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Recurrent Gene Fusions in Prostate Cancer

Chandan Kumar-Sinha^{1,3}, Scott A. Tomlins^{1,3}, and Arul M. Chinnaiyan^{1,2,3,4,5,*}

¹Michigan Center for Translational Pathology, University of Michigan Medical School, Ann Arbor, Michigan 48109

²Howard Hughes Medical Institute, University of Michigan Medical School, Ann Arbor, Michigan 48109

³Department of Pathology, University of Michigan Medical School, Ann Arbor, Michigan 48109

⁴Department of Urology, University of Michigan Medical School, Ann Arbor, Michigan 48109

⁵The Comprehensive Cancer Center, University of Michigan Medical School, Ann Arbor, Michigan 48109

Abstract

The discovery of recurrent gene fusions in a majority of prostate cancers has important clinical and biological implications in the study of common epithelial tumors. Gene fusion and chromosomal rearrangements were previously thought to be the primary oncogenic mechanism of hematological malignancies and sarcomas. The prostate cancer gene fusions that have been identified thus far are characterized by 5' genomic regulatory elements, most commonly controlled by androgen, fused to members of the ETS family of transcription factors, leading to the over-expression of oncogenic transcription factors. ETS gene fusions likely define a distinct class of prostate cancer which may have a bearing on diagnosis, prognosis and rational therapeutic targeting.

Introduction

Prostate cancer is one of the most prevalent malignancies affecting men worldwide, and is the most frequent cancer among American men with an estimated incidence of approximately 220,000 (29% of all cancers in men) and a mortality estimated to be over 27,000 (9% of all male cancer deaths) in 2007¹. An array of treatment modalities are available, including active surveillance, prostatectomy, radiation therapy and androgen ablation therapy, all influenced

*Correspondence to A.M.C.: E-mail: arul@med.umich.edu.

Competing Interest Statement

The University of Michigan has filed for a patent on the detection of gene fusions in prostate cancer, on which S.A.T. and A.M.C. are co-inventors. The diagnostic field of use has been licensed to GenProbe Inc. GenProbe has had no role in the design or experimentation of this study, nor has it participated in the writing of the manuscript. Oncomine and the MCM are freely available to the academic community. The commercial rights to Oncomine and MCM have been licensed to Compendia Bioscience, which A.M.C. co-founded.

A summary of the published literature on the characterization of recurrent gene fusions in prostate cancer. The columns in order from left to right detail the gene fusion, sample cohort the data is derived from, the frequency of the gene fusion in the sample set/observed gene fusion/ total number of samples examined (% gene fusion in the sample cohort), the assay used for detection and characterization of the gene fusions, and the reference.

Databases

Mitelman Database: <http://cgap.nci.nih.gov/Chromosomes/Mitelman>

Oncomine: <http://www.oncomine.org/>

Atlas of Genetics and Cytogenetics: <http://atlasgeneticsoncology.org/index.html>

Entrez gene: <http://www.ncbi.nlm.nih.gov/sites/entrez?db=gene>

COPA package in R: <http://www.bioconductor.org>

COPA package integrated into SAM: <http://www-stat.stanford.edu/~tibs/SAM>

by the use of serum prostate specific antigen (PSA) levels². Even as clinically localized prostate cancer has become highly curable the overall death toll remains high due to recurrence of “cured” cases and progression to hormone refractory metastatic disease, which remains incurable³. Conversely, nonspecific PSA tests result in a large number of false positives for prostate cancer, leading to a *faux*-cancer burden and repeated biopsies⁴. More specific diagnostic modalities, prognostic indicators of progression and a better understanding of prostate cancer biology for treatment of hormone refractory disease are high priorities in prostate cancer research.

Approximately two years ago, our group identified recurrent genomic rearrangements in prostate cancer resulting in the fusion of the 5' untranslated end of *TMPRSS2* (a prostate specific, androgen responsive gene) to *ETS* family genes (oncogenic transcription factors)⁵. The original findings have been rapidly corroborated by several independent groups worldwide and now the focus is on the functional and clinical correlates of prostate cancer in the context of the gene fusions. Emerging experimental evidence suggests that these fusions are key molecular entities driving the development and progression of a unique class of prostate cancers, providing potential avenues for targeted therapy, similar to the *BCR-ABL1* gene fusion in chronic myeloid leukemia (CML).

Gene fusions resulting from chromosomal rearrangements represent the most prevalent form of genetic alterations known in cancers⁶ and, as exemplified by the archetype gene fusion *BCR-Abl1* in CML^{7, 8} they can serve as ideal diagnostic markers^{9–11}, provide insight into tumor biology¹², and most importantly serve as specific therapeutic targets^{13, 14}. Intriguingly, while numerous gene fusions have been described in rare hematological malignancies and even rarer bone and soft tissue sarcomas¹⁵, they are much rarer among epithelial cancers. Gene fusions described among epithelial cancers so far have included *RET-NTRK1* fusions in papillary thyroid carcinoma, *PAX8-PPARG* in follicular thyroid carcinoma, *MECT1-MAML2* in mucoepidermoid carcinoma, the *TFE3-TFEB* in kidney carcinomas, and *BRD4-NUT* in midline carcinomas etc (reviewed¹⁶). Remarkably, recurrent gene fusions have not previously been detected in the most prevalent carcinomas including prostate, breast (with the exception of rare, secretory breast cancers), lung, gastrointestinal and gynecologic tumors¹⁷, despite compelling arguments that predict their occurrence^{15, 18, 19}. The absence of gene fusions in common solid tumors has been attributed to the technical difficulties associated with their cytogenetic analysis. Also, epithelial cancers are thought to be clonally heterogeneous, with causal chromosomal aberrations co-habiting the tissues with clinically irrelevant ones. While cytogenetic analyses help identify ‘physical’ genomic aberrations, recurrent gene fusions in prostate cancer were identified based on gene expression data, bypassing the technical limitations of cytogenetics in solid cancers. This strategy led to the identification of recurrent gene fusions in common solid cancers, close to 50 years after the discovery of Philadelphia chromosome in 1960s.

In this review, we appraise recent progress in the characterization of recurrent gene fusions in prostate cancer. We will highlight the clinical implications of new discoveries, emerging controversies and challenges, as well as future research directions. In addition to serving as potential diagnostic/prognostic markers and therapeutic candidates for a unique class of prostate cancer, the discovery of recurrent rearrangements in prostate cancer affirms a more generalized role for similar chromosomal aberrations in other common epithelial cancers.

Discovering gene fusions with bioinformatics

Cancers are, for the most part, phenotypically and molecularly heterogeneous entities. Thus, characterization of distinct molecular classes with an overarching influence of a single gene or two is clinically and therapeutically significant. For example, in one-quarter to one-third of

all breast cancer cases, amplification and over-expression of the oncogene *HER2* defines an aggressive class that is more likely to metastasize, develop hormone resistance, and respond significantly to *HER2* targeted therapy. Likewise, Philadelphia chromosome positive chronic myelogenous leukemia (CML) typifies 10% of all leukemia cases, where the underlying aberration is a *BCR-ABL1* gene fusion that becomes the focal point of diagnosis, classification, prognostication, therapy, as well as follow up and recurrence monitoring (BOX 1). Other well-defined cancer classes include less than 5% of all breast cancers harboring *BRCA1* or *BRCA2* mutations²⁰, 6% of colon cancers with microsatellite instability or germline mutations characterizing specific clinical classes such as hereditary non-polyposis colorectal cancer (HNPCC), or familial adenomatous polyposis (FAP)^{21, 22} and 10% of non small cell lung cancers harboring sensitizing mutations in *EGFR* that respond to the *EGFR* targeting drug gefitinib²³.

We initiated a systematic identification of candidate oncogenes activated by chromosomal rearrangements or high level copy number changes based on gene expression signatures using an unconventional analytical approach. Cancer gene expression data sets were queried for genes that are highly over-expressed in a subset of samples rather than focusing on those that are widely shared in all samples⁵. To identify such “outlier” genes, we applied a data transformation algorithm, cancer outlier profile analysis (COPA), to all of the 132 gene-expression data sets (comprising >10,000 microarray experiments from various cancers) available in Oncomine (www.oncomine.org^{24–27}), our gene expression compendium (FIG. 1). COPA transformation effectively compresses typical biomarker profiles characterized by a general overexpression of genes in all cancer samples, while accentuating ‘outlier gene’ profiles, characterized by general low expression with marked overexpression in a fraction of the samples. Prioritized outlier genes identified by our systematic approach included several well-known cancer genes involved in recurrent chromosomal aberrations or high level copy number changes associated with their specific cancers. Surprisingly, two genes known to be involved in gene fusions in Ewing’s sarcoma^{28, 29}, namely *ERG* (ETS-related gene) and *ETV1* (ETS variant gene 1), were scored as high ranking outliers in several independent prostate-cancer profiling studies. Akin to Ewing’s sarcoma, where Ewing sarcoma breakpoint region 1 (*EWSR1*)–*ERG* and *EWSR1*–*ETV1* fusion genes are mutually exclusive in different cases²⁸, *ERG* and *ETV1* overexpression were mutually exclusive in prostate cancer. This suggested that elevated expression of *ERG/ETV1* may be a key molecular event in a subset of prostate cancers.

Analysis of prostate cancer samples that overexpress *ERG* and *ETV1* (“outliers”) using exon-walking quantitative PCR revealed overexpression of only their 3’ regions, suggestive of a genetic rearrangement (FIG. 1). This lead us to characterize the 5’ ends of *ERG* and *ETV1* transcripts using a 5’ RNA-ligase-mediated rapid amplification of cDNA ends (RACE) methodology (FIG 1). We discovered that the 5’ ends of *ERG* and *ETV1* are replaced with the 5’ untranslated region of a prostate-specific, androgen responsive, transmembrane serine protease gene (*TMPRSS2*) (21q22.2) in the outlier cases⁵. Fusion transcripts were confirmed by quantitative PCR, and rearrangements at the genomic loci were then assessed in multiple samples by fluorescence in situ hybridization (FISH) on tissue microarrays (FIG. 1). FISH demonstrated that a majority of prostate cancer samples harbor these aberrations. It bears noting that the *TMPRSS2-ETS* fusions are analogous to the *IgH-myc*^{30, 31} and *IgH-bcl2*³² gene fusions in lymphomas. In these cases, no fusion protein is generated but there is massive overexpression of an oncogenic factor under the control of a subverted promoter element¹².

Interestingly, while almost all *ERG* overexpressing tumors were found to harbor *TMPRSS2:ERG* fusion, subsequent investigations identified fewer *TMPRSS2:ETV1* cases than expected based on the frequency of *ETV1* outlier expression. This paradox was investigated with the use of 5’ RACE (FIG. 1) on *ETV1* outlier samples, leading to the characterization of

multiple upstream fusion partners of *ETV1*. These differed in their prostate specificity and androgen responsiveness, presenting a novel level of complexity in the molecular biology of prostate cancers³³. The upstream partners of *ETV1* identified so far include: a prostate specific androgen-induced gene (*SLC45A3*) and an endogenous retroviral element (*HERV-K_22q11.23*) that are functionally analogous to *TMPRSS2*; a prostate-specific, but androgen-repressed gene (*C15orf21*) and a strongly expressed housekeeping gene (*HNRPA2B1*) with no prostate specificity or androgen responsiveness³³. Different rearrangements were characterized in two prostate cancer cell lines with high level *ETV1* expression; LNCaP and MDA-PCa 2B cells were found to have the *ETV1* gene at 7p21 rearranged to a prostate specific region at chromosome 14q13.3–14q21.1. Recently,

Subsequently, we have characterized prostate cancer samples with COPA defined outlier expression of other ETS family genes and discovered more gene fusions such as *TMPRSS2:ETV4*³⁴, *TMPRSS2:ETV5* and *SLC45A3-ETV5*³⁵. Most recently, Hermans et al. identified the prostate specific, androgen induced genes *KLK2* and *CANT1* as additional 5' partners of *ETV4* (cite pubmed ID: 18451133). The COPA methodology of identifying gene expression outliers is available at www.oncomine.org. This algorithm has been implemented in an R package (termed COPA package; available at <http://www.bioconductor.org>)³⁶ and been integrated into Significance Analysis of Microarrays (SAM) methodology by Tibshirani et al. (<http://www-stat.stanford.edu/~tibs/SAM>)³⁷. Recently, COPA has been used to identify genomic alterations associated with the NFκB pathway in multiple myeloma³⁸, supporting the applicability of this methodology for identifying candidates for chromosomal aberration. This gene expression based methodology effectively overcomes the barriers to cytogenetic identification of recurrent chromosomal aberrations associated with solid cancers.

Diversity and frequency of gene fusions in prostate cancer

TMPRSS2:ERG gene fusions have been reported in approximately 50% of over 1500 clinically localized prostate cancer samples analyzed in over two dozen reports published thus far (TABLE 1), reflecting the prevalence of such fusions in PSA screened patient cohorts, with a lower frequency (15%) reported for a population-based cohort (cite Swedish watchful paper). Overall, early- and mid-stage localized prostate cancers and hormone refractory metastatic cancers display *TMPRSS2:ERG* rearrangements in 50% or more cases, whereas high-grade prostatic intraepithelial neoplasia (HGPIN) appear to have a lower frequency of the gene fusions (TABLE 1)^{39–43}. It bears noting that gene fusions involving other ETS family members, primarily *ETV1* but also including *ETV4* and *ETV5*, together likely constitute less than 10% of prostate cancer samples.

Considering the annual incidence (220,000 cases) of prostate cancer in the United States alone, the number of patients with ETS gene fusions likely surpasses the number of patients with *BCR-ABL1* (~7000 patients per year) and several bone and soft tissue sarcomas with individual incidences ranging from a few dozen to a few hundred per year^{1, 15}.

While high throughput FISH assays using tissue microarray sections have been useful in screening large numbers of samples to determine the prevalence of recurrent gene fusions in prostate cancer, the characterization of fusion transcripts by RACE and RT-PCR has revealed a spectrum of fusion transcript variants (FIG. 2; TABLE 1). The most common variants involve *TMPRSS2* exon 1 or 2 fused to *ERG* exon 2, 3, 4 or 5 (ref 5, 39, 40, 44–49). Less frequent combinations include *TMPRSS2* exon 4 or 5 fused to *ERG* exon 4 or 5 (ref 48), and in one case, *TMPRSS2* exon 2 was found fused to inverted *ERG* exon 6–4⁴⁵. These variant fusion transcripts most likely represent alternative splicing variants⁵⁰, and unpublished work from other groups suggests that there are distinct phenotypic effects produced by different isoforms. Lapointe et al. have described two isoforms of *TMPRSS2* involved in fusion with *ERG*: the

reference sequence *TMPRSS2* (exon 1), involved in about 50% of the fusions, and an alternative *TMPRSS2* isoform, mapping 4 kb upstream of the reference sequence, found in 10% of the fusions; approximately 44% of the samples reportedly expressing both the variants⁴⁶. Early efforts to chart the molecular variants have led one group to employ nested RT-PCR to characterize as many as 14 fusion variants⁴⁰ and another 8⁵⁰, with many common variants identified. While the clinical significance of these variants is undefined, some *TMPRSS2:ERG* fusion variants have been associated with prognostic outcomes. For example, Wang *et al*⁵⁰ observed that *TMPRSS2* exon 2 fused with *ERG* exon 4 is associated with aggressive disease, and the fusion transcript with the first in-frame ATG codon present in *ERG* exon 3 was associated with seminal vesicle invasion (which is correlated with poor outcome). Further, other isoforms that highly express fusion mRNAs were also associated with early prostate-specific antigen recurrence.

BCR-ABL1 has well characterized breakpoint clusters in both the genes that determine the nature of fusion genes (BOX 1). With respect to *TMPRSS2:ERG*, Yoshimoto *et al* point to a likely involvement of regions of microhomology (about 300 bp stretches sharing up to 90% homology) in the introns adjacent to fusion exons⁴⁹. Through a systematic mapping of genomic rearrangements in and around the *TMPRSS2* and *ERG* loci using SNP arrays, Liu *et al* correlated the presence of consensus sequences resembling human Alu repeats within the introns of the two genes with the *TMPRSS2:ERG* gene fusion⁵¹.

Most of the fusion genes and transcripts characterized thus far have the protein coding region derived largely from the ETS family gene and in all fusion variants, the ETS domain appears to be retained. Recently, we identified one 'fusion protein', with the first three coding exons (103 amino acids) of an RNA helicase *DDX5*, fused in frame to *ETV4* exon 5, in one prostate cancer with *ETV4* outlier expression (manuscript submitted). However the functional significance of residues from *DDX5* remains unclear.

Clinical, Histological, and Molecular Features

Similar to *BCR-ABL1* positive leukemias^{7, 52}, colon cancers with microsatellite instability^{53, 54}, or breast cancers with *BRCA* mutations^{55, 56}, ETS gene fusions in prostate cancer are associated with specific morphological features and prognoses, as well as specific molecular signatures. A particularly interesting picture is emerging in multi-focal prostate cancers, where different tumor foci in a patient sample have different gene fusion status; however different sites of metastatic prostate cancer from the same patient are uniformly fusion positive or negative.

Association with Morphology

Mosquera *et al* identified five morphological features- blue-tinged mucin, cribriform growth pattern, macronucleoli, intraductal tumour spread, and signet-ring cell- to be significantly associated with prostate tumor samples with *TMPRSS2:ERG* fusion⁵⁷. Samples which harbored three or more of these features were almost always (93%) fusion positive and only 24% of fusion positive samples did not display any of these morphological features⁵⁷. Tu *et al.* also noted a significantly higher frequency of *TMPRSS2:ETS* gene fusions in mucin-positive carcinomas than mucin-negative tumors⁵⁸. Larger studies may help establish the phenotypic associations of the gene fusions (and their variants). A molecular connection between the gene-fusions and specific morphological features will also require follow up studies.

Prognostic Association

TMPRSS2:ERG has been frequently but not unequivocally associated with more aggressive prostate cancers and a poorer prognosis. *TMPRSS2* gene rearrangement has been variously

associated with high pathologic stage⁵⁹ and higher rate of recurrence⁶⁰ in independent cohorts of surgically treated localized prostate cancer cases and the presence of gene fusion has been scored as the single most important prognostic factor^{60, 61}. In a FISH based analysis of 445 cancer cases, not having an *ERG* fusion was found to be a good prognostic factor (90% survival at 8 years) compared to cancers with duplication of *TMPRSS2:ERG* in combination with deletion of 5'-*ERG* (2+Edel) that exhibited very poor cause-specific survival (25% survival at 8 years)⁶². This novel category of '2+Edel' status was found to be an independent of other prognostic factors including Gleason score and serum levels of prostate-specific antigen. In the assessment of gene fusion status in a population-based "watchful waiting" cohort of men with localized prostate cancer- mostly comprising of good or uncertain prognoses, tumors from just 15% of the patients were found to harbor *TMPRSS2:ERG* fusion. Remarkably, this fusion positive subset was significantly associated with prostate cancer specific death⁶³. In another study involving primary prostate cancers and hormone-naïve lymph node metastases, a significant association was observed between *TMPRSS2:ERG* rearranged tumors and higher tumor stage, as well as the presence of metastatic disease involving pelvic lymph nodes⁴¹. Similarly, Rajput *et al* observed more frequent *TMPRSS2:ERG* fusions in moderate to poorly differentiated tumors as compared to well-differentiated tumors⁴⁷. In a cohort of patients treated for clinically localized prostate cancer Nam *et al* observed that *TMPRSS2:ERG* fusion positive subgroup of patients had a significantly higher risk of recurrence (58.4% at 5 years) than fusion negative patients (8.1%)⁶¹. On the other hand many studies have reported an absence of such clinical correlation between the fusions and prognosis. In a report preceding gene fusions discovery, Petrovics *et al* had associated *ERG* overexpressing prostate cancers with several positive prognosticators such as longer recurrence-free survival, well and moderately differentiated stages, lower pathological stage, and negative surgical margins⁶⁴. Similar observations were recently made by Winnes *et al* with respect to gene fusion positive prostate cancers where they report "a clear tendency" for fusion positive tumors to be associated with lower Gleason grade and better survival than fusion negative tumors⁶⁵. Yoshimoto *et al* found no correlation between clinical outcome and presence of gene fusions⁴⁹ and Lapointe *et al* found no significant association between presence of a *TMPRSS2:ERG* fusion and tumor stage, Gleason grade or recurrence-free survival⁴⁶. It may be noted that many of the negative reports have small sample sizes: Yoshimoto *et al*, 15 cases, Winnes *et al*, 50, Liu *et al*, 41, Lapointe *et al*, 63, as compared to the reports of an association: Rajput 196, Perner 136, Nami 26, Nam 165, Mehra 96, Demischelis 111, Attard 445, (TABLE 2). Clearly, more studies, with larger patient cohorts would help resolve specific prognostic association of the gene fusions.

Multifocal prostate cancer

Localized prostate cancer is typically multi-focal with different foci displaying histological and molecular heterogeneity⁶⁷. When the status of gene rearrangements in 93 tumor foci from 43 radical prostatectomy resections were analyzed, 70% of the cases were found rearranged at *TMPRSS2* locus, higher than accounted for by *TMPRSS2:ERG* fusions seen in 55% of the cases⁶⁸. Our study showed that a small percentage of prostate cancers cases might harbor uncharacterized fusion partners driven by the upstream regulatory elements of *TMPRSS2*^{59, 68}. Further, attesting to the heterogeneity of multi-focal cancers, 70% of the cases showed divergent gene rearrangements in the different foci⁶⁸. In another study of *TMPRSS2:ERG* fusions in multi-focal prostate cancers, Barry *et al*. reported that 41% of foci harbored different gene rearrangements⁶⁹. Further, Clark *et al* have reported the detection of *TMPRSS2:ERG* and *TMPRSS2:ETV1* in two different foci of a prostate cancer⁷⁰.

Metastatic prostate cancer

When we surveyed the different metastatic sites of hormone refractory prostate cancer for ETS gene rearrangements, we made two unexpected observations: (1) All the metastatic sites from a patient display identical ETS rearrangement status (fusion positive or negative), and (2) All

of the metastatic prostate cancer sites harboring *TMPRSS2:ERG* were found to be a result of interstitial deletion (EDel) suggesting that EDel is a lethal sub-type of prostate cancer associated with androgen-independent disease⁷¹. Earlier, Attard *et al.* have correlated EDel gene fusions with poorer prognosis⁶² and Perner *et al.*, with high tumor stage and 'metastases to pelvic lymph nodes'⁴¹. Together, these observations strongly suggest that metastatic cancer arises through clonal expansion of malignant cells from a unique primary focus of dissemination, which argues for a careful assessment of different tumor foci of a patient for an accurate prognostication⁷¹ (FIG. 3).

Other Clinical associations

Although gene fusions are *prima facie* acquired somatic mutations, we have observed samples from hereditary prostate cancer (HPC) patients to be three times more likely to harbor *ERG* rearrangements than those from sporadic prostate cancers patients (unpublished observations). Similar familial associations have also been reported with respect to gene-fusion rich myeloproliferative disorders⁷². Our observations could have important clinical implications for the 5–10% of hereditary (as well as 10–20% of familial) prostate cancer patients⁷³. Interestingly, our preliminary observations do not suggest any racial differences in the prevalence of the gene fusions (unpublished observations). Any future studies dealing with these important assessments would need to factor in the multi-focal heterogeneity of the rearrangements, as well as include broader ETS family gene rearrangement screens. Another area of clinical interest would be the assessment of rearrangement status in response to salvage radiotherapy that could facilitate the identification of patients at the risk for recurrence.

Molecular Aberrations associated with Gene Fusions

To elucidate the role of ETS gene fusions in prostate cancer biology (FIG. 4), attempts have been made to define gene expression signatures of ETS-gene over-expressing prostate cancers. A meta-analysis of multiple expression data from 410 human prostate tissue samples (178 normal and 232 tumors and metastases) was performed to identify genes correlated with *ERG* overexpression⁴⁵. A strong association was made with high expression of the *HDAC1* gene and (presumably, consequently) low expression of its target genes. In the same study, an increased expression of WNT-associated pathways and down-regulation of TNF and cell death pathways was also noted. Gene expression profiling of *TMPRSS2:ERG* positive samples lead Setlur *et al.* to define an 87-gene signature-enriched for Estrogen Receptor signaling pathway genes, associated with the gene fusion positive prostate cancer tissues⁷⁴. Follow-up functional studies corroborated the regulation of *TMPRSS2:ERG* expression by estrogenic compounds in an androgen non-responsive prostate cancer cell line harboring the fusion. Increased expression of ER α has been associated with prostate cancer progression, metastasis and androgen resistant phenotype⁷⁵. Taken together, these observations suggest an attractive new therapeutic approach for metastatic prostate cancers that fail androgen ablation therapy (discussed in more detail in a later section).

Since ETS gene fusions appear to account for 50–60% of all prostate cancers, non-ETS fusion cancers may constitute a clinically relevant group as well. We carried out a meta-analysis of three independent gene expression profiling studies^{76–78} for comparison of ETS fusion positive prostate cancers with non-ETS cancers. Importantly, ETS positive and negative tumors had distinct expression, signatures that were maintained across studies and platforms. Using a molecular concepts based analysis (Molecular Concepts Map²⁴), we observed a relative under-expression of genes on chromosomal region 6q21 (for example *FOXO3A* and *CCNC*) in non-ETS prostate cancers⁷⁷. In a subsequent array-CGH analysis from our group, we noted a loss of 6q21 in >45% of non-ETS samples⁷⁹. Recently, Lapointe *et al.* described a deletion at a nearby locus, 6q15 in non-ETS prostate cancer samples⁸⁰. Together, these results suggest that

ETS positive and negative tumors may be fundamentally different types of prostate cancer, driven by distinct genetic aberrations whose effects can be seen in expression signatures.

PIN studies

TMPRSS2:ERG rearrangements have been observed in the prostate cancer precursor tissue, high grade prostatic intraepithelial neoplasia (HGPIN), albeit at a lower frequency. Using a PCR based approach, Cerveira *et al* reported *TMPRSS2:ERG* fusion in 4/19 (21%) of samples examined³⁹, while Perner *et al* reported a very comparable incidence at 5/26 (19.2%) using a FISH based approach⁴². The latter report also indicated that fusion positive HGPIN is almost always present in close proximity of fusion positive cancer tissue. A recent study by Clark *et al* reports HGPIN, as well as some foci resembling low grade PIN (LGPIN), closely associated with fusion positive prostate cancer that harbor *ERG* rearrangements, in as many as six out of nine prostate cancer samples analyzed⁷⁰. Together, these observations strongly suggest that the ETS gene rearrangements may be early events in prostate carcinogenesis.

Functional studies of ETS Gene Fusions in Prostate Cancer

Functional characterization of the role of ETS gene fusions in prostate cancer has primarily focused on assessment of prostate specificity and androgen responsiveness of the fusion genes and the mechanistic role of fusion genes in carcinogenesis. As *TMPRSS2* is prostate specific and strongly induced by androgen,^{81–83} we tested if the *TMPRSS2:ERG* fusion gene is androgen regulated as well. Androgen treatment induced *ERG* expression in prostate cancer cell line VCaP harboring *TMPRSS2:ERG* fusion, but not in LNCaP cells, which are androgen responsive but do not have this fusion⁵. Likewise, there was no induction of *ERG* or *TMPRSS2:ERG* expression in the androgen insensitive NCI-H660 prostate cancer cell line carrying the *TMPRSS2:ERG* fusion⁸⁴. Similarly, Hermans *et al.* found *ERG* expression restricted to the androgen-sensitive human prostate cancer xenografts carrying the *TMPRSS2:ERG* fusion, but not in androgen insensitive samples nor in the samples without the fusion⁴⁴.

Although only *TMPRSS2* has been reported as a 5' fusion partner of *ERG*, additional 5' partners have been identified for *ETV1*, *ETV4* and *ETV5*. These 5' partners include *TMPRSS2*, *SLC45A3*, *HERV-K_22q11.23*, *C15orf21*, *CANT1* and *KLK2*, which are prostate-specific, while *HNRPA2B1* has a ubiquitous housekeeping expression. With respect to androgen regulation, *TMPRSS2*, *SLC45A3*, *HERV-K_22q11.23*, *CANT1* and *KLK2* contribute androgen-inducible sequences, while *C15orf21* is repressed by androgen treatment and *HNRPA2B1* is insensitive to androgens. As androgen ablation therapy is central to advanced prostate cancer management, the divergent androgen responsiveness driving the fusion genes could affect response to therapy and disease progression. This may provide important clues into the biology of hormone insensitive samples as well.

While the upstream regulatory elements of fusion partners dictate prostate-specific, androgen-responsive expression of the ETS genes, an obvious next question is whether these gene fusions are carcinogenic in the prostate. This aspect has been investigated by our group, as well as others, and the results, though not definitive, are very compelling.

To recapitulate the biological effects of aberrant over-expression of *ETV1* in the prostate, truncated *ETV1* was ectopically over-expressed in prostate epithelial cell lines RWPE and PrEC. Surprisingly, we found it did not cause cell transformation and had no effect on cell proliferation or anchorage independent growth; instead, it made the epithelial cells invasive through matrigel assays³³. In a complementary observation, LNCaP cells lost their invasiveness when *ETV1* expression was knocked down^{33, 85}. Consistent with these phenotypic observations, the gene expression signature of *ETV1*-overexpressing RWPE cells

shows an enrichment of molecular concepts (biologically related genes) involved in invasion³³.

To investigate the effects of the gene fusions *in vivo*, we generated transgenic mice over-expressing *ETV1* in prostate epithelium (using a prostate specific, androgen regulated, probasin (*Pb*) promoter⁸⁶). Six out of eight (75%) transgenic mice developed mouse prostatic intraepithelial neoplasia (mPIN), which was variably present in all three prostatic lobes (anterior, ventral and dorsolateral), by 12–14 weeks of age. Consistent with our *in vitro* observations, none of the mPIN foci developed into tumors, which strongly suggests that additional genetic lesions/ environmental factors are required for the development of carcinoma. Virtually identical observations were made in *ERG* over-expression studies *in vitro* as well as in transgenic *Pb-ERG* mice by two independent groups^{87, 88}, and in *ETV5* over-expression studies *in vitro*³⁵.

That transgenic *Pb-ERG* and *Pb-ETV1* mice do not develop frank carcinoma is consistent with clinical observations in human prostate cancer development and progression. Comprehensive FISH analysis showed no *ERG* rearrangements in benign prostate glands, ~20% of PIN lesions (but only intermingling with cancer foci that harbored similar *ERG* rearrangements), ~50% of localized prostate cancers, and ~30% of metastatic prostate cancers⁴². Thus, preceding genetic lesions likely function to deregulate cellular proliferation resulting in PIN, while ETS fusions drive the transition to carcinoma. As metastatic foci from a single case are homogeneous in regards to ETS status, ETS fusions clearly occur before metastasis. We^{33, 87} and others⁸⁹ have speculated that loss of tumor suppressors such as *PTEN* or *NKX3-1* may precede and cooperate with ETS gene fusions to drive cancer development; compound transgenic mice recapitulating these lesions will likely represent an ideal model of human prostate cancer.

Diagnostic, Prognostic, and Predictive Implications of the Gene Fusions

The application of serum measurement of prostate specific antigen (PSA) in prostate cancer diagnosis, prognosis and disease follow-up has had a dramatic impact on prostate cancer management by providing a simple and sensitive primary screening modality to diagnose prostate cancers at a curable stage (reviewed⁴). However, the PSA test exhibits poor specificity, and is often elevated in benign conditions such as prostatitis and benign prostatic hyperplasia^{90, 91}. The diagnosis of indolent prostate cancers has also added to an avoidable cancer burden (reviewed⁹²). Therefore, there is an urgent need for better biomarkers that can distinguish indolent from clinically significant prostate cancer and can reduce the number of unnecessary biopsies (estimated at 70–80%⁹²).

As described in this review, *ETS* positive and negative tumors have distinct morphological features, unique expression signatures, and distinct clinical outcomes. These results suggest that *ETS* negative tumors may harbor other classes of gene fusions, may be driven by distinct molecular mechanisms, or may occur at a different stage of prostate cell differentiation. As prostate cancer is recognized as being heterogenous in both biological and clinical phenotypes, we propose that current data supports two classes of prostate cancer: *ETS* gene fusion positive and *ETS* gene fusion negative. Thus, a sensitive and specific screening test for *ETS* gene fusions, which do not occur in benign prostate tissue or in *ETS* negative cancers, will likely outperform serum PSA for detection of this class of prostate cancer.

Detection of fusions is envisaged from needle biopsies or prostate cells isolated from blood. Recently, Mao et al reported the detection of *TMPRSS2-ERG* fusion from circulating tumor cells⁹³. Since prostate tumor cells are also shed in the urine, PCR based detection of fusion transcripts from urine sediments could provide a noninvasive adjunct to diagnose this class of prostate cancer. We reported sensitive and specific detection of *TMPRSS2:ERG* fusion

transcript from the urine sediments of prostate cancer patients⁹⁴, as well as analyzed the fusion transcript in combination with other putative prostate cancer biomarkers in urine sediments using qPCR⁹⁵. In this discovery study, multiplexed detection of *GOLPH2*, *SPINK1*, *PCA3* and *TMPRSS2:ERG* fusion transcripts outperformed serum PSA or *PCA3* alone in detecting prostate cancer. Combined detection of *TMPRSS2:ERG* and *PCA3* transcripts in urine was reported to provide improved sensitivity of detection of prostate cancer in another study⁹⁶. Future refinement of these tests may lead to a clinical supplement to serum PSA for detecting ETS positive prostate cancer.

As discussed earlier, the inter-focal heterogeneity of prostate cancer gene fusions predicates assessment of multiple needle biopsies⁶⁸. With the continual refinement of clinical association studies, we expect that identification of specific gene fusions will assist in a molecular classification of prostate cancers. For example, as specific fusion subtypes (such as EDe1) are correlated with poorer outcome, patients harboring these events could be considered for more aggressive treatment modalities. Importantly, as indicated by multiple regulatory partners of *EJV1*³³, it is surmised that this information may eventually become integral to therapeutic decision making.

Therapeutic Implications of Prostate Cancer Gene Fusions

Androgen signaling is pivotal to prostate cancer development and survival. “Androgen ablation” therapy continues to be a core treatment modality since the pioneering application of castration and estrogen administration by Charles Huggins in early 1940s^{3, 97, 98}, followed by use of LHRH agonists by Andrew Schally⁹⁹, and more recently replaced by various androgen receptor blockers¹⁰⁰, like cyproterone¹⁰¹, and cyproterone acetate¹⁰² followed by non-steroidal anti-androgens like flutamide¹⁰³, bicalutamide and nilutamide¹⁰⁰. Unfortunately, although androgen ablation provides considerable palliative relief for most patients, it is almost never completely curative, either as a mono-therapy or in combined androgen blockade modalities, and eventually, most recurrent tumors progress to hormone independent disease^{104, 105}.

TMPRSS2:ERG fusion-bearing tumors have been associated with aggressive tumors with lethal phenotype, so it follows that targeting ERG activity in fusion positive samples might offer novel therapeutic avenues for prostate cancer. Discovery of estrogen signaling pathways induced in *TMPRSS2:ERG* positive androgen non-responsive cases may be significant in this respect. Efforts are also underway to identify small molecule inhibitors to ERG activity, as well as to identify downstream targets of ERG protein, that could provide additional therapeutic targets.

Implications for the Future

Genomic rearrangements creating ‘gene fusions’ arguably represent the most common mutation class in human cancers, even though until recently they have been largely characterized in rare hematological and soft tissue malignancies⁶. In this respect, the presence of similar gene fusions in cancers of epithelial origin is remarkable for having eluded discovery till recently. Identification of recurrent gene fusions in prostate cancer by the ‘non-cytogenetic’ approach of gene expression analysis followed by characterization of ‘outlier’ genes represents a novel, albeit one of many possible, technique to explore solid cancers for similar genomic aberrations. The recent report of the presence of *EML4-ALK* fusion transcript in 6.7% (5 out of 75) of non small cell lung cancer (NSCLC) patients, revealed by functional screening of the cancer tissue transcriptome (cDNA expression library)¹⁰⁶, and the discovery of *ALK* and *ROS* fusion proteins in NSCLC through an outlier analysis of the phosphoproteome of cancer tissues and cell lines¹⁰⁷, represent other successful approaches. Genome-wide search for “fusion-transcripts” by next generation sequencing approaches^{108, 109} followed by high

throughput TMA-FISH are likely to be used for future gene-fusion explorations in other common carcinoma. We envision a wider prevalence of gene fusions in prostate cancer, possibly involving other regulatory elements and non-ETS genes that are yet to be discovered (FIG. 5).

An understanding of the diverse regulatory “on/off switches” driving oncogene expression in prostate cancer tissues might lead to clarification of the molecular heterogeneity of multifocal prostate cancer in tandem with the clonally homogenous nature of disseminated foci of metastatic tumors. Recurrent gene fusions appear to be correlated with prostate cancer development and progression, and more studies with larger patient cohorts should identify specific clinical correlates. Continued studies on the relationship between gene fusions, androgen responsiveness, and hormone refractory metastatic disease is crucial as the varying androgen responsiveness of different regulatory elements may drive markedly different responses with androgen deprivation therapy. Considering the research summarized in this review, we propose a molecular classification of prostate cancers--ETS fusion positive and negative--that will likely serve to guide future diagnostic, prognostic and therapeutic advances in prostate cancer.

At a glance

- Approximately 50% prostate cancers from serum PSA-screened cohorts harbor recurrent gene fusions.
- The gene fusions in prostate cancer are characterized by 5' genomic regulatory elements, most commonly controlled by androgen, fused to members of the ETS family of transcription factors.
- *TMPRSS2:ERG* is the most common gene fusion, present in about half of all localized prostate cancers analyzed. *TMPRSS2* also fuses to other ETS family genes such as *ETV1*, *ETV4* and *ETV5* in a small percentage of prostate cancers.
- *ETV1*, *ETV4* and *ETV5* have additional 5' fusion partners, that differ in their prostate specificity and response to androgen.
- Many ETS gene fusion transcript variants have been identified with different 5' and 3' partner sequences, likely with prognostic/ diagnostic implications.
- Prostate cancers harboring *TMPRSS2-ERG* gene fusion display characteristic morphological features of prostate cancers such as macronucleoli, intraductal tumour spread as well as rare blue-tinged mucin, cribriform growth pattern and signet-ring cell.
- *ERG* overexpression imparts invasiveness to prostate cells in vitro and induces plasminogen activation, and matrix metalloprotease pathways.
- ETS gene fusion positive and negative prostate cancers have distinct chromosomal aberrations, expression signatures, morphological features and clinical outcomes, suggesting that they represent fundamentally different classes of prostate cancer.
- Sensitive and specific diagnostic tests and targeted therapeutics will impact the detection and management of ETS positive prostate cancer.

Side Bars

- **PIN:** Prostatic Intraepithelial Neoplasia defines foci of rapidly dividing prostate epithelial cells, believed to be the precursor of prostate cancer. PINs are typically classified as high grade, medium grade and low grade, depending on their level of

differentiation. Approximately one third of men with high grade PIN (HGPIN) develop prostate cancer.

- **Mitelman Database of Chromosome Aberrations in Cancer:** A manually curated database of all published cases of cytogenetic aberrations in cancer along with their clinical associations has been collated by Mitelman *et al*¹¹⁰. This database currently has information on 53,946 cancer cases (November 26, 2007) involving more than 350 gene fusions and other cytogenetic aberrations documented in cancers. (<http://cgap.nci.nih.gov/Chromosomes/Mitelman>).
- **Gene fusion:** A physical linking of two genes (typically accompanied with deletion of portions of the two partner genes) such that they come to share a common regulatory element and/or open reading frames (ORFs), the latter encoding chimeric proteins, for example *BCR-ABL1* or *TMPRSS2-ERG*.
- **ETS:** The **E**26 **T**ransformation **S**pecific family of genes encodes nuclear transcription factors, characterized by DNA binding ETS domains and various protein interaction domains. ETS family proteins are involved in cell growth, signal transduction, cell cycle regulation, apoptosis, hematopoietic, neuronal and myogenic differentiation, and in several human malignancies, such as Ewing's tumors, leukemias and in prostate cancer.
- **TMPRSS2:** An androgen-regulated type II transmembrane serine protease (TTSP) expressed highly in normal prostate epithelium and prostate cancer cells. *TMPRSS2* null mice develop to normal, fertile adulthood¹¹¹.
- **PLAU:** Urokinase Plasminogen Activator is a serine protease that degrades extracellular matrix involved in tumor cell migration and proliferation. *ERG* induces *PLAU* overexpression and inhibition of *PLAU* blocks *ERG* mediated invasion.
- **Androgen ablation:** A core prostate cancer treatment modality that involves severing androgen signaling in prostate cancer either by physical means (castration) or biochemically, by injecting estrogens, or anti Androgens, or Androgen Receptor agonists/ antagonists. Charles Huggins was awarded the Nobel Prize in Physiology or Medicine, 1966 for this development in 1941.
- **Molecular Concepts Map (MCM):** Gene expression signatures of sets of biologically connected genes, such as 'lipid biosynthesis genes' or 'androgen activated genes' etc have been defined as 'molecular concepts' and over 40,000 such concepts have been compiled in Oncomine. The MCM involves interrogating a set of user defined genes (such as an expression signature or genes identified in a functional screen) against all Concepts in the MCM to identify molecular networks enriched in the query set.

BOX 1 Gene fusions and Cancer

- **Recurrent gene fusions in cancer:** Gene fusions represent the most common class of somatic mutations associated with cancer⁶. These may involve the regulatory elements of one gene (often tissue specific) aberrantly apposed to a proto-oncogene, for example, immunoglobulin and T cell receptor regulatory regions fused to *MYC* oncogene in B and T cell malignancies, respectively¹¹². Alternatively, coding regions of two genes get juxtaposed, resulting in a chimeric protein with a new or altered activity; for example the *BCR-ABL1* gene fusion in chronic myelogenous leukemia (CML)^{12, 112} and a subset of acute lymphoblastic leukemia (ALL)^{113, 114}.

- **BCR-ABL1 Paradigm:** *BCR-ABL1* gene fusion on the Philadelphia chromosome (aberrant Chromosome 22) discovered by Nowell and Hungerford in 1961^{10, 115} results from a translocation of the proto-oncogene *c-abl1* from chromosome 9 to the *bcr* gene on chromosome 22; the fusion gene encoding a fusion protein *BCR-ABL1*, in CML^{11, 12}.
 - a. *BCR-ABL1 is a diagnostic marker for CML:* Detection of *BCR-ABL1* fusion transcript in peripheral blood is used to confirm CML diagnosis, monitor cytogenetic remission and residual disease¹¹⁶.
 - b. *BCR-ABL1 fusion gene has many molecular variants:* A wide variety of fusion variants of *BCR-ABL1* are known, as a result of alternative breakpoint regions in *BCR* and in *ABL1*. Depending on the location of breakpoint regions, BCR-ABL1 protein may be 210 kDa (MBcr), 190 kDa (mBcr) or μ Bcr (230kDa). Different fusions have been associated with different disease phenotypes⁵².
 - c. *BCR-ABL1 is pathognomonic for CML:* The BCR-ABL1 fusion protein has tyrosine kinase activity¹¹⁷ which is essential for initiation, maintenance and progression of CML⁷. Transgenic *BCR-ABL1* mice display CML-like myeloproliferative disorders^{118–120}. Transgenic *BCR-ABL1* expression in hematopoietic stem cells induces chronic phase CML in mice^{118, 121}.
 - d. *BCR-ABL1 is a specific therapeutic target:* Imatinib, a small molecule inhibitor of ABL1 tyrosine kinase activity is used as the standard treatment for chronic phase CML^{122–124}.
- **Gene Fusions in Carcinoma- The missing Link:** While leukemias/ lymphomas and bone/soft tissue sarcomas, which together represent only 10% of all human cancers account for more than 80% of all known gene fusions, common epithelial, cancers which account for 80% of cancer related deaths, account for only about 10% of recurrent gene fusions^{15, 19}. This paradox has been challenged by the recent discoveries of gene fusions in prostate and lung cancers.

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FIGURE 1. Cytological and Molecular Techniques used in Gene Fusion Studies

A. Cancer Outlier Profile Analysis (COPA) (described in the main text) was applied to microarray data available Oncomine (B.), and the top “outlier” genes formed our primary candidates to test for potential genomic rearrangements using cytogenetic and molecular techniques. C. The downstream workflow begins with quantitative real time PCR specific to exons from all parts of the gene (Exon-walk PCR), whereby a sharply divergent expression pattern from different parts of the gene suggests that a part of the gene may be split/ rearranged. D. Fusion partners of candidate split genes are identified by cloning the beginning and end of the candidate transcript using rapid amplification of cDNA ends (RACE). 5' RACE is used to identify the 5' fusion partner, and involves ligating an RNA adaptor sequence to the test RNA and carrying out RT-PCR using RNA adaptor specific forward primer and candidate gene specific reverse primer (located close to the split exon). The RACE PCR products are cloned and sequenced to confirm the identity of fusion genes. E. Fusion specific RT-PCR is used to confirm the presence of fusion transcript. F. Finally, the chromosomal rearrangements involved in the gene fusion are characterized by fluorescence in-situ hybridization (FISH) on interphase nuclei. FISH analysis involves hybridization of fluorescently labeled DNA probes corresponding to the candidate target sequences. Since it allows visualization of specific regions of the chromosome based on DNA sequence, it is the method of choice to investigate

the genomic rearrangements like insertion, deletion, and translocation that can split or fuse genes.

In the 'split probe' or 'break apart' assay strategy, two fluorescent probes (for example, green and red) are hybridized to sequences less than 1Mb apart, so that in normal interphase nucleus they appear co-localized. If a genetic rearrangement involves the region spanned by the two probes, the signals are separated and appear distinct (translocation), or a single color signal is seen and the other signal is lost (deletion). In the alternative 'fusion probe' strategy, two probes are localized far apart on the chromosome- or on two separate chromosomes. Two pairs of separate signals are seen in the normal diploid nucleus and in the event of a chromosomal rearrangement leading to gene fusion, the two signals are co-localized. Both these techniques have been used in the gene fusion studies, often in combination.

FIGURE 2. Anatomy of Gene fusions in Prostate Cancer

A cartoon representation of the gene fusions characterized in prostate cancers so far. The 5' fusion partners are depicted on the left side and corresponding 3' partners on the right. Light colors at the ends of the genes depict untranslated exons. The dark colored boxes depict coding exons. The numbers on the boxes identify the base positions of the exons. The arrows represent androgen responsiveness of the fusion genes, arrows pointing up (red) signify androgen mediated upregulation; arrows pointing down (green) represent androgen mediated down regulation of the corresponding gene; the horizontal arrows (blue) represent absence of androgen action on the fusion genes' expression. *TMPRSS2-ETS* gene fusions have been grouped as Type I; other gene fusions which are androgen inducible have been grouped as

Type II, androgen repressed fusion genes make up Type III, Androgen insensitive fusion genes, Type IV, and lastly, the novel situation in prostate cancer cell lines, with ETS genes rearranged to an androgen sensitive location (without the generation of classical gene fusions) has been classified as Type V.

FIGURE 3. Genesis and Progression of Gene Fusions in Prostate Cancer

ETS gene fusions can first be observed in a subset of high grade prostatic intraepithelial neoplasia (HGPINs). Intriguingly, these foci are nearly invariably associated with intermingling foci of carcinoma, suggesting that ETS gene fusions may drive the progression to localized prostate cancer. Alternative lesions likely drive the development of ETS gene fusion negative HGPIN and localized carcinoma. While the same prostate may harbor both fusion positive and negative tumor foci, only a single foci metastasizes to distant organs, as different metastatic sites in the same patient are uniformly fusion positive or negative.

FIGURE 4. Role of Gene Fusions in Prostate Cancer

Androgen mediated upregulation of ETS genes (for example *ERG* or *ETV1*) leads to the activation of ETS target genes that upregulate various “Molecular Concepts” as identified by Molecular Concept Modeling (MCM)^{33, 77, 87} and comprise genes involved in increased protein synthesis, cell adhesion and invasion including Urokinase plasminogen activator (*PLAU*) and several matrix metalloproteases. Preceding genetic lesions, including the loss of a tumor suppressor such as *phosphatase and tensin homolog (PTEN)*, likely drive the development of PIN, while subsequent ETS gene mediated molecular alterations lead to the development of invasive prostate carcinogenesis.

FIGURE 5. Gamut of Gene Fusions in Prostate Cancer

Six major classes of pathognomic gene fusions in prostate cancer are envisioned based on the types of upstream regulatory partners and the nature of the fused genes. These include regulatory regions that are either androgen responsive or androgen repressed or androgen insensitive (housekeeping) fused to ETS family genes or other, yet to be discovered, non-ETS genes

TABLE 1

Recurrent Gene Fusions in Prostate Cancer

Gene Fusion	Sample	Frequency (%)	Fusion Type	Assay	Study
TMPRSS2:ERG	PCA	16/29 (55%)	T1:E4	qRT-PCR/FISH	Tomlins, et al 2005 ¹
TMPRSS2:ETV1	PCA	7/29 (24%)	T1:E4		
TMPRSS2:ERG	PCA, biopsy	14/18 (77%)	T1:E4, T1:E2, T4/5:E4/5	Nested RT-PCR	Soller, et al 2006 ²
TMPRSS2:ETV4	PCA, overexpressing ETS family genes	2/98 (2%)		RACE/qRT-PCR/FISH	Tomlins, et al 2006 ³
TMPRSS2:ERG	PCA, Gleason score 6–9	6/15 (40%)	T1:E5, T2:E5, T1:E4	RT-PCR/FISH	Yoshimoto, et al 2006 ⁴
TMPRSS2:ERG	PCA	58/118 (49%)		FISH/qRT-PCR	Perner, et al 2006 ⁵
TMPRSS2:ERG	PCA	7/18 (41%)			
TMPRSS2:ERG	PCA	35/59 (59%)		RT-PCR	Wang, et al 2006 ⁶
TMPRSS2:ERG	BPH	0/20 (—)	8 different types of fusions		
TMPRSS2:ERG	PCA, T1cN0M0/T2N0M0	17/34 (50%)		RT-PCR/qRT-PCR	Cerviera, et al 2006 ⁷
TMPRSS2:ERG	HGPIN	4/19 (21%)			
TMPRSS2:ERG	BPH	0/13 (—)	T1:E4		
TMPRSS2:ERG	Normal Prostate	0/11 (—)			
TMPRSS2:ERG	PCA	19/26 (73%)		RT-PCR/Nested-PCR	Clark, et al 2007 ⁸
TMPRSS2:ERG	Normal adjacent	8/17 (47%)	T1:E4, T1:E5, T1:E2, T1:E3, etc.-total		
TMPRSS2:ERG	BPH	2/31 (6%)	14 types		
TMPRSS2:ERG	Non-Prostate Normal	0/20 (—)			
TMPRSS2:ERG	Post:DRE Urine	8/19 (42%)	T1:E4 (5) T1:32 (1)	qRT-PCR/FISH	Laxman, et al 2006 ⁹
TMPRSS2:ERG	PCA, Advanced	7/19 (37%)	T2:E6:E4 (1)	RT-PCR/array cGH	Iljin, et al 2006 ¹⁰
TMPRSS2:ETV4	PCA, Advanced	2/18 (11%)	T1/2:E4		
TMPRSS2:ERG	PCA, Xenografts	5/11 (45%)	T1/2:E5	FISH/qPCR	Hermans, et al 2006 ¹¹
TMPRSS2:ETV1	PCA, Xenografts	1/11 (9%)		RT-PCR, Sequencing	Nami, et al 2007 ¹²
TMPRSS2:ERG	PCA, Gleason score 7	11/26 (42%)			
TMPRSS2:ERG	PCA, T1a,b, Ns, M0', Orebro Watchful Waiting Cohort	17/111 (15%)		FISH/qRT-PCR	Demichelis, et al 2007 ¹³
TMPRSS2:ERG	PCA	36/101 (36%)	T2:E4 and T1:E4	FISH/RT-PCR, Sequencing	Rajput, et al 2007 ¹⁴
TMPRSS2:ERG	BHP	0/5 (—)			
TMPRSS2:ERG	PCA	30/56 (54%)		FISH	Mehra, et al 2007 ¹⁵
TMPRSS2:ETV1	PCA	1/53 (2%)			
TMPRSS2:ETV4	PCA	1/58 (2%)			

Gene Fusion	Sample	Frequency (%)	Fusion Type	Assay	Study
TMPRSS2:ERG	PCA	44/63 (70%)	T2:E4 & T2:E4*	RACE/qRT-PCR/FISH	Lapointe, et al 2007 ¹⁶
TMPRSS2:ERG	PCA	120/253 (47%)		FISH	Mosquera, et al 2007 ¹⁷
TMPRSS2:ERG	PCA	18/50 (36%)		RT-PCR	Winnes, et al 2007 ¹⁸
TMPRSS2:ERG	PCA	115/237 (48%)			
TMPRSS2:ERG	Metastases, hormone naive	10/34 (29%)			
TMPRSS2:ERG	Metastases, hormone refractory	3/9 (33%)			
TMPRSS2:ERG	HGPIN	5/26 (19%)		FISH	Perner, et al 2007 ¹⁹
TMPRSS2:ERG	BPH	0/15 (0)			
TMPRSS2:ERG	PIA	0/38 (0)			
TMPRSS2:ERG	Benign Prostate samples	0/47 (0)			
TMPRSS2:ERG	PCA	25/52 (48%)		RT-PCR/FISH	Tu, et al 2007 ²⁰
TMPRSS2:ERG	PCA, Transurethral resection	134/445 (30%)		FISH	Attard, et al 2007 ²¹
HERV_K_22Q11.23:ETV1	PCA and Metastases	-			
SLC45A3:ETV1	PCA and Metastases	-			
C15ORF21:ETV1	PCA and Metastases	-	T1:T4, T2:T4	qRT-PCR/FISH	Tomlins, et al 2007 ²²
HNRPA2B1:ETV1	PCA and Metastases	-			
TMPRSS2:ERG	Prostate Biopsies and Post-DRE urine	17/29 (59%)		RT-PCR	Hessels, et al 2007 ²³
TMPRSS2:ERG	PCA	81/165 (49%)		RT-PCR, Sequencing	Nam, et al 2007 ²⁴
TMPRSS2:ERG	PCA, Multifocal	17/32 (53%)		FISH	Barry, et al 2007 ²⁵

Table 2
Prognostic Associations of TMPRSS2:ERG fusion

	Study reference	ETS gene Status/ Assay	Patient Cohort	Prognostic association
1	Mehra, R. et al. (2007) ⁵⁹	TMPRSS2/ERG rearrangement Break-apart FISH	PCa cases, Surgically treated (n=96)	High pathologic stage
2	Nam, R.K. et al. (2007) ⁶⁶	TMPRSS2:ERG fusion RT-PCR and DNA sequencing	PCa cases, Surgically treated (n=26), Gleason score 7	Higher rate of recurrence Single most important prognostic factor
3	Nam, R.K. et al. (2007) ⁶¹	TMPRSS2:ERG fusion RT-PCR and DNA sequencing	PCa cases, Surgically treated (n=165)	Higher risk of recurrence (58.4% at 5 years). Strong prognostic factor independent of grade, stage and PSA level.
4	Attard, G. et al. (2007) ⁶²	ERG rearrangement Break-apart FISH	PCa cases, transurethral resection specimens, (TMAs, Cohort of conservatively managed patients (no hormone treatment) (n=445)	Very poor cause-specific survival (25% Survival at 8 years) (2+Edel)* compared against ERG rearrangement negative cases (90% survival at 8 years)
5	Demichelis, F. et al. (2007) ⁶³	TMPRSS2:ERG rearrangement Multicolored fusion FISH	PCa cases, Population based watchful waiting cohort (n=111)	Prostate cancer specific death
6	Perner, S. et al. (2006) ⁴¹	TMPRSS2:ERG rearrangement Dual color break-apart FISH	PCa cases, hormone-naïve and hormone-refractory lymph node metastases (n=136)	Higher tumor stage, presence of metastatic disease involving pelvic lymph nodes
7	Rajput, A.B. et al. (2007) ⁴⁷	TMPRSS2:ERG fusion FISH TMPRSS2 Break-apart FISH	PCa cases, Surgically treated Tissue microarrays (TMAs) (n=196)	Moderate to poorly differentiated tumors
8	Petrovics, G. et al. (2005) ⁶⁴	ERG overexpression Microarray, Real-time PCR	PCa cases, Laser Capture microdissected epithelial cells ERG overexpressing tumors	Longer recurrence-free survival, well and moderately differentiated stages, lower pathological stage, and negative surgical margins
9	Winnes, M., et al. (2007) ⁶⁵	TMPRSS2:ERG fusion RT-PCR and DNA sequencing	PCa cases, transrectal ultrasound (TRUS) guided needle biopsies (n=50)	Lower Gleason grade and better survival than fusion-negative tumors
10	Yoshimoto, M. et al. (2006) ⁴⁹	TMPRSS2:ERG fusion RT-PCR and DNA sequencing	PCa cases, Surgically treated (n=15)	No Correlation with clinical outcome
11	Lapointe, J. et al. (2007) ⁴⁶	TMPRSS2:ERG fusion RT-PCR and DNA sequencing TMPRSS2:ERG Fusion FISH	PCa cases, Surgically treated (n=54) Hormone naïve pelvic lymph node metastases (n=9)	No association with tumor stage, Gleason grade or recurrence-free survival