Abstract

Selective kinase inhibitors, which target specific protein kinase activity, are used to identify and inhibit pathways involved in the initiation and maintenance of cancer in solid tumors. A variety of treatments have been identified to target aberrant signaling, and understanding the signaling networks downstream of identified cancer genes is critical. With the increasing number of targets, site-specific drug resistance has been called “oncogene addiction” and demonstrates the acute need to identify and target downstream pathways.

Materials and Methods

Cell lines were cultured in a humidified incubator at 37°C in RPMI-1640 supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (P/S) with or without 2mM L-glutamine. The cell lines were treated with 10µM Gleevec® (PDGFRα), 10µM SU11274 (PDGFRα), 100µM Crizotinib (ALK), 10µM Iressa® (EGFR), 5µM Enzalutamide (AR), or 10µM Docetaxel (c-Met).

Harvesting Samples

Cell lysates were prepared from RTK inhibitor treated and untreated cell lines, proteins were digested with trypsin, and peptides were purified over a C18 micro column. Each purified fraction was then subjected to MS/MS/MS analysis. Data processing was performed using CORE.

Fractionation by IRRP

Samples were subjected to quadruplex reverse phase (qRP) chromatography and selected fractions containing tyrosine kinase substrates were collected (Figure 1). Samples were then reduced and alkylated before being digested. Samples were then labeled with 100 µg of each sample was then labeled with 100µg of TMT (LysC-tagged) and subject to peptide quantification. 100 µg of each sample was then labeled with 100 µg of TMT (LysC-tagged) and subjected to peptide quantification.

Figure 4. RTK driven cell lines profiled through the following spaces: phosphotyrosine, phosphoserine/phosphothreonine, acetylation, methylation, and phospho-tyrosine kinase.

Summary

• Multiple tyrosine kinases are known to be involved in cancer cell proliferation. However, the identification of specific signaling networks downstream of known oncogenes is necessary to optimize therapeutic strategies.

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Figure 6. Pathway diagrams for each cell line were generated using Cytoscape®. These diagrams include proteins with validated identities, including all PTM types, were used for pathway analysis of phospho-tyrosine kinase, acetylation, methylation, and protein tyrosine kinases.