Deep Profiling of Tyrosine Phosphorylation in Gastric Cancer Cells

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Introduction

Protein phosphorylation is known to play a key role in both normal cellular physiology and to be frequently modified in disease states. As such, protein kinases are frequent therapeutic targets in the treatment of cancer and other diseases, and quantitative analysis of phosphorylation changes can be crucial to both mechanism of action studies and biomarker discovery. Cellular levels of tyrosine phosphorylation are significantly lower than serine and threonine and require specific immuno-enrichment for deep profiling. In this study, we combine peptide-level enrichment of phosphotyrosine with a timsTOF Pro to both identify sites of tyrosine phosphorylation and measure changes in that phosphorylation upon treatment of a gastric cancer cell line with a specific tyrosine kinase inhibitor.

Methods

The gastric cancer cell line MKN-45 was treated with and without the c-Met inhibitor SU11274 (1 uM, 2h). Proteins were digested with trypsin and resultant peptides were immunoenriched using the anti-phosphotyrosine antibody P-Tyr-1000 (Cell Signaling Technology, Inc.). 5 IP preparations for control and treated samples were analyzed in duplicate. The resulting extracts were separated by nano HPLC (nanoElute, Bruker) on 250 mm x 75 µm, 1.6 µm (IonOpticks, Australia). 90 min gradients at 400nL/min were analyzed on a trapped ion mobility Q-TOF (timsTOF Pro, Bruker Daltonics) operating in PASEF mode. LFQ data were processed in PEAKS X+ (Bioinformatics Solutions Inc) and Simplifi (ww.simplify.protifi.com) software.

Results

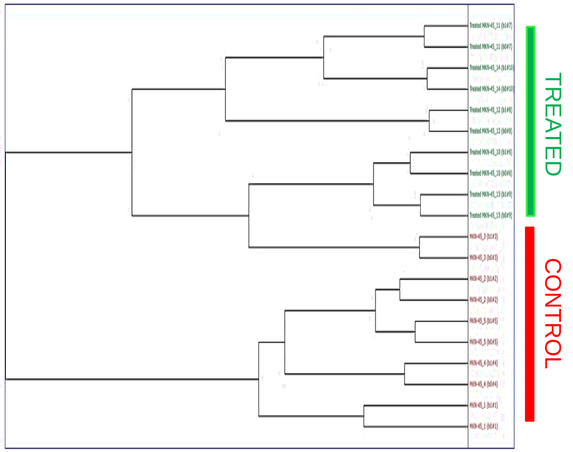

Figure 1. Hierarchical clustering indicates good differentiation of the treated cells compared with the controls

Pathways		Disease
R-HSA-5655291	Signaling by FGFR4	Cancer
R-HSA-5637810	Signaling by EGFRvIII	Cancer
R-HSA-1839117	Signaling by cytosolic FGFR1 fusion mutants	Leukemia
R-HSA-1236382	Signaling by Ligand-responsive EGFR Cancer Variants	Cancer
R-HSA-8853338	Signaling by FGFR3 point mutants	Cancer
R-HSA-2219530	Signaling by Aberrant PI3K	Cancer
R-HSA-5655253	Signaling by FGFR2	Bone development disease, Cancer
R-HSA-5655302	Signaling by FGFR1	Bone development disease, Cancer

Figure 3. A total of 42 pathways were affected for the c-Met inhibitor. 8 disease-associated pathways effected by treatment with SU11274.

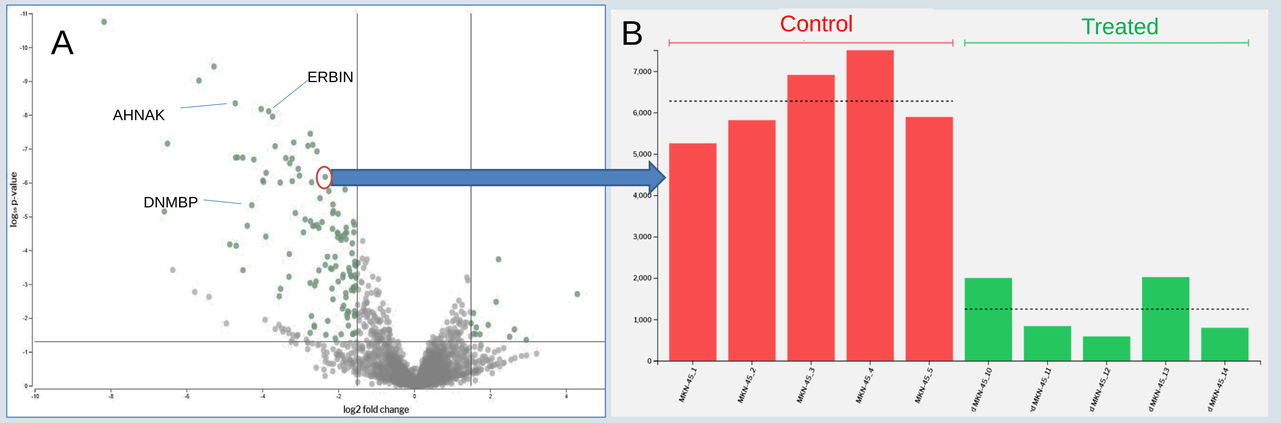

Figure 2. LFQ analysis. A.309 proteins were found to be decreased in the samples treated with SU11274 ($p < 0.05$, log2 fold change>1.5). B. SHIP2_HUMAN protein is down regulated in treated cells (p 6.72e-7, log2 mean fold change = -2.3)

- A total of 5225 protein groups and 38469 unique peptides were identified in this study. 4572 peptides were phosphorylated on a tyrosine residue.
- 309 proteins phosphorylated proteins were found to be down regulated in the cells treated with SU11274.
- 42 pathways were affected by the Met-c inhibitor of which 8 were associated with disease. Most of the pathways associated with disease involved fibroblast growth factor receptors (FGFRs) which are receptor tyrosine kinases.

Conclusions

- PTMScan® Phospho-Tyrosine Rabbit mAb provides very efficient, focused enrichment of modified peptides from human cells
- The timsTOF Pro and PASEF allow deep profiling of phosphotyrosine containing peptides with 4572 unique pY peptides identified in the study indicating the system is an excellent choice for the characterizing and quantifiying low adundance phosphotyrosine modified peptides.

timsTOF Pro