

IPD Project Details

Project ID: IPD6714

Project Title: Delineating the Rb1 interactome data and its modulations during cell cycle progression.

Description: The retinoblastoma (Rb) protein is a potent tumor suppressor which is known to negatively regulate the cell cycle as well as tumor progression. Phosphorylated Rb protein (pRb) has been demonstrated to be in-charge for the key G1 checkpoint, blocking entry into S-phase and thereby the cell growth. This study was designed to capture interacting protein partners of Rb1 as the cell cycle progresses. Rb1 expressing HEK-293 cells were cultured in light, medium and heavy SILAC labels to capture the changes in Rb1 interactome as the cell cycle progressed from G0 to G1S and then to G2 phase, respectively. This data might help in understanding the cell cycle regulatory effect of Rb1 protein and complement the available information on its interacting partners.

Principal Investigator: Dr. Kanury V.S. Rao

PI Affiliation: THSTI - National Chair and Head, Drug Discovery Research Centre
Translational Health Science and Technology Institute, Faridabad, INDIA

Sample Preparation: Sample processing protocol: Stable Rb1 expressing HEK-293 cell line was cultured in light, medium and heavy labelled SILAC media. Light labeled cells were arrested in G0 phase, medium labeled in G2 and heavy labeled in G1S phase.

Peptide Separation: Equal number of differently labeled cells representing each cell cycle phase were pooled, lysed and subjected to AP-MS. Each sample was investigated as three biological replicates.

Protein Characterization: Wiff files generated from AB Sciex 5600 TRIPLE TOF Mass Spectrometer were submitted to protein pilot software, which resulted in 18 group files. Search parameters for database search were as followed: 2 missed cleavages allowed; with cys alkylation as Methyl methanethiosulphonate (MMTS), the "Search Effort" parameter "Thorough ID", which gives us a broad search of various protein modifications. The following parameters were used for identification and quantification of differentially expressed proteins – auto-bias correction for heavy to light ratios; threshold of 1% accepted Global False Discovery Rate from fit (G-FDR-fit) proteins; Minimum protein confidence threshold cut-off of 95% and at least one peptide with 95%

confidence for the relative expression.

Experiment Type: Affinity purification coupled with mass spectrometry proteomics

Species: Data in species_details No Data

Tissue: Data in tissue_details No Data

Cell Type: Data in cell_details No Data

Disease: Unknown No Data

Instrument Details: Data in instrument_details Data in instrument_details

Protein Modifications: 6x(13)C labeled residue

PubMed ID: