TMPRSS2-ERG Gene Fusion Is Rare Compared to *PTEN* Deletions in Stage T1a Prostate Cancer

Kurt W. Fisher,[1] Shaobo Zhang,[1] Mingsheng Wang,[1] Rodolfo Montironi,[2] Lisha Wang,[3] Lee A. Baldrige,[1] Jonas Y. Wang,[1] Gregory T. MacLennan,[4] Sean R. Williamson,[5,6,7] Antonio Lopez-Beltran,[8] and Liang Cheng[1]*

[1]*Department of Pathology, Indiana University School of Medicine, Indianapolis, Indiana*
[2]*Department of Urology, Institute of Pathological Anatomy and Histopathology, Polytechnic University of the Marche Region (Ancona), United Hospitals, Ancona, Italy*
[3]*Michigan Center for Translational Pathology, University of Michigan, Ann Arbor, Michigan*
[4]*Departments of Pathology and Laboratory Medicine, Case Western Reserve University, Cleveland, Ohio*
[5]*Department of Pathology and Laboratory Medicine, Henry Ford Health System, Detroit, Michigan*
[6]*Josephine Ford Cancer Institute, Henry Ford Health System, Detroit, Michigan*
[7]*Department of Pathology, Wayne State University School of Medicine, Detroit, Michigan*
[8]*Faculty of Medicine, Department of Pathology and Surgery, Cordoba University Spain and Champalimaud Clinical Center, Cordoba University, Lisbon, Portugal*

T1a prostate cancers (cancer found incidentally in transurethral resection, <5% of the tissue) are indolent tumors of the transition zone. The overexpression of ERG and the inactivation of PTEN have been shown to be important drivers of carcinogenesis in large series of prostate cancer, but the genetics of transition zone tumors have not been well characterized. We evaluated the status of ERG and PTEN in formalin-fixed paraffin-embedded tissue using immunohistochemical and FISH analysis in 54 T1a transition zone tumors. The protein expression of ERG was determined using a rabbit monoclonal antibody and nuclear staining was scored as positive or negative. The genomic status of *ERG* was determined using three colored FISH using an *ERG-TMPRSS2* tri-color probe set. The protein expression of PTEN was determined using a rabbit monoclonal antibody and cytoplasmic, and nuclear staining was scored as positive or negative. The genomic status of *PTEN* was determined using dual color FISH with a *PTEN* probe and a CEP10 probe. We found *ERG* rearrangement in 2 of 54 tumors (4%), one with protein overexpression by immunohistochemistry. PTEN inactivation was seen in 13 of 54 tumors (24%). Nine of the 13 *PTEN* alleles were inactivated by hemizygous deletion. No homozygous *PTEN* deletion was observed. *PTEN* deletion and *ERG* rearrangement were mutually exclusive. *ERG* rearrangement was rare compared to peripheral zone tumors and to *PTEN* inactivation in T1a transition zone tumors. © 2016 Wiley Periodicals, Inc.

Key words: prostate; transition zone; insignificant cancer; PTEN loss; *TMPRSS2-ERG* rearrangement; fluorescence in situ hybridization; T1a

INTRODUCTION

Adenocarcinomas of the prostate gland arise within different anatomic zones that have varying clinical and molecular characteristics [1,2]. The vast majority of clinically significant prostatic adenocarcinomas arise in the peripheral zone, which have been widely investigated. Transition zone tumors are estimated to make up 16–20% of all prostate tumors, but these tumors have been less extensively studied. Compared to peripheral zone tumors, transition zone tumors have a lower Gleason scores, lower Ki-67 labeling indices, less extraprostatic extension, seminal vesicle invasion, and lymphovascular invasion, suggesting they may have a limited malignant potential [1–4]. However, approximately 20% of transition zone tumors progress to disease that invades beyond the prostate, and approximately 5% have lymph node metastases [5]. Currently, there is no method to predict which transition zone tumors will follow an aggressive course.

Recent discoveries have shown that peripheral zone tumors show a high prevalence of *TMPRSS2-ERG* gene fusions and PTEN inactivation, and the use of these two genetic events may help predict the clinical prognosis. Translocations between the *TMPRSS2-ERG* genes creates a constitutively active transcription factor that is uniquely found in approximately 50% of prostate tumors and thought to be essential for the carcinogenesis in this subset of tumors [6–9]. Large series of prostate cancer estimate PTEN inactivation occurs in approximately 18–23% of all tumors [6,9,10],

*Correspondence to: Department of Pathology and Laboratory Medicine, Indiana University School of Medicine, 350 West 11th Street, IUHPL Room 4010, Indianapolis, IN 46202.

Received 25 March 2016; Revised 30 July 2016; Accepted 4 August 2016

DOI 10.1002/mc.22535

Published online 31 August 2016 in Wiley Online Library (wileyonlinelibrary.com).

© 2016 WILEY PERIODICALS, INC.

and its loss allows for uninhibited activation of the PI3K/Akt/mTOR pathway and additional downstream targets. Mouse models that constitutively overexpressed ERG in a PTEN null background lead to highly penetrant prostate cancer that arises much quicker than PTEN loss alone [11]. *PTEN* deletions have been associated with higher histologic grades, lymph node metastases, and lower overall survival in *TMPRSS2-ERG* gene fusion positive and negative cancers [6,9,12,13]. Prostate cancers that lack *TMPRSS2-ERG* gene fusions and *PTEN* deletion have been shown to have a better prognosis, and the use of both gene rearrangements is the basis of a predictive model of disease reoccurrence [14].

Clinical course of T1a prostate cancers (tumors found incidentally in transurethral resection without clinically suspected tumor, making up <5% of the tissue) is variable with long-term follow-up [15–17]. The status of *TMPRSS2-ERG* fusions and *PTEN*, and have not been well characterized in transition zone T1a tumor. These tumors typically do not receive treatment, but have the risk of progression to clinically significant disease. We assessed the genetic status of *TMPRSS2-ERG* fusions and *PTEN* in order to further understand the biology of prostate cancer in an attempt to find genetic predictors of aggressive behavior. Here, we describe the frequency of PTEN inactivation and *TMPRSS2-ERG* gene fusions, and their relationship in T1a tumors using human prostatic tissue removed by transurethral resection of the prostate (TURP). We analyzed 54 cases of Gleason score 7 or lower adenocarcinoma for the status of *PTEN*, and *TMPRSS2-ERG* using both immunohistochemistry and fluorescence in situ hybridization.

METHODS

Patients

We identified 54 T1a prostate adenocarcinomas by reviewing TURP specimens from participating institutions (Indiana University, Indianapolis, USA; Polytechnic University of the Marche Region, United Hospitals, Ancona, Italy; Case Western Reserve University, Cleveland, OH; Henry Ford Health System, Detroit, MI; Cordoba University, Cordoba, Spain) between 2003 and 2014. All TURP samples with carcinoma diagnoses were reviewed by two anatomic pathologists (KWF and LC) to confirm tumor volume was less than 5% of the resected specimen, meeting the criteria of the American Joint Committee on Cancer for T1a tumor staging. No Gleason grade 8 or higher cancers were found to have less than 5% involvement of a TURP sample. This research was approved by the Institutional Review Board.

Immunohistochemistry

We evaluated the status of ERG and PTEN proteins in formalin-fixed, paraffin-embedded tissue using immunohistochemical staining. Briefly, 4-μm-thick sections were heated in a PT module (DAKO, Carpinteria, CA) in Tris/EDTA (pH 9.0) for 20 min and then cooled down to room temperature. Samples were incubated at 1:200 dilution with PTEN antibody (rabbit monoclonal antibody, clone D4.3 XP, Cell Signaling Technology, Danvers, MA) or ready-to-use ERG antibody (rabbit monoclonal antibody, clone EP111, DAKO) for 30 and 20 min, respectively. This was followed by incubations with DAKO Envision Flex + rabbit linker, Envision HRP, and DAB+ chromogen. All other step followed the manufacturers' provided protocols. A tumor was considered to have PTEN protein loss if the intensity of cytoplasmic staining was markedly decreased or entirely lost in more than 10% of tumor cells compared with surrounding benign glands [18–21]. ERG nuclear staining was scored as positive or negative and any nuclear staining of ERG was considered as indicative of ERG expression [22,23].

Fluorescence In Situ Hybridization

Four-micrometer-thick sections were obtained from formalin-fixed, paraffin-embedded specimen blocks, and deparaffinized with two 15-min washes in xylene, subsequently washed twice with 100% ethanol for 10 min each, and air-dried. The sections were heated at 95°C in 0.1 mM citric acid (pH 6) solution (Invitrogen, Carlsbad, CA) for 10 min, rinsed with distilled water for 3 min, and washed with 2× saline–sodium citrate (SSC) for 5 min. Tissue digestion was performed by applying 0.4 ml of pepsin (Sigma, St Louis, MO) solution (4 mg/ml in 0.9% NaCl in 0.01N HCl) to each slide and incubating the slides in a humidified box for 40 min at 37°C. The slides were rinsed with distilled water for 5 min, washed with 2×SSC for 5 min, and then air-dried.

For *PTEN* copy number assay, a probe cocktail including BAC clones RP11-47D17-gold (3′ *ERG*), RP11-95121-green (5′ *ERG*) and RP11-35C4-aqua (*TMPRSS2*) (Empire Genomics, Buffalo, NY) diluted 1:25 in tDenHyb2 (Insitus, Albuquerque, NM). The genomic status of *ERG* was determined using three colored FISH using a *TMPRSS2* aqua probe, a 5′ *ERG* green probe, and a 3′ gold *ERG* probe. Five microliters of diluted probe were applied to each slide. Coverslips were placed over the slides and sealed with rubber cement. The slides were denatured at 80°C for 10 min and hybridized at 37°C overnight. The coverslips were removed and the slides were extensively washed with two 0.1×SSC/1.5 M urea solutions at 45°C for 20 min, in 2×SSC at 45°C for 10 min, and then in 2×SSC/0.1% NP40 at 45°C for 10 min. Finally, the slides were washed with 2×SC at room temperature for 5 min, air-dried, counterstained with 10 μl DAPI/Antifade (DAPI in Fluorguard, 0.5 g/ml, Insitus, Albuquerque, NM), and sealed with nail polish.

The hybridized slides were observed and documented using a MetaSystem imaging system, and ISIS software (Belmont, MA) under ×100 oil objective. The

images were acquired with a CoolCube 1 camera (MetaSystem) and analyzed with ISIS software. The following filters were used: SP-100 for DAPI, FITC MF-101 for spectrum green, Gold 31003 for spectrum orange, ad, Aqua 31036V2 for spectrum Aqua. Signals from each color channel (probe) were counted under false color, with computerized translation of each color channel into blue, green, red, or aqua. Four sequential focus stacks with 0.3 μm intervals were acquired and integrated into a single image to reduce thickness-related artifacts. For each case, 100 non-overlapping cancer cells nuclei were evaluated. Preparations were considered valid if >90% of the cells showed bright signals. Hemizygous deletion of *PTEN* was defined as ≥50% of tumor nuclei containing one *PTEN* signal, and with the presence of CEP 10 signals. Homozygous deletion of *PTEN* was defined as in ≥30% of tumor nuclei simultaneous loss of both *PTEN* signal, and with the presence of CEP 10 signals [13,24,25]. Cases with *ERG* signal abnormalities in ≥20% of the tumor cell population were considered to be positive [22,26].

Statistical Methods

Fisher exact tests were used to determine the association between PTEN protein expression and allele deletion status. Statistical significance was defined as $P < 0.05$ and all P values were two-sided.

RESULTS

The samples we analyzed were composed of 51 Gleason grade $3+3=6$ and 3 Gleason grade $3+4=7$ prostatic adenocarcinomas, all of which involved less than 5% volume per sample. The average age was 73 years (range: 52–92 years), and the average specimen mass was 58 grams (range: 3–260 g) (Table 1).

We found *ERG* rearrangement by FISH in 2 of 54 tumors (4%), one with corresponding protein overexpression by immunohistochemistry (Figure 1 and Table 1). We did not find any ERG overexpression or *ERG* rearrangements in adjacent benign prostatic glands. We found PTEN protein loss in 13 of 54 (24%) tumors using immunohistochemistry (Figure 2 and Table 2). The PTEN protein loss status was highly correlated with *PTEN* allele deletion detected by FISH method ($P = 0.0001$). Nine of the 13 cases (69%) with PTEN protein loss showed hemizygous deletion of *PTEN* by FISH. No homozygous *PTEN* deletion was observed. We did not find any PTEN protein loss in adjacent benign tissue.

Table 1. Immunohistochemical and FISH Assessment of ERG Status

ERG status	IHC +	IHC −	Total
FISH rearrangement +	1	1	2
FISH rearrangement −	0	52	52
Total	1	53	54

IHC, immunohistochemistry.

DISCUSSION

TMPRSS2-ERG rearrangements can be found in approximately 50% of peripheral zone tumors [6–9] and large series of prostate cancer estimate PTEN inactivation to occur in approximately 18–23% of all tumors [6,9,10]. Using a combination of IHC and FISH, we found that ERG overexpression (4%) is dramatically underrepresented in small T1a transition zone tumors. However, PTEN inactivation (24%) in stage T1a prostate cancer was similar to the most recent estimates of PTEN inactivation in large series of prostate cancer [6,9,10].

It is possible that our study using only immunohistochemistry and FISH studies might underestimate the true incidence of genetic *PTEN* inactivation, but other studies has shown a 75–89% correlation between FISH and IHC [6,18]. A recent study has shown inactivating point mutations of *PTEN* occur in approximately 5% of samples, which would not be detected by FISH and IHC [6]. Even a minor upward adjustment in our estimation of PTEN inactivation in T1a tumors would not change the conclusion that the prevalence is similar to PTEN inactivation seen in peripheral zone tumors. *PTEN* deletion has been associated with a worse prognosis in peripheral zone cancers and the similar frequency of PTEN inactivation in our sample does not seem to be an explanation for the indolent behavior of transition zone tumors [6,9,18,24]. Larger data sets and prospective studies will be needed to assess the prognostic value of PTEN inactivation in transition zone tumors. More recent work has been done to optimize PTEN analysis by immunohistochemistry, and that optimized four color FISH probes have been identified and applied in other cohorts of prostate cancer. These optimized assays have now been applied to a large multicenter cohort with rigorous statistical analysis [21]. The development of a clinical-grade, automated, and cost effective PTEN assay will facilitate further validation of PTEN as an important prognostic and predictive biomarker for prostate cancer.

TMPRSS2-ERG fusion proteins are seen in approximately 50% of prostate cancers, but these were dramatically underrepresented in our sample population. *ERG* is a member of the ETS family, which has 28 unique genes. Of these, *FLI*, *ERG*, *ETV1*, and *ETV4* are commonly deregulated in cancer [27]. *TMPRSS2* and *ERG* are located within three megabases of each other on chromosome 21, and large deletions and less commonly translocations, help to explain the high prevalence of fusions involving *ERG* compared to other ETS family members [28]. ETS family members can also be fused to proteins other than *TMPRSS2*, but represent a tiny fraction of all fusion proteins [29–32]. ERG immunohistochemistry has approximately 85%

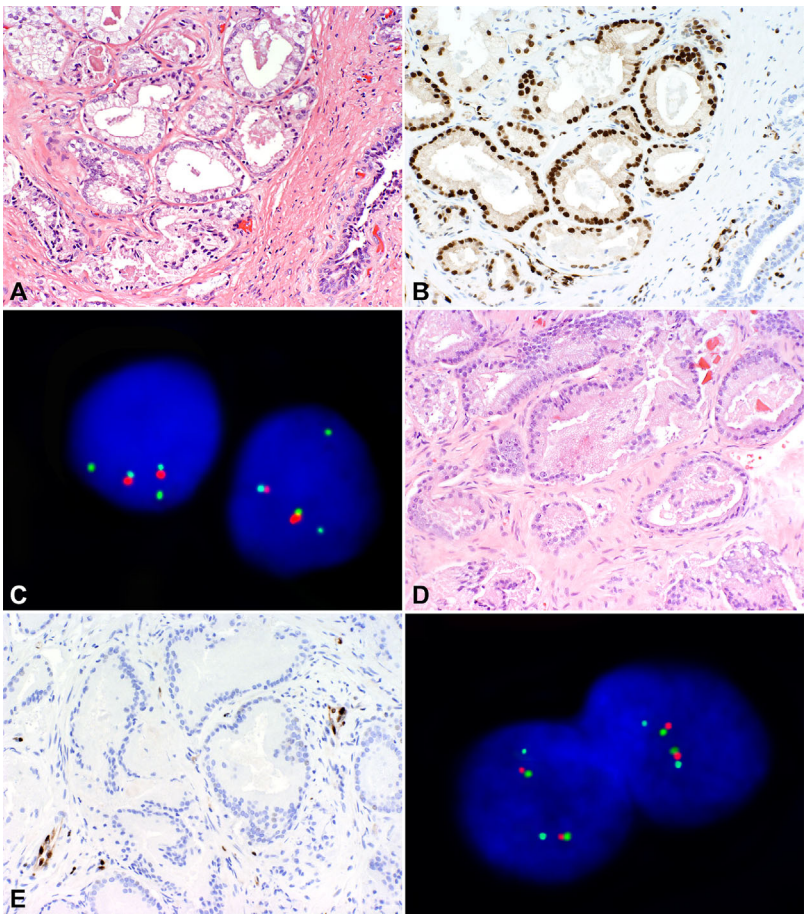

Figure 1. Histological and ERG status by immunohistochemistry and *ERG* FISH in T1a prostate cancer: Only a single example (1 of 54, 2%) of T1a prostate cancer (A) exhibited ERG protein expression by immunohistochemistry (B), and only 2 of 54 (4%) demonstrated *ERG* rearrangement by FISH (C). In each nucleus, one red–green–aqua signal triplet is closely juxtaposed, whereas, the other copy exhibits a widely separated green signal (C). In most T1a prostate cancers (D), neither ERG protein expression (E) nor *ERG* rearrangement was present (F), the latter evidenced by two copies of closely juxtaposed red–green–aqua signals.

sensitivity and specificity for *ERG* fusions confirmed by RT-PCR, and can be used in conjunction with *ERG* FISH to increase confidence in identifying *ERG* fusion positive tumors up to 98.5% [6,7,33,34]. It is possible in our sample population that a different ETS family member or different partner other than TMPRSS2 is involved in rearrangements in some cases; however, this is unlikely to add a substantive fraction of ETS family-rearranged tumors, since other partners have a much lower incidence than *ERG* and *TMPRSS2* was not aberrantly disrupted in any sample.

The best defined role for *TMPRSS2-ERG* fusions appears to be in the initiation of carcinogenesis, as it is found in early lesions and typically homogenously maintained within high grade tumors [22,35–37]. However, there are emerging molecular pathways to carcinogenesis that appear to be mutually exclusive to *ERG* fusions: (i) Speckle-type POZ protein (*SPOP*) is the most commonly mutated gene in prostate cancer and acts as an E3 ubiquitin ligase adaptor that directly binds target proteins, and promotes their cullin 3-dependent ubiquitination and proteolysis [38,39]. Mutations in *SPOP* occur in the substrate binding domain and prevent the interaction with target proteins leading to increased levels of oncogenic steroid receptor co-activator-3 (SRC-3/AIB1), and the androgen receptor [40–42]; (ii) Chromodomain helicase DNA-binding protein 1 (*CHD1*) is a tumor suppressor located at 5q21 that is inactivated, mainly through deletion, in 13–26% of prostate cancers [43–45]. The loss of CHD1 inhibits AR-dependent signaling, which is required for *TMPRSS2-ETS* family gene rearrangements, so CHD1 inactivation and *TMPRSS2*-ETS family gene rearrangements are seldom identified in the same tumor [46,47]. Inactivation of CHD1 forces the developing cancer into a pathway of carcinogenesis that does not require *TMPRSS2*-ETS family rearrangements. Deletion of *CHD1* alone in cell line models of prostate cancer was insufficient to cause invasive carcinoma, and the additional genetic

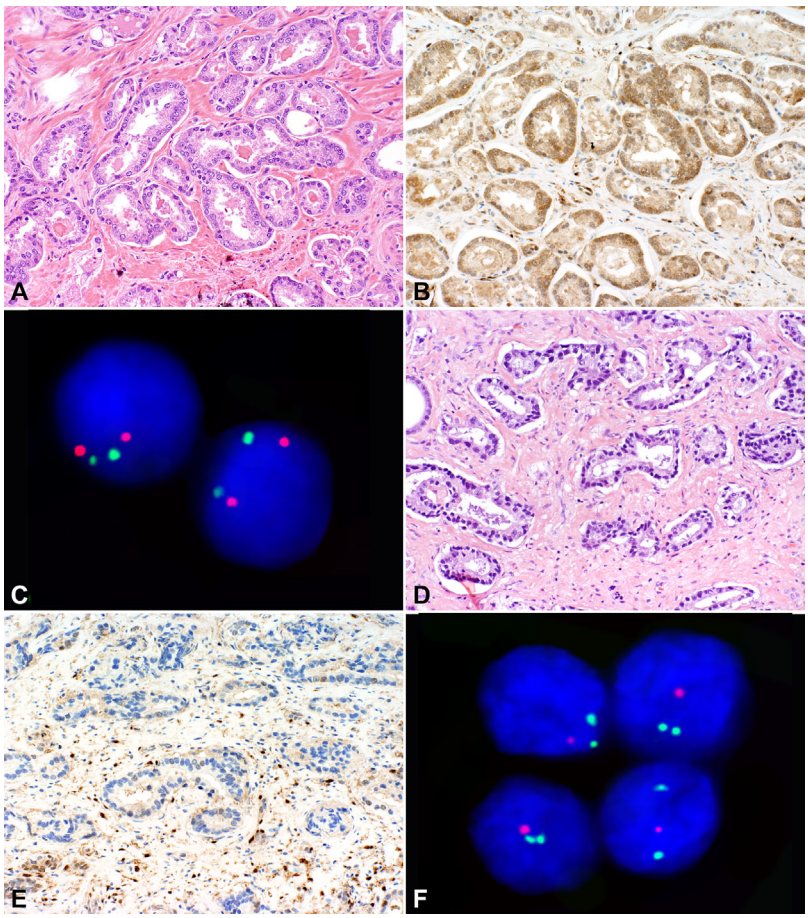

Figure 2. PTEN loss in a subset of T1a prostate cancers: Among the 54 T1a prostate cancers (A), 76% exhibited normal PTEN protein expression (B), and normal *PTEN* copy number (C) as indicated by two red (*PTEN*) and two green (CEP10) signals. A subset of 24% of T1a prostate cancers (D) showed loss of PTEN expression (E), which correlated with hemizygous PTEN deletion as indicated by the loss of one red signal (*PTEN*) and two normal green signals (CEP10).

events required for malignancy remain undetermined [44]; and (iii) Serine peptidase inhibitor, Kalal type 1 (SPINK1) is overexpressed in approximately 6% of all prostate cancers and 10% of *TMPRSS2*-ETS family gene fusion negative cancers [48,49]. SPINK1 overexpression appears largely to be mutually exclusive with *ERG* fusion, and is highly associated with 6q15 and 5q21 deletions, suggesting it represents a unique pathway to carcinogenesis [49]. SPINK1 overexpressing tumors as sensitive to inhibition of EGFR and may be clinically amenable to targeted inhibition of EGFR [50,51].

PTEN inactivation is relatively common compared to ERG rearrangement in T1a prostate cancers. The low prevalence of *TMPRSS2-ERG* gene fusion positive cancers in our study suggests that the alternative molecular pathways to carcinogenesis may play a crucial role in T1a cancers. Further study is needed to define the role of these alternate pathways to tumorigenesis, and assess their role in prognosis and targeted treatment regimens.

Table 2. Immunohistochemical and FISH Assessment of PTEN Status

PTEN status	IHC +	IHC −	Total
Deletion −	41	4	45
Deletion +	0	9	9
Total	41	13	54

IHC, immunohistochemistry.

REFERENCES

1. Bostwick D, Cheng L. Urologic surgical pathology. 3rd edition. Philadelphia, USA: Elsevier; 2014.
2. Andreoiu M, Cheng L. Multifocal prostate cancer: Biologic, prognostic, and therapeutic implications. Hum Pathol 2010; 41:781–793.
3. Pavelić J, Zeljko Z, Bosnar MH. Molecular genetic aspects of prostate transition zone lesions. Urology 2003;62:607–613.
4. Lee JJ, Thomas IC, Nolley R, Ferrari M, Brooks JD, Leppert JT. Biologic differences between peripheral and transition zone prostate cancer. Prostate 2015;75:183–190.

5. Noguchi M, Stamey TA, Neal JEM, Yemoto CEM. An analysis of 148 consecutive transition zone cancers: Clinical and histological characteristics. J Urol 2000;163:1751–1755.
6. Murphy SJ, Karnes RJ, Kosari F, et al. Integrated analysis of the genomic instability of PTEN in clinically insignificant and significant prostate cancer. Mod Pathol 2016;29:143–156.
7. Minner S, Enodien M, Sirma H, et al. ERG status is unrelated to PSA recurrence in radically operated prostate cancer in the absence of antihormonal therapy. Clin Cancer Res 2011;17: 5878–5888.
8. Bismar TA, Yoshimoto M, Vollmer RT, et al. PTEN genomic deletion is an early event associated with ERG gene rearrangements in prostate cancer. BJU Int 2011;107: 477–485.
9. Krohn A, Diedler T, Burkhardt L, et al. Genomic deletion of PTEN is associated with tumor progression and early PSA recurrence in ERG fusion-positive and fusion-negative prostate cancer. Am J Pathol 2012;181:401–412.
10. Troyer DA, Jamaspishvili T, Wei W, et al. A multicenter study shows PTEN deletion is strongly associated with seminal vesicle involvement and extracapsular extension in localized prostate cancer. Prostate 2015;75:1206–1215.
11. Zong Y, Xin L, Goldstein AS, Lawson DA, Teitell MA, Witte ON. ETS family transcription factors collaborate with alternative signaling pathways to induce carcinoma from adult murine prostate cells. Proc Natl Acad Sci USA 2009;106: 12465–12470.
12. Yoshimoto M, Ding K, Sweet JM, et al. PTEN losses exhibit heterogeneity in multifocal prostatic adenocarcinoma and are associated with higher Gleason grade. Mod Pathol 2013;26: 435–447.
13. Sircar K, Yoshimoto M, Monzon FA, et al. PTEN genomic deletion is associated with p-Akt and AR signalling in poorer outcome, hormone refractory prostate cancer. J Pathol 2009;218:505–513.
14. Yoshimoto M, Joshua AM, Cunha IW, et al. Absence of TMPRSS2:ERG fusions and PTEN losses in prostate cancer is associated with a favorable outcome. Mod Pathol 2008;21: 1451–1460.
15. Cheng L, Montironi R, Bostwick DG, Lopez-Beltran A, Berney DM. Staging of prostate cancer. Histopathology 2012;60: 87–117.
16. Cheng L, Bergstralh EJ, Scherer BG, et al. Predictors of cancer progression in T1a prostate adenocarcinoma. Cancer 1999;85:1300–1304.
17. Cheng L, Neumann RM, Blute ML, Zincke H, Bostwick DG. Long-term follow-up of untreated stage T1a prostate cancer. J Natl Cancer Inst 1998;90:1105–1107.
18. Lotan TL, Gurel B, Sutcliffe S, et al. PTEN protein loss by immunostaining: Analytic validation and prognostic indicator for a high risk surgical cohort of prostate cancer patients. Clin Cancer Res 2011;17:6563–6573.
19. Ahearn TU, Pettersson A, Ebot EM, et al. A prospective investigation of PTEN loss and ERG expression in lethal prostate cancer. J Natl Cancer Inst 2016;108:1–9.
20. Ferraldeschi R, Nava Rodrigues D, Riisnaes, et al. PTEN protein loss and clinical outcome from castration-resistant prostate cancer treated with abiraterone acetate. Eur Urol 2015;67: 795–802.
21. Lotan TL, Wei W, Ludkovski O, et al. Analytic validation of a clinical-grade PTEN immunohistochemistry assay in prostate cancer by comparison with PTEN FISH. Mod Pathol 2016;29:904–914.
22. Williamson SR, Zhang S, Yao JL, et al. ERG-TMPRSS2 rearrangement is shared by concurrent prostatic adenocarcinoma and prostatic small cell carcinoma and absent in small cell carcinoma of the urinary bladder: Evidence supporting monoclonal origin. Mod Pathol 2011;24:1120–1127.
23. Schelling LA, Williamson SR, Zhang S, et al. Frequent TMPRSS2-ERG rearrangement in prostatic small cell carcinoma detected by fluorescence in situ hybridization: The superiority of fluorescence in situ hybridization over ERG immunohistochemistry. Hum Pathol 2013;44:2227–2233.
24. Yoshimoto M, Cunha IW, Coudry RA, et al. FISH analysis of 107 prostate cancers shows that PTEN genomic deletion is associated with poor clinical outcome. Br J Cancer 2007; 97:678–685.
25. Korshunov A, Sycheva R, Gorelyshev S, Golanov A. Clinical utility of fluorescence in situ hybridization (FISH) in non-brainstem glioblastomas of childhood. Mod Pathol 2005;18: 1258–1263.
26. Cheng L, Davidson DD, Maclennan GT, et al. Atypical adenomatous hyperplasia of prostate lacks TMPRSS2-ERG gene fusion. Am J Surg Pathol 2013;37:1550–1554.
27. Hollenhorst PC, McIntosh LP, Graves BJ. Genomic and biochemical insights into the specificity of ETS transcription factors. Annu Rev Biochem 2011;80:437–471.
28. Tu JJ, Rohan S, Kao J, Kitabayashi N, Mathew S, Chen YT. 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;20:921–928.
29. Feng FY, Brenner JC, Hussain M, Chinnaiyan AM. Molecular pathways: Targeting ETS gene fusions in cancer. Clin Cancer Res 2014;20:4442–4448.
30. Hernandez S, Font-Tello A, Juanpere N, et al. Concurrent TMPRSS2-ERG and SLC45A3-ERG rearrangements plus PTEN loss are not found in low grade prostate cancer and define an aggressive tumor subset. Prostate 2016;76:854–865.
31. Esgueva R, Perner S, J LaFargue C, et al. Prevalence of TMPRSS2-ERG and SLC45A3-ERG gene fusions in a large prostatectomy cohort. Mod Pathol 2010;23:539–546.
32. Winnes M, Lissbrant E, Damber JE, Stenman G. Molecular genetic analyses of the TMPRSS2-ERG and TMPRSS2-ETV1 gene fusions in 50 cases of prostate cancer. Oncol Rep 2007;17:1033–1036.
33. Eguchi FC, Faria EF, Scapulatempo Neto C, et al. The role of TMPRSS2:ERG in molecular stratification of PCa and its association with tumor aggressiveness: A study in Brazilian patients. Sci Rep 2014;4:5640.
34. Chaux A, Albadine R, Toubaji A, et al. Immunohistochemistry for ERG expression as a surrogate for TMPRSS2-ERG fusion detection in prostatic adenocarcinomas. Am J Surg Pathol 2011;35:1014–1020.
35. Zhang S, Pavlovitz B, Tull J, Wang Y, Deng FM, Fuller C. Detection of TMPRSS2 gene deletions and translocations in carcinoma, intraepithelial neoplasia, and normal epithelium of the prostate by direct fluorescence in situ hybridization. Diagn Mol Pathol 2010;19:151–156.
36. Dai MJ, Chen LL, Zheng YB, et al. [Frequency and transcript variant analysis of gene fusions between TMPRSS2 and ETS transcription factor genes in prostate cancer]. Zhonghua Yi Xue Za Zhi 2008;88:669–673.
37. Mehra R, Han B, Tomlins SA, et al. Heterogeneity of TMPRSS2 gene rearrangements in multifocal prostate adenocarcinoma: Molecular evidence for an independent group of diseases. Cancer Res 2007;67:7991–7995.
38. Barbieri CE, Baca SC, Lawrence MS, et al. Exome sequencing identifies recurrent SPOP, FOXA1 and MED12 mutations in prostate cancer. Nat Genet 2012;44:685–689.
39. Kwon JE, La M, Oh KH, et al. BTB domain-containing speckle-type POZ protein (SPOP) serves as an adaptor of Daxx for ubiquitination by Cul3-based ubiquitin ligase. J Biol Chem 2006;281:12664–12672.
40. Geng C, He B, Xu L, et al. Prostate cancer-associated mutations in speckle-type POZ protein (SPOP) regulate steroid receptor coactivator 3 protein turnover. Proc Natl Acad Sci USA 2013;110:6997–7002.
41. Li C, Ao J, Fu J, 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: 4350–4364.

42. An J, Wang C, Deng Y, Yu L, Huang H. Destruction of full-length androgen receptor by wild-type SPOP, but not prostate-cancer-associated mutants. Cell Rep 2014;6: 657–669.
43. Grasso CS, Wu YM, Robinson DR, et al. The mutational landscape of lethal castration-resistant prostate cancer. Nature 2012;487:239–243.
44. Liu W, Lindberg J, Sui G, et al. Identification of novel CHD1-associated collaborative alterations of genomic structure and functional assessment of CHD1 in prostate cancer. Oncogene 2012;31:3939–3948.
45. Huang S, Gulzar ZG, Salari K, Lapointe J, Brooks JD, Pollack JR. Recurrent deletion of CHD1 in prostate cancer with relevance to cell invasiveness. Oncogene 2012;31:4164–4170.
46. Burkhardt L, Fuchs S, Krohn A, et al. CHD1 is a 5q21 tumor suppressor required for ERG rearrangement in prostate cancer. Cancer Res 2013;73:2795–2805.
47. Demichelis F, Setlur SR, Beroukhim R, et al. Distinct genomic aberrations associated with ERG rearranged prostate cancer. Genes Chromosomes Cancer 2009;48:366–380.
48. Tomlins SA, Rhodes DR, Yu J, et al. The role of SPINK1 in ETS rearrangement-negative prostate cancers. Cancer Cell 2008; 13:519–528.
49. Grupp K, Diebel F, Sirma H, et al. SPINK1 expression is tightly linked to 6q15- and 5q21-deleted ERG-fusion negative prostate cancers but unrelated to PSA recurrence. Prostate 2013;73:1690–1698.
50. Ateeq B, Tomlins SA, Laxman B, et al. Therapeutic targeting of SPINK1-positive prostate cancer. Sci Transl Med 2011;3: 72ra17.
51. Wang C, Wang L, Su B, et al. Serine protease inhibitor Kazal type 1 promotes epithelial-mesenchymal transition through EGFR signaling pathway in prostate cancer. Prostate 2014; 74:689–701.