IPD Project Details

Project ID: IPD9699

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: Equal amount of proteins (~100 ?g) fromcontrol vehicle (BSA) and treated (0.5 mM palmitic acid) conditions were subjected for tryptic digestion (1:20 enzyme-substrate ratio) at 37°C followed by reduction and alkylation.

Peptide Separation: The peptides were labelled by iTRAQ 4-plex Kit (AB Sciex, USA). The experiment was performed with two biological replicates. Control vehicle and PA treated samples were labelled with 114, 115, 116 and 117 iTRAQ tags, respectively.

Protein Characterization: For differential proteomics, proteins identification and relative quantification were performed by ProteinPilot software (v 4.5; AB Sciex, USA) using Paragon algorithm. Proteins were identified with 1% FDR, and the results were exported

to Excel for manual data interpretation.

Experiment Type: Shotgun proteomics

Species: Homo sapiens

Tissue: Hepatocyte (bto:0000575)

Cell Type: Hepatocyte (cl:0000182)

Disease: Unknown

Instrument Details: TripleTOF 5600 (MS:1000932)

Protein Modifications: iodoacetamide derivatized residue

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