IPD Project Details

Project ID: IPD1838

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: HepG2 cells were harvestedin 0, 6, 12, 18, and 24 h of post treatment of palmitic acid, and were subjected for phosphoproteomics analysis.

Peptide Separation: Ten mg of phospho-peptides were enriched with metal oxide affinity enrichment (MOAC) using titanium dioxide beads (TiO2) followed by LC-MS/MS analysis.

Protein Characterization: Temporal phosphoproteomics data set were analysed using Mascot (version 2.3.02). The identification settings were as follows(a) trypsin as a proteolytic enzyme (with up to two missed cleavages); (b) peptide mass error tolerance of 20 ppm; (c) fragment mass error tolerance of 0.20 Da; and (d) carbamido-methylation

of cysteine, oxidation of methionine, deamination of NQ, Phospho (ST), and Phospho (Y) as variable modifications. The pep_score and significance threshold for peptide matches were set to ? 30 and p? 0.05 respectively.

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, phosphorylated residue, deamidated residue, iodoacetamide derivatized residue

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