Review Article
Emergence of ETS transcription factors as diagnostic tools and therapeutic targets in prostate cancer

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Abstract: The discovery of chromosomal translocations in prostate cancer has greatly enhanced our understanding of prostate cancer biology. Genomic rearrangements involving the ETS family of transcription factors are estimated to be present in 50-70% of prostate cancer cases. These rearrangements fuse the ETS factors with promoters of genes that are androgen regulated. Thus, the expression of ETS factors, such as ERG, ETV1, ETV4 and ETV5, is mediated by androgen. *In-vitro* and *in-vivo* studies suggest that overexpression of ETS proteins increase cell proliferation and confer an invasive phenotype to prostate cancer cells. Epidemiological studies demonstrate that ETS-fusion positive patients exhibit tumors corresponding to a more advanced disease. The ability of ETS factors to serve as markers for screening and diagnosing prostate cancer patients is being investigated, and the results have been largely positive to date. Additionally, ETS factors present an excellent opportunity as therapeutic targets and several strategies have been devised to directly target ETS proteins or their binding partners and downstream effectors.

Keywords: Prostate cancer, chromosomal translocation, transcription factor, ETS, TMPRSS2-ERG, ETV1

Introduction

Prostate cancer is the second most leading cause of cancer death in men in the United States, behind only lung cancer. It accounts for 29% of all cancer cases and is responsible for 9% of all cancer deaths (American Cancer Society). Prostate cancer is associated with significant molecular heterogeneity. The mechanisms responsible for disease initiation, progression and metastasis are not yet fully understood. Thus, a better understanding of prostate cancer biology is important for improving prostate cancer screening, developing specific diagnostic tools and ultimately treating the disease.

The discovery of chromosomal translocations in prostate cancer has greatly enhanced our understanding of prostate cancer biology. Chromosomal rearrangements are a common mechanism driving oncogenesis in sarcomas and hematologic malignancies [1]. Recently, fusions involving the erythroblastosis virus E26 transforming sequences (*ETS*) family of transcription factors has been shown to play an

important role in prostate cancer pathogenesis [2]. The *ETS* family of transcription factors consists of a highly conserved group of genes that play important roles in cellular proliferation, differentiation, migration, invasion and angiogenesis [3]. ETS proteins share significant homology with each other and contain a C-terminal *ETS* domain that is involved in DNA-binding [4]. Chromosomal rearrangements involving the ETS family of transcription factors result in truncated ETS proteins that are fused to androgen regulated gene promoters. Thus, the expression of these genes is regulated by androgen in prostate cells harboring *ETS* fusions. The most common fusion product involves the 5' promoter region of *TMPRSS2*, a trans-membrane serine protease that is expressed exclusively in the prostate, fused to the 3' region of *ERG* [2]. This fusion results in the over-expression of a nearly full-length ERG protein. Different studies have shown that the fusion protein is present in approximately 50% of prostate cancer cases. Prostate cells demonstrating androgen dependent ERG over-expression have a molecular signature indicative of an aggressive phenotype [5]. Moreover, the presence of the fusion tran-

Table 1. Summary of published studies demonstrating the frequency of TMPRSS2-ERG fusion in prostate cancer

Authors	Sample size	Technique used to detect rearrangement status	Proportion of ETS-fusion positive patients	Study location	Sample acquisition technique	Cohort selection
Tomlins et al (2005)	29	FISH	23/29 (79.3%)	Ann Arbor, USA	Radical prostatectomy	Localized and metastatic prostate cancer
Perner et al (2006)	118	FISH	58/118 (49.2%)	Boston, USA	Radical prostatectomy	Primary prostate cancer
Perner et al (2006)	17	FISH	7/17 (41.2%)	Boston, USA	Radical prostatectomy	Hormone naïve lymph node metastases
Soller et al (2006)	18	RT-PCR	14/18 (77.8%)	Lund, Sweden	Prostate Biopsy	Prostate adenocarcinoma
Rajput et al (2007)	86	FISH	35/86 (41%)	Vancouver, Canada	Radical prostatectomy	Moderate to poorly differentiated prostate cancers
Nam et al (2007)	26	RT-PCR	11/26 (42.3%)	Toronto, Canada	Radical prostatectomy	Localized prostate cancer
Tu et al (2007)	82	FISH, RT-PCR	36/82 (43.9%)	New York, USA	Radical prostatectomy	Localized and lymph node metastasis
Demichelis et al (2007)	111	FISH	17/111 (15.3%)	Orebro, Sweden	Transurethral resection of prostate	Conservatively managed cohort with prostate cancer
Mosquera et al (2009)	134	FISH	46/100 (46%)	Boston, USA	Prostate Biopsy	Consecutive prostate cancer patients
Miyagi et al (2010)	194	RT-PCR	54/194 (27.8%)	Japan	Radical prostatectomy	Consecutive prostate cancer patients
Rosen et al (2012)	182	FISH	81/182 (44.5%)	Bethesda, USA	Radical Prostatectomy	Patients matched for age, gleason sum and pathologic stage

script has been largely associated with a poor prognosis, lower incidence of recurrence free survival and higher Gleason scores, indicative of a more advanced disease [6]. Fusions involving other *ETS* factors, such as *ETV1* (10% cases), *ETV4* (<1%), *ETV5* (<1%) and *ELK4* (<1%) have also been implicated in prostate cancer [7, 8]. To date, 14 different genes (*TMPRSS2, SLC45A3, C15orf21 CANT1, EST14, FOXP1, HERVK17, FLJ35294, HERV-K, ACSL3 and NDRG1, DDX5, HNRPA2B1, KLK2*) have been identified as fusion partners for *ERG*, *ETV1*, *ETV4*, *ETV5* and *ELK4* [9-12]. Thus, the ETS family of transcription factors represents a novel class of macromolecules that can be exploited for their usefulness as diagnostic tools and therapeutic targets in the treatment of prostate cancer.

Initial discovery

The presence of *ETS* translocations in human prostate cancer was initially reported in a landmark paper published in 2005 [2]. The authors used a bioinformatics approach to probe genes over-expressed across several prostate cancer microarray data sets. They identified *ERG* and *ETV1*, two genes that encode ETS transcription factors and are involved in oncogenic transformations in myeloid leukemia and Ewing's sarcoma [13]. Although the over-expression of ERG in prostate cancer had been previously reported [14], Tomlins *et al* now proposed a mechanism to account for this over-expression. The authors noticed that ERG and ETV1 expression was mutually exclusive and over-expression products included exons 4-7 of the *ETS* factors more commonly than exons 1 and 2. This led them to believe that the over-expression mechanism involved chromosomal rearrangements. 5' RNA ligase-mediated rapid amplification of cDNA ends (RLM-RACE) to obtain the complete transcript of the over-expressed product revealed *TMPRSS2* as the 5' fusion partner. TMPRSS2 is a serine protease expressed in the normal prostate tissue and is strongly induced by androgen in andro-

gen-sensitive prostate cells. Thus, the fusion of 5'-unstranslated region of *TMPRSS2* with 3'-regions of *ERG* or *ETV1* placed these proto-oncogenes under direct regulation by androgen stimulation.

Incidence of ETS fusions

The discovery of chromosomal fusions as a mechanism for ETV1 and ERG over-expression marked a new era in the understanding of prostate cancer biology. Several other groups have, since then, confirmed the original findings of Tomlins *et al.* However, the frequency of gene recombination varies between different studies (15%-78%), probably owing to the sensitivity of the technique used, the number of samples included in the study, the number of fusion variants probed and the tumor stage [15-23]. Earlier studies used fluorescence in-situ hybridization or RT-PCR to probe for recombination between *ERG* and *TMPRSS2*. More recently, ERG expression has been detected by immunohistochemistry using a monoclonal ERG antibody [24].

Using FISH technique, Perner *et al* [15], identified *TMPRSS2-ERG* rearrangements in 49.2% of 118 primary prostate cancers and 41.2% of 18 hormone-naive lymph node metastases. Soon after, Soller *et al* [16] used RT-PCR to detect *TMPRSS2-ERG* in 14 out of 18 cases of prostate adenocarcinoma (78%). Using FISH on prostate cancer tissue microarrays, Rajput *et al* [17] found that 41% of moderate to poorly differentiated tumors (35/86 cases) exhibited *TMPRSS2-ERG* fusion status. However, only 6.7% of well differentiated tumors (1/15 cases) and none of the prostatic hyperplasia cases contained these gene rearrangements (0/5). Other studies by Nam *et al* [18] and Tu *et al* [19] point to a 40%-50% occurrence of gene rearrangement, whereas a study by Demichiles *et al* [20] reported only a 15% occurrence (17/111) of *TMPRSS2-ERG* in a Swedish cohort of men with localized prostate cancer who underwent expectant management. Most of the studies probe for the rearranged product in men who underwent surgery. To determine whether the high frequency observed in these prostatectomy specimens (40-78%) reflects selection bias, Mosquera *et al* [21] determined the prevalence of *TMPRSS2-ERG* fusion status among PSA screened men undergoing prostate biopsy and found that 46% of men with prostate cancer harbored the fusion gene, consistent with the frequency observed in the surgical cohorts. Efforts have also been made to correlate the frequency of gene alterations with ethnic backgrounds. A study of 194 Japanese prostate cancer patients revealed the presence of *TMPRSS2-ERG* rearrangement in only 28% of cases [25]. Another study analyzed the frequency of ERG oncoprotein expression between 91 African-American and 91 Caucasian patients who were matched for age, Gleason score and pathologic state. A markedly greater frequency of ERG expression was found between the index tumors of the Caucasians Americans (63.3%) and those of the African Americans (28.6%) [22].

The correct estimation of *TMPRSS2-ERG* incidence is also complicated by the fact that significant molecular heterogeneity exists between independent tumor foci in multifocal prostate cancer [26, 27]. Minner *et al* performed a study to analyze the extent of heterogeneity for *TMPRSS2-ERG* fusion [23]. They developed a heterogeneity tissue microarray containing samples from 10 different tumor blocks of 190 large prostate cancers. ERG immunostaining was homogeneously positive in 29 prostate cancers (16%), whereas heterogeneous ERG positivity was seen in 74 cancers (42%). Furthermore, ERG heterogeneity was within one tumor focus (intrafocal heterogeneity) in 69 cases (93% of heterogeneous cases) and between different tumor foci (interfocal heterogeneity) in 5 cases (7%). This study shows that ERG heterogeneity exists in a significant portion of prostate cancer cases, and ERG rearrangement status may not be accurately determined by analyzing a single tissue biopsy per patient (**Table 1**).

Although, the individual estimates of fusion occurrence vary widely between different studies, overall the data clearly suggests that the *TMPRSS2*-ERG rearrangement is present in a majority of prostate cancer cases. Further increase in the sensitivity of techniques used and the discovery of new splice variants may lead to an even higher proportion of prostate cancers detected to carry the fusion product.

Mechanism of genomic rearrangement

TMPRSS2 and *ERG* are located 3Mb apart on chromosome 21. The rearrangement between

these two genes can occur either through balanced or unbalanced translocation between two chromosome 21s, or through interstitial deletion (Edel). The literature, to date, suggests that intronic deletion is the more common mechanism for the rearrangement, being responsible for 60%-80% of *TMPRSS2-ERG* cases [5, 15, 19, 28, 29]. The deletion of the intervening region between *TMPRSS2* and *ERG* on chromosome 21, in addition to *ERG* activation, may also lead to loss of intervening genes [30]. However, whether this deletion leads to any additional biological significance resulting in a more aggressive phenotype, is yet to be determined.

Two independent studies have elucidated the mechanism underlying the non-random juxtaposition of *TMPRSS2* and *ERG* in prostate cells [31, 32]. Both studies probe the synergistic ability of androgen signaling coupled with genotoxic stress to induce chromosomal rearrangements. Mani *et al* [31] stimulated the androgen responsive LNCaP prostate cell-line, which does not harbor the *TMPRSS2-ERG* rearrangement, with increasing amounts of dihydrotestosterone (DHT). Using FISH, they observed that the treatment induced proximity between *TMPRSS2* and *ERG* regions on chromosome 21. To determine whether this induced proximity facilitated gene fusions, the authors used radiation as a surrogate for genotoxic stress and observed that DHT stimulated LNCaP cells that were exposed to radiation to harbor the *TMPRSS2-ERG* fusion. Thus, androgen signaling brings the 5' and 3' fusion partners in close proximity, increasing the likelihood of fusions in the presence of agents that cause DNA double stranded breaks.

Lin *et al* [32] used a similar strategy to determine the specific machinery required for the site-specific DNA double-stranded breaks (DSBs). They identified activation-induced cytidine deaminase (AID)/GADD45 and the LINE-1 repeat-encoded ORF2 endonuclease as the enzymes responsible for mediating double-stranded breaks at translocation loci brought in close proximity by ligand bound androgen receptor. Ligation of the DSBs via non-homologous end joining pathway finally results in the generation of rearranged product.

Alternatively spliced forms

The study of *TMPRSS2-ERG* fusion product in prostate cancer is rendered complex by the fact that more than 20 alternatively spliced *TMPRSS2-ERG* isoforms have been identified in prostate cancer [33-36]. These isoforms differ in the location of *TMPRSS2-ERG* junction and are heterogeneous in the presence of various *TMPRSS2* and *ERG* exons. The transcripts range from those encoding full-length ERG protein, N-terminal truncated ERG proteins, ERG isoforms expressing only the DNA binding domain (ETS) and finally those encoding truncated ERG proteins lacking the ETS domain but expressing pointed/SAM domains [36]. The isoforms have been shown to promote proliferation, invasion and motility with variable activities depending on the structure of the 5' region encoding the fusion proteins [34]. Interestingly, the splice variants lacking the ETS domain are more abundantly expressed in prostate cancer patients than those encoding full-length ERG protein [35]. However, recent data suggest increased ratio of full-length over truncated splice forms correlates with a less favorable prognosis. In particular, the fusion isoform involving exon 1-2 of *TMPRSS2* and exon 4-7 of *ERG* is strongly associated with aggressive prostate cancer. The presence of this fusion product is correlated with early recurrence and seminal vesicle invasion [33]. However, the reason for association of particular isoforms with aggressive disease is not known and further investigation is required to answer this phenomena.

Biological function of TMPRSS2-ERG protein

In a physiological setting, ERG expression is mainly restricted to endothelial and hematopoietic cells [37-39]. Its expression pattern underlies the roles of ERG in angiogenesis, endothelial cell differentiation, platelet development, stem-cell function and hematopoiesis. ERG is not expressed in epithelial cells, including the prostatic epithelium [40-42].

In-vitro over-expression of truncated ERG in the same proportion as that found in the majority of prostate cancer patients leads to an increase in migration and cell invasion in primary as well as benign immortalized prostatic epithelial cells [6]. ERG over-expression in these cell lines leads to the activation of several matrix metalloproteinases (MMP3, MMP9, ADAM19) and genes involved in the plasminogen activator pathway (PLAT, PLAU). Both classes of gene products have been directly implicated in sev-

eral cancers [43, 44]. Consequently, ERG knock-down in the *TMPRSS2-ERG* positive prostate cancer cell line VCaP results in decreased expression of these target genes. This selective decrease in gene expression is accompanied by decreased invasion and migration.

The effects of ERG on survival can be attributed partly to its effects on PIM-1 expression [45]. PIM-1 is an oncogene with pro-survival attributes. It is found in several tumors of epithelial and hematological origin, including prostate cancer, and favors genomic instability [46]. ERG also upregulates expression of the chemokine receptor CXCR4, the extracellular matrix protein CRISP3 and the extracellular matrix glycophosphoprotein osteopontin [47-49]. ERG induces the expression of these proteins through binding to ETS binding sequences on the promoter regions of these genes. CXCR4, CRISP3 and osteopontin are key regulators of tumor invasion and metastasis in a variety of cancers [50-52]. ERG also regulates the expression of c-MYC oncogene, which consequently affects cellular morphology and expression of prostate differentiation related genes [53]. Recent data suggests that ERG is implicated in the epithelial-to-mesenchymal transition, epigenetic regulation, inflammation and DNA damage repair pathways. EMT occurs at the invasive front of tumors and produces single migratory cells that are able to escape the primary tumor site. This is concomitant with deregulation of the Wnt pathway [54]. Increased ERG activity is associated with EMT via repression of epithelial-specific genes, such as E-cadherin and cytokeratins, and increased expression of mesenchymal-specific genes, such as *CDH2* and *CDH11* [55]. Loss of E-cadherin is a hallmark of EMT [54]. ERG also upregulates EMT facilitators ZEB1 and ZEB2, which are involved in regulation of E-cadherin, perhaps via SNAIL factors [56]. A genome-wide screen revealed several WNT genes, such as WNT11, WNT2, WNT9A, CCND1, FZD7 and FZD4, as direct targets of ERG [57]. Notably, FZD4 is a direct mediator of WNT signaling and EMT [55]. Wnt-11 promotes neuroendocrine-like differentiation, survival and migration of prostate cancer cells [58].

ERG is implicated in epigenetic regulation through its effects on the transcription of polycomb group protein EZH2. EZH2 is a key ele-ment of the Polycomb Repressive Complex 2 (PRC2) and is responsible for the establishment of the repressive H3K27 methylation marker. ERG concomitantly represses expression of the prostate-specific tumor suppressor NKX3.1 via EZH2 [59]. NKX3.1 is important for prostate cancer initiation and progression as it integrates multiple signaling pathways such as PI3K, Akt, p53 and androgen receptor signaling [60]. *NKX3.1* silencing by ERG and EZH2 leads to increased proliferation and invasion. ERG also positively influences the prostaglandin pathway by modulating levels of 15-hydroxy-prostaglandin dehydrogenase (HPDG) and prostaglandin-E2 (PGE2), directly implicating it in the inflammatory response pathway [61]. Furthermore, ERG activates Nf-kB pathway through toll-like receptor 4, further implicating it in the inflammatory pathway [62].

In-vivo studies with animal models have been equally rewarding in furthering our understanding of ERG activity. Transgenic over-expression of ERG in the mouse prostate results in the development of prostatic intraepithelial neoplasia (PIN). However, it does not lead to carcinoma, thus indicating that ERG is not sufficient for the development of prostate cancer in mice [6]. However, TMPRSS2-ERG knock-down in VCaP xenograft models in SCID mice results in decreased tumor growth *in-vivo* [53]. These results suggest that ETS fusions may occur in the context of other genetic lesions that drive the transition of PIN to prostate cancer or from primary to metastatic disease. To this end, studies by two groups show that *TMPRSS2-ERG* rearrangement co-operates with PTEN loss and activation of PI3K pathway to mediate progression of PIN to prostatic adenocarcinoma [63, 64]. Similar to transgenic ERG mouse models, Pten-deficient mice develop high-grade PIN without progression to invasive cancer. Moreover, Pten-loss is observed in approximately 70% of prostate tumors. Tumors expressing the *TMPRSS2-ERG* genomic rearrangement demonstrate significantly reduced expression of PTEN. Also, ERG over-expression and PTEN loss is correlated across a majority of tumor specimens. Moreover, when PTEN heterozygous mice are crossed with mice that over-express truncated ERG in their prostate, the offspring consistently demonstrated lesions indicative of prostate carcinoma. These findings further stress the fact that ETS fusions function concomitantly in the presence of other genomic alterations to induce malignancy.

Table 2. Summary of published studies demonstrating the association between ERG rearrangement status and clinical outcome

Authors	Sample size	Technique used to detect rearrangement	Association with prognosis	Sample acquisition technique	Cohort selection
Perner et al (2006)	397	FISH	ERG rearrangement is an early molecular event associated with invasion	Radical prostatectomy	Localized prostate cancer, metastasis, hyperplasia, benign tissue
Demichelis et al (2007)	111	RT-PCR	ERG status positively correlates with prostate specific death	Transurethral resection of prostate (TURP)	Conservatively managed cohort with prostate cancer
Nam et al (2007)	26	RT-PCR	ERG is the most important prognostic factor associated with 5-year disease recurrence	Radical prostatectomy	Localized prostate cancer
Nam et al (2007)	165	RT-PCR	ERG is a strong prognostic factor of disease recurrence independent of grade and PSA.	Radical prostatectomy	Localized prostate cancer
Mehra et al (2007)	30	FISH	ERG rearrangement by interstitial deletion leads to a more aggressive phenotype	Rapid autopsy tissue procurement	Androgen-independent metastatic prostate cancer
Attard et al (2008)	445	FISH	Duplication of TMPRSS2-ERG rearrangement is an independent predictor of poor survival	Transurethral resection	Conservatively managed cohort with prostate cancer
Petrovics et al (2005)	114	RT-PCR	ERG rearrangement indicative of lower pathological grade and indicative of recurrence free survival	Laser microdisection	Localized prostate cancer
Winnes et al (2007)	50	RT-PCR	Fusion positive tumors associated with lower gleason grade and better survival	Needle biopsy	Localized prostate cancer
Fine et al (2010)	521	FISH	TMPRSS2-ERG is associated with low gleason scores	Radical prostatectomy	Localized prostate cancer
Pettersson et al (2012)	1180	IHC	ERG rearrangement status is not associated with biochemical recurrence or lethal death	Radical prostatectomy or TURP	Localized and metastatic prostate cancer

Clinical relevance of ERG expression

Recently, the influence of fusion status on clinical outcome has become an area of intense investigation. Several groups have attempted to correlate the presence of *ETS* fusion products with cancer stage and clinical outcomes [15, 18, 20, 65]. Although most studies associate *TMPRSS2-ERG* fusion status with higher Gleason scores, aggressive disease, poor prognosis and decreased incidence of disease free survival, there are differences in the methods, sample size and selection of the prostate patient cohort. Hence, conflicting reports have emerged as a result of these studies.

One of the first studies in this area was performed on a group of prostate cancer patients with localized prostate cancers that were subjected to expectant (watchful waiting) therapy [20]. The study revealed a positive correlation between the presence of the fusion protein and metastasis and prostate cancer specific death. A similar study conducted on patients undergoing radical prostatectomy for localized prostate cancer showed a higher rate of disease recurrence (58.4%) among patients exhibiting the fusion product as compared to patients that were fusion negative (8.1%) [66]. Using a multivariate analysis approach, this group also found that the presence of *TMPRSS2-ERG* is an important prognostic factor associated with disease recurrence [18]. In another study, TMPRSS2-ERG fusion was determined to be an early molecular event associated with invasion and metastasis [67]. Perhaps the most significant association between fusion status and clinical outcome is provided by a FISH based study of 445 prostate cancers from patients who had been conservatively managed [65]. Absence of *ERG* alterations was found to be a favorable indicator of cause specific survival

(90% survival at 8 years). A new category of prostate tumors was also identified, characterized by a duplication of *TMPRSS2-ERG* fusion along with deletion of sequences 5' to ERG. This class of tumors was termed as 2+Edel and patients harboring this mutation exhibited extremely poor cause specific survival (25% survival at 8 years). Moreover, the presence of 2+Edel fusion status as a prognostic factor was found to be independent of Gleason scores as well as serum PSA levels.

Other independent studies have demonstrated either no association or negative correlation between *ERG* fusion status and clinical outcome. In a multifocal prostate cancer tissue microarray based study, no correlation was found between *TMPRSS2-ERG* rearrangement and pathologic stage, Gleason score or recurrence free survival [28]. Similarly, another study ruled out any correlation between fusion status and seminal vesicle invasion or lymph node metastasis. Other groups, on the other hand have reported that fusion-positive tumors are associated with lower Gleason grades and better recurrence free survival [69, 70]. These reports mirror observations made earlier by Petrovics *et al* [14] in a report preceding the discovery of *TMPRSS2-ERG: ERG* rearrangement, where ERG expressing prostate tumors were found to be moderately to well differentiated, while exhibiting a lower pathological grade and indicative of recurrence free survival after radical prostatectomy.

Recently, a large scale study was completed on a cohort of 1,180 men treated with radical prostatectomy. A meta-analysis of prior-research was also conducted as part of this study and it included 5,074 men followed for biochemical recurrence and 2,049 men followed for lethal disease. The results of this study suggest that *TMPRSS2-ERG*, or ERG overexpression, is associated with tumor stage but does not strongly predict recurrence or mortality among men treated with radical prostatectomy [71] (**Table 2**).

As of yet there is no equivocal consensus regarding the presence of fusion products and association with a particular phenotype, although most studies point towards a more aggressive disease state in the presence of genomic rearrangements. Also, it is difficult to compare different studies due to variability in

the method of sample selection (watchful waiting/radical prostatectomy), end-point of studies (higher Gleason score/metastasis/cancer specific death), detection method (FISH/RT-PCR/IHC) and sample size. Interpretation of these studies is further complicated by the significant molecular heterogeneity in prostate cancer. Gene fusions may be present only in certain foci within the prostate and there is no way to determine whether the metastatic node is a direct result of the foci used to assign *TMPRSS2-ERG* rearrangement status.

TMPRSS2-ERG as a biomarker

Monitoring circulating levels of prostate specific antigen (PSA) is the most common form of screening for prostate cancer cases [72]. However, using circulating PSA levels as a biomarker for prostate cancer is complicated by the fact that PSA is also produced by benign prostate epithelia, benign prostatic hypertrophy and infection in the prostate [73]. Hence, PSA has limitations in both sensitivity and specificity. Recently, much interest has been attributed to the detection of *TMPRSS2-ERG* fusion transcripts to supplement or replace PSA testing as the benchmark standard for detecting prostate cancer. Unlike PSA, there is no evidence to suggest that truncated ERG protein is shed from the prostate. Instead, researchers have focused their efforts on detecting ERG fusion transcripts in the urine. As prostate cancer cells are routinely shed in the urine, *TMPRSS2-ERG* fusion transcripts can be easily detected by a non-invasive method [74, 75]. The technique usually involves collecting urine immediately after a digital rectal exam (DRE) as the examination process results in shedding of prostate cells. The urine is sedimented by centrifugation, RNA is extracted and RT-PCR is used to measure the amount of fusion transcript present.

The technique was first used to quantify the amount of *TMPRSS2-ERG* transcripts present in the urine of 19 prostate cancer patients [74]. The presence of fusion transcript in the urine was indicative of fusion status in the prostate of patients. Patients whose urine did not contain the fusion product tested negative for *TMPRSS2-ERG* rearrangement when prostate tissue biopsies were probed by FISH. Another study attempted to quantify *ERG* mRNA in the urine of 237 patients scheduled to undergo

needle biopsy of the prostate [76]. When normalized to PSA mRNA, a higher urine ERG score was found to be associated significantly with prostate cancer on biopsy. Importantly, urine ERG score performed best in men demonstrating PSA levels less than 4ng/ml. A diagnostic marker for this segment of screening populations is particularly important in order to determine which patients should be recommended for biopsy.

Other studies sought to develop a biomarker analysis profile consisting of *TMPRSS2-ERG* and multiple other biomarkers, which would provide superior results compared to conventional PSA testing. Van Gils *et al* [77] studied the diagnostic usefulness of a combination of *TMPRSS2-ERG* transcripts and prostate cancer antigen 3 (PCA3) RNA in urinary sediments after DRE. PCA3 is a non-coding RNA that is specific to the prostate and is over-expressed in prostate cancer [75]. The researchers found that although *TMPRSS2-ERG* fusion transcripts and PCA3 RNA could be detected in the urine with a sensitivity of 37% and 62% respectively, the combination of both markers increased the sensitivity to 73%. Laxman *et al* [78] probed an even wider multiplex of biomarkers as diagnostic tools for the early detection of prostate cancer in a cohort of 234 patients. They found that increased GOLPH2, SPINK1, PCA3 expression and *TMPRSS2-ERG* fusion status were significant predictors of prostate cancer with a sensitivity of 66% and specificity of 76% [76, 78]. *TMPRSS2-ERG* is only found in about 50% of prostate cancer, which renders it highly specific but with low sensitivity. Hence, combining it with more sensitive markers such as PCA3 is a step forward in the right direction. Indeed, a recent multi-center study of 1312 men found that stratification by urine *TMPRSS2-ERG* and PCA3 score is associated with the presence of cancer, tumor volume, and clinically significant cancer in prostatectomy and biopsy patients [79]. Salami *et al* have since developed a multivariable algorithm which combines serum PSA, PCA3, and *TMPRSS2-ERG* and improves prostate cancer prediction with 90% specificity and 80% sensitivity [80].

Other ETS fusions in prostate cancer

Although *TMPRSS2-ERG* rearrangements form the dominant class of fusions found in prostate cancers, rearrangements involving other ETS

family members have also been implicated [81]. *ETV1* fusion products are estimated to be found in approximately 10% of prostate cancer cases. Unlike the ERG proto-oncogene, where the 3'-fusion partner is exclusively *TMPRSS2*, *ETV1* can be rearranged with the 5' untranslated region of several genes. In fact, *TMPRSS2-ETV1* is found in less than 1% of prostate cancer cases. An added level of complexity is introduced by the fact that the 5' *ETV1* partners may or may not be androgen regulated.

In addition, the commonly used prostate cell-lines LNCaP and MDA-PCa 2B, which show the outlier *ETV1* over-expression, only express the wild-type full-length *ETV1* transcript. However, in these cells, the *ETV1* is translocated from chromosome 7 to an intronic region on chromosome 14. This intronic region is prostate specific and is regulated by androgens in its entirety [82].

Similar to ERG, over-expression of ETV1 in benign prostatic epithelial cell-lines results in the induction of a subset of genes involved in migration and invasion [82]. However, unlike ERG, full-length and N-terminal truncated ETV1 seem to have differential activities [10]. Although, both gene products can confer invasiveness when over-expressed in benign prostate epithelia cell-lines, full-length ETV1 appears to be more oncogenic and is able to induce anchorage-independent growth. The mechanism for this difference in activity is not known and further studies are required to investigate the role of full-length and truncated ETV1 proteins on prostate cancer growth and invasion.

Transgenic mice that over-express ETV1 in the prostate develop prostatic intraepithelial neoplasia (PIN), without progression to invasive prostatic adenocarcinoma [83]. Thus, it is likely that, similar to ERG over-expression in mouse prostate, ETV1 fusion products function in the context of pre-existing genomic lesions.

Other ETS family members, such as *ETV4*, *ETV5* and *ELK4* are involved in chromosomal translocations in prostate cancer at a much lesser frequency (<1%). The mechanism driving the expression of these genes is similar, in that, the 5' partner is usually the untranslated region of an androgen-regulated gene. Both *ETV4* and *ETV5* have been shown to fuse with the 5'
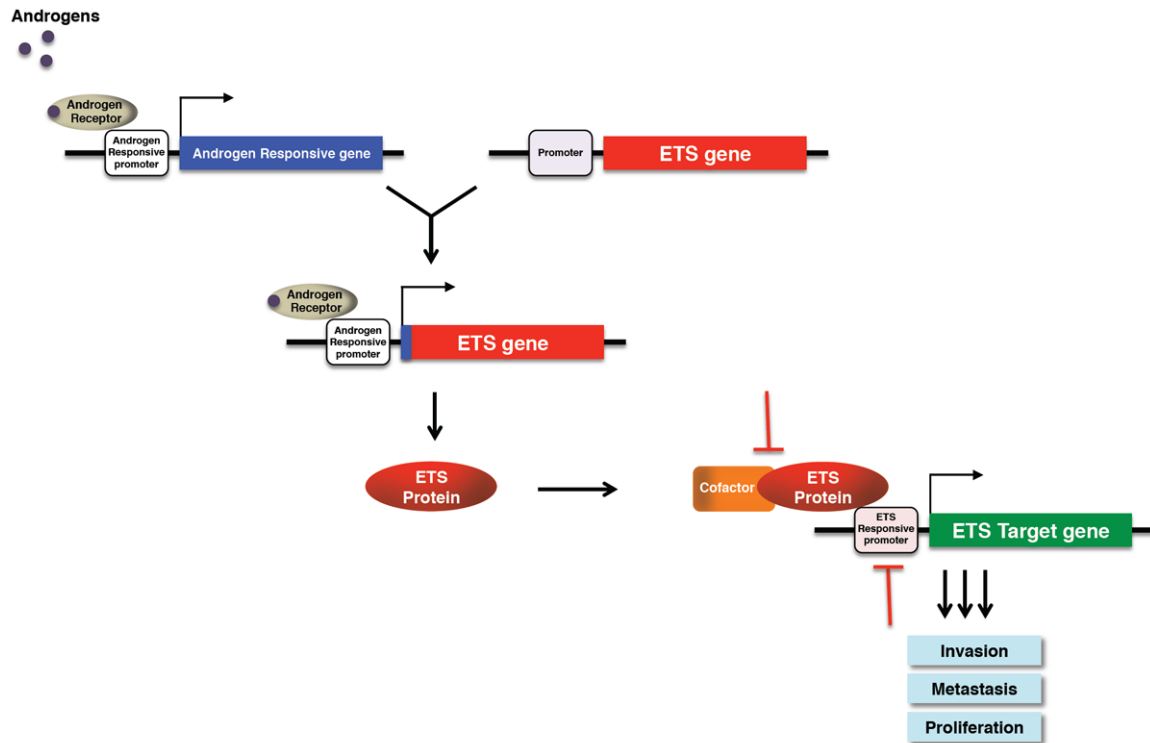
Figure 1. Chromosomal rearrangements involving the ETS family of transcription factors result in truncated ETS proteins that are fused to androgen regulated gene promoters. This lead to androgen regulation of ETS protein expression. ETS proteins regulate the expression of a subset of genes that increase cell proliferation, invasion and metastasis. Inhibition of ETS proteins provide a promising therapeutic target for the treatment of prostate cancer and inhibition of prostate cancer metastasis. These proteins can be targeted by blocking their binding to DNA or inhibiting their interaction with associated cofactors.

untranslated region of *TMPRSS2* in human prostate cancer tissue samples [7, 8]. Additionally, the androgen regulated gene *SLC45A3* can be rearranged with both *ETV5* and *ELK4* [11]. Moreover, *ETV4* can also fuse with *KLK2* and *CANT1,* two prostate specific and androgen regulated genes [10]. In all cases, the fusion leads to an androgen-regulated over-expression of the 3' *ETS* partner. Similar to ERG and ETV1, *in-vitro* studies have shown that the over-expression of these proteins confer an aggressive phenotype to prostate cancer cells, concomitant with a molecular signature that results in the over-expression of a subset of genes involved in invasion.

Unlike other ETS family members, *ELK4* is an endogenously androgen regulated gene. It is expressed in both benign prostate epithelia, as well as prostate cancer samples, although the expression is higher in the latter case [11]. It is yet to be determined what advantages are conferred to the tumor by placing *ELK4* under the

regulation of a different androgen regulated promoter, namely *SLC45A3*. *ELK* and *SLC45A3* are both located on chromosome 1 and it is believed that chromosomal translocation is not the major mechanism responsible for the fusion product. Whether the fusion product is a result of interstitial deletion or trans-splicing is yet to be determined.

Therapeutic applications

Inhibition of ERG protein and ERG pathway provides a promising therapeutic target for the treatment of prostate cancer and inhibition of prostate cancer metastasis. Transcription factors have been historically considered difficult targets due to the complex regulation of their target genes, lack of enzymatic activity and the widespread network of protein binding partners required for their function. However, the successful modulation of transcription factor function in several cancers has now revealed that this large and important class of proteins is indeed "druggable" (**Figure 1**).

It should be noted that ETS proteins contain several regions of intrinsic disorder. Intrinsic disorder accords flexibility and movement to proteins, thus allowing them to participate in multi-protein complexes. These interactions are rapid, yet specific. In the case of transcription factors, disordered regions can implement and co-ordinate multiple functions, thus allowing the cell to regulate DNA binding, protein interactions and context-dependent gene regulation. A quick analysis of ERG amino acid sequence using disorder prediction softwares such as PONDR and DisEMBL reveals that whereas the ETS and SAM domains of ERG are relatively ordered, the remainder of the protein is intrinsically disordered. These intrinsically disordered regions are likely to be critical for ERG function.

We recently showed that YK-4-279, a small molecule inhibitor of EWS-FLI1 in Ewing's sarcoma, robustly inhibits transcription by ERG and ETV1 in prostate cancer cells [84]. Inhibition of ERG and ETV1 activity resulted in reduced migration and invasion of rearrangement-positive prostate cancer cells. Cells lacking rearranged ERG or ETV1 were unresponsive to YK-4-279 treatment. Recently it was demonstrated that ERG transcriptional activity can also be modulated by preventing its binding to the promoter region of target genes. The heterocyclic dithiophene diamidine DB1255 specifically targets a portion of the ERG DNA recognition site by binding to the minor groove of the DNA as a dimer [85]. This leads to reduced ERG transcriptional activity.

Targeting downstream effectors or binding partners of ERG is also an optimal strategy for inhibiting ERG mediated effects on the cell. This can be a rational strategy with a fast track to clinical use, especially in cases where direct inhibitors are already available. A recent study identified phospholipase 2 group VII (*PLA2G7*) as a potential drug target in ERG oncogene positive prostate cancers [86]. PLA2G7 is involved in the cell's response to oxidative stress and promotes cell migration and invasion. Silencing PLA2G7 by lipid-lowering statins sensitizes ERG-positive cells to oxidative stress and reduces proliferation [87]. Recent findings have identified Poly (ADP-Ribose) Polymerase (PARP), a key DNA repair protein, to interact with ERG in a DNA independent manner [88]. PARP1 is shown to be important for ERG protein function and inhibition of PARP1 impairs ERG mediated tumorigenesis and cell invasion. An important clinical advantage of this finding is that several PARP inhibitors are at an advanced stage of clinical testing, and this discovery has opened a wide spectrum of new strategies to target ERG.

Finally, in a recent study, Shao *et al* targeted *TMPRSSS2-ERG* mRNA transcripts using liposomal nanovectors packaged with siRNAs [89]. The targeting siRNAs significantly inhibited tumor growth in a mouse model. The degree of growth inhibition directly correlated with the extent of fusion gene knockdown. Growth inhibition was accompanied with inhibition of angiogenesis, reduced proliferation and an increase in apoptosis of tumor cells.

Concluding remarks

The discovery of *ETS* rearrangements in prostate cancer has greatly enhanced our understanding of prostate cancer pathology and changed our perception regarding the role of chromosomal rearrangements in solid tumor biology. As a diagnostic tool, it has the potential to complement, or even supersede PSA screening as a biomarker for prostate cancer detection. As a therapeutic target, it can be exploited to target primary tumors and prevent metastatic dissemination of tumor cells.

Acknowledgments

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