An integrated Pipeline from SWATH acquisition to MRM^{HR} workflow facilitates Identification and Verification of Prostate Diagnostic Markers

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Introduction

In this study, we developed an effective pipeline to discover and validate tissue protein biomarkers using single-shot short-gradient (15 min) SWATH mass spectrometry, and MRM^{HR} (Figure 1). This pipeline was applied to discover and validate protein biomarkers from several prostate cancer cohorts (Table 1).

Figure 1. Pipeline of prostate cancer biomarkers identification and verification.

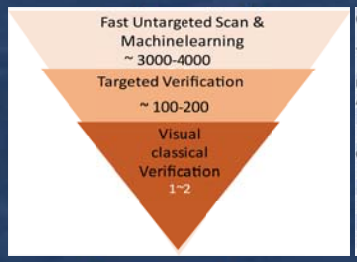

Table 1. demographic and clinical characteristics of the patients from different cohort

Cohorts	PCZA		PCZB		PCZD	
Tissue types	BPH=10	PCa=58	BPH=30	PCa=24	BPH=30	PCa=70
Biological replicates	n=3		n=2		n=1	
Age (yr)						
Median	68	67	69	70	70	67
Range	58-80	52-82	54-82	58-78	60-83	53-81
Gleason score						
Median		7		7		7
Range		6-10		7-9		6-9
3+4		15		11		27
4+3		19		8		30
≥8		17		3		17

Results

We identified 27976 peptide precursors from 4043 SwissProt proteotypic proteins in 204 tissue samples from the PCZA cohort using the S3-SWATH method. The protein matrix was fed into random forest analysis to compute the significance of each protein in differentiating PCa and BPH samples. The best performed 150 proteins in distinguishing PCa and BPH were verified using the same 15-min microflow MRM^{HR} assay in the previous PCZA cohort. The same targeted assay was then used to analyze second independent cohort PCZB (Figure 2).

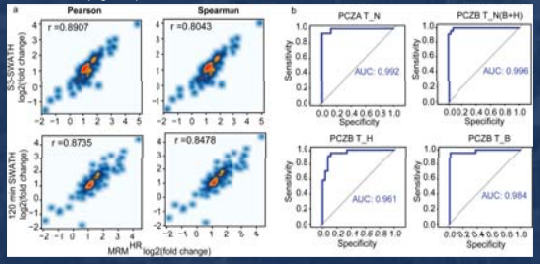

Figure 2. (a) Correlation Coefficient between SWATH and MRMHR in the PCZA cohort. (b) ROC of the PCZB cohort validation by MRM^{HR}.

Then we focused on three protein candidates, PRDX3, COPA, and ECHS1 (Figure 3). PRDX3 is a redox homeostasis dependent protein, is well-known to have significantly higher expression in prostate cancer. COPA is a coatomer that can mediate the biosynthetic protein transport from the endoplasmic reticulum and is associated with cell proliferation, also overexpressed in prostate cancer and its inhibitor can suppress cell cycle and increase apoptosis of PCa. We also found ECHS1, the enoyl-CoA hydratase in mitochondrial, which plays important roles in mitochondrial fatty acid β-oxidation pathway, could be a novel potential biomarker. ECHS1 has been found to be associated withother cancers, while there is no report in prostate cancer.

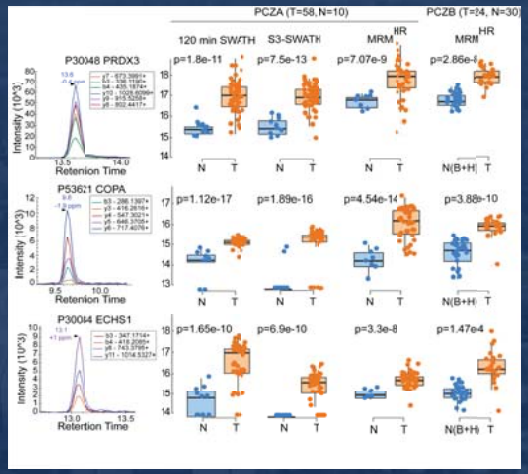

Figure 3. MRM^{HR} validation of potential diagnostic proteins using the PCZA and PCZB. PRDX3 (peptide: +2 DYGVLLEGSGLALR), COPA (peptide: +2 DVAVMQLR), ECHS1 (peptide: +2 EGMTAFVEK) The left panel show the fragment ion extracted ion chromatograms (XICs) for the peptide from each protein. Right panel of boxplots show the peptides quantified in the different data sets.

Finally we selected ECHS1 for immunohistochemistry (IHC) assays in a third cohort PCZD organised in a tissue microarray (TMA). Our integrative SWATH and MRMHR pipeline has identified the ECHS1 as an upregulated protein in tumor tissues compared to the benign ones. In the TMA experiment, we further confirmed the upregulation of ECHS1 in PCa samples (Figure 4).

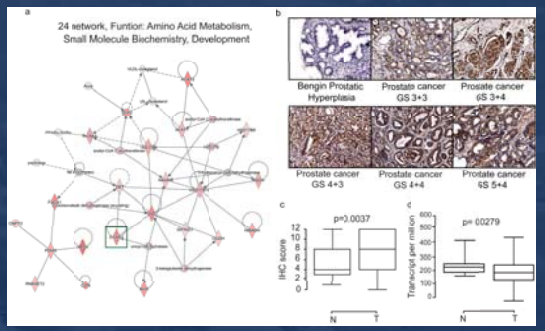

Figure 4. Validation of ECHS1 expression in TMA in a third cohort PCZD. (a) IPA analysis networks include ECHS1 (b)TMA shows positive staining of ECHS1 in prostate cancer tissues. The staining images of representative BPH patients and PCa patients with different Gleason Scores from 6 to 9 were shown. (c) IHC score shows elevated expression of ECHS1 in prostate cancer tissues compared with BPH types . The p value was calculated by using student t-test. (d) Transcript copies were calculated from PCa and BPH tissue types, based on the TCGA dataset. Student t-test was used to calculate the significance.

Conclusions

1. The integrated pipeline combining S3-SWATH acquisition and MRM^{HR} is practical and effective in discovering and verifying diagnostic tissue biomarkers for PCa.
2. ECHS1 is a promising diagnostic biomarker for PCa.

Acknowledgements

The authors thank all collaborators who participated in the procurement of the clinical specimens. This research was supported by the Westlake Startup Grant (to T.G.), Zhejiang Provincial Natural Science Foundation of China (Grant No. LR19C050001 to T.G.), Hangzhou Agriculture and Society Advancement Program (Grant No. 20190101A04 to T.G.).

Competing finacial interests

The research group of T.G. is supported by SCIEX, which provides access to prototype instrumentation, and Pressure Biosciences Inc, which provides access to advanced sample preparation instrumentation.

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