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

Danica Wiredja[1], Yu Liu[1], Giridharan Gokulrangan[1], Daniela Schlatzer[1], Sahar Mazhar[2], Goutham Narla[2], Mark R. Chance[1]

[1] CWRU Center for Proteomics and Bioinformatics, [2] CWRU Institute for Transformative Molecular Medicine

SUMMARY

The development and progression of non-small cell lung cancer involves the coordinate activation of multiple oncogenic pathways, including the MAPK and AKT signaling cascades. Even with the development of various small molecule kinase inhibitors targeting these activated oncogenic 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**.

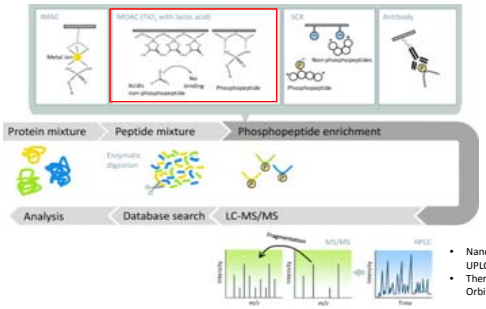
To answer these questions, we performed global phosphoproteomics on two KRAS mutated lung adenocarcinoma cell lines that are responsive to the PP2A activator. Furthermore, we developed two new bioinformatic tools, Protein Set Enrichment Analysis (PSEA) and Protein Interaction Enrichment Network Analysis (PIENA), to extract key perturbed pathways from the datasets. PSEA, through pathway scoring, identifies pathways with uniform directional phosphorylation change 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

Administration of drug (or control) to A549 and H358 Cells → Phosphoproteomic Strategy → Bioinformatic Analysis

Phosphoproteomic Strategy:

Figure 1: Workflow for phosphopetide enrichment, followed by LC-MS/MS

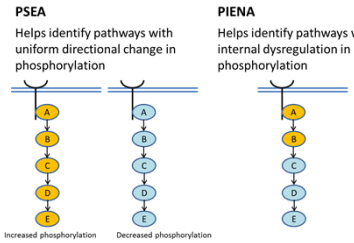


- NanoAquity UPLC
- Thermo Velos Orbitrap

METHODS, cont'd

Bioinformatic Analysis:

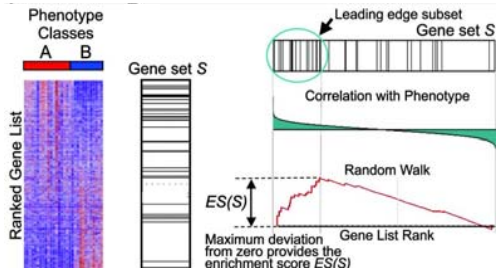
Figure 2a: Overview of the goals for PSEA and PIENA pathway extraction tools



PSEA: Protein Set Enrichment Analysis; based on the original approach by Subramanian et al., 2005

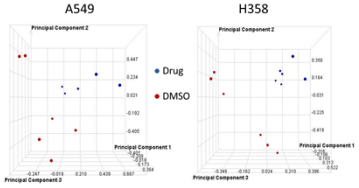
PIENA: Protein Interaction Enrichment Network Analysis; based on the original approach by Liu et al., 2012

Figure 2b: Pathway scoring system, as described by Subramanian et al., 2005



RESULTS

Figure 3: PCAs and general statistics of both cell lines reveal good separation between drug and control groups and good coverage



	# phospho-peptides	# phospho-proteins
A549	3000	1606
H358	2897	1619
Shared	1865	1143

Work of Danie Schlatzer and Giri Gokulrangan

Figure 4: Select pathways identified by PSEA and PIENA

A549

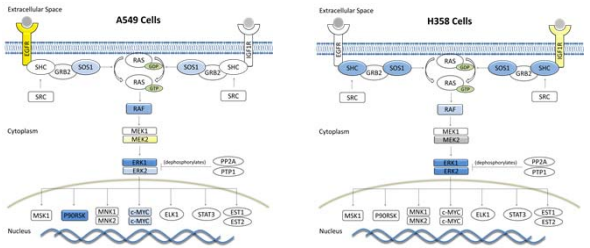
	Pathway	Database	P-value
PSEA	Meiosis	Reactome	0.016
	Glycosaminoglycan metabolism	Reactome	0.000
	P53	Biocarta	0.022
	E2F regulation of DNA replication	Reactome	0.006
PIENA	ERK	Biocarta	0.002
	MTORC 1	Reactome	0.000
	Mitotic G1-S Phase	Reactome	0.010
	MAPK TRK	PID	0.011
	PKB (AKT)	Reactome	0.015

H358

	Pathway	Database	P-value
PSEA	PML	Biocarta	0.010
	HDAC Class III	PID	0.010
	Antigen proteasome degradation	Reactome	0.017
	Oocyte meiosis	KEGG	0.000
PIENA	HER2	Biocarta	0.000
	IL2RB	Biocarta	0.020
	GNRH	KEGG	0.016
	ERK	Biocarta	0.000
	Double strand break repair	Reactome	0.012

RESULTS, cont'd

Figure 5: PIENA identified ERK as the only pathway significant in both cell lines with $p \leq 0.01$



	ERK pathway p-value	# peptide pairs	pathway coverage
A549	0.002	55	32%
H358	0	21	28%

Blue = decreased fold change in drug vs. control
Yellow = increased fold change
Grey = fold change = 1

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

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2. Liu et al., BMC Syst Biol. 2012 Jun 13;6:65