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 *TMRPSS2–ERG* and other *ETS* gene fusions in prostate cancer.

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

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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 &

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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 in ~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 PEA3-subfamily that contain an N-terminal acidic trans-activation 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 defined. Recently, the first Chromatin immuno-precipitation (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

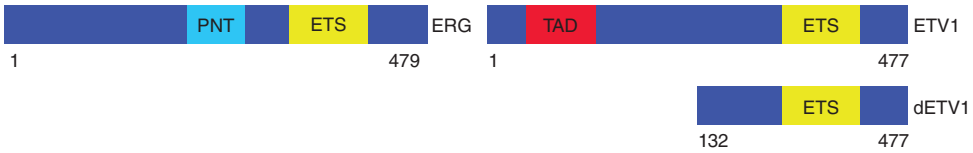

Figure 1
Schematic presentation of the ETS transcription factors ERG, ETV1, and truncated ETV1. ETS, ETS DNA-binding domain; PNT, pointed protein–protein interaction domain; TAD, acidic transactivation domain.

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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*. 2008*a*). *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*.

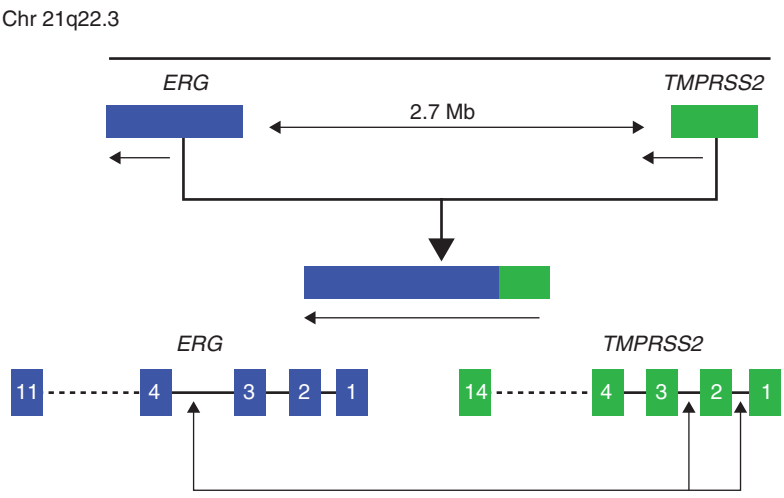

Figure 2
Schematic presentation of the *TMPRSS2–ERG* fusion on chromosome band 21q22.

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Table 1 ETS gene fusions in prostate cancer

5′ Fusion partner	Prostate specific	Androgen regulated	ETS partner	Frequency (%)
TMPRRS2 (chr 21q)	+	+	ERG (chr 21q)	50
SLC45A3 (chr 1q)	+	+		<1
NDRG1 (chr 8)	+/−	+		<1
SLC45A3 (chr 1q)	+	+	FLI1	<1
TMPRRS2 (chr 21q)	+	+	ETV1 (chr 7p)	<1
SLC45A3 (chr 1q)	+	+		<1
FOXP1 (chr 3p)	ND	ND		<1
EST14 (chr 14q)	+	+		<1
HERVK17 (chr 17p)	+			<1
*HERV-K*_22q11.23	+	+		<1
C15ORF21 (chr 15q)	+	+ (down)		<1
HNRPA2B1 (chr 7p)	−	−		<1
ACSL3 (chr 2q)	+/−	+		<1
TMPRRS2 (chr 21q)	+	+	ETV4 (chr 17q)	<1
KLK2 (chr 19p)	+	+		<1
CANT (chr 17q)	+	+		<1
DDX5 (chr 17q)	−	−		<1
TMPRRS2 (chr 21q)	+	+	ETV5 (chr 3q)	<1
SLC45A3 (chr 1q)	+	+		<1
[a]*SLC45A3* (chr 1q)	+	+	ELK4 (chr 1q)	<1

ND, not determined.
[a]Read-through transcript.

2008*b*). *ETS* gene fusions in prostate cancer seem mutually exclusive, but in multifocal disease more than one fusion event can be found. *ERG* is predominantly fused to *TMPRSS2*, but *ETV1, ETV4*, and *ETV5* have multiple fusion partners that all are located on different chromosomes (Table 1; Tomlins *et al*. 2006, 2007, Attard *et al*. 2008*a*, Han *et al*. 2008, Helgeson *et al*. 2008, Hermans *et al*. 2008*a*,*b*, Clark & Cooper 2009, Rubin *et al*. 2011). Interestingly, two of the fusion partners are endogenous retroviral *HERV-K* sequences that are apparently insignificant in the normal prostate. A gene encoding a non-coding RNA, denoted EST14, can also be a more frequent fusion partner.

Most of the fusion partners of the ETS genes *ETV1*, *ETV4* and *ETV5* are androgen-upregulated and display prostate-specific expression (Table 1). There are, however, exceptions. The *ETV1* fusion partner *C15ORF21* is downregulated by androgens and *HNRPA2B1* and *DDX5* are housekeeping genes. Remarkably, expression of *HNRPA2B1* is regulated by a dual-specific CG-rich promoter that cannot be methylated and always maintains an open chromatin structure (Antoniou *et al*. 2003, Lindahl-Allen & Antoniou 2007).

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*. 2008*a*, 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*. 2008*a*, 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

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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, 2008*b*, Hermans *et al*. 2008*a*, 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, 2008*b*, 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*. 2008*b*). However, in other studies, *TMPRSS2–ERG* GEMs did not even develop PIN. Among the progeny from crossbreeding *Erg* 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 *NCALD*, 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* knockout 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 (dETV1) (Fig. 1), or as a full-length protein, due to translocation of the complete gene to a different genomic region (Hermans *et al*. 2008*a*, 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*. 2008*a*).

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*. 2008*a*).

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, ETVI 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

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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*. 2008*b*, Perner *et al*. 2006). However, this was not confirmed in other studies (Lapointe *et al*. 2007, Saramaki *et al*. 2008, Gopalan *et al*.

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

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

RP, radical prostatectomy; FISH, fluorescence *in situ* hybridization; TURP, transurethral resection of the prostate; LN, lymph node.
[a]*ERG* overexpression.
[b]Higher recurrence rate, no survival analysis.
[c]More early recurrences, no survival analysis.
[d]Longer BRFS for *TMPRSS2(exon0)–ERG* (multivariate level).

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2009). It also 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.* 2008*b*). 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 *TMPRSSS2–ERG* expression and response to a specific non-surgical treatment are limited. We studied 71 hormone-naïve 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.* 2008*a*).

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

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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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