IPD Project Details

Project ID: IPD4755

Project Title: Molecular insight into palmitic acid-induced hepatocellular carcinoma cell death.

Description: The accumulation of lipid is a histological and biochemical hallmark of obesity-associated hepato-steatosis.Moreover, growing evidenceindicatesthathigher free fatty acids (FFAs) level in hepatocytes affects a myriad of biological processes leading to excessive metabolic imbalance, increased reactive oxygen species (ROS), deregulated autophagy, and impairment of mitochondrial and ER stress, that collectively drives cell death. Lipotoxicity and cell death mechanisms have been studied for many years. However, the molecular signals that link these two events during lipid stress remain poorly understood. From the very beginning, to systematically study hepato-lipotoxicity, HepG2 treated with PA providentially recapitulates the global lipotoxic responses, including insulin resistance to hepatocyte death. Therefore, using this cell-based model system, we pursued a comprehensive, differential quantitative approach where measurements of protein dynamics are analysed by mass spectrometry. Given that indispensable information, successive temporal phosphoproteomics dynamics are allowed us to in-depth analysis of lipotoxicity associated mechanistic network of cell death more precisely.

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Sample Preparation: AIF-immuno-precipitation followed by LC-MS/MS were executed in control vs palmitic acid treated condition at 24 h. Both the experiment was performed with three biological replicates.

Peptide Separation: All the mass spectrometric analysis was performed using TripleTOF 5600 (SCIEX).

Protein Characterization: The MS/MS raw spectrum from interactomics study was run on a MaxQuant (v1.6.1.0) with default settings against Human database. The identification settings were as follows (a) trypsin as a proteolytic enzyme (b) Oxidation (M); Acetyl (Protein N-term);Carbamidomethyl (C);Deamidation (NQ) set as variable modifications. For quantification LFQ algorithm were applied.

Experiment Type: Affinity purification coupled with mass spectrometry proteomics

Species: Homo sapiens

Tissue: Hepatocyte (bto:0000575)

Cell Type: Hepatocyte (cl:0000182)

Disease: Unknown

Instrument Details: TripleTOF 5600 (MS:1000932)

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

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