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Review – Prostate Cancer

ETS Gene Fusions in Prostate Cancer: From Discovery to Daily Clinical Practice

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Abstract

Context: In 2005, fusions between the androgen-regulated transmembrane protease serine 2 gene, *TMPRSS2*, and E twenty-six (ETS) transcription factors were discovered in prostate cancer.
Objective: To review advances in our understanding of ETS gene fusions, focusing on challenges affecting translation to clinical application.
Evidence acquisition: The PubMed database was searched for reports on ETS fusions in prostate cancer.
Evidence synthesis: Since the discovery of ETS fusions, novel 5′ and 3′ fusion partners and multiple splice isoforms have been reported. The most common fusion, *TMPRSS2:ERG*, is present in approximately 50% of prostate-specific antigen (PSA)–screened localized prostate cancers and in 15–35% of population-based cohorts. ETS fusions can be detected noninvasively in the urine of men with prostate cancer, with a specificity rate in PSA-screened cohorts of >90%. Reports from untreated population-based cohorts suggest an association between ETS fusions and cancer-specific death and metastatic spread. In retrospective prostatectomy cohorts, conflicting results have been published regarding associations between ETS fusions and cancer aggressiveness. In addition to serving as a potential biomarker, tissue and functional studies suggest a specific role for ETS fusions in the transition to carcinoma. Finally, recent results suggest that the 5′ and 3′ ends of ETS fusions as well as downstream targets may be targeted therapeutically.
Conclusions: Recent studies suggest that the first clinical applications of ETS fusions are likely to be in noninvasive detection of prostate cancer and in aiding with difficult diagnostic cases. Additional studies are needed to clarify the association between gene fusions and cancer aggressiveness, particularly those studies that take into account the multifocal and heterogeneous nature of localized prostate cancer. Multiple promising strategies have been identified to potentially target ETS fusions. Together, these results suggest that ETS fusions will affect multiple aspects of prostate cancer diagnosis and management.

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1. Introduction

Prostate cancer is characterized by mutations in genes that promote cancer (ie, oncogenes) or that prevent cancer (ie, tumor-suppressor genes). These mutations include insertion, deletion, or substitution of single nucleotides and chromosomal gains, losses, or rearrangements. Recurrent mutations often disrupt genes that play a causal role in cancer development and can be exploited for diagnosis, disease subclassification, prognosis, and therapy. Many causal cancer genes have been identified through the analysis of recurrent chromosomal rearrangements and resulting gene fusions, which characterize leukemias, lymphomas, and sarcomas [1]. The prototypic example is a rearrangement between chromosomes 9 and 22, which results in fusion of the breakpoint cluster region gene, *BCR*, and the receptor tyrosine kinase gene, *ABL*, and characterizes chronic myelogenous leukemia (CML). This finding led to the development of imatinib, which inhibits the *BCR:ABL* gene fusion product and has revolutionized the treatment of CML [2]. In contrast, recurrent rearrangements had not been identified in common epithelial tumors until 2005, when gene fusions involving members of the E twenty-six (ETS) family of transcription factors were reported in prostate cancer [3]. In this review, we summarize the discovery of ETS fusions and how their discovery has advanced our understanding of prostate cancer biology. We also appraise current research and future challenges with regard to translating this discovery into clinical practice.

1.1. Discovery of ETS fusions in prostate cancer

Previous chromosomal rearrangements were identified using karyotyping, which has limited resolution and is difficult to apply to epithelial cancers. Recently, DNA microarrays, which simultaneously monitor the expression of thousands of genes, have transformed cancer research by providing gene-expression profiles for many cancer types. DNA microarrays, however, had not been used to identify rearrangements and causal cancer genes. The discovery of ETS fusions began with the hypothesis that marked overexpression of an oncogene from chromosomal amplification or rearrangement should be evident in DNA microarray data but not necessarily by traditional analytical approaches. While heterogeneous patterns of oncogene activation are observed in the majority of cancer types (eg, the v-erb-b2 erythroblastic leukemia viral oncogene homolog 2 gene, *ERBB2*, being amplified in approximately 25% of breast cancers), traditional microarray analysis methods prioritize genes commonly activated across a class of cancer samples, and these methods will fail to find such oncogene expression profiles (Fig. 1). Thus, a novel bioinformatics algorithm called the Cancer Outlier Profile Analysis (COPA) was developed to analyze DNA microarray data for *outlier genes* (those markedly overexpressed in a subset of cases) [3].

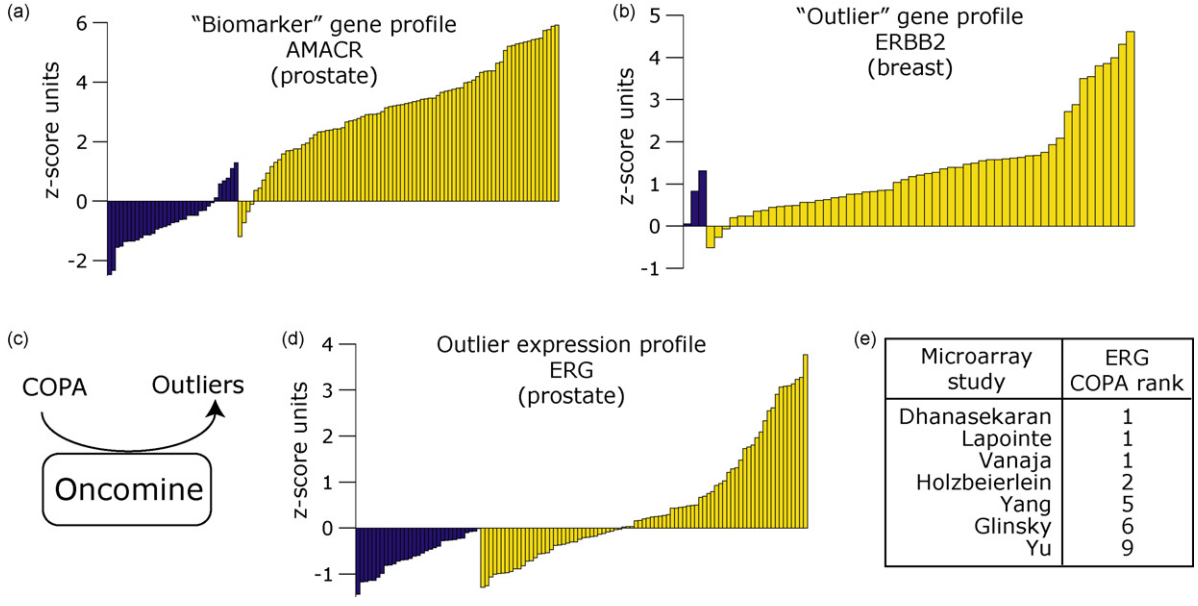

Fig. 1 – Nomination of ETS genes as outliers in prostate cancer gene expression data. Standard analysis of expression profiling data prioritizes genes with a biomarker profile, characterized by generalized overexpression in cancer samples (orange bars), compared to benign samples (blue bars). (a) The biomarker profile of the alpha-methylacyl-CoA racemase gene, *AMACR*, is shown from a prostate cancer profiling study. *AMACR* expression in each sample is shown in *z*-score units (standard deviations from the median). (b) The outlier profile of the v-erb-b2 erythroblastic leukemia viral oncogene homolog 2 gene, *ERBB2*, in a breast cancer profiling study shows marked overexpression in only a fraction of cancer samples. (c) A bioinformatics algorithm called the Cancer Outlier Profile Analysis (COPA) was designed to identify genes with outlier profiles (similar to *ERBB2* in breast cancer). COPA was applied to the Oncomine (www.oncomine.org) database of cancer expression profiling data, to prioritize outliers across cancer types. (d–e) ETS gene family members, the v-ets erythroblastosis virus E26 oncogene homolog gene, *ERG*, and the ETS variant 1 gene, *ETV1*, were identified as high-ranking outliers across multiple prostate cancer profiling studies: (d) The outlier expression profile of *ERG* is shown from a prostate cancer profiling study (compare to a); (e) The rank of *ERG* (out of all measured genes) in multiple prostate cancer profiling studies (identified by the last name of the first author) in the Oncomine database.

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COPA was applied to Oncomine (www.oncomine.org), a database of microarray-expression profiling studies and correctly identified several outlier profiles for genes in specific cancer types with known recurrent rearrangements or amplifications. *ERBB2*, for example, was prioritized as a high-ranking outlier in multiple breast cancer data sets. In several independent prostate cancer studies, COPA identified strong outlier profiles for the v-ets erythroblastosis virus E26 oncogene homolog gene, *ERG*, and the ETS variant 1 gene, *ETV1* (Fig. 1), two ETS family transcription factors that are involved in recurrent rearrangements in Ewing's sarcoma and leukemias [4]. *ERG* and *ETV1* invariably showed mutually exclusive outlier profiles, suggesting a redundant role in prostate cancer. Characterizing cases with *ERG* or *ETV1* outlier expression led to the identification of fusions of the 5′ untranslated region of the prostate-specific androgen-induced transmembrane protease serine 2 gene, *TMPRSS2*, to the respective ETS gene [3]. The fusion of *TMPRSS2* with *ERG* or *ETV1* only occurred in cases with overexpression of the respective ETS gene, and fusions were not detectable in benign prostate tissues. Using fluorescence in situ hybridization (FISH), more than half of a prostate-specific antigen (PSA)–screened cohort of prostatectomy samples had ETS rearrangements, confirming their existence at the chromosomal level. Analysis of *TMPRSS2:ERG*-positive and *TMPRSS2:ERG*-negative prostate cancer cell lines showed that the *TMPRSS2:ERG* fusion resulted in androgen-regulated expression of *ERG*. Thus, the androgen-responsive elements that normally restrict the expression of *TMPRSS2* to the prostate drove the aberrant overexpression of 5′ truncated ETS oncogenes.

2. Evidence acquisition

The PubMed database was searched for reports on ETS fusions in prostate cancer. Search terms *ETS*, *ERG*, or

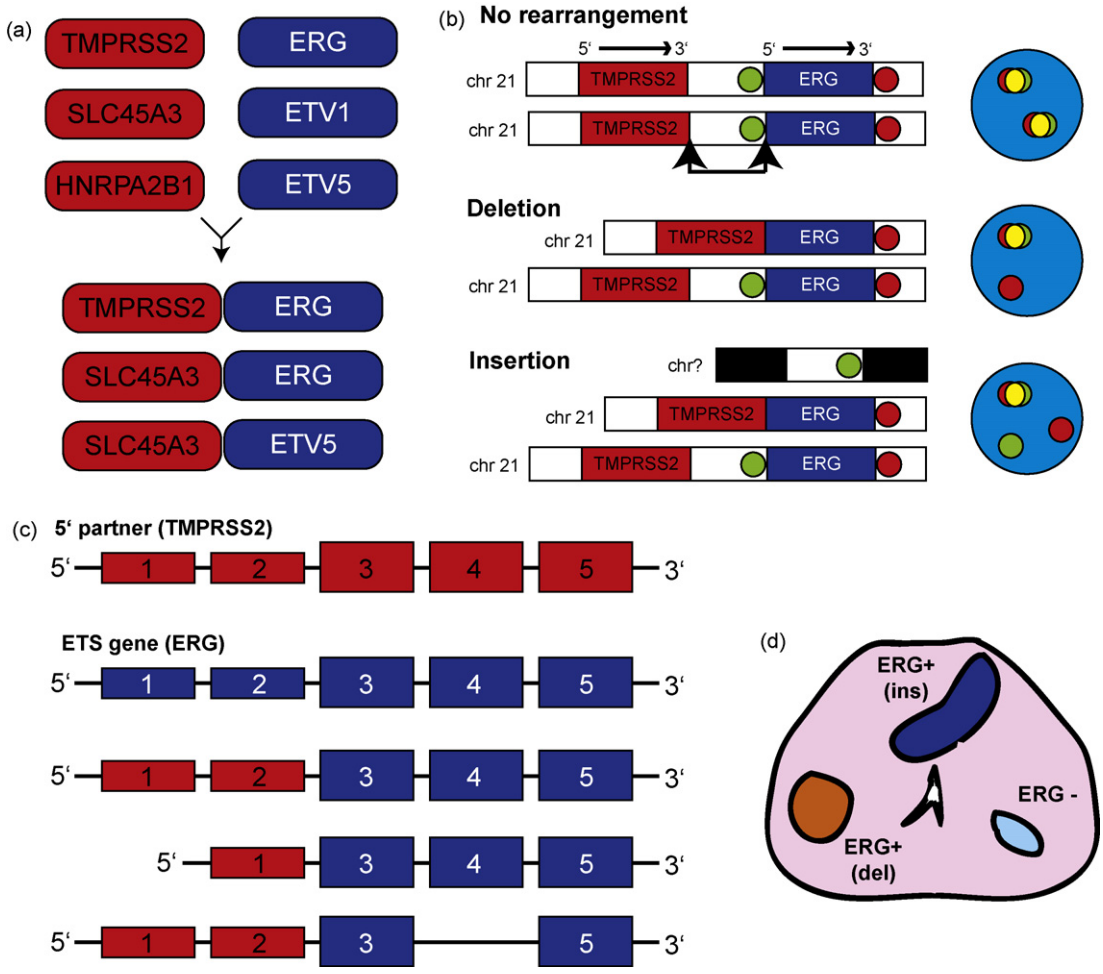

Fig. 2 – Complexity of ETS gene fusions in prostate cancer. (a) Multiple 5′ partners (red) and ETS genes (blue) have been identified. (b) Two genomic mechanisms of *TMPRSS2:ERG* gene fusions have been identified. The prostate-specific androgen-induced transmembrane protease serine 2 gene, *TMRPSS2*, and the v-ets erythroblastosis virus E26 oncogene homolog gene, *ERG*, are located approximately 3 megabases (Mb) apart on chromosome 21, and fusion can occur either through deletion of the intervening genomic region (arrows) or insertion of the intervening region to another chromosome. Stylized results obtained by fluorescence in situ hybridization (FISH) using probes located 5′ (green) and 3′ (red) to *ERG* are shown to the right of the structural diagrams, with colocalization of 5′/3′ *ERG* probes indicated in yellow. (c) Multiple fusion transcript isoforms have been characterized. Stylized structures for *TMPRSS2* (red) and *ERG* (blue) are shown. Noncoding and coding exons are shown in small and large boxes, respectively. Transcripts differ in the location of the junction between the 5′ partner and the ETS gene, as well as the included exons. (d) Localized prostate cancer is commonly multifocal, with several distinct appearing foci of cancer. A single prostate can contain foci without ETS gene rearrangements, foci with *TMPRSS2:ERG* fusion through deletion (del), or foci with *TMRPSS2:ERG* fusion through insertion (ins).

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TMPRSS2 were combined with *prostate* to identify relevant reports.

3. Evidence synthesis

Since the discovery of *TMPRSS2*:ETS fusions, progress has been made in several areas that will affect their translation to clinical application. These areas include identifying additional 5′ and 3′ fusion partners, determining the prevalence of ETS fusions, determining the role of distinct fusion transcript isoforms, understanding the timing and function of ETS fusions in prostate cancer, and understanding ETS fusions in the context of multifocal disease.

3.1. Additional 5′ and 3′ fusion partners

After the initial report of ETS fusions, subsequent studies have identified novel 5′ and 3′ partners (Fig. 2a). Screening additional microarray data sets for ETS gene outlier expression led to the identification of fusions involving the ETS variant 4 gene, *ETV4*, and the ETS variant 5 gene, *ETV5* [5,6]. A FISH-based study of all 27 ETS genes did not reveal additional recurrent rearrangements, suggesting that most 3′ ETS gene partners have been identified [7]. Yet two reports have identified transcripts from the fusion of the solute carrier family 45, member 3 gene, *SLC45A3* and the ETS-domain protein (serum response factor accessory protein 1) gene, *ELK4*, due to transcriptional read-through of these adjacent genes on chromosome 1q32 [8,9]. This finding suggests that other ETS genes may be involved in fusions.

Several studies have demonstrated that ETS fusion–positive and ETS fusion–negative cancers have distinct transcriptional profiles [10–13], suggesting that they represent fundamentally different diseases. This distinction has been exploited in an effort to identify novel biomarkers of ETS fusion–negative prostate cancers. Specifically, multiple groups have identified the serine peptidase inhibitor, Kazal type 1 gene, *SPINK1*, as an overexpressed outlier in a subset of ETS-negative cancers [14,15]. *SPINK1* outlier expression has been associated with shorter biochemical recurrence–free survival, and *SPINK1* knockdown in 22RV1 prostate cancer cells (ie, *SPINK1* outlier-expression cells) attenuates cell invasion, suggesting a functional role.

In addition to *TMPRSS2*, subsequent studies have identified at least 12 additional 5′ partners involved in ETS fusions [7,16–19] (Fig. 2a). These novel 5′ fusion partners appear to contribute mostly to *ETV1*, *ETV4*, *ETV5,* and *ELK4* gene fusions, and a recent report by Attard et al demonstrated that almost two-thirds of patients with *ETV1* fusions have 5′ fusion partners that are yet uncharacterized [16]. In conclusion, while additional ETS genes involved in rearrangements or fusions are likely to be rare, it is probable that either a few prominent or many rare, 5′ partners remain to be discovered. While the expression of most 5′ partners are induced by androgen (including *TMPRSS2*), several 5′ partners are ubiquitously expressed across tissues [19].

3.2. Prevalence of ETS fusions in prostate cancer

Determining the prevalence of ETS fusions is complicated by a lack of completeness of the 5′ and 3′ partners, the detection method, and the characteristics of the clinical cohort assessed. Reverse-transcriptase polymerase chain reaction (RT-PCR), for example, can only detect specific fusion isoforms with known 5′ partners. *TMPRSS2:ERG* is by far the most common subtype of ETS fusions (approximately 85% of all ETS fusion–positive samples [7,20,21]), it is often the only subtype examined, and it can be used to estimate ETS fusion prevalence. A recent review assessing >25 published studies with approximately 1500 cases found that *TMPRSS2:ERG* fusions have been reported in approximately 50% of prostate cancers, reflecting the prevalence in PSA-screened cohorts from North America, Europe, and Asia [20]. Since that time, nine additional studies [7,22–29] with 718 cases representing similar cohorts have reported a *TMPRSS2:ERG* prevalence of 44%, consistent with previous results. Similarly, a multi-institution study of *for cause* needle biopsies (ie, elevated PSA level or abnormal digital rectal examination [DRE]) found that 46 of 100 biopsies with cancer had *TMPRSS2:ERG* rearrangements (Mosquera et al, in press). Results from three population-based cohorts with >750 cases have been published, with *TMPRSS2:ERG* prevalences of 15% (clinical stage T1a–b) [30], 30% (T1–3) [31] and 35.5% (T1–3) [32]. Presently, the reasons for different prevalence in population and PSA-screened cohorts are unclear, although clinical T1 stage cancers in both population-based cohorts have the lowest *TMPRSS2:ERG* prevalence (15% and 17% [30,31]).

Assessing *ETV1*, *ETV4*, and *ETV5* fusions is best accomplished by FISH, given the multiple 5′ partners. The largest studies suggest that, together, they account for approximately 5–10% of PSA-screened prostate cancers [7,16,21]. Attard et al, for example, identified *ETV1* gene rearrangements in 5.4% of the population-based cohort of 429 patients with approximately 30% *TMPRSS2:ERG* prevalence [16].

3.3. Identification of multiple rearrangement mechanisms and fusion-transcript isoforms

Additional complexity is introduced by both the rearrangement mechanism and the multiple fusion-transcript isoforms (Fig. 2b–c). *TMPRSS2* and *ERG* are located <3 megabases (Mb) apart on chromosome 21, and *TMPRSS2:ERG* fusions can occur either through interchromosomal insertion [33] or through deletion of the intervening region on chromosome 21 (approximately 50% by each mechanism [7,10,21,28,29,31,32,34–41]) (Fig. 2b). Furthermore, while the most common *TMPRSS2:ERG* fusion-transcript isoform consists of exon 1 of *TMPRSS2* fused to exon 4 of *ERG*, >20 *TMPRSS2:ERG* isoforms have been reported [3,22,34,38,42–46]. Heterogeneity has been identified in the location of the *TMPRSS2:ERG* fusion junction and in the exons of *ERG* present in the fusion transcript (Fig. 2c). The presence of certain isoforms (such as the isoform consisting of exons 1–2 of

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TMPRSS2 fused to exon 4 of *ERG*) was shown by Wang et al to be associated with features of aggressive prostate cancer [45]. Follow-up in vitro experiments have shown that distinct fusion junctions and variably present *ERG* exons have different effects on proliferation and invasion in cell-line models [46].

3.4. The role of ETS fusions in prostate cancer development

Studies focusing on the timing of ETS fusions and in vitro and in vivo functional studies are rapidly increasing our knowledge of the role of ETS fusions in prostate cancer. In addition to their importance for guiding clinical translation, these studies are also revealing basic principles of prostate cancer biology.

ETS fusions were initially identified in both clinically localized and hormone-refractory metastatic prostate cancer, but they were undetectable in benign prostate tissue [3]. Other studies, however, have reported the presence of ETS fusions in benign prostate tissue and candidate precursor lesions such as high-grade prostatic intraepithelial neoplasia (PIN) [24,36,42,47–49]. At the transcript level, ETS fusions can be detected by both quantitative and nonquantitative techniques, and at the chromosomal level, ETS rearrangements can be detected using FISH.

Although *TMPRSS2:ERG* transcripts have been reported rarely in benign prostate tissues [24,42], quantitative studies have never demonstrated overexpression of *ERG* or *TMPRSS2:ERG* in benign tissue to the level seen in cancer. No published FISH-based studies have reported ETS gene rearrangements in benign prostate glands, proliferative inflammatory atrophy (PIA), or benign prostatic hyperplasia [36]. Thus, while the significance of detectable ETS fusion transcripts in benign prostate tissue in the absence of overexpression and chromosomal rearrangements is presently unclear, they are likely to have minimal function in that setting, and they are likely to minimally affect clinical translation for diagnosis.

Regarding the role of ETS fusions in PIN, profiling defined prostate cell populations isolated by laser capture microdissection demonstrated that PIN and prostate cancer share similar transcriptional profiles [13]. But in specimens from patients with matched PIN and prostate cancer, ETS fusions were only observed in cancer (using marked ETS gene overexpression as a surrogate). This finding led to the hypothesis that in these cases, ETS fusions mediated the transition from PIN to invasive cancer. Subsequent studies demonstrated that approximately 20% of PIN lesions harbor *ERG* rearrangements [24,36,47,49], compared with approximately 50% of localized cancers. Importantly, all PIN lesions with *ERG* rearrangements analyzed using FISH also had intermingling cancer foci with *ERG* rearrangements [36,49]. Some *ERG* rearrangement–positive cancers, however, had *ERG* rearrangement–negative paired PIN lesions [36,49]. These studies support ETS fusions driving the transition from PIN to invasive prostate cancer, and they support at least a subset of PIN lesions as prostate cancer precursors. They also support an alternative pathway where ETS-positive cancer does not require a preceding PIN lesion.

Tissue-based studies have been complemented by in vitro and in vivo studies. Overexpression of truncated *ERG*, *ETV1*, *ETV5*, or various *TMPRSS2:ERG* isoforms in primary or immortalized benign prostatic epithelial cells increases cell migration and invasion in all reported models [6,12,18,19,46,50]. Similarly, knockdown of *ERG* or *TMPRSS2:ERG* in the vertebral cancer of the prostate (VCaP) cell line (*TMPRSS2:ERG* positive) or *ETV1* in the human prostate adenocarcinoma cell line (LNCaP) (*ETV1* rearrangement positive) similarly decreases invasion [12,19,46,51]. In vitro studies have produced conflicting results with regard to the role of ETS fusions in proliferation. The results are likely to have been influenced by the following factors: the length of time that proliferation was measured, whether overexpression/knockdown was done transiently or stably, the efficiency of overexpression/knockdown, and the specific models used [6,12,18,19,46,50,51]. Two groups showed that *TMPRSS2:ERG* knockdown in VCaP resulted in decreased tumor growth using in vivo orthotopic [46] or subcutaneous [51] murine models.

In vivo recapitulation of ETS fusions by prostate-specific expression of truncated *ERG* or *ETV1* in mice resulted in the development of PIN but not carcinoma [12,19,50]. Additionally, overexpression of truncated *ERG*, truncated *ETV1*, or *TMPRSS2:ERG* failed to transform primary or immortalized benign epithelial cells [12,19,46], although expression of *ETV1* was transformed into immortalized nontumorigenic PNT2C2 prostate epithelial cells [18]. Thus, tissue and in vitro studies support a role for ETS fusions in mediating the transition to invasive cancer. These results are probably from PIN lesions in a subset of cases, and several in vitro studies also support a role for ETS fusions in proliferation. ETS fusions, however, have not transformed primary or immortalized prostate epithelial cells, and in vivo recapitulation in the mouse results in the development of PIN, not cancer. These results suggest that ETS fusions occur in the context of other genetic lesions, such as those that drive the transition from benign epithelium to PIN. All in vitro and in vivo overexpression systems reported to date do not harbor additional genetic lesions, and it is likely that models combining ETS fusions with other lesions will be needed to accurately model prostate cancer development.

Additional evidence for ETS fusions in the transition to invasive cancer is provided by the similar incidence of *ERG* rearrangements in clinically localized prostate cancer and both hormone-naïve cancer (33% and 46%) [36,44] and castration-resistant metastatic prostate cancer (CRPC) (33%, 37%, and 37%) [27,52,53]. This evidence suggests that ETS lesions are not continually selected for during metastatic progression. A FISH-based study by Mehra et al, which assessed ETS fusions in a cohort of 27 men who died of CRPC, found that 52% of the men had *TMPRSS2* or ETS gene rearrangements and all metastatic deposits from a single patient were uniformly ETS fusion positive or ETS fusion negative [52].

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3.5. ETS fusions in the context of prostate cancer multifocality

Localized prostate cancer is a multifocal disease, with several discrete tumor foci present in the same prostate gland. In contemporary series, approximately 75% of whole-mount radical prostatectomy specimens have multiple cancer foci [54]. Recently, several groups have examined ETS fusions in the context of multifocal cancer. All groups showed that from 41% to 67% of cases harbor individual cancer foci that differ with regard to the presence of ETS fusions or fusion mechanism (ie, through deletion or insertion) [24,37,39,48] (Fig. 2d). ETS fusions are present in almost all cells in an individual focus when present, suggesting that multiple clonal cancers are developing in a single prostate; thus, the majority of men truly develop multiple cancers in their prostate. Combined with the observation that all metastatic foci in an individual patient are uniformly positive or negative for ETS fusion, this suggests that only one focus is seeding metastatic deposits. Almost all biomarker studies in prostate cancer are performed using a single tissue section or tissue microarray core from the *dominant focus* (usually defined as the largest focus or the focus with the highest Gleason pattern); however, presently there is no way of knowing whether the dominant focus is also the focus seeding metastases. Beyond influencing the diagnostic potential of ETS fusions, this issue probably confounds prognostic studies involving ETS fusions and other biomarkers.

3.6. Translation of ETS fusions to clinical practice

To advance the care of men with prostate cancer, the discovery of ETS fusions must be translated to clinical practice. In this section, we discuss the challenges and prospects for clinical translation of ETS fusions, including diagnosis, prognosis, therapeutic targeting and follow-up (Fig. 3).

3.6.1. The use of ETS fusions for early diagnosis of prostate cancer

Since their discovery, ETS fusions have been promoted as specific prostate cancer biomarkers, with potential use for diagnosing prostate cancer. In this section we review two of the most promising areas: early diagnosis and diagnostic dilemmas.

Beyond the technical development of an ETS fusion–based test, additional issues and controversies are inherent in the application of an early detection test for prostate cancer [55–57]. Widespread use of serum PSA screening in the United States has led to increased detection of prostate cancer and a shift in stage at diagnosis [55–57]. But clinically insignificant prostate cancer is common, and early detection leads to overdiagnosis [55–57]. Importantly, recent results from randomized trials in Europe and the United States did not conclusively answer whether PSA screening improves prostate cancer survival [58,59], and longer follow-up is needed.

The protein product of almost all ETS fusions, including the most common *TMPRSS2:ERG* isoform, is a truncated ETS protein rather than a chimeric protein. In our experience, commercially available ETS antibodies show poor specificity for individual ETS family members, and improved reagents are needed. Additionally, unlike PSA, there is no evidence that the protein products of ETS fusions are secreted. Precedents for the use of noncoding transcripts for the early detection of prostate cancer have been provided by *PCA3* [60], which is a noncoding transcript that can be detected in post-DRE urine and is being used clinically as a prostate cancer biomarker [61]. Hence, most early research on ETS fusions has focused on detection of the fusion transcript in urine (Fig. 3).

In 2006, Laxman et al reported the detection of *TMPRSS2:ERG* transcripts by quantitative RT-PCR in post-DRE urine from 42% of men with prostate cancer [62]. In a follow-up study, Laxman et al demonstrated that a panel of four transcript biomarkers, including *TMPRSS2:ERG*, prospectively collected from post-DRE urine, outperformed

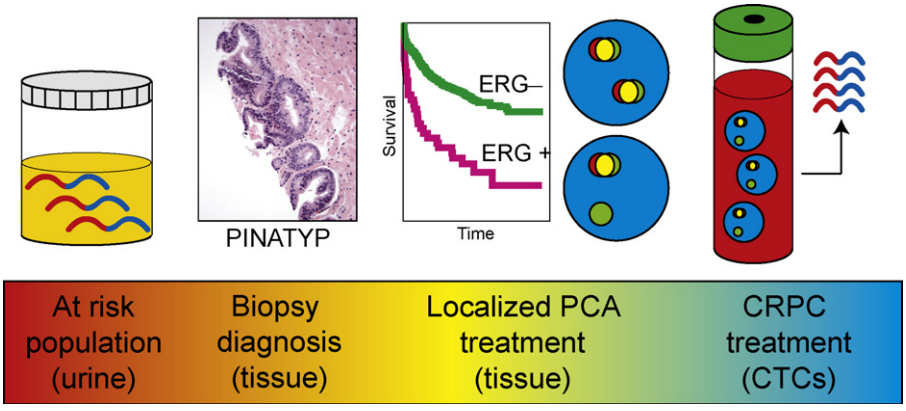

Fig. 3 – Translation of ETS gene fusions to clinical practice. Potential applications of ETS gene fusions during the clinical course of prostate cancer are shown. Detection of fusion transcripts in the urine of at-risk men can be used for the early detection of prostate cancer. Determination of ETS gene fusion status in biopsy tissue may be able to stratify precursor lesions and diagnostically difficult cases. ETS fusion status and specific fusion mechanisms and/or transcripts may be used to influence treatment decisions for localized prostate cancer, including the choice of active surveillance versus definitive treatment. ETS fusion status and transcript expression in circulating tumor cells may be used to indicate ongoing androgen signaling in the castration resistant metastatic stage. CRPC castration-resistant prostate cancer; CTCs = circulating tumor cells; PCA = prostate cancer; PINATYP = prostatic intraepithelial neoplasia and adjacent small atypical glands.

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serum PSA screening or *PCA3* detection alone for the prediction of prostate cancer in 234 men undergoing for cause biopsy or prostatectomy [63]. Recently, promising results were reported by Hessels et al, who used a combination of RT-PCR and Southern blotting to prospectively detect *TMPRSS2:ERG* in post-DRE urine from 108 men undergoing for cause biopsy [64]. They found that detection of *TMPRSS2:ERG* had a sensitivity of 37%, a specificity of 93%, and a positive predictive value of 94%. Groskopf et al reported interim results from a multi-institution study that used transcription-mediated amplification to detect *TMPRSS2:ERG* in prospectively collected post-DRE urine from 208 men undergoing for cause biopsy. In two independent cohorts, the authors found sensitivities of 32% and 51% and specificities of 93% and 91%, respectively.

These results suggest that a commercially available urine test for *TMPRSS2:ERG* is technically feasible. Multiple studies suggest that such a test in PSA-screened cohorts has a sensitivity of 30–50% with specificity >90%, consistent with the tissue prevalence of *TMPRSS2:ERG* fusions in these populations. One of the most obvious benefits of this test is likely to be increased specificity compared with PSA screening, which is not surprising given that PSA level is specific for prostate tissue but not for prostate cancer. Yet this also means that the theoretical maximum sensitivity, regardless of the cut-off used, is likely to be equal to the incidence of *TMPRSS2:ERG* fusion cancers in the population being tested. Testing for *TMPRSS2:ERG*, however, may detect the approximately15–20% of men who harbor prostate cancer but have a normal DRE and a PSA level <4.0 ng/ml, including the substantial proportion of these men who harbor high-grade Gleason disease [65,66]. Presently, it is unclear how the multifocal nature of prostate cancer will affect urine-based testing.

A urine-based assay for *TMPRSS2:ERG* is likely to be clinically available before important questions regarding its application as an early detection test will be answered, similar to serum PSA. Hence, obvious future areas of research are likely to mirror those of serum PSA and include population-based studies in which all patients receive a prostate biopsy, regardless of *TMPRSS2:ERG* result. Improvements in multiplexing may also increase the sensitivity of urine-based testing without sacrificing specificity.

3.6.2. The use of ETS fusions in diagnosis of prostate cancer from biopsy

The initially reported specificity of ETS gene rearrangements for prostate cancer has also prompted research that may affect the diagnosis of prostate cancer using needle biopsy. The FISH-based study by Mosquera et al showed that the presence of *ERG* gene rearrangements in PIN always implied the presence of cancer [49], and FISH-based assessment of isolated PIN has been envisioned to identify men with adjacent cancer that was not sampled or are undergoing the transition to invasive prostate cancer. Additionally, FISH-based assessment of isolated atypical glands suspicious for carcinoma or isolated high-grade PIN and adjacent small atypical glands (PINATYP) may aid in predicting which men have cancer and whether PINATYP represents adjacent cancer from outgrowths off the high-grade PIN. Hence, research is ongoing as to whether the presence of ETS fusions can be used to stratify these lesions (Fig. 3).

3.6.3. Association of ETS fusions and clinically aggressive prostate cancer

As described above, an early detection test is beneficial if it reduces cause-specific mortality, which has not been conclusively answered for serum PSA testing. Additionally, PSA testing has led to the overdiagnosis of cancer that never would have presented clinically. Thus, an active area of research has been to identify biomarkers of aggressive prostate cancer that could be utilized to stratify patients before definitive treatment or that could guide more aggressive treatment. Several reports of associations between ETS fusions and features of aggressive prostate cancer have been published from two types of cohorts: population-based *watchful waiting* cohorts and retrospective prostatectomy series. A review of the literature demonstrates that studies have reported associations between ETS fusions and both more and less aggressive clinical courses. In this review, we hope to clarify this confusion but concede that large population-based studies are needed.

At the population level, three studies assessing ETS fusions have been reported [30–32], although one was limited by a small number of events [32]. Demichelis et al reported on a population-based study of 111 Swedish men with T1a–T1b prostate cancer diagnosed by transurethral resection of the prostate (TURP) for symptomatic benign prostatic hyperplasia. These men were not identified by PSA screening, and they were followed without curative treatment (ie, watchful waiting); the study end point was development of distant metastases or prostate cancer-specific death [30]. The authors observed a significant association between the presence of *TMPRSS2:ERG* fusion and metastases or disease-specific death, although this association was not significant when adjusted for Gleason score [30]. Similarly, in a cohort of 445 men with T1–T3 prostate cancer diagnosed by TURP and conservatively managed, Attard et al found significant associations between *ERG* rearrangements and increased Gleason score, higher clinical stage, and higher baseline serum PSA level [31]. The authors found that, compared to men without *TMPRSS2:ERG* rearrangements, men with *TMPRSS2:ERG* fusions through deletion had significantly worse prostate cancer–specific and overall survival. Men with *TMPRSS2:ERG* fusions through deletion with two or more copies of the 3′ region of *ERG* (referred to as "2+EDel") had the worst survival rate (25% at 8 yr vs 90% in *ERG* rearrangement–negative cancers) [31].

Retrospective studies examining associations between ETS fusions and features of aggressiveness or outcome following radical prostatectomy have also been reported. These studies have produced conflicting results; for example, several have found associations on univariate or multivariate analysis between ETS fusions and features of aggressive prostate cancer including higher Gleason grade,

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increased stage, or decreased PSA recurrence-free survival [21,35,41,67–69]. Other studies have reported no association with aggressive features or recurrence-free survival [22,26,29,34,40,44], while others found association with lower Gleason grade [23,29] or increased recurrence-free survival [27].

In addition to obvious cohort differences, additional factors are likely to contribute to the different results observed between the watchful waiting and prostatectomy studies, as are the conflicting results between prostatectomy cohorts. Both types of studies, as reported, have not addressed the multifocality of localized prostate cancer, and as described above, there is currently no way of knowing whether the focus used for assigning ETS fusion status is the focus that would go on to metastasize or cause a recurrence of rise in PSA level after treatment. Additionally, it is difficult to directly compare population-based studies, in which cancer was diagnosed by TURP in the absence of widespread PSA screening and patients were managed expectantly, to prostatectomy cohorts, in which patients were diagnosed by biopsy in the presence of PSA-screening, treated with prostatectomy, and followed for biochemical recurrence. While the former studies describe the natural history of ETS fusion–positive cancers, the latter explore outcome after intervention. Additionally, the end points reported in the population studies (ie, prostate cancer specific and overall survival) and the prostatectomy cohorts (ie, recurrence of rise in PSA level) are distinct. Porter et al, for example, reported that in a radical prostatectomy cohort with 25 yr of follow-up, 45.5% of men diagnosed with clinically localized prostate cancer in the pre–PSA-screening era had PSA recurrence postprostatectomy but only 18.5% died of prostate cancer [70]. Thus, while PSA recurrence following surgery is associated with prostate cancer-specific death, the majority of men with PSA recurrence will die of other causes. Therefore, we would argue for caution in overinterpreting the results of studies using PSA recurrence as an end point.

Evidence from both watchful waiting cohorts [31] and prostatectomy series [28,41] suggest that *TMPRSS2:ERG* fusion through deletion, particularly in the presence of gain of *ERG*, may be associated with more aggressive cancer (Fig. 3). In addition to the watchful waiting study reported by Attard et al [31], Perner et al reported that *TMPRSS2:ERG* fusion through deletion was significantly associated with high tumor stage and presence of pelvic lymph node metastases in a prostatectomy series [41]. In the study of men who died of CRPC, in which all metastatic foci from a single case were uniformly ETS fusion positive or negative, all 10 of the 27 cases that were *TMPRSS2:ERG* positive represented fusion through deletion [52].

In summary, based on two large observational clinical studies with long-term follow-up, we would argue that, left untreated, *TMPRSS2-ERG* prostate cancer will run a more aggressive clinical course than fusion-negative cancer (Fig. 3). In the setting of surgical or other interventions immediately following diagnosis, data are insufficient to make any reasonable conclusions. Emerging evidence suggests that *TMPRSS2:ERG* cancers with fusion through deletion and gain of *ERG* may be particularly aggressive. Overexpression of *ERG* has also been associated with poor clinical outcome in acute myelogenous leukemia [71], and some of the genes located in the 3-Mb area of deletion between *TMPRSS2* and *ERG* (eg, *HMGN1*) have been proposed as tumor suppressors [41].

Population-based studies suggest that men with *TMPRSS2:ERG*-positive cancers have more aggressive cancers; early detection in urine may reduce disease mortality. Additionally, whether ETS fusions are independent prognostic factors for an early detection test is not as important as detecting aggressive cancers. A urine-based test should be able to detect the presence of any fusion-positive foci in a man with prostate cancer. Obviously, prospective trials correlating urine, biopsy, natural history, and outcome following prostatectomy will be needed to further explore associations between ETS fusion status and outcome.

3.6.4. *ETS fusions as therapeutic targets*

In addition to their potential as specific biomarkers, ETS fusions are attractive therapeutic targets (Fig. 4). Research into targeting the 5′ or 3′ end of ETS fusions, as well as downstream targets, is ongoing. Most 5′ ETS fusion partners, for example, including *TMPRSS2*, are androgen responsive, and it is likely that current and future therapeutic strategies that target androgen signaling [72,73] may function at least in part through inhibition of ETS fusions (Fig. 4). In phase 1 and 2 trials of abiraterone acetate, a small-molecule inhibitor of cytochrome P (CYP) 17, Attard et al found that 32 of 77 (41%) men with CRPC had ERG rearrangements that could be detected in circulating tumor cells (CTCs) by FISH prior to treatment. Importantly, 12 of 15 patients (80%) who had a $\geq$90% decline in PSA level on abiraterone had an ERG rearrangement, compared with 20 of 62 patients (32%) who did not have a $\geq$90% decline in PSA level [53,74]. In this cohort, a decline in PSA level was associated with decreased CTCs and an increased survival rate, and phase 3 trials incorporating fusion status are ongoing. This suggests that ETS gene-fusion status may be used as a prospective read-out of androgen dependence in the CRPC state.

These results are important in light of a recent study by Hermans et al, who reported that ETS fusions were relevant in localized cancer but were bypassed in "androgen receptor negative" CRPC (based on the absence of fusion transcript expression in cases with genomic evidence of rearrangement) [75]. Results from Attard et al [53] provide further evidence to suggest that most CRPCs, including those with ETS fusions, remain dependent on androgen signaling [53,73]. These results suggest that men with gene fusions driven by androgen-regulated 5′ partners may be responsive to antiandrogen treatment, and future trials should be designed to interrogate gene fusion status and expression (as a read-out for androgen signaling) to allow for correlation with clinical response (Fig. 3). A recent study by Setlur et al suggests that estrogen signaling may also drive the expression of *TMPRSS2*:ETS fusions [11], suggest-

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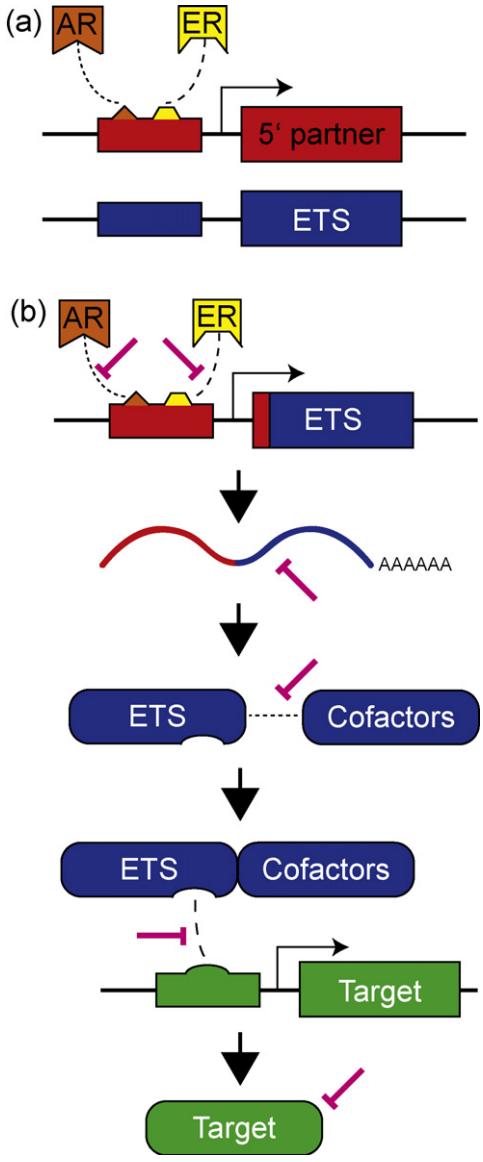

Fig. 4 – Potential therapeutic targeting of ETS gene fusions. (a) In the absence of ETS gene fusions, interaction of the androgen receptor (AR) and/or the estrogen receptor (ER) with response elements in the regulatory region (small box) drive the expression of 5′ partners (such as the prostate-specific androgen-induced transmembrane protease serine 2 gene, *TMPRSS2*). (b) With ETS gene fusions, these interactions now drive the expression of chimeric ETS gene transcripts and can be targeted by interfering with androgen or estrogen signaling. Chimeric ETS gene transcripts may be targeted using short interfering RNA (siRNA). Encoded ETS proteins interact with cofactors to regulate transcription of target genes, and this interaction may be targeted with small molecules. ETS genes bind to specific DNA sequences present in the regulatory region of downstream targets, which may be targeted by small molecules. Some downstream target proteins that are required for the phenotypic effects caused by ETS gene fusions may also be targeted.

ing an additional potential mechanism for targeting ETS fusions (Fig. 4).

In respect to targeting the 3′ end of ETS fusions (Fig. 4), nuclear transcription factors have conventionally been considered poor drug targets. Screening for compounds that inhibit binding of the ETS domain to target DNA or block interactions with required cofactors may prove challenging, but a novel method to identify inhibitors of ETS fusions in Ewing's sarcoma was recently demonstrated by Stegmaier et al [76]. The authors identified an expression signature of *EWS:FLI* inhibition in Ewing's sarcoma cell lines and then screened a small-molecule library with a high-throughput, ligation-mediated amplification assay to identify compounds that produced a similar *EWS:FLI* inhibition signature. This approach identified cytosine arabinoside as a modulator of *EWS:FLI* [76].

Given their specificity for prostate cancer, ETS fusions appear to be ideal candidates for emerging RNA interference–based therapies [77], and knockdown of *TMPRSS2:ERG* in fusion-positive cells has been shown to inhibit tumor growth in xenograft assays [46,51] (Fig. 4). Finally, as deregulated transcription factors, ETS fusions are likely to drive prostate cancer development through induction or repression of downstream target genes. Hence, a potential therapeutic strategy is to target such required downstream targets (Fig. 4). This strategy has been applied in principle to ETS fusions in Ewing's sarcoma by Smith et al, who used expression profiling of in vitro *EWS:FLI* knockdown and reexpression to identify *NKX2.2* as an *EWS:FLI*-regulated gene that is necessary for transformation [78].

4. Conclusions

In addition to the clinical applications reviewed in this paper, ETS fusions have the potential to affect other aspects of prostate cancer management; however, research in these areas is in its infancy or has not yet been reported. It is unclear, for example, whether ETS fusions may be used for monitoring disease recurrence. Similarly, no reports of association between ETS fusions and outcome after radiation therapy or adjuvant androgen deprivation therapy have yet been published.

Fundamental issues about ETS fusions remain poorly understood and will likely drive and shape their translation to clinical practice. The mechanism driving ETS gene rearrangements, for example, particularly the relationship to androgen-regulated 5′ partners, is unknown. The relationship between preceding and/or concurrent epigenetic and genetic events (eg, those that initiate the development of PIN) and ETS fusions are uncharacterized. Finally, driving mutations or similar fusions in ETS-negative cancers have not been well characterized, and a large subset of prostate cancers may harbor fusions not involving ETS genes. Novel analytical techniques combined with continued technological improvements in high-density exon, single-nucleotide polymorphism and comparative genomic hybridization microarrays, and next-generation sequencing are beginning to provide insight into these issues [8,32,79–84].

In summary, our understanding of ETS fusions has increased rapidly since their discovery. With regard to clinical translation, population-based studies suggest that ETS fusion–positive cancers have a more aggressive natural history and support early detection based efforts. Presently, conflicting results have been reported regarding an prognosis of ETS fusion–positive versus ETS fusion–negative cancers after surgical resection. The first clinical applications of ETS fusions will likely be for early diagnosis and

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diagnosis and/or stratification of difficult diagnostic cases on needle biopsy. Finally, several strategies for therapeutically targeting ETS fusions have been identified and are currently being pursued.

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Study concept and design: Tomlins, Bjartell.
Acquisition of data: Tomlins.
Analysis and interpretation of data: Tomlins.
Drafting of the manuscript: Tomlins, Rubin.
Critical revision of the manuscript for important intellectual content: Bjartell, Chinnaiyan, Jenster, Nam, Rubin, Schalken.
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Supervision: Chinnaiyan.
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