[UCI]I804:24011-200000265837

2016년 8월

박사학위논문

한국인 전립샘암의 발생위치에 따른 분자면역학적 표현형과 임상병리학적 특징

조 선 대 학 교 대 학 원

의 학 과

임 샤 론

한국인 전립샘암의 발생위치에 따른 분자면역학적 표현형과 임상병리학적 특징

Molecular and Immunohistochemical Phenotyping and Clinicopathologic Characteristics according to the Zonal Distribution in Korean Prostatic Adenocarcinoma

2016년 8월 25일

조 선 대 학 교 대 학 원

의 학 과

임 샤 론

한국인 전립샘암의 발생위치에 따른 분자면역학적 표현형과 임상병리학적 특징

지도교수 이 미 자

이 논문을 의학 박사학위신청 논문으로 제출함

2016년 4월

조 선 대 학 교 대 학 원

의 학 과

임 샤 론

임샤론의 박사학위 논문을 인준함

위원장 조선대학교 교수 기 근 홍 (인)

위 원 조선대학교 교수 김 철 성 (인)

위 원 조선대학교 교수 임 성 철 (인)

위 원 성균관대학교 교수 권 기 영 (인)

위 원 조선대학교 교수 이 미 자 (인)

2016년 6월

조 선 대 학 교 대 학 원

목 차

표목차

도목차

초 록

한국인 전립샘암의 발생위치에 따른 분자면역학적 표현형과 임상병리학적 특징

임샤론

지도교수 : 이미자

조선대학교 대학원 의학과

목적 : 해부학적인 구조로 분류하면 대다수의 전립선 종양은 주변부(peripheral zone)에서 발생하며 일부는 이행부(transition zone)에서 발생한다. 서양에서 발생하는 이행부의 종양은 고유한 형태학적 특징과 예후의 차이를 보이며 대부분 전립선의 전방에 나타나는 것으로 알려져 있다. 이러한 차이가 한국인의 전립샘암에서도 적용되는지 확인하고자 하였다.

재료 및 방법 : 근치적 전립선 절제술 검체를 종양의 위치와 발생 구역에 따라 분류하였고 이에 대하여 각각 분자병리학적 및 임상병리학적 특징을 조사하기 위하여 ERG, SPINK1, SPOP, PTEN 그리고 AR-V7 에 대한 면역 화학 염색, PTEN 결손을 확인하기 위한 fluorescence in situ hybridization 검사를 시행하였다. 종양의 발생 위치에 따라 검사결과의 차이가 있는지 교차 분석을 통하여 검증하였다.

결과 : 전체 환자의 31.9 % 에서 전방 경향이 나타났으며 전방위주 종양은 주로 이행부에서 발생하였다. 외과적 절제면은 특히 기저부와 전방부가 종양에 포함되는 양상을 보였다. 종양 발생 위치가 전방부인 경우에 PTEN 결손과 SPOP

음성의 비율이 높았고 또한 구역별로 비교하면 이행부에 위치하였을 때 AR-V7이 양성으로 나타나는 비율이 통계적으로 유의하였다. 이러한 결과로 미루어 보아 종양의 발생 위치와 구역 분포가 한국인의 전립샘암의 분자면역학적 차이에 기여한다고 판단하였다.

결론 : 종양의 전방 경향과 구역 분포에 따라서 서양의 연구와는 구별되는 분자면역학적 그리고 임상병리학적 차이를 확인 할 수 있었다. 이러한 차이를 유발하는 원인이 무엇인지 서양과 구별되는 관점에서 더욱 연구한다면 한국인의 전립샘암에 대한 이해를 고취하여 향후 적절한 치료와 예후 예측에 기여 할 수 있을 것이라 생각된다.

핵심단어 : 한국인 전립샘암, 분자면역학적 표현형, 임상병리학적 특징, 전방경향, 구역분포

Ⅰ. INTRODUCTION

Prostate cancer (PCa) has experienced a rapid rise in incidence in Korea and other Far East Asian countries possibly as a result of westernization of lifestyles and widespread implementation of prostate specific antigen (PSA) screening, however, general understanding and management of PCa patients in that region is still based on the previous data accumulated mostly from Caucasian patients [1, 2].

Considering that PCa is heavily ethnicity-dependent, it is imperative to gain clear insight into salient features unique to each ethnic group and one of the features with possible ethnic difference is the zonal distribution of PCa [3]. PCa is reported to be more common in peripheral zone (PZ), while transition zone (TZ) harbors a fraction of tumors mostly in indolent forms, which is the basis of rationale for the widely accepted scheme of prostate biopsy in current clinical practice [4, 5]. Recently a report on the African American (AA) PCa patients found that higher proportion of aggressive tumors than Caucasians are located in anterior aspect of prostate which is largely occupied by TZ [3]. Thus, it is worthwhile to elucidate the zonal distribution of PCa and related histologic features to provide the foundation for the continuation or modification of current management of PCa patients in Korean cohort.

The difference of molecular characteristics has been at least partially attributed to zonal location of tumors and one of the molecular differences reported between ethnic groups is TMPRSS2-ETS family gene fusion, which shows relatively low incidence in Asian patients and possibly in TZ tumors [6-9]. TMPRSS2-ETS family gene fusion, found in 50-70% of PCa in initial studies, is one of the most common genetic changes across all human malignancy and its prognostic significance has been debated and not clearly established [10, 11]. However, mapped in relatively early stage of tumorigenesis, it is employed as one of the key determinants in many proposed models of molecular phenotyping of PCa [10]. Other relatively popular elements of molecular classification of PCa include SPOP mutation, SPINK1 over-expression or PTEN aberration, which show complete or partial mutual exclusiveness with TMPRSS2-ETS family gene fusion [12].

Some of the other biomarkers of potential clinical relevance are related to androgen signaling axis, which is responsible for the growth and maintenance of one of the most hormone sensitive tumor in men [13]. Known as the most common form of splice variants of androgen receptor, androgen receptor variant 7 (AR-V7) is expected to be a useful predictive or prognostic marker for advanced cases of PCa and it is worthwhile to assess its pattern of expression in PCa and analyze the difference dependent on zonal location [12].

Hence, the aim of this study is to analyze the intraprostatic zonal distribution of PCa in Korean patients focusing on the characteristics of TZ- located or anterior-predominant tumors (APT) and to elucidate the molecular or immunophenotypic features related to the zonal location. Subsequently, it is expected that the collections of knowledge gleaned from this study would be implemental in fine-tuning the management of PCa in Korean patients who may show different clinicopathologic features compared to Caucasians.

II. MATERIALS AND METHODS

A. Clinicopathologic evaluation

A retrospective study was conducted with 273 radical prostectomy specimens which were diagnosed of prostatic adenocarcinoma with available medical records were enrolled during August 2014 to June 2015 by the institutional pathology databases of Samsung Medical Center. By reviewing the clinical information and pathologic reports of the enrolled patients, we confirmed the patient's age, underlying disease, PSA level, whether receive anti-hormonal therapy, as well as tumor size, stage, Gleason score and status of resection margin. This study protocol was approved by the Institutional Review Board of the Samsung Medical Center. The modified the 2005 International Society of Urological Pathology was utilized for Gleason scoring. Tumor stage was decided depend on 7th Edition of the AJCC Cancer Staging [14]. All specimens were undergone formalin fixation overnight after inking four different colors in each quadrant along the outer surface. The prostate were performed entirely embedded and mapping processing, the seminal vesicles were amputated at their junction with the prostate and submitted separately. For review of whole-mount tissue sections, we drew imaginary line at the midpoint of the prostatic urethra along the horizontal axis. Based on this line, we designated anterior-predominance (AP) of largest index tumor and evaluated about zonal distribution and tumor location (Figure 1). Specimens in which tumor nodules located in the concurrent anterior and posterior areas were near same amount were not designated "anterior-predominant" and

excluded from present research. Pathologic review of slides was conducted by 2 genitourinary pathologists.

B. Tissue microarray construction

For further molecular and immunohistochemical investigation, out of the original 273 review cases 24 cases were excluded owing to insignificant cancer or inappropriate specimen. In refining 249 cases, representative formalin-fixed, paraffin-embedded (FFPE) blocks for tissue microarray (TMA) were selected, by prior reviewing hematoxylin and eosin (H&E) - stained slides. According to manual tissue microarrayer (Accumax, ISU Abxis, Seoul, Korea), two signature tissue cores (2.0mm) were obtained from each tumor and replaced into two recipient paraffin blocks, which were used for IHC and FISH.

C. Immunohistochemical stain (ERG, SPINK1, SPOP, PTEN and AR V7)

The primary antibodies for ETS-related gene (ERG), serin peptidase inhibitor Kazal 1 (SPINK1), speckle-type POZ protein gene (SPOP), phosphatase and tensin homolog (PTEN), androgen receptor splice variant 7 (AR-V7) was prepared for IHC, which were performed with Bond-max autoimmunostainer (Leica Biosystem, Melbourne, Australia) using Bond™ Polymer refine detection, DS9800 (Vision Biosystems, Melbourne, Australia) according to the standard manufactured protocol. Details of primary antibodies and the immunostaining protocols are summarized in Table 1. In brief, 4-μm sections from FFPE were deparaffinized and undergone immersion with citrate/EDTA buffer (pH 6.0-8.0) for 8-9 minutes at room temperature (RT) for antigen retrieval. Endogenous peroxidase was inactivated via incubation with

hydrogen peroxide for 5 minutes at RT. The slides were then incubated for 30 minutes at RT with primary antibodies. Next, secondary antibodies (OmniMap anti-Rabbit HRP; Tucson, AZ, USA) and chromogenic substrate Diaminobenzidine (ChromoMap DAB; Tucson, AZ, USA) were applied for visualization during 15 and 8 minutes, each, at RT. Vascular endothelial cells or normal prostatic glands were used as positive internal control. All slides were evaluated blindly using a microscope (BX50; Olympus Corporation, Tokyo, Japan) each pathologists.

D. Immunohistochemistry interpretation and criteria

ERG staining was considered as positive when nuclear, strong (2 positive or 3 positive) and diffuse (more than 90% of tumor cells) expression was observed. Positivity of SPINK1 was determined with moderate (2 positive) to strong (3 positive) cytoplasmic staining at least 10% of cancer cells [15]. SPOP was assessed with positive when showed any intensity except no staining in 20-100 % of tumor cell proportion [16]. PTEN protein was evaluated comparing of benign prostatic glands. Partial or complete deletion was defined as cytoplasmic staining intensity less than non-tumor glandular lesion in more than 30% of tumor cells or absence of staining in 90% of cancer cells [15]. The immunostaining of AR-V7 was performed by multiplying of independent two methods: intensity score and quantity score, as previously estimated [13, 16]. The intensity score was measured as 0, no staining; 1, mild staining; 2, moderate staining; 3, remarkable staining. The proportion of positive cells was categorized as 0, ≤1%; 1, ≤10%; 2, ≤50%; 3, ≤80% and 4, over 80%. Those cores with more than 2 final score were considered positive AR-V7.

E. Fluorescence in situ hybridization for PTEN

The fluorescence in situ hybridization (FISH) analysis for PTEN deletion was performed in 4-μm sections of each TMA slides which were stained with 4′, 6-diamidino-2-phenylindole dihydrochloride (DAPI), using commercially available PTEN deletion specific probe (Vysis LSI, PTEN 10q23/CEP 10 dual Color Probes, Abbott Molecular, Abbott Park, IL, USA). At least 100 non-overlapping tumor cells with inter-phase nuclei were counted, and the cut-off value for positive PTEN deletion was defined as >50% of tumor cells showing one orange -PTEN single copy, two green signal -CEP 10 pattern indicative of a PTEN deletion (Figure 3). The signal was detected by analyzing software of Isis Fluorescence Imaging System (MetaSystems).

F. Statistical analysis

Statistical analysis was conducted using SPSS 23.0 statistical software (SPSS, Chicago, IL, USA). The associations between AP or zonal distribution and clinicopathologic parameters, as well as the associations between AP or zonal distribution and IHC results or deletion of PTEN were assessed using Pearson χ^2 test and Fisher's exact test. P-value with less than 0.05 was considered as significant value.

III. RESULTS

A. Correlation between clinicopathologic parameter

Among total cases (273), tumors occurred predominantly in the anterior location in 87 (31.9%) of the specimen. Comparative results for parameters about pathologic and clinical features between APT and non-APT are summarized in Table 2. There was no difference between age, tumor size, stage, Gleason score, growth pattern and extra-prostatic extension (EPE) in the two groups. In terms of zonal distribution, APT was demonstrated with higher incidence of transition zone (P value: <0.001), especially, 164 (60.07%) out of 273 cases located in single zone of PZ or TZ only, 90.9% (10/11) of TZ only tumor appeared in APT with significant value. On the contrary, 86.3% of PZ tumor (132/153) predominantly involved in non-anterior PZ (NPZ). Twenty-four both zone tumors (24/109) were existed more broad area in TZ than PZ, and their AP rate is 83.3% (20/24). Eighty-five both zone tumors (85/109) were involved predominant PZ, and their non-AP rate is 57.6% (49/85). Except 179 cases out of entire cohort (n=273) demonstrated with involved resection margins at least one area and recognized foci of positive margins were specific area - base (P value: 0.046) and anterior area of body (P value: 0.024), especially in the cases of AP. The rates of positive resection margin in APT were significantly higher (42.5%) than those with non-APT tumors (30.64%).

B. Correlation between immunohistochemistry and zonal distribution

AR-V7 staining was detected in 78 cases, above all 60 specimens (76.9%) were

PZ and 18 specimens (23.1%) were from TZ which was statistically significant (P value: 0.050). ERG staining was detected in 40 cases, among them 32 specimens (80.0%) were PZ and 8 specimens (20.0%) were from TZ. PTEN deletion was detected in 101 cases, out of them 79 specimens (78.2%) were PZ and 22 specimens (21.8%) were from TZ. SPINK1 staining was detected in 24 cases, mainly 20 specimens (83.3%) were PZ and 4 specimens (16.7%) were from TZ. SPOP staining was detected in 123 cases, majority of all 103 specimens (83.7%) were PZ and 20 specimens (16.3%) were from TZ (Table 3).

C. Correlation between immunohistochemistry and anterior-predominance

AR-V7 staining was detected in 78 cases, out of them 29 specimens (37.2%) were APT and 49 specimens (62.8%) were from non-APT. ERG staining was detected in 40 cases, among them 12 specimens (30.0%) were APT and 28 specimens (70.0%) were from non-APT. PTEN deletion was detected in 101 cases, above all 49 specimens (48.5%) were APT and 52 specimens (51.5%) were from non-APT which was statistically significant (P value: 0.005). SPINK1 staining was detected in 24 cases, among them 9 specimens (37.5%) were APT and 15 specimens (62.5%) were from non-APT. SPOP staining was detected in 123 cases, out of them 38 specimens (30.9%) were APT and 85 specimens (69.1%) were from non-APT which was statistically significant (P value: <0.001) (Table 4).

D. Association between PTEN FISH and tumor location

PTEN deletion was detected in 80 cases with FISH performed, out of them 62 specimens (77.5%) were PZ and 18 specimens (22.5%) were from TZ, also 36 out of

80 specimens (45%) were APT and 44 specimens (55%) were from non-APT (Table 5).

E. Concordance in PTEN deletion between IHC and FISH

PTEN deletion with FISH method identified in 80 cases, among them 54 cases (67.5%) were corresponds of IHC staining. PTEN deletion with IHC staining detected in 101 cases, out of them 54 cases (53.5%) were corresponds of FISH result. Concordance rate was 57.55% (99/172) that is higher levels of discrepancy - 41.86% (72/172) - , which were result of analysis statistically significant (P value: 0.05) (Table 6).

F. Immunophenotype in respect of tumor location

AR-V7 positivity was significantly associated with zonal distribution, respectively TZ tumor showed strong correlation more than PZ tumor. In contrast to AR-V7, other markers had no significant relationship with zonal distribution. PTEN deletion was significantly associated with AP, respectively APT showed considerable connection more than non-APT. SPOP positivity showed similar tendency with AP, respectively non-APT showed significant association more than APT. In contrast to PTEN and SPOP, other markers had no significant relationship with AP. The results of IHC suggest immunophenotype organizing of anterior-predominance (AP) and zonal distribution. First, anterior peripheral zone (APZ) subtype strong likelihood AR-V7 negative, PTEN deletion and SPOP negative. Second, anterior transition zone (ATZ) subset had more possibility of AR-V7 positive, PTEN deletion and SPOP negative. Third, Non-anterior peripheral zone (NPZ) subgroup had good prospect of AR-V7 negative, PTEN no deletion and

SPOP positive. Last, Non-anterior transition zone (NTZ) subtype showed significant concurrence as AR-V7 positive, PTEN intact and SPOP positive (Figure 2).

IV. DISCUSSION

Several previous studies have description about the clinicopathologic difference TZ compared with PZ [5, 17]. Most investigators had matched conclusion that TZ tumors should be considered distinctly from PZ tumors due to the former have more indolent behavior and lower percent higher Gleason score more than 4 [4, 17, 18]. In the recent investigation with RP specimen analysis, APT had an aspect of rise in frequency with variable value by papers from 15% to 23.3% during about 4.5 years [7]. In present research, we focused on anteriority and topographical relationship with subcategorizing zonal distribution of APT. According to zonal origin of prostate cancer, tumors involving mainly TZ more than PZ have distinct pathologic features such as higher potential positive surgical margins in the apico-anterior area and bladder neck [5]. But, these explanations not coupled with our data. The point of present research was AP, which is identified relatively strong association of TZ and demonstrated that involved anterior-basal prostate surgical margins. In our review, a series of studies including present article could be possibly informative in regarding preoperative planning [5]. Also, discrepancies with previous studies that agreements which are TZ tumor had lower Gleason score and anterior tumor more frequent in PZ [4, 7]. Ahmadie et al, in a research of 197 anterior predominant tumors, have demonstrated that there were more centered on PZ, instead of TZ origin [7]. In paragraph about differences in EPE between APT and non-APT, his group had significant result that anterior sited tumors be considered as lower rates

of EPE than posterior located tumors, with regardless of zones [7]. Intriguingly, we were unable to explain that statistical differences in EPE between APT and non-APT. In a literature about zonal molecular signature of Sinnott et al, they acquired supportive conclusion that tumor derived zone importantly contributes to gene expression discriminating between TZ and PZ molecular signature properties [19]. But the distinctiveness from our research is the subgroups of tumor sample, representatively transurethral resection of prostate (TURP) and RP specimen were used to demonstrate gene expression difference between TZ and PZ tissue each other [19]. There was single molecular methodology in this study which was PTEN FISH, using this tool we were revealed no molecular differences of PTEN deletion associating AP or zonal distribution.

Androgen receptors (AR) have essential functions in the tumorigenesis of PCs. The ligand-binding domain of AR gene displays to be dispensable for AR transcriptional activity, so its deletion had followed by continuous activation of AR transcription ability [20]. There are more than 20 AR splice variants, most of all AR-V7 is one of the most frequent and famous with association of local recurrence and poor prognosis and it is potential factor of aggressive behavior according to zonal distribution [20, 21]. Genomic distinction between different population also reported, TMPRSS2-ERG gene fusion was observed correlation with PTEN deletion in western country, it is not supportive in Chinese PCs [22]. OF note, approximately 10% of PCs show SPINK1 alteration, which could be detected with immunohistochemical stain and it is appears mutually exclusive with ERG expression [15].In vitro, Knockdown of SPOP gene related with tumor growth and invasion, recognizing that function of SPOP gene as tumor suppressor gene, to

date [23].Among variable molecular alteration in PCs, ERG, PTEN and SPINK1 are acquiring acceptance as prognostic biomarkers, however, it is limited Caucasians and not fit Korean [24].

In aspect of ethnic features, our cohort was composed of Korean male, exclusively. This hereditary homogeneity is fairly important, in that view of confirmed ethnic variation [12]. Recently, a report suggests that African-American (AA) patients appear more aggressive behavior and tendency anterior localization [3]. Otherwise, the risk of high grade PCs (more than Gleason score 8) or aggressive stage PCs is frequent in Korean patients of recent retrospective multi-racial study [25] In examination of Faisal et al, between different race -AA and Caucasian-American (CA) have distinct ERG, ETS, and SPINK1 expression pattern, anterior tumors also had lower expression of Androgen receptor (AR) signaling genes [12]. ERG rearrangement is showing higher frequency in CA than AA patients, ETS related alterations are occurring in more 40% of CA men [12, 26]. In same report, anterior tumor more likely to be ERG negative and SPINK1 negative with regard of race [12]. These findings differ from Korean patients showing other pattern which was not supportive meanwhile known as. As mentioned in prior, AR-V7 expression showed association with zonal distribution, especially TZ tumor was revealed higher frequency of the marker. PTEN deletion and SPOP negativity were more likely to be associated with APT. In contrast to these three antibodies, ERG and SPINK1 had no significant relationship with tumor location.

We analyzed IHC and PTEN FISH data with location-based with target of AP and zonal distribution. This study demonstrates association among AR-V7, PTEN and

SPOP immunostain profile in relation to tumor location which were classified into four subgroups which were anterior PZ (APZ), anterior TZ (ATZ), non-anterior PZ (NPZ) and non-anterior TZ (NTZ).

V. CONCLUSION

We evaluated correlation immunophenotyping and anatomical tumor location which were categorizing on a basis of anterior predominant tumors (APT) and zonal distribution. Although prognostic predictive value and treatment response were not investigated in our study, phenotype of IHC antibodies - AR-V7, PTEN and SPOP was demonstrates molecular differences in terms of topographical relationship exclusively Korean racial group. Further research will followed for clarifying causative factors driving molecular heterogeneity including contribute to subset differences by ethnic group and tumor location. In addition, figure out the discrimination of two groups of Asian and other race is interesting issue of future study.

REFERENCES

1. Jung, K.W., et al., *Cancer statistics in Korea: incidence, mortality, survival, and prevalence in 2009.* Cancer Res Treat, 2012. **44**(1): p. 11-24.

2. Daniyal, M., et al., *Epidemiology, etiology, diagnosis and treatment of prostate cancer.* Asian Pac J Cancer Prev, 2014. **15**(22): p. 9575-8.

3. Sundi, D., et al., *Pathological examination of radical prostatectomy specimens in men with very low risk disease at biopsy reveals distinct zonal distribution of cancer in black American men.* J Urol, 2014. **191**(1): p. 60-7.

4. Greene, D.R., et al., *A comparison of the morphological features of cancer arising in the transition zone and in the peripheral zone of the prostate.* J Urol, 1991. **146**(4): p. 1069-76.

5. Iremashvili, V., et al., *Prostate cancers of different zonal origin: clinicopathological characteristics and biochemical outcome after radical prostatectomy.* Urology, 2012. **80**(5): p. 1063-9.

6. Dong, J., et al., *TMPRSS2:ETS fusions and clinicopathologic characteristics of prostate cancer patients from Eastern China.* Asian Pac J Cancer Prev, 2014. **15**(7): p. 3099-103.

7. Al-Ahmadie, H.A., et al., *Anterior-predominant prostatic tumors: zone of origin and pathologic outcomes at radical prostatectomy.* Am J Surg Pathol, 2008. **32**(2): p. 229-35.

8. Magers, M.J., et al., *Clinicopathologic characteristics of anterior prostate cancer (APC), including correlation with previous biopsy pathology.*

Med Oncol, 2015. **32**(11): p. 249.

9. Mygatt, J., et al., *Anterior tumors of the prostate: clinicopathological features and outcomes.* Prostate Cancer Prostatic Dis, 2014. **17**(1): p. 75-80.

10. Tu, J.J., et al., *Gene fusions between TMPRSS2 and ETS family genes in prostate cancer: frequency and transcript variant analysis by RT-PCR and FISH on paraffin-embedded tissues.* Mod Pathol, 2007. **20**(9): p. 921-8.

11. Yoshimoto, M., et al., *Three-color FISH analysis of TMPRSS2/ERG fusions in prostate cancer indicates that genomic microdeletion of chromosome 21 is associated with rearrangement.* Neoplasia, 2006. **8**(6): p. 465-9.

12. Faisal, F.A., et al., *Racial Variations in Prostate Cancer Molecular Subtypes and Androgen Receptor Signaling Reflect Anatomic Tumor Location.* Eur Urol, 2015.

13. Hornberg, E., et al., *Expression of androgen receptor splice variants in prostate cancer bone metastases is associated with castration-resistance and short survival.* PLoS One, 2011. **6**(4): p. e19059.

14. Frederick, L., et al., *AJCC cancer staging manual*. Vol. 1. 2002: Springer Science & Business Media.

15. Bhalla, R., et al., *Novel dual-color immunohistochemical methods for detecting ERG-PTEN and ERG-SPINK1 status in prostate carcinoma.* Mod Pathol, 2013. **26**(6): p. 835-48.

16. Zhao, H., et al., *Transcript levels of androgen receptor variant AR-V1 or AR-V7 do not predict recurrence in patients with prostate cancer at indeterminate risk for progression.* J Urol, 2012. **188**(6): p. 2158-64.

17. Noguchi, M., et al., *An analysis of 148 consecutive transition zone*

cancers: clinical and histological characteristics. J Urol, 2000. **163**(6): p. 1751-5.

18. Shannon, B.A., J.E. McNeal, and R.J. Cohen, *Transition zone carcinoma of the prostate gland: a common indolent tumour type that occasionally manifests aggressive behaviour.* Pathology, 2003. **35**(6): p. 467-71.

19. Sinnott, J.A., et al., *Molecular differences in transition zone and peripheral zone prostate tumors.* Carcinogenesis, 2015. **36**(6): p. 632-8.

20. Sun, S., et al., *Castration resistance in human prostate cancer is conferred by a frequently occurring androgen receptor splice variant.* J Clin Invest, 2010. **120**(8): p. 2715-30.

21. Guo, Z., et al., *A novel androgen receptor splice variant is up-regulated during prostate cancer progression and promotes androgen depletion-resistant growth.* Cancer Res, 2009. **69**(6): p. 2305-13.

22. Mao, X., et al., *Distinct genomic alterations in prostate cancers in Chinese and Western populations suggest alternative pathways of prostate carcinogenesis.* Cancer Res, 2010. **70**(13): p. 5207-12.

23. Li, C., et al., *Tumor-suppressor role for the SPOP ubiquitin ligase in signal-dependent proteolysis of the oncogenic co-activator SRC-3/AIB1.* Oncogene, 2011. **30**(42): p. 4350-64.

24. Noh, B.J., et al., *Prognostic value of ERG, PTEN, CRISP3 and SPINK1 in predicting biochemical recurrence in prostate cancer.* Oncol Lett, 2016. **11**(6): p. 3621-3630.

25. Jeong, I.G., et al., *Differences in the aggressiveness of prostate cancer among Korean, Caucasian, and African American men: A retrospective cohort*

study of radical prostatectomy. Urol Oncol, 2016. **34**(1): p. 3.e9-14.

26. Magi-Galluzzi, C., et al., *TMPRSS2-ERG gene fusion prevalence and class are significantly different in prostate cancer of Caucasian, African-American and Japanese patients.* Prostate, 2011. **71**(5): p. 489-97.

Table 1. Antibodies and staining protocol

Antibody	Clone	Cat. No	Vendor	Dilution	Buffer
ERG	EPR3864	# 2805-1	Epitomics	1:100	EDTA buffer, pH 9.0
SPINK1	4D4	H00006690-M01	abnova	1:200	citrate buffer, pH 6.0
SPOP	N1C1	GTX106942	GeneTex	1:200	citrate buffer, pH 6.0
PTEN	138G6	#9559	Cell Signaling	1:100	EDTA buffer, pH 8.0
AR-V7	EPR15656	ab198394	Abcam	1:200	EDTA buffer, pH 8.0

ERG, ETS-related gene; SPINK1, serin peptidase inhibitor Kazal 1; SPOP, speckle-type POZ Protein gene; PTEN, phosphatase and tensin homolog; AR-V7, androgen receptor splice variant 7

Table 2. Relationship between clinicopathologic parameters and anterior predominance (%)

Anterior predominance	APT (n=87)	Non-APT (n=186)	p-value
Age			0.175
≤65	34 (27.6)	89(72.4)	
>66	53 (35.3)	97 (64.7)	
Zonal distribution*			<0.001
TZ only (n=11)	10(90.9)	1 (9.1)	
PZ only (n=153)	21 (13.7)	132 (86.3)	
TZ>PZ (n=24)	20(83.3)	4(16.7)	
PZ>TZ (n=85)	36(42.4)	49(57.6)	
Size (cm)			0.186
≤1	5 (33.3)	10 (66.7)	
>1 and ≤2	21 (22.6))	72 (77.4)	
>2 and ≤3	37 (35.2)	68 (64.8)	
>3 and ≤4	20 (39.2)	31(60.8)	
>4 and ≤5	4 (44.4)	5(55.6)	
Stage			0.128
pT2	58 (35.4)	106 (64.6)	
pT3	29(26.6)	80(73.4)	
Gleason score			0.256
6	12 (46.2)	14 (53.8)	
≥7	73 (30.3)	168 (69.7)	
Growth pattern			0.117
Infiltrative	47 (29.9)	110 (70.1)	
Nodular	37 (38.1)	60 (61.9)	
Scattered	3 (15.8)	16 (84.2)	
RMs*			0.046
Apex	6 (28.6)	15 (71.4)	
Base	3 (75.0)	1 (25.0)	
Body	8 (29.6)	19 (70.4)	
AB/ BB	20 (47.6)	22 (52.4)	
Negative	50 (27.9)	129 (72.1)	
Body RMs*			0.024
Anterior	14 (60.9)	9 (39.1)	

Posterolateral	8 (25.0)	24 (75.0)	
multifocal	4 (33.3)	8 (66.7)	
EPE			0.179
Extensive	19 (25.3)	56 (74.7)	
Focal	6 (24.0)	19 (76.0)	
No	62 (35.8)	111 (64.2)	

* P value ≤ 0.05

APT, anterior-predominant tumors; TZ, transition zone; PZ, peripheral zone; RMs, resection margins; AB, apico-body; BB, basal-body; EPE, extraprostatic extension

Table 3. Relationship between immunohistochemical result and zonal distribution (%)

Zonal distribution	PZ (n=139)	TZ (n=33)	p-value
AR-V7†*			0.050
Positive	60 (76.9)	18 (23.1)	
Negative	79 (84.9)	14 (15.1)	
ERG			0.881
Positive	32 (80.0)	8 (20.0)	
Negative	107 (81.1)	25 (18.9)	
PTEN†			0.543
Deletion	79 (78.2)	22 (21.8)	
No deletion	59 (84.3)	11 (15.7)	
SPINK1			0.735
Positive	20 (83.3)	4 (16.7)	
Negative	119(80.4)	29(19.6)	
SPOP			0.123
Positive	103(83.7)	20 (16.3)	
Negative	36 (73.5)	13 (26.5)	

PZ, peripheral zone; TZ, transition zone; ERG, ETS-related gene; SPINK1, serin peptidase inhibitor Kazal 1; SPOP, speckle-type POZ Protein gene; PTEN, phosphatase and tensin homolog; AR-V7, androgen receptor splice variant 7

* P value ≤ 0.05

†; One case was not applicable

Table 4. Relationship between immunohistochemical result and anterior predominance (%)

Anterior predominance	Present (n=68)	Absent (n=104)	p-value
AR-V7†			0.589
Positive	29 (37.2)	49 (62.8)	
Negative	39 (41.9)	54 (58.1)	
ERG			0.159
Positive	12(30.0)	28 (70.0)	
Negative	56 (42.4)	76 (57.6)	
PTEN†*			0.005
Deletion	49 (48.5)	52 (51.5)	
No deletion	18 (25.7)	52 (74.3)	
SPINK1			0.826
Positive	9 (37.5)	15 (62.5)	
Negative	59(39.9)	89(60.1)	
SPOP*			<0.001
Positive	38(30.9)	85 (69.1)	
Negative	30 (61.2)	19 (38.8)	

ERG, ETS-related gene; SPINK1, serin peptidase inhibitor Kazal 1; SPOP, speckle-type POZ Protein gene; PTEN, phosphatase and tensin homolog; AR-V7, androgen receptor splice variant 7

* P value ≤ 0.05

†; One case was not applicable

Table 5. Association of FISH results with zonal distribution and anterior predominance (%)

	FISH		p-value
	Deletion (n=80)	No deletion (n=92)	
Zonal distribution			0.303
PZ	62 (44.6)	77 (55.4)	
TZ	18 (54.5)	15 (45.5)	
Anterior predominance			0.172
Present	36 (52.9)	32 (47.1)	
Absent	44 (42.3)	60 (57.7)	

PZ, peripheral zone; TZ, transition zone

* P value ≤ 0.05

Table 6. Concordance in PTEN deletion between immunohistochemical result and FISH

(%)

Immunohistochemistry †	FISH		Total
	Deletion (n=79)	No deletion (n=92)	
Deletion	54 (53.5)	47 (46.5)	101
No deletion	25 (35.7)	45 (64.3)	70
p-value			0.05*

* P value ≤ 0.05

†; One case was not applicable

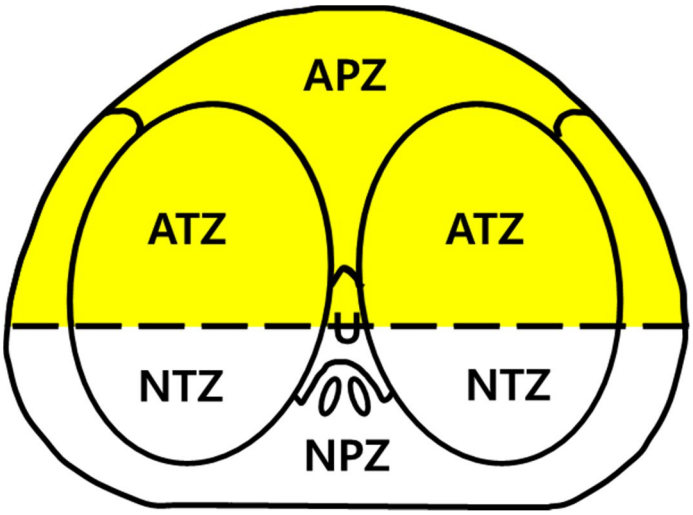

Figure 1. Schematic division with imaginary line that across the urethra (U) in axial section at midlevel. Assignment of anterior-predominance (AP) of largest index in case of located in yellow area, upper half. Anterior predominant tumors (APT) were located in anterior peripheral zone (APZ) or anterior transition zone (ATZ). Lower half of divisional line (white area) is composed of non-anterior peripheral zone (NPZ) and non-anterior transition (NTZ).

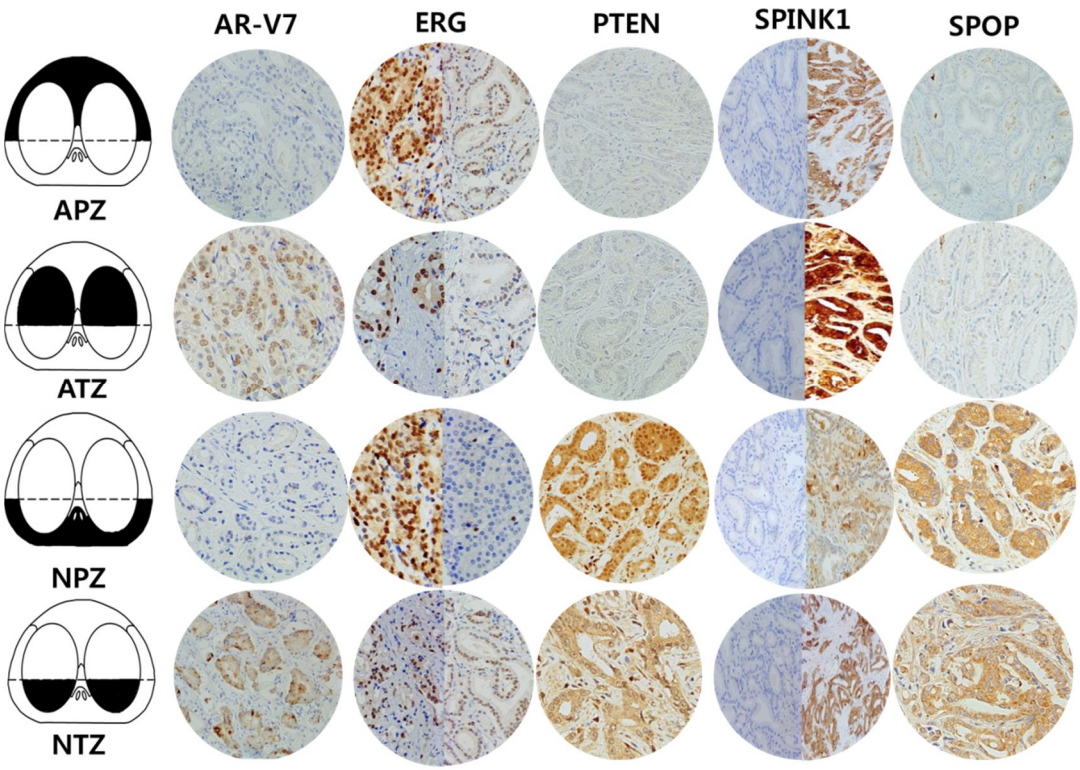

Figure 2. Immunophenotype of AR-V7, ERG, PTEN, SPINK-1 and SPOP according to tumor location. Anterior peripheral zone (APZ) relatively revealed AR-V7, negative; PTEN deletion; SPOP, negative. Anterior transition zone (ATZ) recognized AR-V7, positive; PTEN deletion; SPOP, negative. Non-anterior peripheral zone (NPZ) showed AR-V7, negative; PTEN no deletion; SPOP, positive. Non-anterior transition zone (NTZ) displayed AR-V7, negative; PTEN deletion; SPOP, negative.

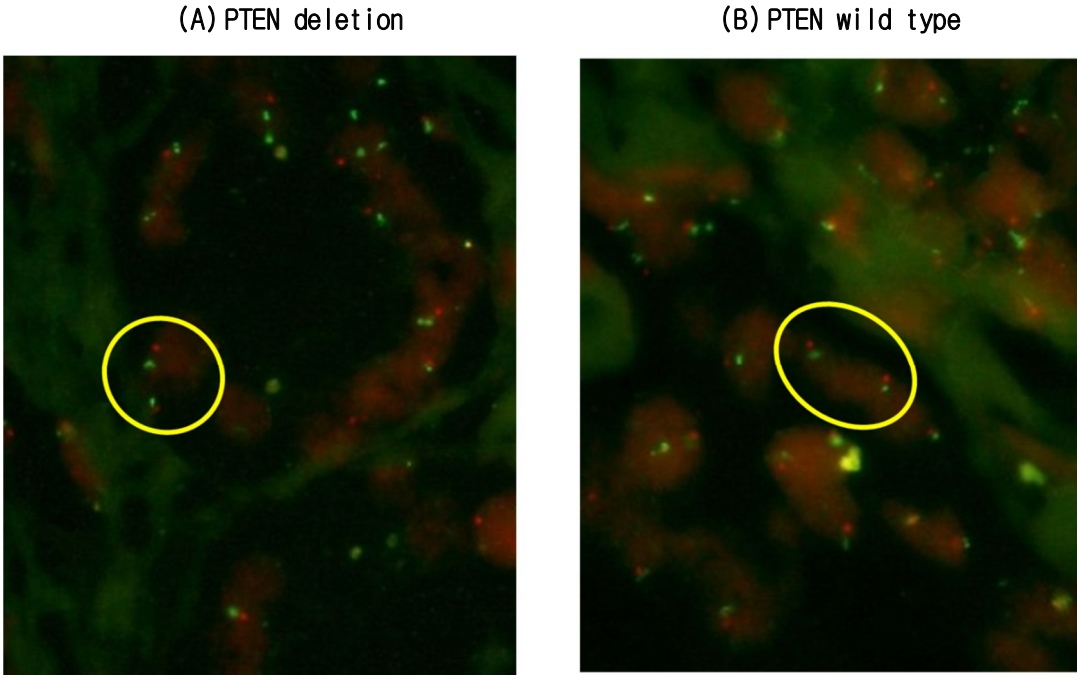

Figure 3. Representative PTEN deletion image with FISH method reveals single orange signal (A), in contrast to normal control - two orange signal (B).