

# Revealing Systematic Proteomic Remodeling through Library-Free DIA-MS Analysis of a Novel Progressive Prostate Cancer Cell Model

Daniel G. Delafield<sup>1</sup>, Hannah M. Miles<sup>2</sup>, Teresa Liu<sup>3</sup>, William A. Ricke<sup>2,3,4</sup>, Lingjun Li<sup>1,2</sup>

<sup>1</sup>Department of Chemistry, <sup>2</sup>Division of Pharmaceutical Sciences, <sup>3</sup>Department of Urology, <sup>4</sup>George M. O'Brien Center for Benign Urology Research, University of Wisconsin-Madison



## Overview

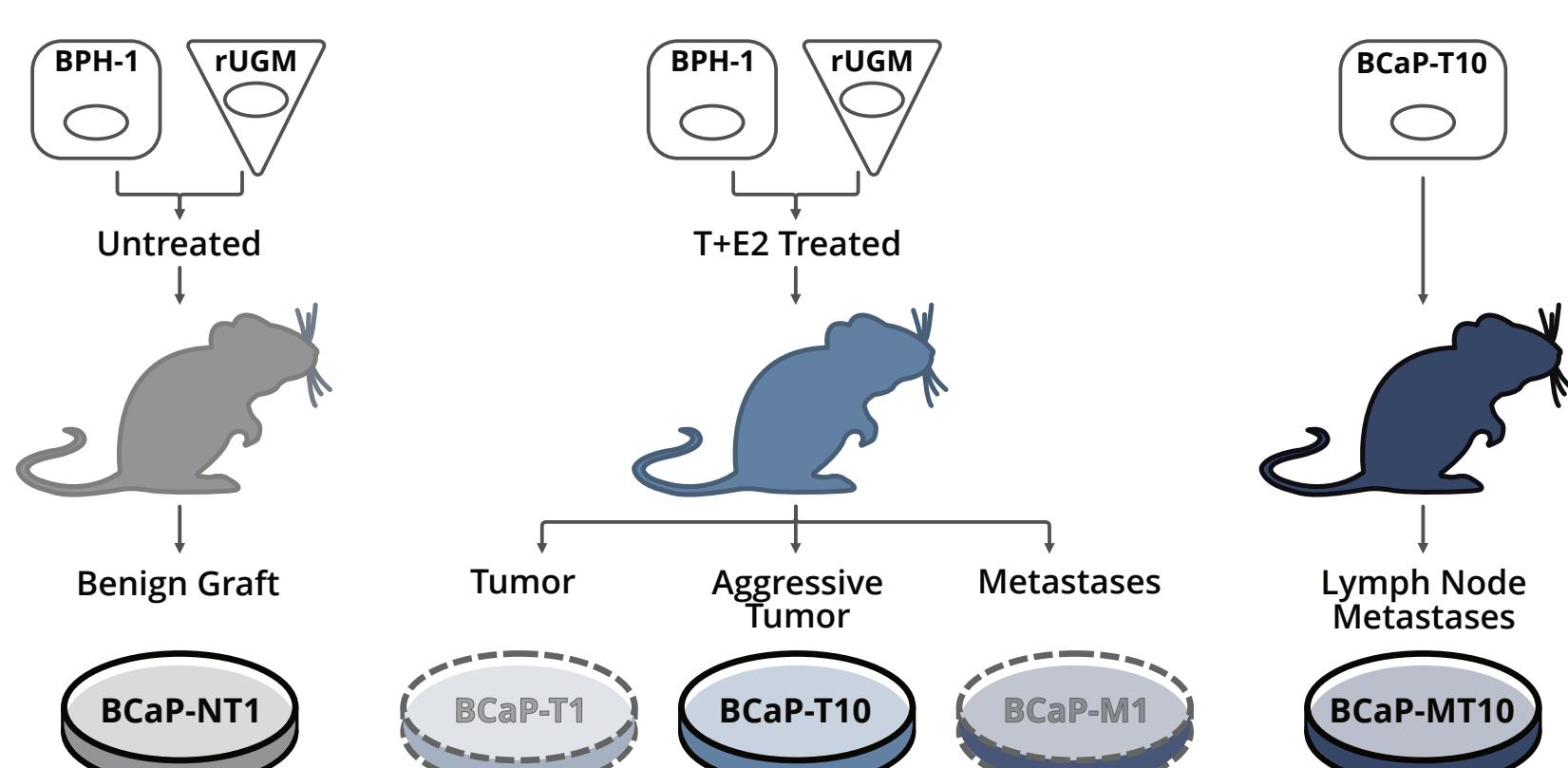
This work utilizes high-throughput, library-free data-independent mass spectrometry to identify protein communities specific to prostate cancer progression. Utilizing a novel progressive prostate cancer cell model that mimics human disease we cluster 1,245 proteins into 6 diagnostic patterns that reveal cancer diagnoses, malignancy progression, and differentiation of cancer phenotype.

## Introduction

Prostate cancer (PCa) is the second leading form of cancer in men and is commonly associated with late detection, incorrect diagnoses, and ineffective monitoring<sup>1</sup>. Biomolecular investigations are critical to uncovering useful therapeutic targets, as well as the ability to correctly diagnose, stratify and monitor cancer severity.

Using a novel prostate cancer cell model (BCaP)<sup>2</sup> that mimics the discrete genetic and molecular characteristics of human cancer stages, we provide early reports on the proteomic reorganization that accompanies PCa malignancy and progression. Utilizing data-independent acquisition mass spectrometry (DIA-MS), we reveal 1,245 dysregulated proteins across 6 diagnostic patterns that reveal targets for disease diagnoses, severity assignment and therapeutic monitoring.

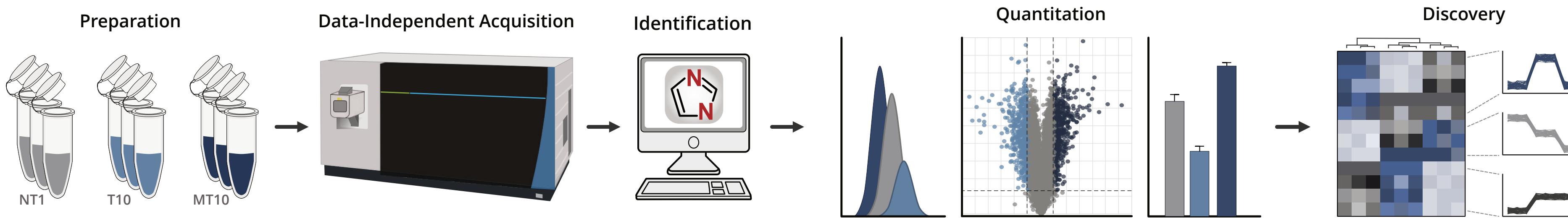
## Progressive PCa Model



Built on early reports that demonstrate circulating hormone levels can inspire carcinogenesis<sup>3</sup>, the benign prostate hypertrophy-derived cancer progression (BCaP) model<sup>2</sup> has been validated as a mimic of human cancer.

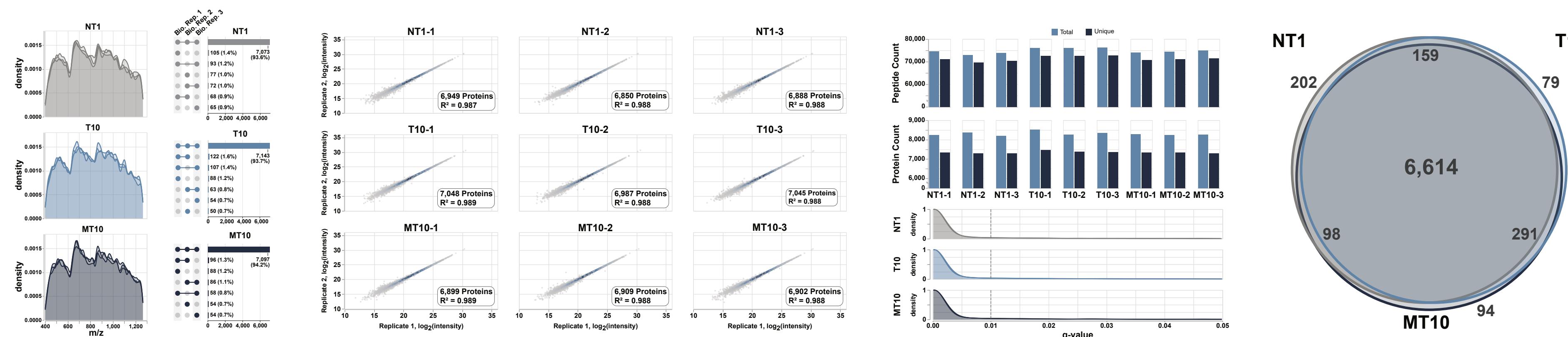
This model utilizes renal grafts of with or without treatment to produce 5 unique lines, ranging from benign to aggressive malignancy. Here we utilize benign (NT1), aggressive tumorigenic (T10) and aggressive metastatic tumorigenic (MT10) for proteomic evaluation.

## Workflow

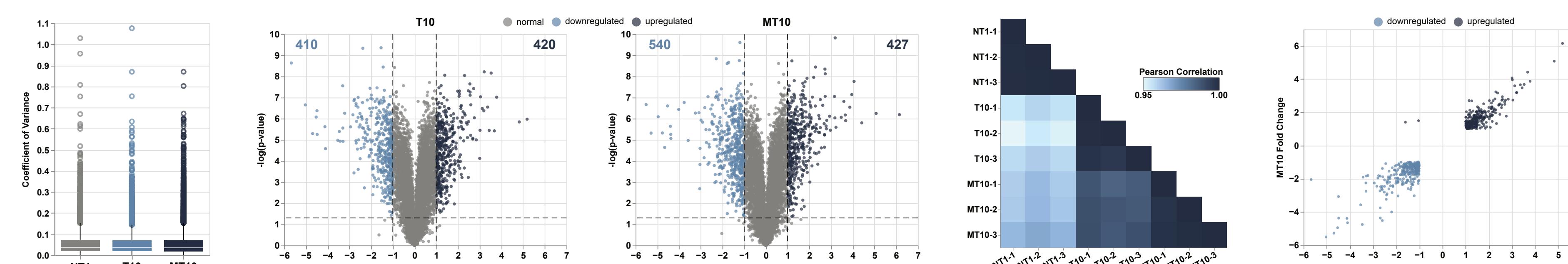


Biological triplicates of each cell line were subjected to standard bottom-up preparation prior to duplicate measurement through DIA-MS. DIA-NN<sup>4</sup> was used to perform library-free identification and protein quantitation. Proteins were filtered for confidence and ubiquity prior to custom data analysis.

## Discovery, Validation, Quantitation

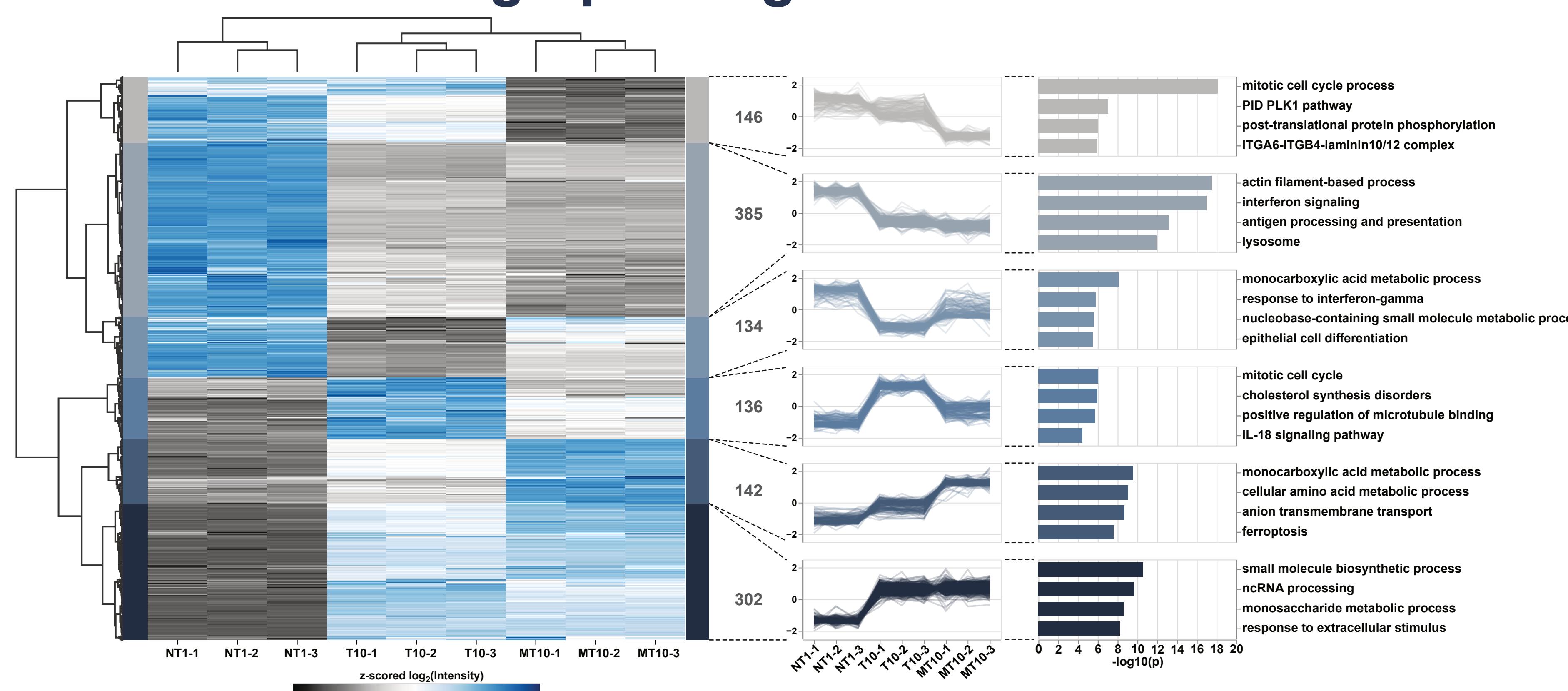


Match between runs (MBR) enabled highly-reproducible precursor and protein identification (a), quantitative estimates highly conserved between technical replicates (b). Proteotypic peptides were mapped to proteins, and filtered to a strict 1% protein-level FDR (c). A final collection of 6,614 proteins (d) were identified and quantified in every sample and utilized for later analysis.



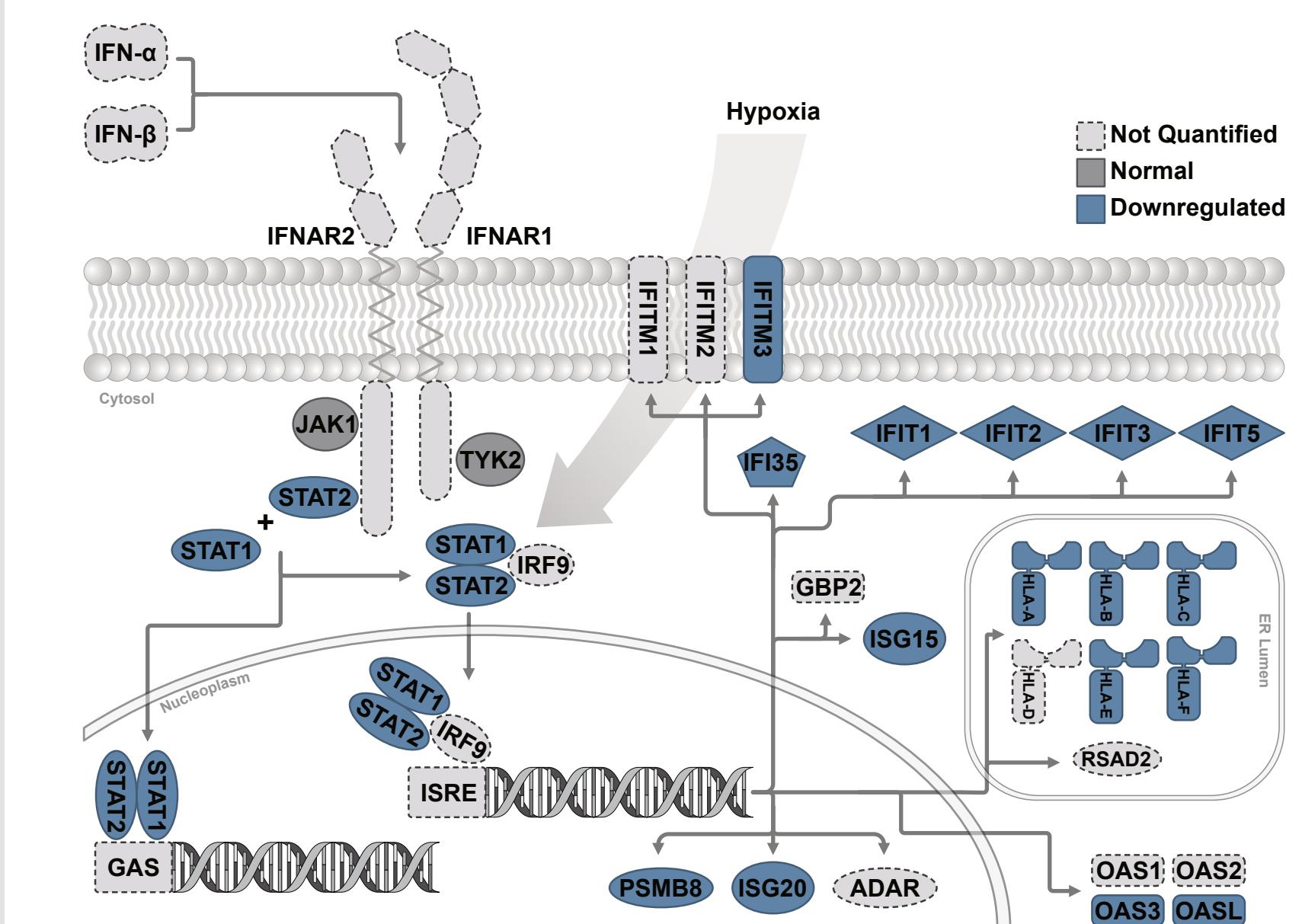
Low variance (mean=5.7–6.0%) in abundance estimates (a) rationalized a 1-fold change abundance threshold. 1,245 proteins were dysregulated across aggressive cell lines (b) with similar quantities suggesting phenotypic relationship. Pearson correlation (c, d) confirms these protein measurements correlate to PCa progression and highlight the potential for disease state fingerprinting.

## Biomolecular Fingerprinting

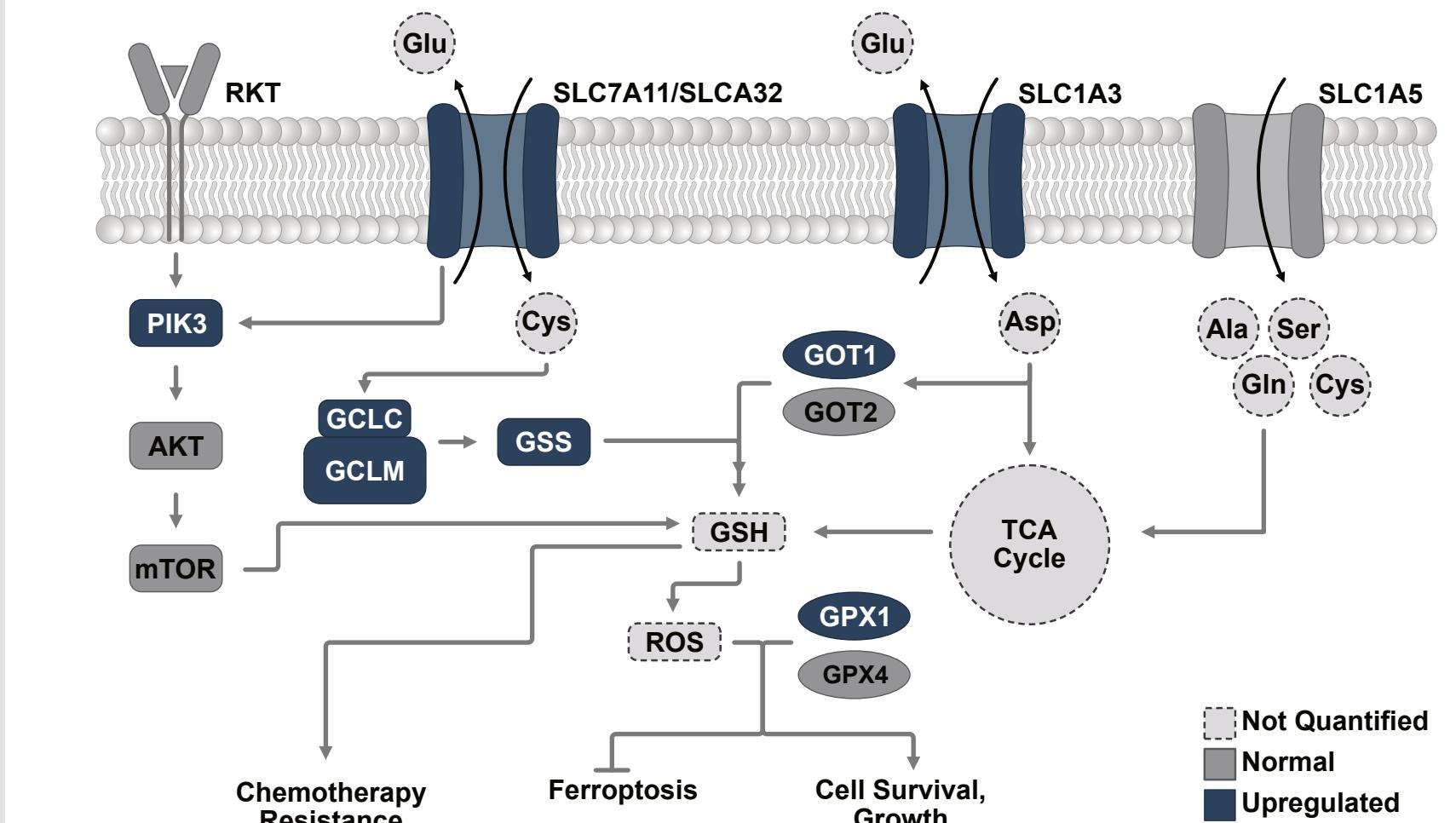


Column-wise hierarchical clustering enabled facile discrimination of disease state, suggesting the uniqueness of protein expression in each sample. This large protein panel was further partitioned through row-wise clustering, revealing 6 distinct diagnostic patterns related to malignancy, cancer progression, and phenotype. Gene ontology of each cluster reveals protein communities related to cancer progression, highlighting kinetochore reorganization and small molecule biosynthesis.

## Dysregulated Pathways



We established the importance of protein measurements by comparison to RNA microarray quantitation (not shown). These analyses revealed the STAT pathway to be significantly suppressed in advanced cancer types and suggest a permanent hypoxic phenotype. Of note, this dysregulation was observed only at the protein level, whereas transcriptomic analyses were normal.



To better understand the role of metabolic activity within cancer development, we manually inspected these protein communities and revealed a concerted upregulation of glutathione (GSH)-related proteins. Our data implicate the increased production of GSH and clearance of reactive oxidative species as a cause of increased survival. Similarly, other cancers have demonstrated resistance to treatment through increased GSH.

## Conclusion

Our investigation of a novel progressive cell model revealed proteomic alterations specific to cancer development. We stratified these proteins into diagnostic clusters and validate their relevance to prostate cancer.

We posit the diagnostic patterns presented here may be further investigated to validate putative biomarkers, reveal protein panels for accurate diagnosis, and provide a protein-centric approach to severity assessment.

## Contact

Graham Delafield  
Ph.D. Candidate, University of Wisconsin-Madison  
delafield@wisc.edu  
grahamdelafield.com

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## References

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