Case Study: Phosphoproteomics of Colorectal Cancer Metastasis

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Phosphorylation is commonly altered in disease states such as cancer.

Mass spectrometry is the most popular method to understand global phosphorylation changes.
Most deaths occurring from colorectal cancer occur from metastasis

Progression of cancer is related to invasive cellular behavior, migration, angiogenesis, and resistance to apoptosis

These processes are regulated by phosphorylation

Hanahan and Weinberg, Cell (2000), 100, 57-70
The SW480 and SW620 are isogenic cell lines often used to study colorectal cancer metastasis.

SW480 cells were derived from an adenocarcinoma and the SW620 lines were derived from a lymph node metastasis.

Both cell lines exhibit chromosomal instability.
Methods Employed

- SW480 and SW620 cells were cultured with SILAC for quantification and lysed with a urea buffer.

- Phosphopeptides were enriched with both IMAC and TiO$_2$ beads followed by high pH fractionation.

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Quantitative Phosphoproteomics Results

- Phosphoproteomic data was searched using MaxQuant and localization probabilities were filtered to be 75% or greater.
- 1,759 phosphosites quantified mapped to 1,114 phosphoproteins.
Quantitative Phosphoproteomics Results

- Many phosphosites and phosphopeptides were observed to be differentially expressed.

- Red values are phosphopeptides expressed more in SW620, blue values were expressed more in SW480 cells.
A network analysis of phosphosites showed clusters in mitosis, cell cycle functions, mRNA biogenesis, and nuclear pore protein complexes.

Perturbation observed in mitotic signaling is consistent with the more aggressive growth of the SW620 cells.

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Conclusions

• SW620 cells have differential regulation in cellular adhesion, mitosis, cytoskeletal structure, mRNA transport, and translations

• Phosphoproteomics can provide information about cellular signaling events and incorporation into kinase signaling networks

• Specific sites of phosphorylation can be quantified within a sample