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Original Article

SPOP mutation in prostate cancers in Korean population: variation in its mutation frequency among ethnic groups

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Abstract: Speckle-type POZ protein, SPOP, mediates *SRC-3* oncogene ubiquitination and proteolysis in human cancer. Frequent *SPOP* mutation is observed in exome sequencing of prostate cancer (Pca) in western population. We performed *SPOP* mutation analysis and investigated its protein expression of PCa in Korean population. We selected 108 cases of prostatectomy specimen for PCa at Samsung Medical Center in Seoul, Korea from 1995 to 2006. All cases were sequenced by Sanger sequencing to analyze *SPOP* somatic mutations in paraffin-embedded tissue. In addition, we also applied SPOP immunohistochemistry (IHC) on the tissue microarray blocks for 108 cases. All cases were successfully sequenced. Three missense mutations, p.Phe102Cys in one case and double mutation with p.Phe125Val and p.Glu145 Lys in another case, were identified (1.85%). p.Phe102Cys and p.Phe125Val have been previously reported in the literature; however, p.Glu145 Lys was newly discovered in this study. All but one case were successfully stained with IHC. Thirty two (32.7%) out of 107 cases showed SPOP expression loss and loss of SPOP expression was not correlated with mutation status. In conclusion, we identified three missense mutations including double mutation in 108 PCa in Korean population. The incidence (2/108, 1.85%) is lower than previous studies which had done in western population.

Keywords: SPOP protein, mutation, prostate cancer

Introduction

Prostate cancer (PCa) is the second most frequent cancer among men worldwide and has been increasing in nearly all countries including Korean due to westernization [1-3]. Given the increasing incidence of PCa and technological developments in genomics, there has been increased interest in molecular diagnosis and classification of PCa. There are new attempts at PCa classification according to genomic alteration. The main division of molecular classification is gene fusion involving *ETS* family member accounting for up to 80% of PCa [4, 5]. Whole exome sequencing in PCa which had performed in western population has revealed a number of somatic mutations including Speckle-type POZ protein (*SPOP*) gene [6-8].

Analysis *SPOP* mutations in Korean population were done in two studies with incidence of 4.4% (2/45) and 6.9% (6/87) [9, 10]. We performed *SPOP* mutation analysis on more expanded cohort, 108 surgically excised PCa specimens and determined its protein expression by immunohistochemistry (IHC).

Materials and methods

Patients and samples

One hundred eight cases of radical prostatectomy for PCa at Samsung Medical Center in Seoul, Korea from 1995 to 2006 were selected. Cases with incomplete resection or neoadjuvant treatment (radiotherapy or hormonal therapy) were excluded. Clinical data were reviewed based on electronic medical records, and pathologic diagnoses were reviewed by three pathologists (N. Yoon, Y. Choi and G. Kwon). The modified the 2005 International Society of Urological Pathology was utilized for

Table 1. Clinicopathologic parameters of the 108 patients with prostate carcinoma

Clinicopathologic parameters		Mean (range)
Age		65 (44-78)
		No (%) of patients
Gleason score	6	9 (8.3%)
	7	77 (71.3%)
	8-10	22 (20.4%)
T stage	2	75 (69.4%)
	3a	22 (20.4%)
	3b	8 (7.4%)
	4	3 (2.8%)

Table 2. Primer sequences of *SPOP* gene

SPOP Exon	Sequence
Exon 6	F1: 5-TCCAGTTCTATCAAAATGGATGC-3
	R1: 5-ACGCAAAAACCAGATCAAAG-3
	F2: 5-TCCAGTTCTATCAAAATGGATGC-3
	R2: 5-ACGCAAAAACCAGATCAAAGC-3
	F3: 5-TTTTCTATCTGTTTTGGACAGG-3
	R3: 5-CAAGCCACAACTTGTCAGTG-3
Exon 7	F1: 5-GTTGTGGCTTTGATCTGGTTT-3
	R1: 5-ACTCCACTTGGGGCTTTTTC-3
	F2: 5-CAAGTTGTGGCTTTGATCTGG-3
	R2: 5-CCACTTGGGGCTTTTTCTTA-3
	F3: 5-TTTGCGAGTAAACCCCAAAG-3
	R3: 5-CTCATCAGATCTGGGAACTGC-3

Gleason grading. Tumor stage was determined according to 7th Edition of the AJCC Cancer Staging Manual. Clinicopathological data including age, Gleason score and pathologic stage are summarized in **Table 1**. The study protocol was approved by the Samsung Medical Center Institutional Review Board.

Tissue microarray construction

We selected representative formalin-fixed, paraffin-embedded (FFPE) blocks for TMA from archived pathology files of Samsung Medical Center preparation by reviewing hematoxylin and eosin (H&E)-stained slides. Using a manual tissue microarrayer (Accumax, ISU Abxis, Seoul, Korea), two representative tissue cores (0.2 cm in diameter) were taken from each tumor and placed into two recipient paraffin blocks.

Immunohistochemistry

The SPOP rabbit monoclonal antibody (ERG-3864, Epitomics, Burlingame, CA, USA, dilution 1:100) was used for IHC, which was performed using a BenchMark XT (Roche/Ventana Medical Systems, Tucson, AZ, USA) according to the manufacturer's instructions. In brief, 4-μm sections of FFPE tissues were deparaffinized and were immersed in Tris/Borate/EDTA buffer (pH 8.0-8.5) for 8 minutes at room temperature to retrieve the antigen. Endogenous peroxidase was quenched via incubation with hydrogen peroxide for 5 minutes at room temperature. The sections were incubated for 32 minutes at room temperature with primary antibodies. The secondary antibody (OmniMap anti-Rabbit HRP, Tucson, AZ, USA) and chromogenic substrate Diamiobenzidine (ChromoMap DAB; Tucson, AZ, USA) were applied for 16 and 8 minutes, respectively, at room temperature. Normal prostate glands in each core were used as a positive internal control. The SPOP protein was expressed in the cytoplasm of normal prostate epithelium or tumor cells. If the intensity of tumor cells is weaker than normal epithelium, the case was judged as presence of SPOP expression loss.

Extraction of DNA

Two 5-μm-thick unstained slides were obtained from representative blocks of FFPE samples. Original H&E slides were reviewed to identify the region of tumor and tumor tissues were microdissected from corresponding areas of unstained slides. Additional H&E slides were prepared after obtaining the slides for DNA extraction and reviewed to confirm the presence of tumor. Genomic DNA was isolated from FFPE tumor samples using a ReliaPrep™ FFPE gDNA extraction kit (Promega, Madison, WI, USA). The concentration of DNA was determined by a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies).

Sequencing of SPOP

PCR was performed in 20-μl reactions containing 100 ng of template DNA, 2 μl×10 buffer, 0.25 mmol/L deoxynucleoside triphosphate (dNTP), 10 pmol primers and 1.25 U Taq DNA polymerase (iNtRON, Seoul, Korea). Primer pairs were used to amplify the coding sequences of SPOP exons 6 and 7 (**Table 2**). The thermal cycling conditions were 5 min at 95°C, followed by 40 cycles of denaturation at 94°C for 30 s, annealing at 58°C for 30 s, and extension at 72°C for 30 s. PCR products were subjected to electrophoresis on 2% agarose gels and were purified with a QIA quick PCR purification kit (Qiagen, Hilden, Germany). Bidirectional

Table 3. Features of cases with *SPOP* missense mutation

Case	Age	Histology	Gleason score	TNM stage	Mutation type	Amino acid change (Codon change)
1	63	Adenocarcinoma	3+4=7	T2aN0M0	Missense	p.Phe102Cys (c.305T>G)
2	66	Adenocarcinoma	3+4=7	T2aN0M0	Missense	p.Phe125Val (c.373T>G)
					Missense	p.Glu145 Lys (c.433G>A)

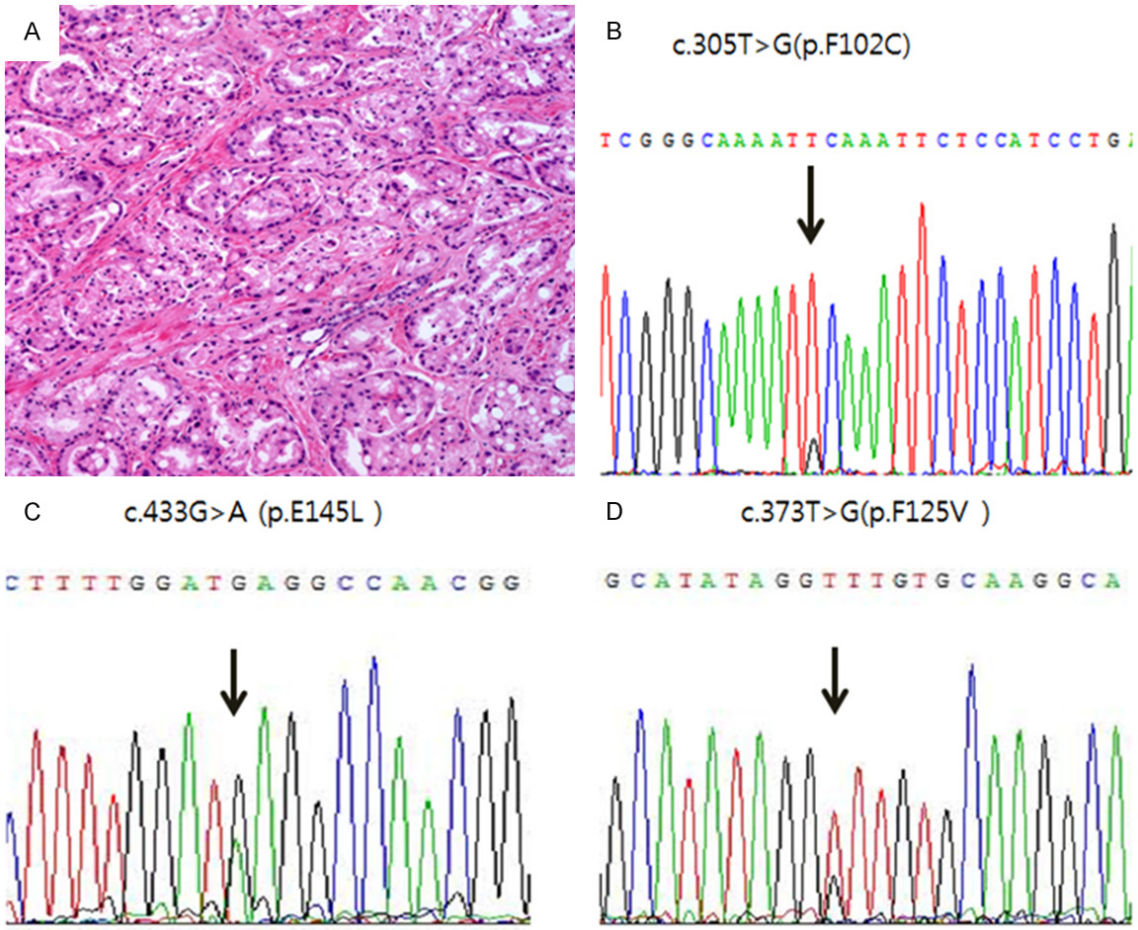

Figure 1. The pathologic features (A) and results from Sanger sequencing (B) of the first case with *SPOP* mutation. On microscopic analysis, the tumor showed well-formed atypical glands with some fused structures. *SPOP* DNA sequence revealed a missense mutation (p.Phe102Cys) in the first case. Chromatogram showed the double missense mutations (p.Phe125Val and p.Glu145 Lys) in the second case (C and D).

sequencing was performed using the BigDye Terminator v1.1 kit (Applied Biosystems) on the ABI 3130XL genetic analyzer (Applied Biosystems). The results were regarded as mutation positive if a mutation was detected in both the forward and reverse Sequencher version 4.10.1 (Gene Codes Corporation, Ann Arbor, MI, USA) along with a manual review of chromatograms.

Statistical analysis

Statistical analysis was performed using SPSS 20.0 statistical software (SPSS, Chicago, IL, USA). Correlation analyses between the *SPOP* mutation and clinicopathologic parameters were done using the x^2-test or Fisher's exact test. A *p*-value of < 0.05 was regarded as significant.

Results

SPOP mutation

The tumor content was approximately 70% on average after microdissection. All cases are successfully sequenced demonstrating clear

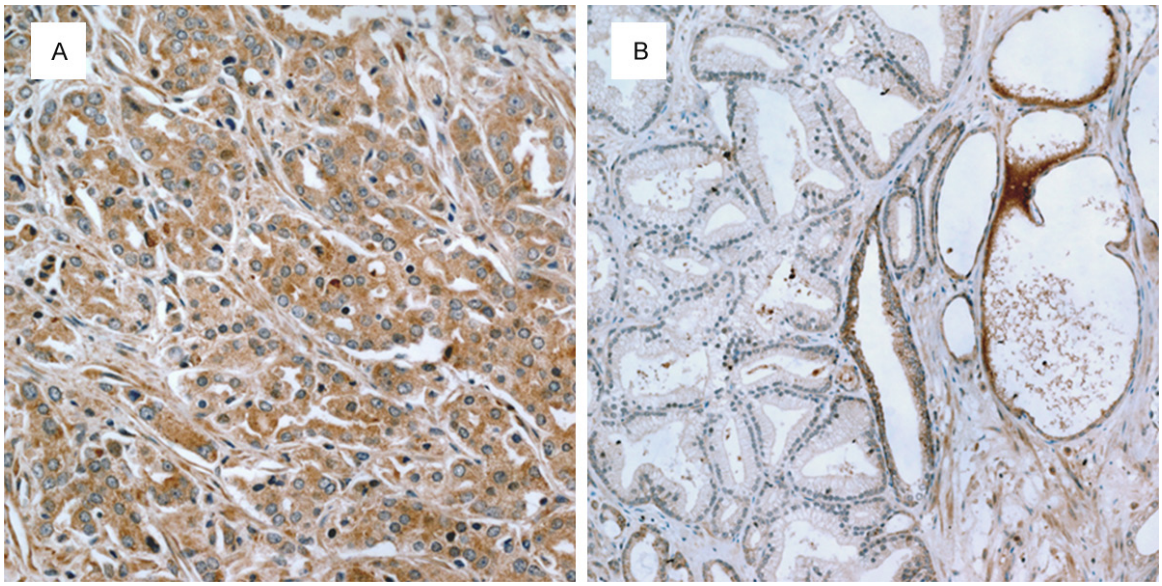

Figure 2. The SPOP immunohistochemistry result. A. SPOP is well expressed in cytoplasm of cancer cells. B. SPOP expression loss is identified in cancer glands whereas adjacent benign glands show positive staining.

signal peaks. The results from sequencing the *SPOP* gene are summarized in **Table 3**.

Three missense mutations, p.Phe102Cys (p.F102C) in one case, and double mutation with p.Phe125Val (p.F125V) and p.Glu145 Lys (p.E145K) in another case, were identified (1.85%) (**Figure 1**). The patient with the p.F102C mutation was 63 years old, with a PSA level of 12.51 ng/ml at the time of initial diagnosis. The patient's primary Gleason grade was 3 and secondary Gleason grade was 4, giving a total score of 7. The pathologic stage was T2c with no lymph node metastasis. The patient who had double mutation with the p.F125V and p.E145K mutations was 66 years old, with a PSA level of 7.93 ng/ml at the time of initial diagnosis. The primary Gleason grade was 3 and the secondary Gleason grade was 4, giving a total score of 7. The pathologic stage was T2a, with no lymph node metastasis. There was no metastasis or recurrence during the follow-up periods of 80 and 98 months, respectively. As for clinicopathologic parameters, there was no significant correlation with the *SPOP* mutations.

SPOP IHC

All but one case were successfully stained with IHC. Thirty two (32.7%) out of 107 cases showed expression loss in tumor cells compared to normal epithelium (**Figure 2**). No association was noted between *SPOP* mutation and protein expression. Two cases with *SPOP* mutation was well expressed SPOP IHC.

Discussion

SPOP is Bric-a-brac/Tramtrack/Broad complex (BTB) protein that is a constituent of Cul3-based ubiquitination [10, 11]. The *SPOP* gene consists of two domains, the N-terminal MATH domain, which directly binds to steroid receptor coactivator-3 (SRC-3), and the C-terminal BTB/POZ domain, which directly binds to Cul3 [12]. Overexpression and overactivation of SRC-3 occur in many human cancers, and SPOP protein regulates SRC-3-mediated oncogenic signaling and tumorigenesis by promoting its degradation [12, 13]. It is speculated that *SPOP* in human cancers, including PCa, functions as a tumor suppressor [10, 12, 13]. *SPOP* mutation contribute to PCa development by altering the steady-state levels of key component in the androgen-signaling pathway [14].

We screened for somatic mutations in exons six and seven of the *SPOP* gene in 108 PCa because all of the *SPOP* mutations previously reported occurred within these two exons [6, 9, 10]. Three somatic missense mutations were found in two cases: p.F102C in one case, and double mutation with p.F125V and p.E145K in another case. These two cases have no distinctive clinicopathologic characteristics com-

pared with the case without *SPOP* mutation. Although p.F102C and p.F125V have been previously reported in the literature, p.E145K was newly discovered in this study [6, 12]. Especially, p.F102C and p.F125V SPOP mutants are known to exert a gain-of-function oncogenic effect by increasing SRC-3 protein levels and AR transcriptional activity above baseline [12]. p.F102 and p.F125 are revealed to be frequently mutated site in previous study [9]. Three mutations of this study are gathered in MATH domain in coincidence with previous works [6, 9, 10]. SPOP mutants in PCA weaken the interaction between SPOP and SRC-3 and the coactivator function of SRC-3 on AR transcriptional activity, thus inhibiting ubiquitination and degradation of SRC-3 [12].

In our study, *SPOP* mutation in PCa is identified in about 1.85% of all cases, which is lower than the 3.5-15% reported previously in studies including multi-organ cohorts [6, 7, 9]. This result can add a new data to mutation incidence rate of *SPOP* gene especially in Asian population. In several Korean series, the frequencies of *SPOP* mutation in PCa are similar or relatively lower than western group [9, 10].

PCa exhibits different molecular characteristics across ethnic group [15]. Recent studies assessed the difference in prevalence of *ERG* rearrangements and revealed that Asian and African American showed lower frequency than Caucasian Men [16, 17]. More specifically, *ERG* rearrangements have been revealed lower frequency in Korean, Japanese and Chinese than in Caucasian men [18-20]. Khani *et al* compared prevalence of molecular alteration in PCa between African American and Caucasian men revealed that there are distinctive differences in frequency of SPINK1 overexpression, *ERG* rearrangement and *PTEN* deletion between two ethnic groups. They also found that *SPOP* mutation was less frequent in African American (4.5%) than in Caucasian (10.3%) [15]. In addition, the study that we investigated in same cohort [22] revealed no mutation in *MED12* exon 26 which were frequently observed in western population with 5.4% [6].

Aside from genetic heterogeneity among ethnic groups, the technical difference may contribute to difference of mutation prevalence. Exome sequencing which performed by Barbieri et al is known to be more sensitive method compared to Sanger sequencing performed in the study by Kim MS et al and ours [6, 21].

In summary, Sanger sequencing for *SPOP* mutations in FFPE of 108PCa specimens in Korean population reveals lower frequency than western population. Two (1.85%) cases had missense mutation and one out of two had double mutation. In addition, 32.7% cases showed loss of expression of SPOP protein in IHC but not correlated with *SPOP* mutation.

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