Phosphoproteomic and Bioinformatic Characterization of the Signaling Alterations in Response to a PP2A Activator in Lung Cancer

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SUMMARY

The development and progression of non-small cell lung cancer involves the coordinate activation of multiple oncopgenic pathways, including the MAPK and AKT signaling cascades. Even with the development of various small molecule kinase inhibitors targeting these activated oncopgenic pathways, their long-term effectiveness is limited by crosstalk and the development of acquired resistance, highlighting the need to inhibit multiple signaling cascades simultaneously. Phosphatases are biological antagonists of kinases and have an incredibly diverse range of signaling targets, making them ideal targets for targeted activation using small molecule based approaches. Data suggests that a novel series of compounds that were derived from reverse engineering of the tricyclic neuroleptics have anti-cancer effects by directly binding and activating the serine/threonine phosphatase (PP2A). While this enzyme is known to dephosphorylate key oncogenic signaling proteins, the spectrum of perturbations in the phosphoproteome of cancer cells induced by these compounds is unknown.

To answer these questions, we performed global phosphoproteomics on two KRAS mutated lung adenocarcinoma cell lines that are responsive to the PP2A activator. Furthermore, we developed two new bioinformatic tools, Protein Set Enrichment Analysis (PSEA) and Protein Interaction Enrichment Network Analysis (PIENA), to extract key perturbed pathways from the datasets. PSEA, through pathway scoring, identifies pathways with uniform directional change in phosphorylation in all protein members of that set. Alternatively, PIENA extracts pathways with internal dysregulation by looking for intensity differences between phosphopeptide pairs. Aside from detecting many other signaling cascades classically implicated in cancer, our methods notably identified the ERK pathway as significantly dephosphorylated in both cell lines. These results not only demonstrate the advantage of this proteomic-bioinformatic pipeline in providing unbiased and global identification of key drivers of signaling, but they also contribute to a more thorough understanding of this novel PP2A activator’s anticancer effects.

METHODS, cont’d

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RESULTS

Figure 3: PCA analysis and general statistics show good separation between drug and control groups and good coverage

Figure 4: Select pathways identified by PSEA and PIENA

Figure 5: PSEA identified ERK as the only pathway significant in both cell lines with p ≤ 0.01

CONCLUSIONS

• Phosphoproteomic analysis was performed on both A549 and H358 KRAS mutated cells using TRC-794 vs. DMSO
• Results from both cell lines were reproducible and show good separation between drug and control (PCA data)
• Pathway identification analysis was performed on the entire dataset and revealed a common dysregulation through the ERK pathway in both cell types (PIENA data)

Further Analysis:
• Validation by Western blot for the ERK pathway
• Protein-protein interaction network analysis of the phosphoproteomic dataset to identify alterations not captured by canonical pathways

ACKNOWLEDGMENTS

CWRU Center for Proteomics and Bioinformatics
Case Comprehensive Cancer Center P50 Grant
Clinical and Translational Science Collaborative (CTSC) UL1-TR-000439 Grant
Institute for Transformative Molecular Medicine
Harrington Discovery Institute / University Hospitals
Medical Scientist Training Program (MSTP) T32 Grant

REFERENCES