Am J Transl Res 2009;1(4):341-351
www.ajtr.org/AJTR905002

Review Article

ETS gene fusions and prostate cancer

Wei Huang, Michelle Waknitz

Department of Pathology and Laboratory Medicine, University of Wisconsin-Madison, 600 Highland Avenue, Madison WI 53792, USA

Received May 10, 2009; accepted May 20, 2009; available online May 25, 2009

Abstract: Chromosomal rearrangements are common genetic alterations in solid tumors and hematologic neoplasias. Recently, gene fusions between erythroblastosis virus E26 transforming sequence (ETS) family of transcription factors and androgen-regulated, prostate-specific TMPRSS2 gene were detected in about 50% of prostate cancers. Further studies have shown a diversity of TMPRSS2:ETS hybrid transcripts and heterogeneity of the fusion genes in multifocal prostate cancer. The role of these gene fusions in prostate carcinogenesis, the protein products associated with the variant fusion transcripts and their association with tumor morphology, stage, and clinical outcomes have also been studied. Additional data have demonstrated ETS gene fusions as a potential biomarker for diagnosing and stratifying prostate cancer patients. The following summarizes these recent advances.

Key words: Prostate cancer, ETS, TMPRSS2, gene fusion

Introduction

Prostate cancer is the most common age-related cancer for men in the U.S. and after lung cancer, it is the leading cause of cancer-related deaths among men in the U.S. [10]. The key molecular events involving initiation and progression of prostate cancer remain largely unknown. Many molecular alterations in prostate cancer have been discovered in recent years due to increasingly sophisticated molecular technologies. A few gene products such as AMACR [12, 13], Prostate Cancer Antigen 3 (PCA3) [15, 16] and GSTP1 [19-21] have shown promising value in clinical application for prostate cancer screening and diagnosis; whereas many molecular alterations identified so far still lack substantial supporting evidence. A recent discovery of gene fusions involving E twenty-six (ETS) genes and androgen-regulated, prostate-specific TMPRSS2 gene in about 50% of prostate cancers [22], a triumphant example of bioinformatic analysis [1], demonstrates for the first time, recurrent chromosomal rearrangements in an epithelial carcinoma-prostate adenocarcinoma. The prevalence in population based-cohorts is 15% to 36 % [9, 23, 24] for unknown reasons. These findings not only were the first demonstration of recurrent chromosomal rearrangements in an epithelial carcinoma, but also make this the most prevalent gene fusion known in any solid tumor [26]. ETS fusions are less common in prostatic intraepithelial neoplasia (PIN), and they cooperate with PI3-kinase pathway to cause carcinogenesis [27].

The possibility that ETS gene fusions could play as an important role in prostate carcinogenesis as BCR-ABL fusion in chronic myelogenous leukemia has caused great excitement among prostate cancer researchers.

TMPRSS2 and ETS gene fusions are identified in about half of prostate adenocarcinomas

Applying a powerful bioinformatics tool- the Cancer Outlier Profile Analysis (COPA) to the Oncomine database, a compendium of 132 gene expression data sets representing 10,486 microarray experiments, Tomlins et al first identified several outlier profiles for genes

Table 1. Eighteen reported ETS gene fusion variants in human prostate with a fusion transcript present

5'-end	3'-end (ETS)	Androgen-regulated	Prostate-specific	references
TMPRSS2	**ERG**	**↑**	**yes**	**[1-8]**
ACSL3	ETV1	↑	yes	[9]
C15orf21	ETV1	↑	yes	[9]
CANT1	ETV1	↓	yes	[11]
EST14	ETV1	↑	yes	[14]
FLJ35294	ETV1	↑	yes	[11]
HERV-K_22q11.23	ETV1	↑	yes	[17]
HNRPA2B1	ETV1	unchanged	no	[18]
HERVK17	ETV1	↑	yes	[14]
SLC45A3	ETV1	↑	yes	[18]
TMPRSS2	ETV1	↑	yes	[1-3]
CANT1	ETV4	↑	yes	[25]
DDX5	ETV4	↑	yes	[11]
FLJ35294	ETV4	↑	yes	[11]
KLK2	ETV4	↑	yes	[28]
TMPRSS2	ETV4	↑	yes	[4, 25, 29]
SCLC45A3	ETV5	↑	yes	[17]
TMPRSS2	ETV5	↑	yes	[17]

in specific cancer types in which recurrent rearrangement or high-level amplification is known to occur [1]. In several independent data sets, COPA identified strong outlier profiles in prostate cancer for ETS-related gene (ERG) (21q22.3) and *ETV1* (7p21.2), two genes that encode ETS family transcriptional factors. In further analysis of the joint expression profiles of *ERG* and *ETV1* across several prostate cancer data sets, they found that ERG and ETV1 invariably showed mutually exclusive outlier profiles and overexpression in prostate cancer, supporting their hypothesis that ERG and ETV1 are similarly involved in prostate cancer development. To determine the mechanism responsible for ERG and ETV1 overexpression, they characterized the RNA from prostate cancer samples using real-time quantitative PCR (qPCR). qPCR consistently showed a loss of the 5' region of ERG or ETV1 for cases with marked overexpression of the 3' end. Next, RNA ligase mediated rapid amplification of cDNA ends (RLM-RACE) was used to characterize the 5' end of the ERG or ETV1 transcripts in such samples. Sequencing determined that the 5' end of ERG or ETV1 was consistently replaced with the 5' untranslated region of the prostate-specific gene, transmembrane protease, serine 2 (TMPRSS2; 21q22.2). The fusion between TMPRSS2 and EGR or ETV1 was subsequently confirmed in a separate group of clinically localized prostate cancer samples using qPCR. Furthermore, FISH analysis on a set of 29 prostate cancer cases selected independently of any known ERG or ETV1 expression, shows that 23 of 29 (79%) had TMPRSS2-ETV1 fusions or ERG rearrangement.

TMPRSS2:ETS fusions were then confirmed by other groups with reported frequencies from 15% to 77.8% in different cohorts [24, 26, 29, 30].

Gene fusion variants

Since the first report of gene fusions between TMPRSS2 and ERG or ETV1 in 2005, more gene fusion variants involving ETS family transcriptional factors, ERG (21q22.3), ETV1 (7p21.2), ETV4 (17q21.31) and ETV5 (3q28) have been reported (**Table 1**). Multiple groups have confirmed the largest proportion of fusions involve TMPRSS2-ERG (45-70%) [6, 29, 31-33].

Diversity of TMPRSS2-ERG hybrid transcripts

TMPRSS2-ERG gene fusions have recently been reported to be present in a high proportion of human prostate cancers. Clark et al first reported considerable diversity in the precise structure of TMPRSS2-ERG hybrid transcripts found in human prostates [34]. In their study, fourteen distinct hybrid transcripts were characterized, each containing different

combinations of sequences from the TMPRSS2 and ERG genes. The transcripts include two that are predicted to encode a normal full-length ERG protein, six that encode N-terminal truncated ERG proteins and one that encodes a TMPRSS2-ERG fusion protein. Interestingly, distinct patterns of hybrid transcripts were found in samples taken from separate regions of individual cancer-containing prostates, suggesting that TMPRSS2-ERG gene fusions may develop independently in different regions of a single prostate.

Wang et al recently characterized in detail the expression of TMPRSS2-ERG fusion mRNAs and correlated the isoforms and expression levels with clinical outcome in cancers from men undergoing radical prostatectomy [6]. They found that there was significant variation in the alternatively spliced isoforms expressed in different cancers. Expression of an isoform in which the native ATG in exon 2 of the TMPRSS2 gene is in frame with exon 4 of the ERG gene, was associated with clinical and pathologic variables of aggressive disease. Expression of other isoforms, in which the native ERG ATG in exon 3 was the first in-frame ATG, was associated with seminal vesicle invasion, and was correlated with poor outcome following radical prostatectomy. Cancers not expressing these isoforms tended to express higher levels of fusion mRNAs, and in this group, higher expression levels of fusion mRNA were present in cancers with early prostate-specific antigen recurrence. Thus, both the isoform of TMPRSS2-ERG expressed and its expression level may affect prostate cancer progression.

Fusion proteins and their functions

TMPRSS2-ERG gene fusions have been extensively studied in prostate cancer. However, virtually nothing is known about the nature of full-length transcripts and encoded proteins. Hu et al studied qualitative and quantitative features of full-length TMPRSS2-ERG transcripts in prostate cancer [35]. In their study, full-length TMPRSS2-ERG transcripts were cloned and sequenced from a cDNA library generated from pooled RNA of six TMPRSS2-ERG fusion-positive prostate tumors. The encoded ERG proteins were analyzed in HEK293 cells. Copy numbers of TMPRSS2-ERG splice variants were determined by RT-PCR in laser capture microdissected prostate cancer cells. Two types of TMPRSS2-ERG cDNAs were identified: type I, which encodes full-length prototypical ERG protein (ERG1, ERG2, ERG3), and type II, encoding truncated ERG proteins lacking the ETS domain (ERG8 and a new variant, TEPC). In microdissected prostate tumor cells from 122 patients, relative abundance of these variants was in the following order: ERG8 > TEPC > ERG 3 > ERG1/2 with combined overexpression rate of 62.3% in prostate cancer. Increased ratio of type I over type II splice forms correlated with a less favorable pathology and outcome. These findings may enhance the utility of ERG as a biomarker and therapeutic target in prostate cancer, and perhaps may also explain some of the reported variation of TMPRSS2-ERG frequencies and associated outcomes in different cohorts.

TMPRSS2-ERG gene fusions found in the majority of prostate cancers show significant heterogeneity in the 5' region of the alternatively spliced fusion gene transcripts. However, Wang et al found that there was also significant heterogeneity within the coding exons as well [36]. There is variable inclusion of a 72-bp exon and other novel alternatively spliced isoforms. To assess the biological significance of these alternatively spliced transcripts, Wang's group expressed various transcripts in primary prostatic epithelial cells (PrEC) and in an immortalized PrEC line, PNT1a. The fusion gene transcripts promoted proliferation, invasion, and motility with variable activities that depended on the structure of the 5' region encoding the TMPRSS2-ERG fusion and the presence of the 72-bp exon. Cotransfection of different isoforms further enhanced biological activity, mimicking the situation in vivo, in which multiple isoforms are expressed. Finally, knockdown of the fusion gene in VCaP cells resulted in inhibition of proliferation in vitro and inhibition of tumor progression in an in vivo orthotopic mice model. Their results indicate that TMPRSS2-ERG fusion isoforms have variable biological activities promoting tumor initiation and progression and are consistent with some previous clinical observations indicating that certain TMPRSS2-ERG fusion isoforms are significantly correlated with more aggressive disease.

Heterogeneity of gene fusions in multifocal prostate adenocarcinoma

Prostate cancer is a multifocal disease, and the origins as well as molecular mechanisms of multiple cancer foci remain elusive with respect to prostate cancer initiation or progression. As TMPRSS2 is a common 5' partner of ETS gene fusions, Mehra et al recently mapped TMPRSS2 rearrangement by fluorescence in situ hybridization (FISH) to study the origin and molecular basis of multifocal prostate cancer heterogeneity [37]. TMPRSS2 rearrangement was evaluated by FISH on a tissue microarray representing 93 multifocal prostate cancers from 43 radical prostatectomy resections. They found 70% (30 of 43) of the cases showed TMPRSS2 rearrangement, including 63% through deletion (loss of the 3' TMPRSS2 signal), 27% through translocation (split of 5' and 3' TMPRSS2 signals), and 10% through both mechanisms in different tumor foci. Of the 30 TMPRSS2 rearrangements, 30% showed concordance in all tumor foci, whereas 70% were discordant in at least one focus. In TMPRSS2 gene rearrangement cases, the largest (index) tumor showed rearrangement 83% of the time. Pathologic stage, size, or Gleason grade of the multifocal prostate cancer did not correlate with overall TMPRSS2 rearrangement. These results suggest that multifocal prostate cancer is a heterogeneous group of diseases arising from multiple, independent clonal expansions.

Clark et al also found distinct patterns of hybrid transcripts in samples taken from separate regions of individual cancer-containing prostates while studying the diversity of TMPRSS2-ERG hybrid transcripts [34]. In another study, Clark et al also reported that in cancers containing ERG alterations, the observed pattern of changes was often complex. Different categories of ERG gene alteration were found either together in a single cancerous region or within separate foci of cancer in the same prostate slice. In some cases the juxtaposition of particular patterns of ERG alterations suggested possible mechanisms of tumor progression. Prostate cancers harboring ERG alterations commonly contained cancerous foci that lacked rearrangements of the ERG gene. A single trans-urethral resection of a prostate specimen examined harbored both ERG and ETV1 gene rearrangements demonstrating that the observed complexity may, at least in part, be explained by multiple ETS gene alterations arising independently in a single prostate [38].

Similarly, Barry et al reported heterogeneity of TMPRSS2-ERG fusion in multifocal prostate cancer. From 80 consecutive radical prostatectomy specimens, they identified 32 cases with multiple spatially separate tumors. They assessed two to three tumor foci for TMPRSS2-ERG fusion using an ERG break-apart interphase fluorescence in situ hybridization assay in each case. They found that individual tumor foci showed homogeneity for fusion status (intrafocal clonal homogeneity). However, between foci, heterogeneity for fusion status was seen in 13 (41%) of the 32 cases. Of these 13, 5 (38%) had foci of carcinoma with no fusion and foci with fusion by deletion, 4 (31%) had foci with no fusion and foci with fusion through insertion, 2 (15%) had foci with fusion through insertion and foci with fusion through deletion, and 2 (15%) showed three distinct foci of carcinoma showing no fusion, fusion through insertion, and fusion through deletion [8].

Similar findings were reported by Furusato et al.[39]. Their study revealed there were two types of TMPRSS2-ERG fusions, type A and type C, in which TMPRSS2 fuses to exons 4 or 5 respectively, of ERG. Quantitative expression of TMPRSS2-ERG fusion type A and C_transcripts was analyzed in benign, tumor and PIN areas, selected from whole-mount radical prostatectomy slides. TMPRSS2-ERG expression was correlated with clinicopathological features. Overall, 30 of 45 (67%) patients exhibited TMPRSS2-ERG fusion transcripts in at least one tumor focus. Of the 80 tumor foci analyzed, 39 had TMPRSS2-ERG fusion (type A only: 30, type C only: 2, both types A and C: 7), with predominant detection of the TMPRSS2-ERG fusion type A (27/30, 90%) in the index tumors. Of 14 PIN lesions, 2 were positive for type A fusion. Frequent presence of the TMPRSS2-ERG in index tumors suggests critical roles of ERG alterations in the onset and progression of a large subset of prostate cancer. These results showed that the fusion type A, but not C, in benign glands or PIN may give rise to carcinoma, according to Furusato.

Association with cancer morphology

Mosquera et al first conducted a study to assess the association between TMPRSS2 –

ERG gene fusions and morphological phenotype of prostate cancer[31]. TMPRSS2-ERG fusion status was studied in 227 prostate cancer cases using an ERG break-apart FISH assay. The 227 cases of clinically localized prostate cancer were from five hospital-based radical prostatectomy cohorts. The prostate cancer samples were embedded in five tissue microarrays (TMAs). One to 12, 0.6 mm in diameter TMA biopsy cores (median 3) were randomly taken from the dominant tumor nodule. Inclusion in this study required at least one assessable TMA histospot in step sections for the hematoxylin and eosin (H&E) and fluorescence *in situ* hybridization (FISH) slides. Morphological features were evaluated while blinded to the *TMPRSS2-ERG* fusion status. Two reviewers assessed each tumor for presence or absence of eight morphological features. Five morphological features were found associated with TMPRSS2-ERG fusion prostate cancer: blue-tinged mucin, cribriform growth pattern, macronucleoli, intraductal tumor spread, and signet-ring cell features, all with p-values < 0.05. Only 24% (n=30/125) of tumors without any of these features displayed the TMPRSS2-ERG fusion. By comparison, 55% (n=38/69) of cases with one feature (RR=3.88), 86% (n=38/44) of cases with two features (RR=20.06), and 93% (n=14/15) of cases with three or more features (RR=44.33) were fusion positive (p<0.001). These findings suggest a significant link between a molecular alteration in prostate cancer and distinct phenotypic features. The authors postulate that the biological effect of TMPRSS2-ERG overexpression may drive pathways that favor these common morphological features, and these features may be helpful in diagnosing TMPRSS2-ERG fusion prostate cancer, which may have both prognostic and therapeutic implications.

Rajput et al reported the frequency of rearrangements involving TMPRSS2, ERG, or ETV1 genes in prostate cancer of varying Gleason grades through fluorescence in situ hybridization (FISH) on prostate cancer tissue microarrays (TMAs) [40]. FISH positive cases were confirmed by RT-PCR and DNA sequence analysis. A total of 106/196 (54.1%) cases were analyzed by FISH. None of the five benign prostatic hyperplasia cases analyzed exhibited these gene rearrangements. TMPRSS2-ERG fusion was found more frequently in moderate to poorly differentiated tumors (35/86, 40.7%) than in well differentiated tumors (1/15, 6.7%, p = 0.017). TMPRSS2-ETV1 gene fusions were not detected in any of the cases tested.

While studying the incidence, pathological features and clinical parameters of TMPRSS2-ERG gene fusion in a cohort of 196 Canadian men treated by radical prostatectomy for localized prostate cancer, Darnel et al found that 41% of the patients showed positive gene fusion status in their prostate cancer [41]. Using break-apart FISH assay to indirectly assess the fusion of TMPRSS2-ERG, they found that the TMPRSS2-ERG gene fusion status was homogenous within a single cancer focus and 82% of fusion positive prostate cancer was present in Gleason score (GS) 6 or 7 vs. 14% in GS 8 (p = 0.004). Moreover, TMPRSS2-ERG fusion was present in 42% of Gleason pattern 3 vs. 27% of Gleason pattern 4 (p = 0.014).

Some studies have also found clonal ERG rearrangements both in high grade prostatic intraepithelial neoplasia (PIN) and in atypical in situ epithelial lesions consistent with the diagnosis of low grade PIN [27]. It has been postulated that that ERG gene alterations represent an initiating event that promotes clonal expansion to form regions of epithelial atypia. The complex patterns of ERG alteration found in prostatectomy specimens have important implications for the design of experiments investigating the clinical significance and mechanism of development in individual prostate cancers [2, 38, 42, 43].

Association with clinical outcomes

The impact of ETS gene fusions on prostate cancer stage and clinical outcomes has been studied recently by several groups. The results seem inconsistent. In six studies, they were unfavorable, but in two there was no association and in two they were favorable.

Unfavorable outcomes

Wang et al [6] found that 59% of clinically localized prostate cancers express the TMPRSS2/ERG fusion gene, confirming the initial observations of high frequency expression of this fusion mRNA in prostate cancer. There was significant variation in the alternatively spliced isoforms expressed in different cancers. As noted earlier, expression of an isoform, in which the native ATG in exon 2 of the TMPRSS2 gene is in frame with exon

4 of the ERG gene, was associated with clinical and pathologic variables of aggressive disease. Expression of other isoforms, in which the native ERG ATG in exon 3 was the first in-frame ATG, was associated with seminal vesicle invasion, and is correlated with poor outcome following radical prostatectomy. Cancers not expressing these isoforms tended to express higher levels of fusion mRNAs, and in this group, higher expression levels of fusion mRNA were present in cancers with early prostate-specific antigen recurrence. They concluded both the isoforms of TMPRSS2:ERG fusions expressed and expression level may affect prostate cancer progression.

Demichelis et al studied a population-based cohort of men with localized prostate cancers followed by expectant (watchful waiting) therapy [24]. They identified a statistically significant association between TMPRSS2:ERG fusion and prostate cancer specific death (P<0.01). qRT-PCR demonstrated high ETS-related gene (ERG) expression to be associated with TMPRSS2:ERG fusion (P<0.005) although the association was not significant when adjusted for Gleason score. These data suggest that TMPRSS2:ERG fusion prostate cancers may have a more aggressive phenotype, possibly mediated through increased ERG expression.

Nam et al [44] tested for the presence of the TMPRSS2:ERG gene fusion product among 26 patients who underwent surgery for clinically localized prostate cancer using RT-PCR and direct DNA sequencing, and evaluated its prognostic significance. All 26 patients had cancers of the same histologic grade (Gleason score 7). The fusion protein was present within prostate cancer tumor cells in eleven patients (42.3%). Nine patients experienced biochemical disease relapse (elevated PSA) after a mean follow-up of 12 months (range 1 to 48 months). Patients with the fusion protein had a significantly higher rate of recurrence (5-year recurrence rate 79.5%) compared to patients who lacked the fusion protein (five-year recurrence rate 37.5%, p = 0.009). In multivariate analysis, the presence of gene fusion was the single most important prognostic factor.

In a larger cohort study using a similar approach, Nam et al [30] examined prostate cancer specimens from 165 patients who underwent surgery for clinically localized prostate cancer between 1998 and 2006. They found that the fusion gene was expressed within the prostate cancer cells in 81 of 165 (49.1%) patients. Of the 165 patients, 43 (26.1%) developed prostate-specific antigen (PSA) relapse after a mean follow-up of 28 months. The subgroup of patients with the fusion protein had a significantly higher risk of recurrence (58.4% at 5 years) than did patients who lacked the fusion protein (8.1%, P<0.0001). In a multivariable analysis, the presence of gene fusion was the single most important prognostic factor; the adjusted hazard ratio for disease recurrence for patients with the fusion protein was 8.6 (95% CI=3.6-20.6, P<0.0001) compared to patients without the fusion protein. They concluded that among prostate cancer patients treated with surgery, the expression of TMPRSS2-ERG fusion gene is a strong prognostic factor and is independent of grade, stage and PSA level.

Attard et al [45] reported that cancers lacking ERG alterations exhibited favorable cause-specific survival (90% survival at 8 years). However, they identified a novel category of prostate cancers, characterized by duplication of the fusion of TMPRSS2 to ERG sequences together with interstitial deletion of sequences 5' to ERG (called '2+Edel'), which by comparison exhibited extremely poor cause-specific survival (hazard ratio=6.10, 95% confidence ratio=3.33-11.15, P<0.001, 25% survival at 8 years). In multivariate analysis, '2+Edel' provided significant prognostic information (P=0.003) in addition to that provided by Gleason score and prostate-specific antigen level at diagnosis. Other individual categories of ERG alteration were associated with intermediate or good prognosis. They conclude that determination of ERG gene status, including duplication of the fusion of TMPRSS2 to ERG sequences in 2+Edel, allows stratification of prostate cancer into distinct survival categories.

Using fluorescence *in situ* hybridization (FISH), Perner et al [5] identified the TMPRSS2:ERG rearrangements in 49.2% of 118 primary prostate cancers and 41.2% of 18 hormone-naive lymph node metastases. The FISH assay detected intronic deletions between ERG and TMPRSS2 resulting in TMPRSS2:ERG fusion in 60.3% (35 of 58) of the primary TMPRSS2:ERG prostate cancers and 42.9% (3 of 7) of the TMPRSS2:ERG hormone-naive lymph node

metastases. A significant association was observed between TMPRSS2:ERG rearranged tumors through deletions and higher tumor stage and the presence of metastatic disease involving pelvic lymph nodes. Using 100K oligonucleotide single nucleotide polymorphism arrays, a homogeneous deletion site between *ERG* and *TMPRSS2* on chromosome 21q22.2-3 was identified with two distinct subclasses distinguished by the start point of the deletion at either 38.765 or 38.911 Mb. The deletion as cause of TMPRSS2:ERG fusion is associated with clinical features for prostate cancer progression compared with tumors that lack the TMPRSS2:ERG rearrangement.

No association

In a recent study by Mehra et al [37], TMPRSS2 rearrangement was evaluated by FISH on a tissue microarray representing 93 multifocal prostate cancers from 43 radical prostatectomy resections. 70% (30 of 43) of the cases showed TMPRSS2 rearrangement, including 63% through deletion (loss of the 3' TMPRSS2 signal), 27% through translocation (split of 5' and 3' TMPRSS2 signals), and 10% through both mechanisms in different tumor foci. Of the 30 TMPRSS2 rearranged cases, 30% showed concordance in all tumor foci, whereas 70% were discordant in at least one focus. In TMPRSS2 rearranged cases, the largest (index) tumor was rearranged 83% of the time. However, pathologic stage, size, or Gleason grade of the multifocal prostate cancer did not correlate with overall TMPRSS2 rearrangement.

Rouzier et al [33] retrospectively analyzed a large series of formalin-fixed, paraffin-embedded samples including 55 prostate carcinomas and 11 benign prostate tumors. They identified the fusion gene TMPRSS2-ERG by RT-PCR in 40/55 carcinomas (72%). Their study demonstrates that the detection of ETS fusion gene by RT-PCR is feasible on formalin-fixed and paraffin-embedded samples, but no significant association between the presence of the fusion gene and any clinical feature, such as preoperative serum prostate-specific antigen (PSA) level (PSA>20 or PSA< or =20), pTNM stage including capsule invasion, seminal vesicle invasion, and lymph nodes metastases, or recurrence was observed in this series.

Favorable outcomes

Winnes et al [46] analyzed a series of 50 prostate cancer samples for expression of TPRSS2-ERG and TMPRSS2-ETV1 fusion transcripts. RT-PCR analysis revealed TMPRSS2-ERG fusion transcripts in 18 of the 50 tumors (36%). None of the tumors expressed a TMPRSS2-ETV1 fusion. However, the frequency of ERG-fusions in the present study is somewhat lower than previously observed, indicating heterogeneity with regard to expression of ETS-gene fusions in subsets of prostate cancers. Moreover, clinical follow-up studies showed a clear tendency that fusion-positive tumors were associated with lower Gleason grade and better survival than fusion-negative tumors.

Saramaki et al [47] assessed the presence of the TMPRSS2:ERG rearrangement by RT-PCR and FISH in 19 prostate cancer xenografts and 7 prostate cancer cell lines. The expression of ERG was studied in the xenografts and cell lines and in 49 freshly frozen clinical prostate samples by qRT-PCR. The frequency of the TMPRSS2-ERG fusion in clinical prostate cancer (n = 253) on tissue microarrays was assessed by three-color fluorescence in situ hybridization. They found that seven of 19 (37%) of the xenografts overexpressed ERG and had TMPRSS2-ERG rearrangement. Two xenografts, representing small cell carcinomas, also contained the fusion but did not express ERG. In clinical tumor specimens, the overexpression of ERG was associated with the rearrangement (P = 0.0019). Fifty of 150 (33%) of the prostatectomy specimens and 28 of 76 (37%) of the hormone-refractory prostate cancers on the tissue microarrays carried the TMPRSS2-ERG rearrangement. They found that TMPRSS2-ERG rearrangement was associated with longer progression-free survival in patients treated by prostatectomy (P = 0.019), and according to multivariate analysis, it was an independent predictor of favorable outcome (relative risk, 0.54; 95% confidence interval, 0.30-0.98). Also, the fusion was not associated with Gleason score, pT stage, diagnostic prostate-specific antigen, or cell proliferation activity in prostatectomy specimens or with the AR gene amplification in hormone-refractory tumors.

Implication and applications

The role of ETS gene fusions in prostate

carcinogenesis is not fully understood. The expression ERG is low or undetectable in benign prostate epithelial cells while the prevalence of ERG overexpression in prostate cancer cells due to TMPRSS2:ERG fusions is high. Most ERG fusion partners, including TMPRSS2, are androgen sensitive, and the fusions might be inhibited by targeting androgen signaling [48].

These findings suggest causal roles of ERG protein in prostate carcinogenesis. Evidence suggests that ETS gene fusions may be an initial trigger point causing ETS gene overexpression in early prostate cancer development [1, 24, 38, 49]. Many reports have shown that ETS gene fusions are associated with prostate cancer progression and clinical outcomes (discussed above). Based on supporting data, although still at early stage, ETS gene fusions may be used as biomarkers for early detection of prostate cancer in urine samples [50-53], for pretreatment risk stratification, or as therapeutic targets for drug development and treatment.

Summary

The data accumulated thus far have confirmed the importance of ETS gene fusions in prostate cancer development and progression. To date, 18 variants of ETS gene fusions have been reported. TMPRSS2-ERG is the most common fusion detected in prostate cancer (45-70%), with great diversity in the precise structure of the TMPRSS2-ERG hybrid transcript found in human prostates. Different TMPRSS2-ERG variants are detected in multifocal prostate cancer indicating clonal heterogeneity of this cancer, which may explain, in part, some of the variations in reported association with outcomes. Further population studies of gene fusion variants and clinical outcomes are needed to clarify this inconsistency, taking into careful consideration the multifocal, clonally heterogeneous nature of prostate cancer. Otherwise, sampling bias can obscure test results. The roles of ETS gene fusions in prostate carcinogenesis, the ETS gene fusion transcripts and their protein functions also need to be further delineated. Nonetheless, ETS gene fusions seem promising as biomarkers for prostate cancer detection.

Please address correspondences to: Wei Huang, MD, Department of Pathology and Laboratory Medicine, University of Wisconsin-Madison, 600 Highland Avenue, Madison WI 53792, USA, E-mail: whuang23@wisc.edu

References

[1] Tomlins SA, Rhodes DR, Perner S, Dhanasekaran SM, Mehra R, Sun XW, Varambally S, Cao X, Tchinda J, Kuefer R, Lee C, Montie JE, Shah RB, Pienta KJ, Rubin MA and Chinnaiyan AM. Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. Science 2005; 310: 644-648.

[2] Cerveira N, Ribeiro FR, Peixoto A, Costa V, Henrique R, Jeronimo C and Teixeira MR. TMPRSS2-ERG gene fusion causing ERG overexpression precedes chromosome copy number changes in prostate carcinomas and paired HGPIN lesions. Neoplasia 2006; 8: 826-832.

[3] Hermans KG, van Marion R, van Dekken H, Jenster G, van Weerden WM and Trapman J. TMPRSS2:ERG fusion by translocation or interstitial deletion is highly relevant in androgen-dependent prostate cancer, but is bypassed in late-stage androgen receptor-negative prostate cancer. Cancer Res 2006; 66: 10658-10663.

[4] Iljin K, Wolf M, Edgren H, Gupta S, Kilpinen S, Skotheim RI, Peltola M, Smit F, Verhaegh G, Schalken J, Nees M and Kallioniemi O. TMPRSS2 fusions with oncogenic ETS factors in prostate cancer involve unbalanced genomic rearrangements and are associated with HDAC1 and epigenetic reprogramming. Cancer Res 2006; 66: 10242-10246.

[5] Perner S, Demichelis F, Beroukhim R, Schmidt FH, Mosquera JM, Setlur S, Tchinda J, Tomlins SA, Hofer MD, Pienta KG, Kuefer R, Vessella R, Sun XW, Meyerson M, Lee C, Sellers WR, Chinnaiyan AM and Rubin MA. TMPRSS2:ERG fusion-associated deletions provide insight into the heterogeneity of prostate cancer. Cancer Res 2006; 66: 8337-8341.

[6] Wang J, Cai Y, Ren C and Ittmann M. Expression of variant TMPRSS2/ERG fusion messenger RNAs is associated with aggressive prostate cancer. Cancer Res 2006; 66: 8347-8351.

[7] Yoshimoto M, Joshua AM, Chilton-Macneill S, Bayani J, Selvarajah S, Evans AJ, Zielenska M and Squire JA. 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: 465-469.

[8] Barry M, Perner S, Demichelis F and Rubin MA. TMPRSS2-ERG fusion heterogeneity in multifocal prostate cancer: clinical and biologic implications. Urology 2007; 70: 630-633.

[9] Attard G, Clark J, Ambroisine L, Mills IG, Fisher G, Flohr P, Reid A, Edwards S, Kovacs G, Berney D, Foster C, Massie CE, Fletcher A, De

Bono JS, Scardino P, Cuzick J and Cooper CS. Heterogeneity and clinical significance of ETV1 translocations in human prostate cancer. Br J Cancer 2008; 99: 314-320.
[10] Jemal A, Siegel R, Ward E, Hao Y, Xu J, Murray T and Thun MJ. Cancer statistics, 2008. CA Cancer J Clin 2008; 58: 71-96.
[11] Han B, Mehra R, Dhanasekaran SM, Yu J, Menon A, Lonigro RJ, Wang X, Gong Y, Wang L, Shankar S, Laxman B, Shah RB, Varambally S, Palanisamy N, Tomlins SA, Kumar-Sinha C and Chinnaiyan AM. A fluorescence in situ hybridization screen for E26 transformation-specific aberrations: identification of DDX5-ETV4 fusion protein in prostate cancer. Cancer Res 2008; 68: 7629-7637.
[12] Rubin MA, Zhou M, Dhanasekaran SM, Varambally S, Barrette TR, Sanda MG, Pienta KJ, Ghosh D and Chinnaiyan AM. alpha-Methylacyl coenzyme A racemase as a tissue biomarker for prostate cancer. Jama 2002; 287: 1662-1670.
[13] Zhou M, Chinnaiyan AM, Kleer CG, Lucas PC and Rubin MA. Alpha-Methylacyl-CoA racemase: a novel tumor marker over-expressed in several human cancers and their precursor lesions. Am J Surg Pathol 2002; 26: 926-931.
[14] Hermans KG, van der Korput HA, van Marion R, van de Wijngaart DJ, Ziel-van der Made A, Dits NF, Boormans JL, van der Kwast TH, van Dekken H, Bangma CH, Korsten H, Kraaij R, Jenster G and Trapman J. Truncated ETV1, fused to novel tissue-specific genes, and full-length ETV1 in prostate cancer. Cancer Res 2008; 68: 7541-7549.
[15] de Kok JB, Verhaegh GW, Roelofs RW, Hessels D, Kiemeney LA, Aalders TW, Swinkels DW and Schalken JA. DD3(PCA3), a very sensitive and specific marker to detect prostate tumors. Cancer Res 2002; 62: 2695-2698.
[16] Hessels D, Klein Gunnewiek JM, van Oort I, Karthaus HF, van Leenders GJ, van Balken B, Kiemeney LA, Witjes JA and Schalken JA. DD3(PCA3)-based molecular urine analysis for the diagnosis of prostate cancer. Eur Urol 2003; 44: 8-15; discussion 15-16.
[17] Helgeson BE, Tomlins SA, Shah N, Laxman B, Cao Q, Prensner JR, Cao X, Singla N, Montie JE, Varambally S, Mehra R and Chinnaiyan AM. Characterization of TMPRSS2:ETV5 and SLC45A3:ETV5 gene fusions in prostate cancer. Cancer Res 2008; 68: 73-80.
[18] Tomlins SA, Laxman B, Dhanasekaran SM, Helgeson BE, Cao X, Morris DS, Menon A, Jing X, Cao Q, Han B, Yu J, Wang L, Montie JE, Rubin MA, Pienta KJ, Roulston D, Shah RB, Varambally S, Mehra R and Chinnaiyan AM. Distinct classes of chromosomal rearrangements create oncogenic ETS gene fusions in prostate cancer. Nature 2007; 448: 595-599.
[19] Yegnasubramanian S, Kowalski J, Gonzalgo ML, Zahurak M, Piantadosi S, Walsh PC, Bova GS, De Marzo AM, Isaacs WB and Nelson WG. Hypermethylation of CpG islands in primary and metastatic human prostate cancer. Cancer Res 2004; 64: 1975-1986.
[20] Nakayama M, Gonzalgo ML, Yegnasubramanian S, Lin X, De Marzo AM and Nelson WG. GSTP1 CpG island hypermethylation as a molecular biomarker for prostate cancer. J Cell Biochem 2004; 91: 540-552.
[21] Lee WH, Morton RA, Epstein JI, Brooks JD, Campbell PA, Bova GS, Hsieh WS, Isaacs WB and Nelson WG. Cytidine methylation of regulatory sequences near the pi-class glutathione S-transferase gene accompanies human prostatic carcinogenesis. Proc Natl Acad Sci U S A 1994; 91: 11733-11737.
[22] Kumar-Sinha C, Tomlins SA and Chinnaiyan AM. Recurrent gene fusions in prostate cancer. Nat Rev Cancer 2008; 8: 497-511.
[23] FitzGerald LM, Agalliu I, Johnson K, Miller MA, Kwon EM, Hurtado-Coll A, Fazli L, Rajput AB, Gleave ME, Cox ME, Ostrander EA, Stanford JL and Huntsman DG. Association of TMPRSS2-ERG gene fusion with clinical characteristics and outcomes: results from a population-based study of prostate cancer. BMC Cancer 2008; 8: 230.
[24] Demichelis F, Fall K, Perner S, Andren O, Schmidt F, Setlur SR, Hoshida Y, Mosquera JM, Pawitan Y, Lee C, Adami HO, Mucci LA, Kantoff PW, Andersson SO, Chinnaiyan AM, Johansson JE and Rubin MA. TMPRSS2:ERG gene fusion associated with lethal prostate cancer in a watchful waiting cohort. Oncogene 2007; 26: 4596-4599.
[25] Tomlins SA, Mehra R, Rhodes DR, Smith LR, Roulston D, Helgeson BE, Cao X, Wei JT, Rubin MA, Shah RB and Chinnaiyan AM. TMPRSS2:ETV4 gene fusions define a third molecular subtype of prostate cancer. Cancer Res 2006; 66: 3396-3400.
[26] Soller MJ, Isaksson M, Elfving P, Soller W, Lundgren R and Panagopoulos I. Confirmation of the high frequency of the TMPRSS2/ERG fusion gene in prostate cancer. Genes Chromosomes Cancer 2006; 45: 717-719.
[27] King JC, Xu J, Wongvipat J, Hieronymus H, Carver BS, Leung DH, Taylor BS, Sander C, Cardiff RD, Couto SS, Gerald WL and Sawyers CL. Cooperativity of TMPRSS2-ERG with PI3-kinase pathway activation in prostate oncogenesis. Nat Genet 2009; 41: 524-526.
[28] Hermans KG, Bressers AA, van der Korput HA, Dits NF, Jenster G and Trapman J. Two unique novel prostate-specific and androgen-regulated fusion partners of ETV4 in prostate cancer. Cancer Res 2008; 68: 3094-3098.
[29] Mehra R, Tomlins SA, Shen R, Nadeem O, Wang L, Wei JT, Pienta KJ, Ghosh D, Rubin MA, Chinnaiyan AM and Shah RB. Comprehensive assessment of TMPRSS2 and ETS family gene

aberrations in clinically localized prostate cancer. Mod Pathol 2007; 20: 538-544.
[30] Nam RK, Sugar L, Yang W, Srivastava S, Klotz LH, Yang LY, Stanimirovic A, Encioiu E, Neill M, Loblaw DA, Trachtenberg J, Narod SA and Seth A. Expression of the TMPRSS2:ERG fusion gene predicts cancer recurrence after surgery for localised prostate cancer. Br J Cancer 2007; 97: 1690-1695.
[31] Mosquera JM, Perner S, Demichelis F, Kim R, Hofer MD, Mertz KD, Paris PL, Simko J, Collins C, Bismar TA, Chinnaiyan AM and Rubin MA. Morphological features of TMPRSS2-ERG gene fusion prostate cancer. J Pathol 2007; 212: 91-101.
[32] Tu JJ, Rohan S, Kao J, Kitabayashi N, Mathew S and 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.
[33] Rouzier C, Haudebourg J, Carpentier X, Valerio L, Amiel J, Michiels JF and Pedeutour F. Detection of the TMPRSS2-ETS fusion gene in prostate carcinomas: retrospective analysis of 55 formalin-fixed and paraffin-embedded samples with clinical data. Cancer Genet Cytogenet 2008; 183: 21-27.
[34] Clark J, Merson S, Jhavar S, Flohr P, Edwards S, Foster CS, Eeles R, Martin FL, Phillips DH, Crundwell M, Christmas T, Thompson A, Fisher C, Kovacs G and Cooper CS. Diversity of TMPRSS2-ERG fusion transcripts in the human prostate. Oncogene 2007; 26: 2667-2673.
[35] Hu Y, Dobi A, Sreenath T, Cook C, Tadase AY, Ravindranath L, Cullen J, Furusato B, Chen Y, Thangapazham RL, Mohamed A, Sun C, Sesterhenn IA, McLeod DG, Petrovics G and Srivastava S. Delineation of TMPRSS2-ERG splice variants in prostate cancer. Clin Cancer Res 2008; 14: 4719-4725.
[36] Wang J, Cai Y, Yu W, Ren C, Spencer DM and Ittmann M. Pleiotropic biological activities of alternatively spliced TMPRSS2/ERG fusion gene transcripts. Cancer Res 2008; 68: 8516-8524.
[37] Mehra R, Han B, Tomlins SA, Wang L, Menon A, Wasco MJ, Shen R, Montie JE, Chinnaiyan AM and Shah RB. Heterogeneity of TMPRSS2 gene rearrangements in multifocal prostate adenocarcinoma: molecular evidence for an independent group of diseases. Cancer Res 2007; 67: 7991-7995.
[38] Clark J, Attard G, Jhavar S, Flohr P, Reid A, De-Bono J, Eeles R, Scardino P, Cuzick J, Fisher G, Parker MD, Foster CS, Berney D, Kovacs G and Cooper CS. Complex patterns of ETS gene alteration arise during cancer development in the human prostate. Oncogene 2008; 27: 1993-2003.
[39] Furusato B, Gao CL, Ravindranath L, Chen Y, Cullen J, McLeod DG, Dobi A, Srivastava S, Petrovics G and Sesterhenn IA. Mapping of TMPRSS2-ERG fusions in the context of multi-focal prostate cancer. Mod Pathol 2008; 21: 67-75.
[40] Rajput AB, Miller MA, De Luca A, Boyd N, Leung S, Hurtado-Coll A, Fazli L, Jones EC, Palmer JB, Gleave ME, Cox ME and Huntsman DG. Frequency of the TMPRSS2:ERG gene fusion is increased in moderate to poorly differentiated prostate cancers. J Clin Pathol 2007; 60: 1238-1243.
[41] Darnel AD, Lafargue CJ, Vollmer RT, Corcos J and Bismar TA. TMPRSS2-ERG fusion is frequently observed in gleason pattern 3 prostate cancer in a canadian cohort. Cancer Biol Ther 2009; 8:
[42] Teixeira MR. Chromosome mechanisms giving rise to the TMPRSS2-ERG fusion oncogene in prostate cancer and HGPIN lesions. Am J Surg Pathol 2008; 32: 642-644; author reply 644.
[43] Mosquera JM, Perner S, Genega EM, Sanda M, Hofer MD, Mertz KD, Paris PL, Simko J, Bismar TA, Ayala G, Shah RB, Loda M and Rubin MA. Characterization of TMPRSS2-ERG fusion high-grade prostatic intraepithelial neoplasia and potential clinical implications. Clin Cancer Res 2008; 14: 3380-3385.
[44] Nam RK, Sugar L, Wang Z, Yang W, Kitching R, Klotz LH, Venkateswaran V, Narod SA and Seth A. Expression of TMPRSS2:ERG gene fusion in prostate cancer cells is an important prognostic factor for cancer progression. Cancer Biol Ther 2007; 6: 40-45.
[45] Attard G, Clark J, Ambroisine L, Fisher G, Kovacs G, Flohr P, Berney D, Foster CS, Fletcher A, Gerald WL, Moller H, Reuter V, De Bono JS, Scardino P, Cuzick J and Cooper CS. Duplication of the fusion of TMPRSS2 to ERG sequences identifies fatal human prostate cancer. Oncogene 2008; 27: 253-263.
[46] Winnes M, Lissbrant E, Damber JE and 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.
[47] Saramaki OR, Harjula AE, Martikainen PM, Vessella RL, Tammela TL and Visakorpi T. TMPRSS2:ERG fusion identifies a subgroup of prostate cancers with a favorable prognosis. Clin Cancer Res 2008; 14: 3395-3400.
[48] Chen Y, Sawyers CL and Scher HI. Targeting the androgen receptor pathway in prostate cancer. Curr Opin Pharmacol 2008; 8: 440-448.
[49] Rostad K, Mannelqvist M, Halvorsen OJ, Oyan AM, Bo TH, Stordrange L, Olsen S, Haukaas SA, Lin B, Hood L, Jonassen I, Akslen LA and Kalland KH. ERG upregulation and related ETS transcription factors in prostate cancer. Int J Oncol 2007; 30: 19-32.
[50] Laxman B, Tomlins SA, Mehra R, Morris DS, Wang L, Helgeson BE, Shah RB, Rubin MA, Wei JT and Chinnaiyan AM. Noninvasive detection of TMPRSS2:ERG fusion transcripts in the urine of men with prostate cancer. Neoplasia 2006;

8: 885-888.
[51] Laxman B, Morris DS, Yu J, Siddiqui J, Cao J, Mehra R, Lonigro RJ, Tsodikov A, Wei JT, Tomlins SA and Chinnaiyan AM. A first-generation multiplex biomarker analysis of urine for the early detection of prostate cancer. Cancer Res 2008; 68: 645-649.
[52] Hessels D, Smit FP, Verhaegh GW, Witjes JA, Cornel EB and Schalken JA. Detection of TMPRSS2-ERG fusion transcripts and prostate cancer antigen 3 in urinary sediments may improve diagnosis of prostate cancer. Clin Cancer Res 2007; 13: 5103-5108.
[53] Tomlins SA, Bjartell A, Chinnaiyan AM, Jenster G, Nam RK, Rubin MA and Schalken JA. ETS Gene Fusions in Prostate Cancer: From Discovery to Daily Clinical Practice. Eur Urol 2009;