IPD Project Details

Project ID: IPD9042

Project Title: p53 amyloid induced cellular transformation and tumor formation in the mouse xenograft model

Description: p53 amyloid formation is predicted to be involved in cancer initiation, but the direct evidence of how altered p53 acts as an oncogene is lacking. Cells with p53 amyloids show enhanced survival, apoptotic resistance with increased proliferation and migration rates. Proteomic profiling of cells containing p53 aggregates suggests that p53 amyloid formation triggers aberrant expression of pro-oncogenes while downregulating the tumor-suppressive genes. We propose that wild-type p53 amyloid formation can potentially contribute to the initiation of tumor development.

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Sample Preparation: Proteins were extracted from p53 core fibril and peptide seeds treated, and untreated (control) MCF 10A cells by using RIPA buffer. For 8plex iTRAQ isobaric mass tag labeling, 40 ?g of each sample was reduced with 5 mM DTT at 37 °C for 60 min, and alkylated with 20 mM IAA at room temperature for 30 min in dark.

Peptide Separation: Protein digestion (trypsin-to-substrate ratio of 1:20) was performed overnight at 37 °C. In the T1 generation, control, peptide treated, and core fibril treated samples were labeled with mass tags 113 and 115, 114, and 116 respectively. Similarly, in the T5 generation, control, peptide treated, and core fibril treated samples were labeled with 117 and 119, 118, and 121 respectively. The labeling of isobaric mass tags was performed at room temperature for 2h. All labeled peptides were pooled together and fractionated by basic reverse phase chromatography. The fractions were combined into 10 pools, vacuum dried followed by desalted using C18 fast-flow tips. The mass spectrometric data were acquired using TripleTOF 5600 (SCIEX).

Protein Characterization: All raw files (.wiff) were converted to Mascot generic file (mgf) format using Peak View (version 1.2.0.3, SCIEX). ProteinPilot (version 4.5, SCIEX) with the Paragon algorithm was used for protein identification and relative iTRAQ quantification. Proteins were identified against the UniProt human-reviewed database containing only canonical sequences. The search parameters were set as follows:

iTRAQ 8plex (peptide labeled); IAA Cysteine alkylation; digestion enzyme Trypsin. Peptides and proteins were validated at < 1% false discovery rate (FDR) and with unused score > 1.3 (which corresponds to > 95% confidence). The cutoff value for up-regulation and down-regulation of proteins was set to > 1.3 and < 0.8 respectively with p-value ? 0.05.

Experiment Type: Shotgun proteomics

Species: Homo sapiens

Tissue: Mammary gland (bto:0000817)

Cell Type: Mammary gland epithelial cell (cl:0002327)

Disease: Breast cancer (doid:1612)

Instrument Details: TripleTOF 5600 (MS:1000932)

Protein Modifications: iTRAQ8plex-116 reporter+balance reagent acylated residue, iodoacetamide derivatized residue

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