

Targeted Quantification of Mutant SPOP Proteins in Prostate Cancer

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Overview

- Missense mutations in speckle-type POZ protein (SPOP) contribute to prostate cancer development by altering the steady-state levels of key components in the androgen-signaling pathway.
- Selected reaction monitoring (SRM)-based targeted quantification enables multiplexed and isoform-specific detection of mutations at protein level.
- SRM assays have been developed for seven most frequent SPOP mutations in the meprin and TRAF (Tumor necrosis factor receptor-associated factor) homology (MATH) domain and two peptides in the non-mutated region.
- PRISM (high-pressure, high-resolution separations coupled with intelligent selection and multiplexing)-SRM¹ enables the quantification of low abundance endogenous SPOP mutant peptides in the wild type and mutant cell lines.

Introduction

- Somatic mutation at SPOP gene has been identified frequently (up to 15%) in human prostate cancers and is mutually exclusive with prevalent ETS fusions (approximately 50%).
- The vast majority of the research on SPOP mutation has been performed using genomic techniques, such as whole exome-sequencing.
- However, the qualitative and quantitative analyses of mutant SPOP at the protein level are still very limited.
- The potential of the mutant SPOP protein as a biomarker for diagnosis, prognosis, or target therapy of prostate cancer remains largely unknown.
- To address this issue, targeted proteomics assays using high sensitivity SRM mass spectrometry approaches have been developed for quantifying mutant SPOP proteins in prostate cancer.

Methods

- SRM assays were developed for the seven most frequent mutations of SPOP protein and its non-mutated region.
- Purified recombinant SPOP protein (OriGene) was used to aid initial test on digestion and peptide selection using a ThermoScientific Velos Orbitrap instrument; surrogate peptides were synthesized by Thermo Scientific.
- Enzymatic digestion was carried out by using Arg-C instead of trypsin to preserve mutation sites, followed by reduction and alkylation.
- Protein digests were either analyzed directly by LC-SRM or fractionated with target peptide fractions of interest selected and analyzed using PRISM-SRM (see Figure 1).
- Peptide mixtures at concentration of 0.2 µg/µL and 1.0 µg/µL with spike-in heavy standards were analyzed using LC-SRM (1 µg loading) and PRISM-SRM (45 µg loading) analysis, respectively.
- Four different 293T cell lines, with and without expression of the three most frequent SPOP mutations in prostate cancer (Y87N, F102C or F133V), were analyzed to evaluate the validity and performance of these assays.
- SRM data were generated on Waters nanoACQUITY UPLC coupled to ThermoScientific TSQ Vantage triple quadrupole mass spectrometer and analyzed by Xcalibur and Skyline software.

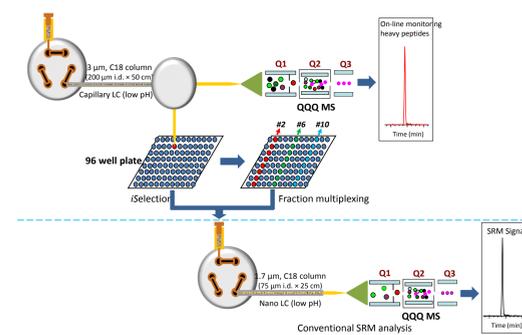


Figure 1. PRISM-SRM workflow.

Results



Figure 2. Comparison of Arg-C and Asp-N digestion of SPOP. Red-font amino acids: mutation sites; red bars: spectral counts.

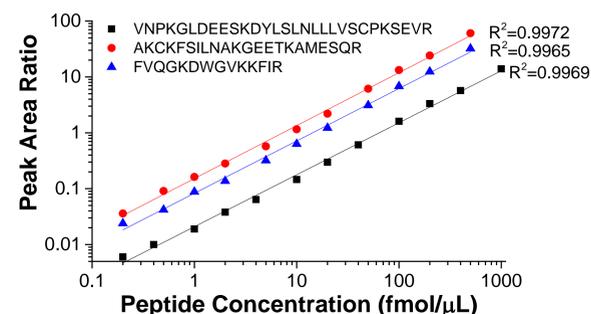


Figure 3. Response curve of three SPOP mutant peptides.

Table 1. Quantification of mutant peptides in 293T cells expressing WT and mutant (Y87N, F102C and F133V) SPOP proteins (fmol/µg).

Cell line	VNP KGLDEESKDYL S L N L L L V S C P K S E V R	AKCKFSILNAKGEETKAMESQR	FVQGDWGVKKFIR	LADELGG LWENSR	SLASAQC PFLGPPR
	Y87N	F102C	F133V	WT	WT
WT	1.29	N.Q.	2.43	6.17	21.36
Y87N	61.39	37.67	43.60	179.67	434.64
F102C	98.4	10.86	56.68	188.44	467.78
F133V	4.09	N.Q.	1.82	16.50	29.11

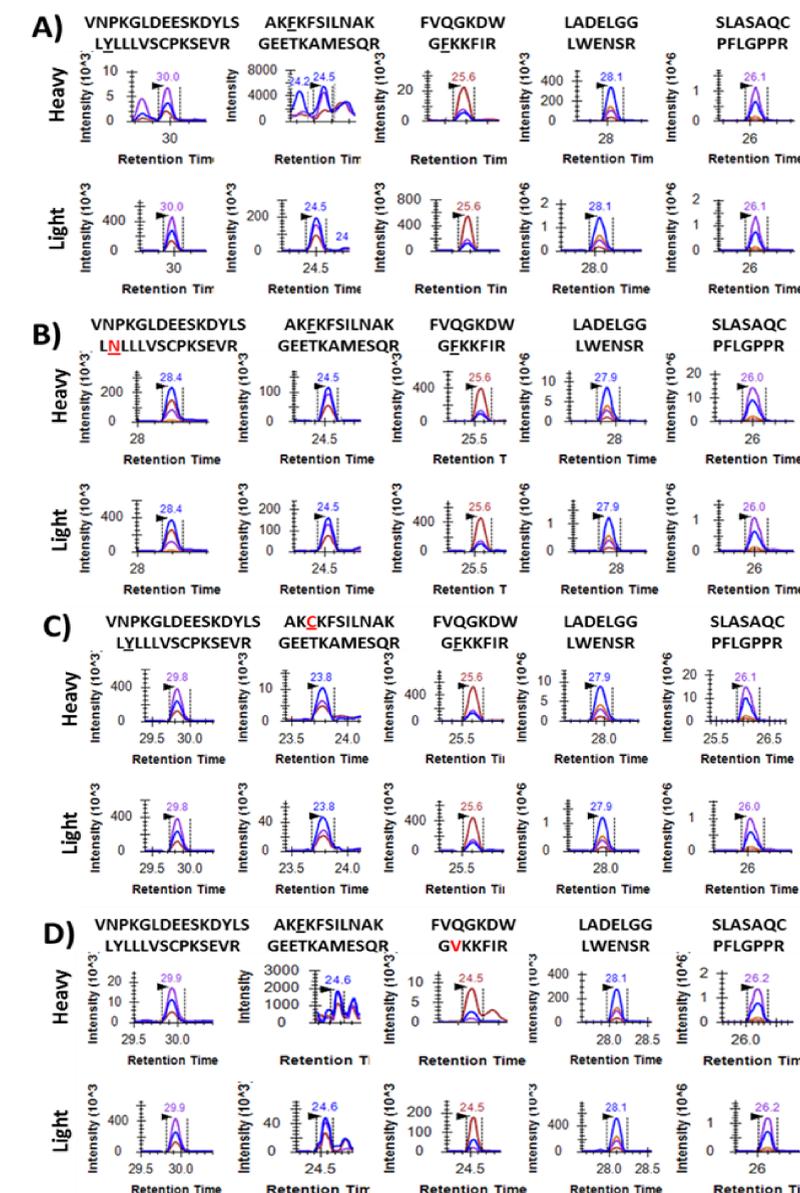


Figure 4. PRISM-SRM analysis of SPOP peptides in 293T cells expressing WT (A) and mutant Y87N (B), F102C (C) and F133V (D) SPOP proteins.

Conclusions

- Arg-C is superior in generating SPOP mutant peptides for SRM analysis compared to other proteases (e.g., Asp-N).
- The resulting Arg-C peptides are longer and more hydrophobic than tryptic peptides (and contain multiple Lys); however, all the mutation PRISM-SRM assays showed a tested linear dynamic range of 3 orders of magnitude, and the LOQs range from 0.5 to 1.0 fmol/µg of total protein in the cell lysate.
- PRISM-SRM provides increased sample loading and reduced background/interference, resulting in improved sensitivity in detection of the SPOP mutation peptides.
- Applying PRISM-SRM assays to analyze 293T cells with the expression of WT and three most frequent SPOP mutations in prostate cancer led to confident detection of all three SPOP mutations in the positive cell lines, but not in the negative cell lines.
- SRM assays enabled multiplexed, isoform-specific detection of mutant SPOP proteins (and other protein candidates such as TMPRSS2:ERG fusion proteins^{2,3}), which holds great potential in biomarker verification for prostate cancer.

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