ETS fusion genes in prostate cancer

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Abstract

Prostate cancer is very common in elderly men in developed countries. Unravelling the molecular and biological processes that contribute to tumor development and progressive growth, including its heterogeneity, is a challenging task. The fusion of the genes ERG and TMPRSS2 is the most frequent genomic alteration in prostate cancer. ERG is an oncogene that encodes a member of the family of ETS transcription factors. At lower frequency, other members of this gene family are also rearranged and overexpressed in prostate cancer. TMPRSS2 is an androgen-regulated gene that is preferentially expressed in the prostate. Most of the less frequent ETS fusion partners are also androgen-regulated and prostate-specific. During the last few years, novel concepts of the process of gene fusion have emerged, and initial experimental results explaining the function of the ETS genes ERG and ETV1 in prostate cancer have been published. In this review, we focus on the most relevant ETS gene fusions and summarize the current knowledge of the role of ETS transcription factors in prostate cancer. Finally, we discuss the clinical relevance of TMPRSS2–ERG and other ETS gene fusions in prostate cancer.

Key Words
- prostate cancer
- gene fusion
- androgen regulation
- ETS gene
- prostate specific
- translocation

Introduction

Prostate cancer is the most frequent malignancy and the second most common cause of cancer-related death in men in the USA and in other countries with a Western lifestyle (Siegel et al. 2013). Almost all prostate cancers are adenocarcinomas and it is generally accepted that prostate cancers develop from a precursor stage denoted as prostate intraepithelial neoplasia (PIN; DeMarzo et al. 2003). Growth patterns of tumors can be very different and heterogeneous, reflected in the so-called Gleason grade (Lotan & Epstein 2010). Similar to other tumors, prostate cancer growth is driven by the accumulation of genetic and epigenetic alterations. One of the earliest genetic alterations in prostate cancer is overexpression of the ERG oncogene, which occurs in over 50% of prostate cancers (Tomlins et al. 2005, Hermans et al. 2006, 2009, Soller et al. 2006). The overexpression of ERG is in the majority of tumors driven by fusion of the ERG gene with transmembrane protease, serine 2 (TMPRSS2), a prostate-specific and androgen-regulated gene that maps very close to ERG on the same chromosome. This gene fusion has never been found in normal prostate but is present in tumor adjacent to PIN (Cerveira et al. 2006, Mosquera et al. 2008, Park et al. 2010, van Leenders et al. 2011). ERG is a member of the large family of ETS transcription factors (Hollenhorst et al. 2011).

Localized prostate cancer can be cured by surgical removal of the prostate or by local radiotherapy, but approximately 30% of treated patients show recurrences. It is well established that the growth of prostate cancer depends on male steroid hormones, androgens. Therefore, the treatment of choice of metastasized prostate cancer is one of various types of endocrine therapy, all aiming at the inhibition of the function of the androgen receptor (AR), the intracellular molecular target of androgens (Feldman & Feldman 2001, Scher & Sawyers 2005, Lonergan &
Tindall 2011). The AR is a member of the family of ligand-dependent nuclear receptor transcription factors. Although many prostate tumors show an initial response to endocrine therapy, within 1–3 years essentially all tumors become resistant to the therapy and patients develop a disease now described as castration-resistant prostate cancer (CRPC). Remarkably, in CRPC the AR still plays a prominent role. Androgen signaling in CRPC can be modified by many different mechanisms, including amplification and mutation of the AR gene.

In this review, the role of ERG and other ETS transcription factors in prostate cancer is described. The focus is on the mechanism of ETS overexpression and on the clinical relevance of ERG and other ETS genes.

Functions of ETS transcription factors

The founding member of the ETS family of transcription factors, v-ets, was originally discovered as part of the GAG–MYB–ETS fusion protein of the transforming virus E26 that induces leukemia in chickens. The ETS family is composed of approximately 27 members, that all share high homology in their evolutionary conserved DNA-binding domain, the ETS domain, that is in the C-terminal part of the protein. Homology in other parts of the proteins is limited (Fig. 1; Oikinawa & Yamada 2003, Seth & Watson 2005, Hollenhorst et al. 2011).

The 85-amino-acid ETS domain forms a helix–turn–helix DNA-binding structure that recognizes a GGAA/T core consensus sequence, the ETS binding site, in the regulatory regions of target genes. Small differences in the composition of flanking sequences of the binding site contribute to the specificity of ETS binding (Wei et al. 2010, Hollenhorst et al. 2011). A second conserved domain present in a subset of ETS factors is the pointed domain (PNT). This 65–85 amino acid helix–loop–helix domain functions in protein–protein interactions. In addition to the ETS- and PNT-domains, activation and repression domains have been postulated for most ETS factors.

On the basis of their overall structural composition and on the homology in the ETS domain, ETS transcription factors can be separated into ~11 subfamilies (Oikinawa & Yamada 2003, Hollenhorst et al. 2011). ERG is, together with FLI1, a member of the ERG-subfamily and ETV1, ETV4, and ETV5 are the members of the PEA3-subfamily that contain an N-terminal acidic transactivation domain (TAD; Oh et al. 2012). ETS proteins can function not only as transcription activators but also as repressors. Many directly or indirectly ETS-regulated genes have been identified. Recently, the first Chromatin immunoprecipitation (ChIP)-chip and ChIP-seq data have been published that identify and compare the binding sites of different ETS transcription factors in different cell lines and tissues (Wei et al. 2010, Hollenhorst et al. 2011). A wide variety of overlapping and more specific binding sites have been documented.

ETS transcription factors can play crucial roles in many biological processes, including cellular proliferation, differentiation, apoptosis, tissue remodeling, angiogenesis, metastasis, and transformation. Deregulated expression of ETS genes has been described in leukemia’s and solid tumors (Seth & Watson 2005). Moreover, overexpression of ETS genes, most commonly ETS1, ETS2, ETV1, and ETV4 has been observed in breast, colon, lung, and prostate cancers. In general, overexpression of ETS genes was associated with advanced stage of the disease. More recently, it has been found that ETV1 in concert with activating KIT mutations plays a prominent role in gastrointestinal stromal tumors (Chi et al. 2010). As mentioned earlier, ERG is the most frequently overexpressed ETS gene in prostate cancer. ERG overexpression is found in both early- and late-stage prostate cancer (CRPC) (Tomlins et al. 2005, Soller et al. 2006, Hermans et al. 2009).

Fusion of TMPRSS2 to ERG and other ETS gene fusions in prostate cancer

ETS genes are frequently involved in gene fusions, resulting in the synthesis of chimerical proteins or altered expression of the ETS protein. ETS fusion genes have been detected in Ewing’s sarcoma and in leukemia (Bohlander 2005, Khoury 2005, Hollenhorst et al. 2011). Fusion of the
Ewing’s sarcoma gene (EWS) to FLI1 occurs in over 90% of Ewing’s sarcoma. This gene fusion leads to the production of a chimerical protein, linking the N-terminal region of EWS to the ETS-domain of FLI1. EWS–ERG fusions are detected in approximately 5% of Ewing’s sarcoma. In rare cases EWS is linked to other ETS genes. The first exons of EWS encode a strong transactivation domain. The chimerical protein produced not only modulates the expression of ETS target genes, but probably also induces the expression of novel genes. In leukemia many different fusion genes involving the ETS gene TEL (ETV6) have been described.

In 2005, frequent overexpression of ERG in prostate cancer was observed (Petrovics et al. 2005). Later that year, it was discovered that the mechanism underlying this overexpression was the recurrent genomic rearrangement between the first exon(s) of TMPRSS2 and the ERG oncogenes (Fig. 2; Tomlins et al. 2005). This latter finding was rapidly confirmed and extended by others, and it is now generally accepted that over half of prostate cancers harbor the TMPRSS2–ERG gene fusion as the most frequent genomic alteration (Kumar-Sinha et al. 2008; Table 1).

TMPRSS2 is an androgen-regulated gene that is preferentially expressed in the prostate (Hermans et al. 2009). TMPRSS2 is located on chromosomal band 21q22. ERG maps also 21q22 in the same orientation, at a distance of approximately 3 Mb (Fig. 2). The fusion of the androgen- and prostate-specific regulating sequences and first exon(s) of TMPRSS2 to the coding sequences of ERG resulted in the androgen-regulated overexpression of ERG. Fusion of TMPRSS2 to ERG can occur by two mechanisms: the genomic region between the two genes can be lost by interstitial deletion, which is the case in approximately 60% of the fusion-positive tumors, or it can be the result of more complex genomic rearrangements involving chromosome 21q22 and presumably other chromosomes (Hermans et al. 2006, Perner et al. 2006). TMPRSS2–ERG has never been detected in normal prostate or in benign prostatic hyperplasia (Cerveira et al. 2006, Park et al. 2010, van Leenders et al. 2011). So, TMPRSS2–ERG is a very specific prostate cancer biomarker, although TMPRSS2–ERG has been found in approximately 20% of PIN lesions (Cerveira et al. 2006, Mosquera et al. 2008). More recently, ERG overexpression has been detected by immunohistochemistry in a much higher percentage of PIN (Park et al. 2010, van Leenders et al. 2011). So, the formation of TMPRSS2–ERG is an early event in prostate carcinogenesis. It remains to be established whether the gene fusion plays a role in PIN to cancer progression or can even play a role in earlier stages of prostate cancer development. At a low frequency, ERG overexpression is not caused by fusion to TMPRSS2, but by fusion to SLC45A3 or NDRG1, two other androgen-regulated genes that are preferentially expressed in the prostate (Table 1; Esgueva et al. 2010). These two fusion partners do not map to 21q22, indicating that chromosomal proximity is important but not essential for the fusion event.

ETV1 is overexpressed in 5–10% of prostate cancers (Tomlins et al. 2005, Hermans et al. 2008a). ETV1 gene fusions lead to overexpression of a truncated ETV1 protein that lacks the N-terminal TAD domain (Fig. 1). In a low percentage of tumors, structurally and functionally related ETV4 or ETV5 is overexpressed due to gene fusion (Tomlins et al. 2006, Helgeson et al. 2008, Hermans et al.

Figure 2
Schematic presentation of the TMPRSS2–ERG fusion on chromosome band 21q22.
ETV1 is overexpressed not only as a fusion gene but also as a full-length mRNA, due to translocation of the complete gene (Tomlins et al. 2007, Hermans et al. 2008a, Gasi et al. 2011). Several full-length ETV1 translocations are to a specific region of chromosome 14 that also contains EST14. Recently, we mapped a full-length ETV1 translocation to chromosome 4 (Gasi et al. 2011), but for most translocations of the complete gene the chromosomal region of translocation has not yet been studied. Identification of the characteristics of these regions will be very helpful in addressing the question as to whether or not there are common mechanisms of full-length ETV1 overexpression. In a small percentage of prostate tumors, the ETS gene ELK4 is overexpressed due to cis-splicing of the flanking SLC45A3 gene (Rickman et al. 2009, Zhang et al. 2012). The finding that the expression of ETS transcription factors is mutually exclusive in clinical prostate cancers might not necessarily indicate a similar function. In a small proportion of ETS-negative samples, overexpression of SPINK1 has been described, and more recently, a mutually exclusive mutation of SPOP has been identified (Tomlins et al. 2008a, Barbieri et al. 2012). However, a direct association between ETS genes and SPINK1 or SPOP has not yet been found.

### Mechanism of gene fusion

Probably, the genomic proximity of TMPRSS2 and ERG is an important determinant in explaining the high frequency of TMPRSS2–ERG fusion as compared with other ETS gene fusions. Although all other ETS gene fusion events are between genes that map on different chromosomes or at a long distance on the same chromosome, it has been postulated that these ETS fusions might also be facilitated by nuclear proximity of the fusion partners. This is presumed to be accomplished by looping out of genomic regions under certain cell growth conditions, facilitating the expression of the fusion partners (Lin et al. 2009, 2012, Mani et al. 2009).

As described earlier, most ETS-fusion partners share the properties of androgen-upregulated and prostate-specific expression. So, the mechanisms of regulation of expression of the fusion partner seem to be a second important determinant in the fusion event (Lin et al. 2009, 2012, Mani et al. 2009). Regulation of expression might contribute to nuclear proximity. It has also been postulated that binding of an activated AR to genes encoding fusion partners plays an active role in the fusion process. Evidence has been provided that the AR is instrumental in induction of genomic breaks by recruiting enzymes such as DAX1 and HNRPA2B1 to form a complex that facilitates genomic breakage and the recruitment of repair machinery (Lindahl-Allen & Antoniou 2007).

### Table 1  ETS gene fusions in prostate cancer

<table>
<thead>
<tr>
<th>5’ Fusion partner</th>
<th>Prostate specific</th>
<th>Androgen regulated</th>
<th>ETS partner</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TMPRSS2 (chr 21q)</td>
<td>+</td>
<td>+</td>
<td>ERG (chr 21q)</td>
<td>50</td>
</tr>
<tr>
<td>SLC45A3 (chr 1q)</td>
<td>+</td>
<td>+</td>
<td></td>
<td>&lt;1</td>
</tr>
<tr>
<td>NDRG1 (chr 8)</td>
<td>+</td>
<td>+</td>
<td></td>
<td>&lt;1</td>
</tr>
<tr>
<td>SLC45A3 (chr 1q)</td>
<td>+</td>
<td>+</td>
<td>FLI1 (chr 1q)</td>
<td>&lt;1</td>
</tr>
<tr>
<td>TMPRSS2 (chr 21q)</td>
<td>+</td>
<td>+</td>
<td>ETV1 (chr 7p)</td>
<td>&lt;1</td>
</tr>
<tr>
<td>SLC45A3 (chr 1q)</td>
<td>+</td>
<td>+</td>
<td></td>
<td>&lt;1</td>
</tr>
<tr>
<td>FOXP1 (chr 3p)</td>
<td>ND</td>
<td>ND</td>
<td></td>
<td>&lt;1</td>
</tr>
<tr>
<td>EST14 (chr 14q)</td>
<td>+</td>
<td>+</td>
<td></td>
<td>&lt;1</td>
</tr>
<tr>
<td>HERVK17 (chr 17p)</td>
<td>+</td>
<td>+</td>
<td></td>
<td>&lt;1</td>
</tr>
<tr>
<td>HERV-K, 22q11.23</td>
<td>+</td>
<td>+</td>
<td></td>
<td>&lt;1</td>
</tr>
<tr>
<td>C10ORF21 (chr 15q)</td>
<td>+</td>
<td>(down)</td>
<td></td>
<td>&lt;1</td>
</tr>
<tr>
<td>HNRPA2B1 (chr 7p)</td>
<td>−</td>
<td>−</td>
<td></td>
<td>&lt;1</td>
</tr>
<tr>
<td>ACSL3 (chr 2q)</td>
<td>+/−</td>
<td>+</td>
<td>ETV4 (chr 17q)</td>
<td>&lt;1</td>
</tr>
<tr>
<td>TMPRSS2 (chr 21q)</td>
<td>+</td>
<td>+</td>
<td></td>
<td>&lt;1</td>
</tr>
<tr>
<td>KLK2 (chr 19p)</td>
<td>+</td>
<td>+</td>
<td></td>
<td>&lt;1</td>
</tr>
<tr>
<td>CANT (chr 17q)</td>
<td>+</td>
<td>+</td>
<td></td>
<td>&lt;1</td>
</tr>
<tr>
<td>DDX5 (chr 17q)</td>
<td>−</td>
<td>−</td>
<td></td>
<td>&lt;1</td>
</tr>
<tr>
<td>TMPRSS2 (chr 21q)</td>
<td>+</td>
<td>+</td>
<td>ETV5 (chr 3q)</td>
<td>&lt;1</td>
</tr>
<tr>
<td>SLC45A3 (chr 1q)</td>
<td>+</td>
<td>+</td>
<td>ELK4 (chr 1q)</td>
<td>&lt;1</td>
</tr>
<tr>
<td>*SLC45A3 (chr 1q)</td>
<td>+</td>
<td>+</td>
<td></td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

ND, not determined.
*Read-through transcript.
as topoisomerase II β, or cytidine deaminase (CDA), and ORF2 endonuclease (Lin et al. 2009, 2012, Haffner et al. 2010, 2011). However, the experimental conditions used to investigate the mechanisms of chromosomal proximity and of induced DNA damage were rather complex, and follow-up studies should confirm and extend these earlier observations.

**Biological and molecular functions of ETS proteins in prostate cancer**

The main function of ERG and other ETS proteins in prostate cancer is not well understood. ETS overexpression alone seems insufficient to induce prostate cancer. In vitro studies showed that overexpression of ERG or ETV1 in immortalized, non-tumorigenic epithelial prostate cells increased cell migration and invasion (Tomlins et al. 2007, 2008b, Hermans et al. 2008a, Klezovitch et al. 2008, Wang et al. 2008), and knockdown of ERG or ETV1 in prostate cancer cell lines slowed invasion (Tomlins et al. 2007, 2008b, Sun et al. 2008, Wang et al. 2008).

In genetically modified mice (GEMs), overexpression of ERG or ETV1 resulted in the development of PIN but not of invasive cancer (Klezovitch et al. 2008, Tomlins et al. 2008b). However, in other studies, TMPRSS2–ERG GEMs did not even develop PIN. Among the progeny from crossbreeding Egr mice with Pten-knockout mice, PIN and micro-invasive cancer were observed (Carver et al. 2009, King et al. 2009, Baena et al. 2013, Chen et al. 2013). Witte and colleagues provided additional evidence that ERG can cooperate with several different oncogenes or tumor suppressor genes in the development of mouse prostate tumors (Zong et al. 2009).

By comparison of global gene expression data for clinical prostate cancer samples with and without ERG overexpression, pathways associated with ERG overexpression have been identified. Data obtained by Iljin et al. (2006) indicated a role of the WNT pathway in ERG-associated prostate cancer and showed high expression of HDAC1 in ERG-overexpressing tumor samples. Also, activation of the transforming growth factor β (TGFβ) pathway has been associated with ERG overexpression (Brase et al. 2011). Although the data reported in different studies are variable, a consistent association with ERG overexpression, of more than ten genes, including CACNA1D, TDRD1, PLA2G7, and NCA1D, has been found (Iljin et al. 2006, Jhavar et al. 2008, Taylor et al. 2010, Brase et al. 2011, Boormans et al. 2013). This does not mean that these genes are direct ERG target genes. They might be indirectly regulated by ERG or they might represent a common prostate cell type in which TMPRSS2–ERG fusion occurred. Recently, TDRD1 has been identified as the first directly ERG-regulated gene (Paulo et al. 2012, Boormans et al. 2013). The mechanism of expression of other ERG-associated genes remains to be investigated.

Important initial results regarding the molecular effect of ERG overexpression in prostate cancer have been published (Yu et al. 2010). It has been shown by whole-genome ChIP-seq that there is overlap between genomic regions that bind AR and ERG. As a result, ERG overexpression can interfere with and modify the expression of AR-regulated genes. A model has been proposed in which ERG overexpression inhibits AR-regulated differentiation and stimulates dedifferentiation mediated by the H3K27 methyltransferase polycomb gene EZH2. In a Pten knock-out GEM prostate cancer model, ERG overexpression changed the AR cistrome (Chen et al. 2013).

Our knowledge of the biological and molecular effects of ETV1 overexpression in prostate cancer is more limited. As described earlier, due to gene fusion, ETV1 can be overexpressed as a truncated protein lacking the N-terminal TAD domain (dTETV1) (Fig. 1), or as a full-length protein, due to translocation of the complete gene to a different genomic region (Hermans et al. 2008a, Gasi et al. 2011). In in vitro studies, full-length ETV1 is a strong transcriptional activator, but dETV1 is much less active. Although both variants similarly induce migration and invasion in non-tumor prostate cells, only the full-length protein seems capable of inducing anchorage-independent growth in in vitro assays (Hermans et al. 2008a).

The relatively low percentage of clinical prostate cancer samples with ETV1 overexpression complicates the elucidation of the possible role of these different forms in prostate cancer growth. Although MMPs and the UPA/UPAR system have been described as ETV1-associated genes, a clear global picture of ETV1-regulated gene expression is still lacking (de Launoit et al. 2006, Hermans et al. 2008a).

An important remaining question is whether ERG and ETV1 affect prostate cancer development by the same mechanism. This might not be the case, although ERG and ETV1 are both members of the same ETS family. ERG and ETV1 at least partially interact with overlapping binding sites, but might have different effects on target gene expression. ERG negatively regulates AR-regulated gene expression and ETV1 has the opposite effect (Baena et al. 2013). As an example, although ERG inhibits PSA expression, ETV1 seems to stimulate PSA expression (Shin et al. 2009, Yu et al. 2010). Paulo et al. (2012) recently suggested that there are both specific and shared
targets of ETV1 and ERG. Applying unsupervised clustering of mRNA from primary clinical samples, we observed that ETV1-positive and ERG-positive tumor samples clustered separately (Boormans et al. 2013, Gasi Tandefelt et al. 2013). So, molecular evidence for a common mechanism of ERG and ETV1 in human prostate cancer is limited.

Heterogeneity of prostate cancer

Because localized prostate cancer can be a multifocal disease, tumors have been tested for ETS gene fusions in different cancerous foci within one prostate. In approximately half of the cases, individual tumor foci differed according to the presence of ETS rearrangements or fusion mechanism (deletion or translocation; Barry et al. 2007, Mehra et al. 2007). Because ERG gene fusion is an early event, it confirmed and extended the general assumption that the majority of men develop multiple cancers in their prostate. Metastatic prostate cancer foci in one individual, however, displayed identical ETS rearrangement, showing that only one tumor focus seeded metastatic deposits (Mehra et al. 2008, Liu et al. 2009, Guo et al. 2012).

ETS fusions as diagnostic and prognostic markers of prostate cancer

Because ERG fusion transcripts are present in approximately 50% of prostate tumors, it is obvious that the presence of ERG fusion transcripts in prostate tissue or in urine or overexpression of ERG protein detected by immunostaining in prostate biopsies can be an important robust diagnostic marker of prostate cancer in a large subgroup of patients. Absence of ERG is not informative. The prognostic significance of TMPRSS2–ERG gene fusion is controversial and contradictory results have been reported (Table 2). The discrepancies might be the result of the differences in the patient populations studied, the techniques used to detect gene fusions and the effect of treatment on the examined tumor samples. Originally, Petrovics et al. (2005) found that patients with high expression levels of ERG showed a slower progression than patients with tumors without ERG overexpression. After the discovery of recurrent gene fusions TMPRSS2–ERG was more frequently to be found correlated with poor clinical outcome (Table 2; Wang et al. 2006, Demichelis et al. 2007, Nam et al. 2007, Attard et al. 2008b, Perner et al. 2006). However, this was not confirmed in other studies (Lapointe et al. 2007, Saramaki et al. 2008, Gopalan et al. 2009).

Table 2 Original studies on clinical relevance of TMPRSS2–ERG fusion gene in prostate cancer

<table>
<thead>
<tr>
<th>Reference</th>
<th>n</th>
<th>Tissue</th>
<th>Technique</th>
<th>TMPRSS2–ERG (%)</th>
<th>Follow-up (median)</th>
<th>Fusion-positive vs fusion-negative cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A) Cancer-specific (CSS) and overall survival (OS)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gopalan et al. (2009)</td>
<td>521</td>
<td>RP</td>
<td>FISH</td>
<td>42</td>
<td>7.9 years</td>
<td>No difference in OS (univariate level)</td>
</tr>
<tr>
<td>FitzGerald et al. (2008)</td>
<td>214</td>
<td>RP/TURP</td>
<td>FISH</td>
<td>35.5</td>
<td>12.3 years</td>
<td>No difference in CSS (multivariate level)</td>
</tr>
<tr>
<td>Attard et al. (2008b)</td>
<td>445</td>
<td>TURP/biopsies</td>
<td>FISH</td>
<td>30</td>
<td>7.5 years</td>
<td>Poorer CSS and OS (multivariate level)</td>
</tr>
<tr>
<td>Demichelis et al. (2007)</td>
<td>111</td>
<td>TURP/Millin prostatectomy</td>
<td>FISH</td>
<td>15</td>
<td>9.1 years</td>
<td>Poorer CSS (univariate level)</td>
</tr>
<tr>
<td>(B) PSA recurrence-free survival (PRFS)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Petrovics et al. (2005)</td>
<td>114</td>
<td>RP</td>
<td>Quantitative RT-PCR</td>
<td>62&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NS</td>
<td>Longer PRFS (univariate level)</td>
</tr>
<tr>
<td>Saramaki et al. (2008)</td>
<td>150</td>
<td>RP</td>
<td>FISH</td>
<td>33</td>
<td>5.5 years</td>
<td>Longer PRFS (multivariate level)</td>
</tr>
<tr>
<td>Nam et al. (2007)</td>
<td>165</td>
<td>RP</td>
<td>RT-PCR</td>
<td>42</td>
<td>1.7 years</td>
<td>Poorer PRFS (multivariate level)</td>
</tr>
<tr>
<td>Perner et al. (2006)</td>
<td>118</td>
<td>RP</td>
<td>FISH</td>
<td>49</td>
<td>NS</td>
<td>–&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Wang et al. (2006)</td>
<td>59</td>
<td>RP</td>
<td>RT-PCR</td>
<td>59</td>
<td>NS</td>
<td>–&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lapointe et al. (2007)</td>
<td>63</td>
<td>RP LN</td>
<td>RT-PCR</td>
<td>70</td>
<td>2.0 years</td>
<td>No difference in PRFS</td>
</tr>
<tr>
<td>Hermans et al. (2009)</td>
<td>67</td>
<td>RP</td>
<td>Quantitative RT-PCR</td>
<td>66</td>
<td>10.2 years</td>
<td>No difference in PRFS&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

RP, radical prostatectomy; FISH, fluorescence in situ hybridization; TURP, transurethral resection of the prostate; LN, lymph node.

<sup>a</sup>ERG overexpression.

<sup>b</sup>Higher recurrence rate, no survival analysis.

<sup>c</sup>More early recurrences, no survival analysis.

<sup>d</sup>Longer BRF5 for TMPRSS2(exon0)–ERG (multivariate level).
It was suggested that a subgroup of patients who had gene fusion with an interstitial deletion between TMPRSS2 and ERG (so-called ‘class Edel’) had poorer clinical outcome than gene-fusion-negative patients or than patients with TMPRSS2–ERG gene fusion without loss of the genomic region between the two genes (Attard et al. 2008b). Alternatively, it is possible that the poor survival associated with a copy number increase of chromosome 21 reflected generalized aneuploidy and genomic instability. We showed that patients expressing TMPRSS2–ERG fusion transcripts starting at an alternative first exon had better outcomes after radical prostatectomy than patients carrying tumors that only expressed TMPRSS2(exon1)–ERG (Hermans et al. 2009) and confirmed this finding in a completely independent patient cohort (Boormans et al. 2011). In the largest series reported thus far, more than 1100 radical prostatectomy specimens were evaluated for ERG overexpression using immunohistochemistry (Pettersson et al. 2012) and ERG overexpression was studied in correlation with biochemical recurrence and metastases-and cancer-specific survival. In the study population, 49% of the patients overexpressed ERG and although this overexpression was associated with a higher pathological T-stage, no association was found between ERG overexpression and survival in this cohort (median follow-up 12.6 years). In addition, the authors carried out a meta-analysis including analysis of prostate tissues or urine samples from more than 10,000 patients. The vast majority of the cases were primary tumors. Again no association between ERG overexpression and/or TMPRSS2–ERG gene fusion and clinical outcome was observed.

In almost all studies exploring the correlation between ERG gene fusion and clinical outcome, ERG status was assessed on surgically treated specimens. Data on association of TMPRSS2–ERG expression and response to a specific non-surgical treatment are limited. We studied 71 hormone-naive prostate cancer lymph node metastases. Although you might expect an important role for TMPRSS2-ERG in the success of endocrine treatment because of the androgen-regulation of TMPSS2 expression, in this group of patients no association between TMPRSS2-ERG expression and time to development of castration-resistant disease was detected (Boormans et al. 2010). ERG-positive tumors in CRPC patients who were treated with the inhibitor of testosterone synthesis abiraterone acetate were more frequent in patients who responded well to the therapy than in patients who did not show a good response (Attard et al. 2009). ERG status alone was not sufficient to explain sensitivity to abiraterone, but these results indicated that ETS gene fusions remained dependent on androgen signalling, despite the castration-resistant stage of the disease. Whether gene fusion status of the tumor has implications for the timing and the choice of endocrine therapy remains to be clarified further.

Although TMPRSS2–ERG overexpression might not be a tumor progression marker, it remains a strong novel therapeutic target because of its prostate cancer specificity and its overexpression in many stages of tumor development. So far, no specific inhibitors of ERG function have been described. In a recent report two independent cohorts of over 100 patients were treated with external beam radiotherapy (Dal Pra et al. 2013). Although preclinical studies predicted that TMPRSS2–ERG tumors might be more sensitive to radiation (Brenner et al. 2011), the presence of the gene fusion showed no association with biochemical recurrence-free survival in the clinical study. So, a simple extrapolation of experimental data to the clinical setting seems not to be possible and other factors not included in the clinical analyses so far contribute to clinical behavior.

In a recent study, we have identified in a group of prostate cancer patients who showed ERG overexpression in the tumor, two subgroups with very different clinical outcomes (Gasi Tandefelt et al. 2013). A 36-gene signature was generated that could predict rapid clinical progression in this group of ERG-positive patients. Using this predictor it was not possible to separate ERG-negative patients into two clinically relevant subgroups. We presumed that the ERG-positive patient group was more homogeneous, facilitating the identification of groups of genes that cooperate with ERG in tumor progression. No doubt, the ERG-negative group was genetically more heterogeneous, making selection of subgroups more difficult. In ETS-negative samples, there is evidence that SPINK1 overexpression was an independent predictor of clinical progression (Tomlins et al. 2008a).

**Concluding remarks**

The finding of ETS gene fusions in prostate cancer has been a major step in increasing our knowledge of the molecular and biological mechanisms of development and progressive growth of the disease. The postulated mechanisms of gene fusion and molecular function of ETS genes are of high general interest. Further exploration of proposed mechanisms will contribute to understanding of the processes of genomic rearrangements and oncogene heterogeneity in general. The gene fusions are also of the utmost importance in clinical prostate cancer. At the moment, ERG overexpression is already instrumental in the diagnosis of the disease. Moreover, elucidation of
the mechanisms of ETS gene expression and function increases the opportunity for finding new therapeutic targets for early and late stage prostate cancer (CRPC).

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review.

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References


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