequentially eluted in 80% and 100% ACN, with 0.1% formic acid. The eluted samples were vacuum dried and stored at -80°C until further use.

Protein Characterization: MaxQuant software (v1.6, Max Planck Institute of Biochemistry, Germany) was used for peptide/protein identification and quantitation. Data generated from MS/MS spectra, in the form of .wiff files were searched against the UniProtKB human protein database (20,214 entries, Aug 2017) with search parameters selected as follows: 4 maximum missed cleavages for tryptic digestion; cysteine modified by carbamidomethyl as fixed modification; oxidation (M) and acetylation (Protein N-term) as variable modifications; taxonomy- Homo sapiens; Triple TOF 5600 instrument. Mass tolerances for the precursor and fragment ions were 0.05 and 0.1 Da, respectively. Global FDR of 1% was fixed as threshold for protein identification. Proteins were quantified using at least one unique peptide, and match between runs was selected to increase the quantifiable values across samples. LFQ data were used for further analysis and comparisons across variant groups. Statistical analysis was carried out on Perseus (v2.0.7.0, Max Planck Institute of Biochemistry). Peak intensities were log2 transformed to improve normality and missing values imputed using normal distribution with width of 0.3 and down shift of 1.8. Next, the data was normalized by median subtraction. The quality of raw data was assessed using Pearson's coefficient and PCA plot statistics. Perseus was used to generate PCA projections and calculate Pearson's r coefficient between samples. Raw values were plotted on GraphPad Prism (v8). Statistical comparison between groups was performed using two-tailed, unpaired Student's t-test. Threshold adjusted p-value (<0.05) was used to define statistical significance. The mean abundance of >1.5-fold in PTB vs TB was considered significant. The FDR- adjusted p-value (q value) was less than 0.05. Normalised intensity of each significant DEP was plotted against sample POGs in longitudinal fashion to visualize their trajectory from preterm towards term deliveries.

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: Abnormality of prenatal development or birth No Data

Instrument Details: Data in instrument_details Data in instrument_details

Protein Modifications: monohydroxylated residue, iodoacetamide derivatized residue

