Commentary

Complementing Genomics and Transcriptomics: Phosphoproteomics Illuminating Systems Biology in Prostate Cancer

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Abstract

Integrating phosphoproteomics with traditional genomics and transcriptomics provides a more comprehensive overview of the signaling networks in advanced prostate cancer for immediate pre-clinical and future clinical use. Our recent publication introduces computational approaches for integrating the phosphoproteome, specifically with the intent of identifying important kinase signaling networks in advanced stage prostate cancer.

Keywords

Phosphoproteomics, castration-resistant prostate cancer, precision medicine, kinases, systems biology
Prostate cancer is the most common male cancer and second leading cause of cancer related death of men in the United States. Although treatable at early stages, prostate cancer can ultimately progress into a metastatic and lethal state. Successful first line treatment options vary from radiation and surgical removal of the prostate (prostatectomy) to physical (orchiectomy) and chemical (e.g., Leuprolide) castration that block the androgen/androgen receptor axis that fuels the tumor. Unfortunately, a majority of patients will eventually develop resistance to these treatments and the disease progresses into a castration-resistant form usually concomitant with metastases, termed metastatic castration resistant prostate cancer (mCRPC). This has led to the development of second generation anti-androgen drugs such as enzalutamide and abiraterone acetate with greater efficacy. However, this vicious cycle of resistance and treatment continues as the disease develops new ways of evading therapy.  

Previous studies have largely focused on the mutational and transcriptional landscape of mCRPC to identify new markers of resistance with mixed results. These studies and others have also provided evidence that the mutation rates in prostate cancer and subsequently mCRPC are relatively low compared to other cancers. It is therefore imperative to identify novel approaches that will improve our understanding of treatment resistance in advanced prostate cancer in order to better tailor treatment options for patients. One concept is the implementation of phosphoproteomics that enable the identification of pathway activity in lieu of mutational burden.

We previously used immunohistochemistry staining to show increased tyrosine phosphorylation in mCRPC patient tumor samples when compared to treatment naïve or locally confined prostate cancer, suggestive of heightened kinase activity in these more aggressive tumor types. We followed up our findings by performing unbiased shotgun phosphopeptide enrichment coupled to quantitative mass spectrometry (known as phosphoproteomics) to aid in the identification of these kinases in mCRPC samples. Based on our previous work and the paucity of mutations in mCRPC, we predicted that integration of the phosphoproteome will
complement analyses from genomic and transcriptomic studies to aid in the identification of potential pathway drivers in this disease (Fig. 1), which is what the findings suggest in our recent publication in Cell®.

We procured treatment naïve prostate cancers and mCRPC tumor samples via an IRB-approved rapid autopsy program from the University of Michigan and utilized phosphoproteomics to generate a compendium of phosphotyrosine (pY) and phosphoserine/phosphothreonine (pS/pT) peptides. To identify differential kinase activity between the sample types, we applied the master regulator inference algorithm (MARINa) which infers the differences in kinase activity based on differential phosphorylation of its targets®. We also applied MARINa to the patients’ transcriptomic data and identified aberrantly activated transcription factor master regulators in mCRPC.

To integrate our phosphoproteomic data with other omic datasets, we used the Tied Diffusion through Interacting Events (TieDIE) algorithm®. This pathway-based method expands upon heat diffusion strategies, such as the HotNet algorithm®, to integrate information from several different sources. We employed TieDIE to integrate our phosphoproteomic data with genomic data, transcriptomic data, and a priori knowledge from pathway databases® and generated a cohort-level scaffold network for mCRPC. Importantly, integration of the phosphoproteomic data enhanced and in some cases validated the pathway networks provided by genomic or transcriptional analyses. We observed that the AKT/mTOR/MAPK signaling pathway was significantly enriched in the integrated analysis but marginally enriched when phosphoproteomic data was excluded. Proteins involved in other cancer hallmarks, including cell cycle pathway, DNA repair pathway, and nuclear receptor pathway were also enriched when we included the phosphoproteomic input.® These results provide compelling evidence for the inclusion of the phosphoproteome towards identifying potential therapeutic targets in metastatic CRPC.

In an attempt at personalized medicine via dataset integration, we used a sample-specific version of MARINa called the Virtual Inference of aberrant Protein activity by Enriched Regulon...
analysis (VIPER)⁹. We used the VIPER software to analyze individual patient transcriptome and phosphoproteome data and summarized it into transcriptional and kinase master regulators, respectively, which was applied to a pre-established cohort-level scaffold network to generate personalized integrated network models for each patient. To visualize a patient’s network model in a simpler manner, we created the Phosphorylation-based Cancer Hallmarks using Integrated Personalized Signatures (pCHIPS) diagram, which can be accessed online (https://sysbiowiki.soe.ucsc.edu/pchips)⁵. This tool illustrates patient variability via differential pathway activation. It can also reveal the differences in patient pathway networks pre- and post-treatment. In our study, we made use of the individual pCHIPS and created a kinase hierarchy to prioritize targetable kinases in the patients we were able to analyze.

While our network-based approach may demonstrate future clinical utility, we see our strategy having a more immediate contribution towards advancing pre-clinical research in mouse models and cell lines. While the current use of genomics and transcriptomics has identified key signaling networks in prostate cancer², ³, we believe that the addition of phosphoproteomics will complement well with these technologies to increase discovery and action of novel targets in this disease. Integration of phosphoproteomics will allow us to better interpret the systems biology of current pre-clinical models, thus identifying additional kinases or their substrates to test as targets for therapy. Current mouse models typically originate from genomic studies that identify key driver genes or tumor suppressors of interest¹⁰. The phosphoproteome would provide additional possibilities that include both the kinases and their downstream substrates as specific players in this disease. Overall, this would allow us to create more specific and representative mouse models of prostate cancer for use in pre-clinical research.

In summary, we have shown in a panel of mCRPC patients that integration of phosphoproteomic data complements well with traditional genomics studies and yields a more complete map of the signaling network in this disease. The Cancer Genome Atlas (TCGA) has revealed an enormous amount of genomic information in cancer, including prostate cancer, but
there are instances where the genomic data is insufficient to determine the next plan of action. For example, cancers with low mutation frequencies often provide few actionable targets. However, phosphoproteomics can help to bridge that gap and aid in the identification of tangible targets for therapy. Ultimately, it is the functional aspect of the signaling networks that are both drivers and targets in cancer, hence the necessity to understand more about pathways at the protein level. Programs such as the Clinical Proteomic Tumor Analysis Consortium (CPTAC) in collaboration with the Cancer Moonshot have recognized the importance of linking genomic data with functional proteomic data. It will therefore be necessary to re-focus our strategy on a more global effort that encompasses analyses from all areas of research. We hope this new computational approach will provide a foundation to encourage system biologists to develop novel algorithms that would utilize the increase in phosphoproteomic knowledge.

**Disclosure of potential conflicts of interest**

No potential conflicts of interest were disclosed.

**Acknowledgements**

We thank all the co-authors from the Drake, Paull et al. paper (*Cell* 2016), especially members of the Dr. Owen N. Witte and Dr. Joshua M. Stuart laboratories and the University of Michigan rapid autopsy program. The computational algorithm described in this manuscript was developed by Dr. Evan O. Paull in the laboratory of Dr. Stuart (University of California, Santa Cruz). Due to citation limitations, we were not able to cite all of the important work that has contributed to the identification of new targets in mCRPC.

**Funding**

LCC is supported by the National Institute of General Medical Sciences of the National Institutes of Health under award number T32 GM008339. JMD is supported by the Department of
Defense Prostate Cancer Research Program W81XWH-15-1-0236, Prostate Cancer Foundation Young Investigator Award, and by a grant from the New Jersey Health Foundation.

Author contributions

VMT, LCC, and JMD wrote the manuscript.
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Figure 1. Value of Multi-Omic Integration. Focusing on singular approaches such as genomics (A), transcriptomics (B), proteomics (C), or phosphoproteomics (D) reveals context dependent pathways within a given tumor type. Adopting integrated, multi-omic analysis (E) can unveil the overall systems biology of the entire tumor and provide new mechanisms for therapeutic intervention.