IPD Project Details

Project ID: IPD7455

Project Title: Phosphoproteomic Analysis of Lethal Castration Resistant Prostate Cancer Reveals Patient but not Metastatic Site Heterogeneity of Tyrosine Kinase Activation

Description: Tissue lysis was performed as previously described (Drake, J.M., et al. Oncogene-specific activation of tyrosine kinase networks during prostate cancer progression. Proc Natl Acad Sci U S A 109, 1643-1648 (2012)) Briefly, greater than 300 mg of frozen tumor mass was homogenized and sonicated in urea lysis buffer (20 mM HEPES pH 8.0, 9 M urea, 2.5 mM sodium pyrophosphate, 1.0 mM beta-glycerophosphate, 1% N-octyl glycoside, 2 mM sodium orthovanadate). Total protein was measured using the BCA Protein Assay Kit (Thermo Scientific/Pierce) and 25 mg of total protein was used for phospho-proteomic analysis. Phospho-tyrosine peptide enrichment and liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis was performed as previously described (Drake, J.M., et al. Oncogene-specific activation of tyrosine kinase networks during prostate cancer progression. Proc Natl Acad Sci U S A 109, 1643-1648 (2012); Rubbi, L., et al. Global phosphoproteomics reveals crosstalk between Bcr-Abl and negative feedback mechanisms controlling Src signaling. Sci Signal 4, ra18 (2011); Graham, N.A., et al. Glucose deprivation activates a metabolic and signaling amplification loop leading to cell death. Molecular systems biology 8, 589 (2012)) Phospho-peptides were identified using the Proteome Discoverer software (version 1.3.0.339, Thermo Fisher Scientific). MS/MS fragmentation spectra were searched using SEQUEST against the Uniprot human reference proteome database with canonical and isoform sequences (downloaded January 2012 from uniprot.org). Search parameters included carbamidomethyl cysteine (*C) as a static modification. Dynamic modifications included phosphorylated tyrosine, serine, or threonine (pY, pS, pT, respectively) and oxidized methionine (*M). The Percolator node of Protein Discoverer was used to calculate false-discovery rate (FDR) thresholds and the FDR for the datasets was adjusted to 1% (version 1.17, Thermo Scientific). The Percolator algorithm uses a target-decoy database search strategy and discriminates true and false identifications with a support vector machine. The PhosphoRS 2.0 node was used to more accurately localize the phosphate on the peptide44. Only phospho-peptides with at least one phospho-tyrosine assignment with a reported probability above 20% were considered. MS2 spectra for all reported phosphopeptides are available under the PRIDE accession numbers.

Principal Investigator: Dr. Medicharla Venkata Jagannadham

PI Affiliation: Chief Scientist, CSIR-Centre for Cellular and Molecular Biology, Hyderabad-500007, India

Sample Preparation: 22Rv1 cells were grown in RPMI medium supplemented with l-glutamine, FBS, and nonessential amino acids (NEAAs). LNCaP, DU145, and C4-2 cells were grown in DMEM supplemented with l-glutamine, FBS, and NEAA. See details in reference(s):

Peptide Separation: Frozen tissues from the Rapid Autopsy program were sent overnight on dry ice for phosphotyrosine peptide analysis. See details in reference(s):

Protein Characterization: Data analysis was performed as previously described. See details in reference(s):

Experiment Type: Bottom-up

Species: Data in species_details No Data

Tissue: Unknown No Data

Cell Type: Unknown No Data

Disease: Unknown No Data

Instrument Details: Data in instrument_details Data in instrument_details

Protein Modifications: monohydroxylated residue, phosphorylated residue, iodoacetamide derivatized residue

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