IPD Project Details

Project ID: IPD2593

Project Title: Phosphoproteome profile of Human Brain Hemisphere

Description: This project is a continuation of our previous study which resembles Interregional and Inter-hemisphere Proteome of the Human Brain. The current study aims to generate a multiregional phosphoproteome map of 12 neuroanatomical regions from both the hemisphere. The analysis of the raw data was done using MaxQuant and Perseus software. In addition to this phosphopeptides showing equal to and more than 0.75 localization probability and intensities inall the replicates were selected for further analysis. The hemisphere and region specificphosphoproteome expression has also been studied further to understand the functional aspect using in-silico tools. Finally, the data has also been integrated with the previously published proteomic expression in the brainprot (http://www.brainprot.org/) to drive the scientific community in neurobiology research.

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Sample Preparation: Brain tissue samples derived from the 12 different regions were homogenized in a lysis buffer supplemented with protease and phosphatase inhibitors. The homogenates were spun down at for 1 h at 15?. Protein quantitation was performed with the Bradford assay kit (Bio?Rad). Six hundred micro grams of protein were used to obtain the phosphorylated fractions from the brain regions. For protein digestion, the reduction was performed with the addition of DTT to a final concentration and incubation at RT for 30 min. Subsequent alkylation with iodoacetamide was performed for 30 min in the dark at room temperature. An additional reduction step was carried out, allowing the reaction to stand at room temperature for 30 min.

Peptide Separation: The mixture was diluted to 0.6 M urea using MilliQ?water, and after the addition of trypsin (Promega) (enzyme: protein, 1:50 w/w), the sample was incubated at 37? for 16 h. Digestion was quenched by acidification (pH < 6) with acetic acid. After protein enzymatic cleavage, peptide cleaning was performed using Pierce™ Peptide Desalting Spin Columns (ThermoFisher). To obtain the phosphorylated peptide fractions, the High?Select™ TiO2 Phosphopeptide Enrichment Kit (Thermo Scientific)

was used according to the manufacturer's instructions. Phospho-enriched samples were then dried and ready for the MS analysis.

Protein Characterization: The raw files were analysed with MaxQuant (v2.0) against UniProt Human Proteome Database (Proteome ID: UP000005640). Trypsin was selected for digestion with a limit of 2 missed cleavages. Carbamidomethylation of Cysteine (+57.021464 Da) was set as the fixed modification, though oxidation of methionine (+15.994915 Da) was set as the variable alteration. Additional modifications like phospho STY modification were added. The base length for a peptide was set to 7AA. Distraction mode was set to "randomize", and the sort of distinguished peptides was set to "unique+razor". Furthermore, normalization and fold change of raw intensities were calculated using Perseus software (v1.6.15.0).

Experiment Type: Shotgun proteomics

Species: Homo sapiens - 9606

Tissue: Brain (bto:0000142)

Cell Type:

Disease: Unknown

Instrument Details: Orbitrap Fusion (MS:1002416)

Protein Modifications: phosphorylated residue

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