

Application of an antibody-independent, highly sensitive PRISM-SRM proteomics approach for monitoring TMPRSS2-ERG fusion proteins in prostate cancer cell lines and tumor tissues

Jintang He¹, Xuefei Sun¹, Tujin Shi¹, Athena A. Schepmoes¹, Thomas L. Fillmore², Vladislav A. Petyuk¹, Fang Xie¹, Rui Zhao², Marina A. Gritsenko¹, Feng Yang¹, Naoki Kitabayashi³, Sung-Suk Chae³, Mark A. Rubin³, Javed Siddiqui^{4,5}, John T. Wei⁴, Arul M. Chinnaiyan⁵, Wei-Jun Qian¹, Richard D. Smith¹, Jacob Kagan⁶, Sudhir Srivastava⁶, Tao Liu¹, Karin D. Rodland¹, and David G. Camp¹

¹Biological Sciences Division and ²Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory, ³Department of Pathology and Laboratory Medicine, Weill Cornell Medical College, ⁴Departments Urology and ⁵Michigan Center for Translational Pathology, University of Michigan Medical School, ⁶Division of Cancer Prevention, National Cancer Institute



Pacific Northwest
NATIONAL LABORATORY

Overview

- PRISM-SRM assays allow for highly sensitive detection and accurate quantification of TMPRSS2-ERG fusion protein products in prostate cancer cell lines and tumor tissues.
- At least two groups of ERG protein isoforms are simultaneously expressed in prostate cancer cells and tumor tissues at widely variable levels.
- ERG expression is highly correlated with TMPRSS2-ERG gene rearrangement.

Introduction

- Fusions between the transmembrane protease serine 2 (TMPRSS2) and ETS related gene (ERG) have been identified in ~50% of prostate cancer cases and represent one of the most specific biomarkers that define a distinct molecular subtype of prostate cancer.
- We recently developed and applied an antibody-independent PRISM¹ (high-pressure high-resolution separations with intelligent selection and multiplexing)-SRM (selected reaction monitoring) strategy for quantifying low abundance TMPRSS2-ERG fusion protein products in prostate cancer cell lines and tumor tissues.
- Our results suggest that at least two groups of ERG protein isoforms are simultaneously expressed in prostate cancer cells and tumor tissues at widely variable levels.

Methods

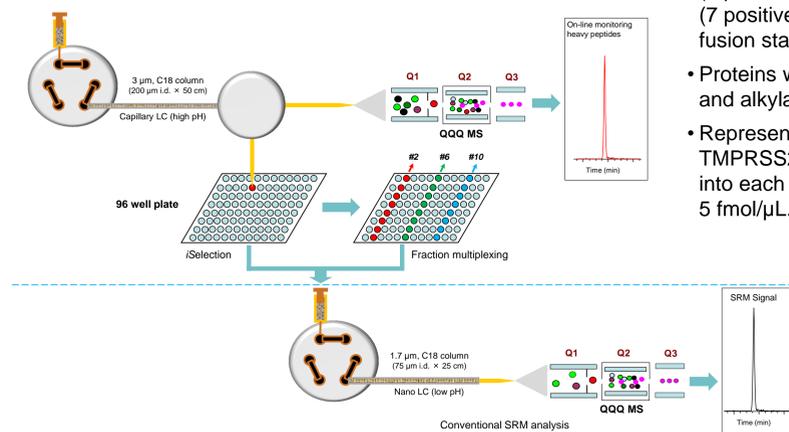


Figure 1. Schematic depicting PRISM-SRM workflow.

PRISM-SRM detection of TMPRSS2-ERG fusion protein products in prostate cancer cells or tissues

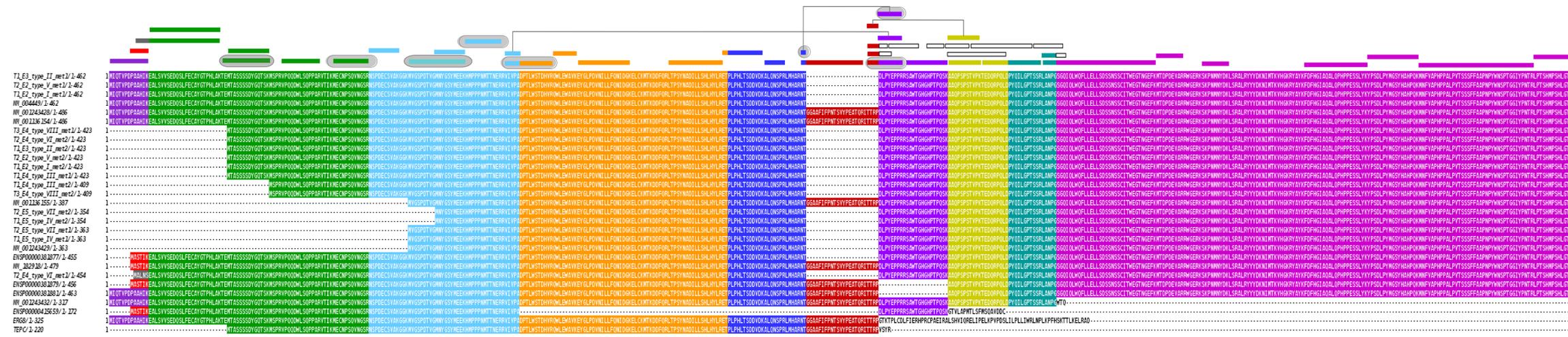


Figure 2. Mapping of the peptides selected for SRM analysis of various isoforms of TMPRSS2-ERG fusion protein products. The exons are color coded; grey shading [] denotes peptides detected in prostate cancer cell lines or tumor tissues by PRISM-SRM.

Experimental:

- TMPRSS2-ERG fusion protein SRM assays were developed using synthetic peptides.
- Samples comprised six prostate cancer cell lines (2 positive and 4 negative) and twelve tumor tissues (7 positive and 5 negative); all with genomics-level fusion status confirmation.
- Proteins were extracted from each sample, reduced and alkylated, and further digested with trypsin.
- Representative heavy peptide standards of various TMPRSS2-ERG fusion protein isoforms were spiked into each sample (1 μg/μL) to a final concentration of 5 fmol/μL.

- Tryptic digests were separated using high resolution basic pH RPLC (PRISM) followed by LC-SRM analysis of each target peptide enriched fraction.
- SRM data acquired on a TSQ Vantage mass spectrometer (Thermo Scientific) were analyzed using Xcalibur and Skyline.

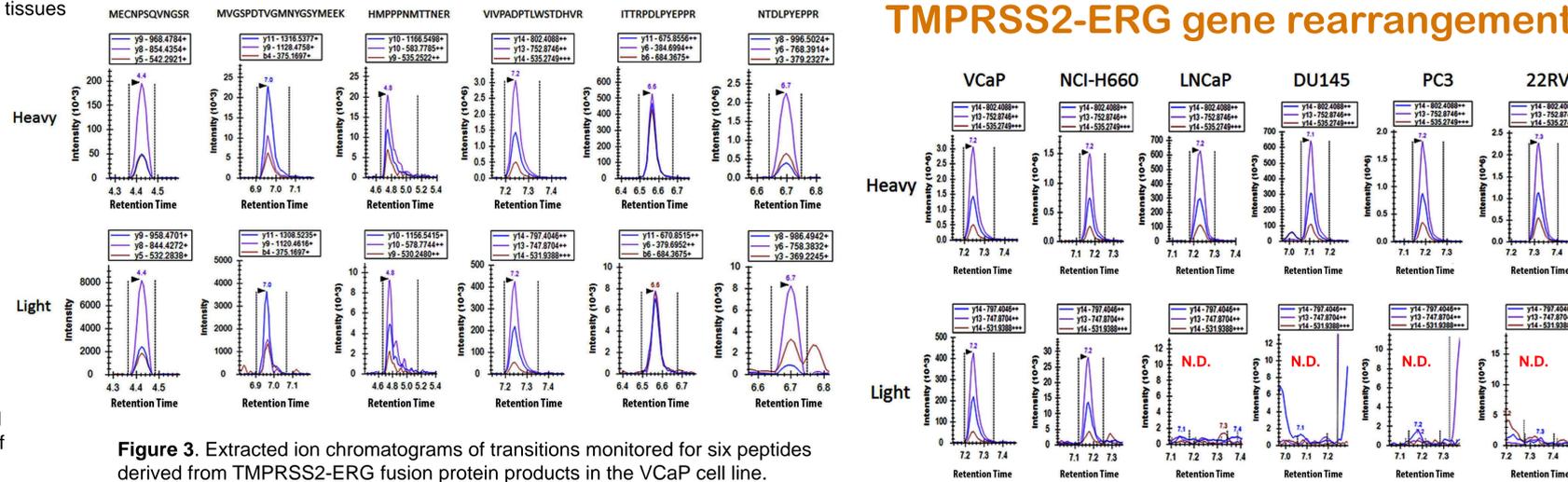


Figure 3. Extracted ion chromatograms of transitions monitored for six peptides derived from TMPRSS2-ERG fusion protein products in the VCaP cell line.

ERG expression is correlated with TMPRSS2-ERG gene rearrangement

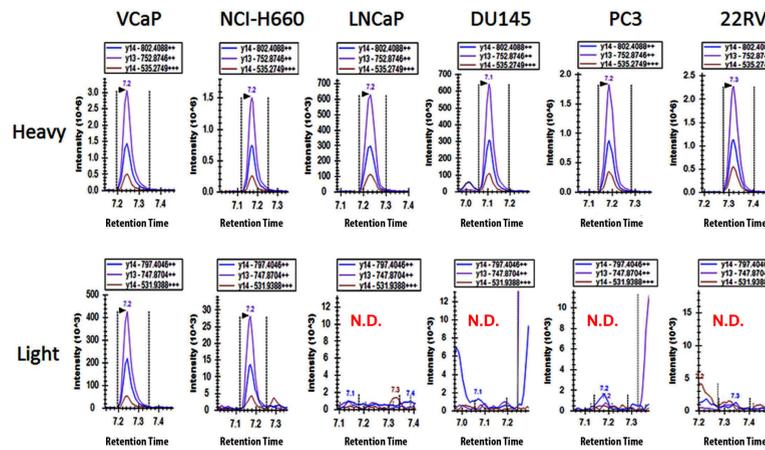


Figure 4. Extracted ion chromatograms of transitions monitored for ERG-derived peptide VIVPADPTLWSTDHVR in both TMPRSS2-ERG fusion positive (VCaP and HCl-H660) and TMPRSS2-ERG fusion negative (LNCaP, DU145, PC3, and 22RV1) prostate cancer cell lines. N.D. – not detected.

Table 1. Quantification of ERG peptides in prostate cancer cell lines.

Sequence	amol/μg of total protein					
	VCaP	NCI-H660	LNCaP	DU145	PC3	22RV1
MECNPSQVNGSR	189					
MVGSPTDVTGMNYSYMEEK	618					
HMPPPNMTTNER	1240	84				
VIVPADPTLWSTDHVR	726	98				
ITTRDPLVEPPR	73					
NTDLPVEPPR	17					

Table 2. Quantification of ERG peptides in prostate tumor tissues

Sequence	amol/μg of total protein						
	PT1	PT2	PT3	PT4	PT5	PT6	PT7
TEMTASSSDYQTSK							287
MVGSPTDVTGMNYSYMEEK	339	43		80	382	117	546
HMPPPNMTTNER	1210	169	19	78	760	543	762
VIVPADPTLWSTDHVR	48	NQ		NQ	30	74	35
ITTRDPLVEPPR	31	6					
NTDLPVEPPR	26	6		NQ			

Conclusions

- PRISM-SRM allows for detection and quantification of TMPRSS2-ERG fusion at the protein level.
- Multiple isoforms of TMPRSS2-ERG fusion protein products (i.e. truncated ERG protein) are simultaneously expressed in prostate cancer cell lines and tumor tissues at widely variable levels.
- ERG protein expression is highly correlated with TMPRSS2-ERG gene rearrangement.
- Future quantitative analyses of TMPRSS2-ERG fusion protein products in large cohorts of prostate tumor specimens should provide additional information on the biomarker potential of TMPRSS2-ERG protein products, e.g., stratifying prostate cancer risk.

Acknowledgments

Portions of this work were supported by the National Cancer Institute Early Detection Research Network Interagency Agreement (Y01-CN-05013-29) and the National Institute of General Medical Sciences (8 P41 GM103493-10). Proteomics portions of this work were performed in the Environmental Molecular Sciences Laboratory, a U.S. Department of Energy/BER national scientific user facility at Pacific Northwest National Laboratory in Richland, Washington.

Reference

- Shi T, et al. *Proc Natl Acad Sci USA* 109:15395-15400 (2012).

CONTACT: Jintang He, Ph.D.
Biological Sciences Division
Pacific Northwest National Laboratory
E-mail: jintang.he@pnnl.gov