Verification of prostate cancer genomics biomarker candidates at protein level using SRM-MS

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Overview

- Selected reaction monitoring (SRM) assays have been developed for 52 prostate cancer candidate biomarkers selected from previous transcriptomics studies, and tested in prostate tissue samples.
- PRISM (high-pressure, high-resolution separations coupled with intelligent selection and multiplexing)-(SRM)[1] enables highly sensitive, multiplexed quantification of low abundance protein candidates.
- Thirteen proteins are significantly different between benign control and tumor samples. Six proteins show potential as prognostic prostate cancer biomarker.
- This approach holds great potential for rapidly translating genomics-based discovery candidates into protein-based biomarkers.

Introduction

- Targeted MS-based proteomics such as SRM provides an antibody-independent strategy for sensitive, specific and multiplexed verification of genomics biomarker candidates at the protein level.
- In this study, 52 biomarker candidates for prostate cancer derived from genomics data including: prostate cancer prognosis associated genes, prostate cancer associated genes that were up-regulated in transcriptomics studies, and other cancer related genes (e.g., the ERG isoforms[2, 3] and ETV1), were analyzed.
- SRM was used to identify a panel of differentially abundant proteins with the potential to discriminate between aggressive and indolent forms of prostate cancer.

Methods

- PRISM-SRM[1] assays have been developed for protein quantification.
- Proteins were extracted from tissue sample, reduced, alkylated and digested by trypsin. Heavy peptide standards were spiked into each sample (0.2 µg/µL for LC-SRM, 1 µg/µL for PRISM-SRM) at a final concentration of 10 fmol/µL.
- Protein digests were fractionated with target peptide fractions of interest selected using PRISM. 96 fractions were concatenated into 24 fractions. LC-SRM was then used for analyzing each individual target peptide fraction (see **Figure 1**).
- Cohort I including 10 high Gleason-score (7-9) prostate tumors and 10 benign prostatic hyperplasia (BPH) OCT-embedded tissue samples
- Cohort II with 10 metastasis (MET), 10 biochemical recurrence (BCR) and 10 no progression with 10+ years follow-up FFPE tissue samples, were analyzed using PRISM-SRM, respectively.
- SRM data acquired on a TSQ Vantage mass spectrometer (Thermo Scientific) were analyzed using Xcalibur and Skyline.

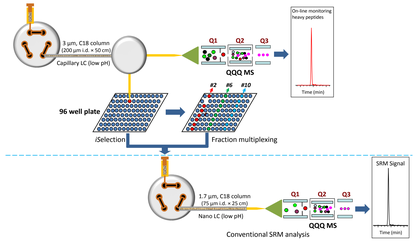

Figure 1. PRISM-SRM workflow.

Results

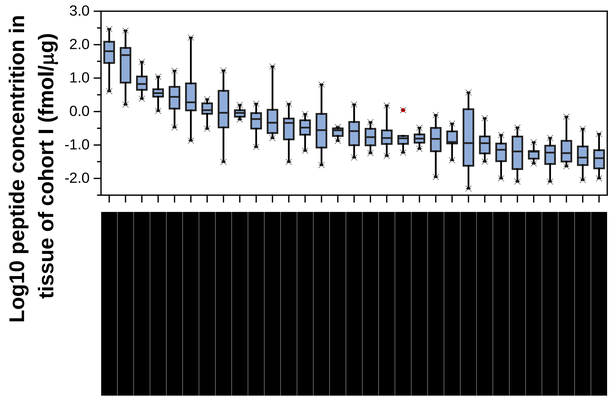

Figure 2. Quantification of the protein biomarker candidates in the Cohort I OCT-embedded prostate issue samples by PRISM-SRM.

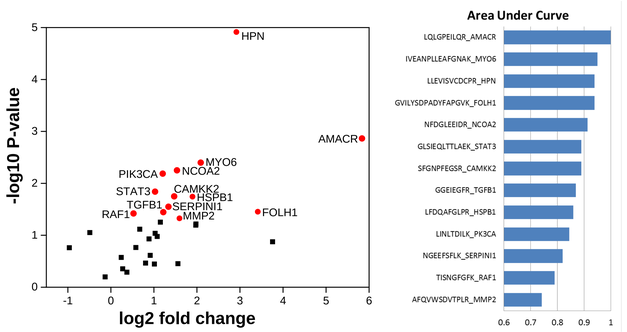

Figure 3. Quantification of individual proteins in BPH tissues and prostate tumors with Gleason scores 7-9. Thirteen proteins with *p*-value<0.05 are colored in red (left panel). The area under the ROC curve (AUC) values are shown for the 13 proteins (right panel).

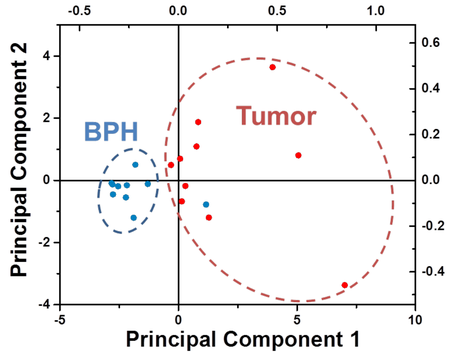

Figure 4. PCA of the 13 proteins with significant difference in abundance (*p*-value<0.05) between the BPH tissue and prostate tumor with Gleason scores 7-9.

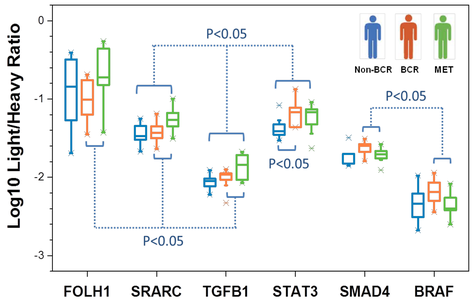

Figure 5. Boxplot of $\log_{10}$ light/heavy ratio of proteins with significant difference in abundance (*p*-value<0.05) between the no progression, BCR and MET groups in Cohort II FFPE samples.

Conclusions

- Overall, PRISM-SRM analyses of all the patient tissue samples enabled the detection of 48 out of 52 biomarker candidates, suggesting extremely low level of expression of the remaining 4 genes (HXC6, OSTP, TWST1, and ERG8); in comparison regular LC-SRM can only detect 21 of these candidates at the protein level.
- In the 10 high Gleason-score tumors and 10 BPH controls, 13 proteins were differentially abundant with *p*-value<0.05: HPN, AMACR, MYO6, NCOA2, PK3CA, STAT3, CAMKK2, HSPB1, SERPINI1, FOLH1, TGFB1, RAF1, and MMP2.
- In the 10X10X10 FFPE sample analysis, there were three proteins discriminating between “metastatic progression” and “no progression” tumors, one protein discriminated between BCR and “no progression” tumors, and five proteins discriminated between metastatic progression and BCR tumors (*p*-value<0.05).
- PRISM-SRM provides a highly sensitive method for multiplexed quantification and rapid screening of potential biomarker candidates at the protein level. This approach holds great potential for rapidly translating genomics-based discovery candidates into protein-based biomarkers.

Acknowledgements

This work was funded by NCI Early Detection Research Network Interagency Agreement (ACN15006-001). Samples were analyzed using capabilities developed under the NIH grants DP2OD006668, P41GM103493, U24CA160019, and U24CA210955. This project was performed in the Environmental Molecular Sciences Laboratory, a U.S. Department of Energy (DOE) Office of Biological and Environmental Research (OBER) national scientific user facility on the Pacific Northwest National Laboratory (PNNL) campus. PNNL is a multiprogram national laboratory operated by Battelle for the DOE under contract DE-AC05-76RL01830.

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