Deep Insight Section
Review

TMPRSS2:ETS gene fusions in prostate cancer

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I. Background
Prostate cancer (CaP) is the most commonly diagnosed male malignancy and a leading cause of cancer deaths in developed countries. With one in six men diagnosed, CaP remains a serious global public health issue (Jemal et al., 2008). CaP is a clinically heterogeneous disease, with manifestations ranging from a rapid and often fatal progression, to the typical, indolent disease which remains relatively insignificant to a patient's health.

Clinicopathological criteria, including Gleason grading, are not sufficient to differentiate men whose tumours require immediate and aggressive therapy from those that would suffice with vigilant clinical observation, thereby causing the latter group enormous amounts of unnecessary treatment (Yao and Lu-Yao, 2002). In this regard, the emerging data on the genetics of CaP hold great promise not only in stratifying this heterogeneous group of patients, but also in providing the groundwork for future development of targeted therapy.
I. i. Prostate cancer oncogenomics

Advances in cytogenetics and genomics facilitated the characterization of common genomic alterations in CaP, which are predominantly characterized by deletions (10q, PTEN; 13q, RB1; 8p, NKX3.1 (a prostate-specific tumour suppressor); 5q; 2q; 17p; and less commonly 6q; 7p; 16q; 18q) with only a small number of recurrent gains (8q, MYC; and chromosome 7). More complex patterns as well as an accumulation in the number of genomic gains and amplifications (Xq11.2-q12, androgen receptor (AR)) emerge as the disease advances. Genomic rearrangements leading to the formation of TMPRSS2:ETS gene fusions and deletion of the PTEN tumour suppressor are the two most frequent alterations observed in CaP (Tomlins et al., 2005; Yoshimoto et al., 2007; Yoshimoto et al., 2008). The TMPRSS2:ERG gene fusion is the principle genomic alter and a characteristic signature in approximately half of prostatic malignancies.

II. Discovery of TMPRSS2:ETS fusion genes in prostate cancer

The discovery of the TMPRSS2:ERG gene fusion exemplifies the current shift in the strategy in cancer genomics from experimental to bioinformatics approaches. By surveying 132 gene-expression CaP datasets from the Oncomine database (Compendia Bioscience) using the data transformation algorithm cancer outlier profile analysis (COPA), Chinnaiyan and colleagues identified genes with aberrant expression profiles in a subset of samples (Tomlins et al., 2005). COPA allowed the systematic investigation of cancer-related genes known to participate in chromosomal rearrangements in haematological malignancies and sarcomas. Two mutually exclusive Erythroblastosis virus E26 transformation-specific (ETS) transcription factors, ETS variant 1 (ETV1, 7p21.3) and ETS-related gene (ERG, 21q22.2), were identified as high-ranking outliers in several independent gene-expression profiling datasets. Exon-walking quantitative PCR (qPCR) of patient samples found the 3' regions of ERG and ETV1 to be overexpressed but the corresponding 5' regions were absent. 5' RNA ligase-mediated rapid amplification of cDNA ends identified the 5' end of these transcripts as the promoter sequences belonging to the prostate-specific, androgen regulated transmembrane protease, serine II (TMPRSS2, 21q22.3).

III. Frequency of TMPRSS2:ETS gene fusions in prostate cancer

This breakthrough study reported that 79% of radical prostatectomy (RP) samples harboured a fusion of the 5' untranslated region (UTR) of TMPRSS2 with the coding sequences of either ERG or ETV1 (Tomlins et al., 2005). Interestingly, just months prior, Petrovics et al. (2005) reported that ERG was the most commonly overexpressed oncogene in CaP by microarray and qPCR analysis. Subsequent studies showed that the frequency of the TMPRSS2:ERG fusion gene (or ERG rearrangement) is exceptionally variable and inconsistent in the literature, ranging from 27% to 79% in radical prostatectomy (RP) and biopsy samples, generally from prostate-specific antigen (PSA) screened cohorts (Tomlins et al., 2005; Yoshimoto et al., 2008; Mehra et al., 2007b; Watson et al., 2009; Barwick et al., 2010; Magi-Galluzzo et al., 2010). Some of the discrepancies in frequency relate to differences in the patient cohort (race as well as global geographical location or PSA-screened versus population based) or the type of specimen examined, as well as the technique used to detect the fusion gene. This concept is clearly illustrated when comparing population based cohorts, which have a lower reported frequency of 15-35%, as compared to RP cohorts (Attard et al., 2008a; Demichielis et al., 2007; Fitzgerald et al., 2008). The TMPRSS2:ERG gene fusion is found in approximately half of Caucasian patients, with a lower reported frequency in African-American men and is less common in Asian cohorts (Mosquera et al., 2009; Magi-Galluzzo et al., 2010; Lee K et al., 2010; Miyagi et al., 2010). An excellent example of discrepancies based on patient populations was demonstrated in a study that compared patients from the United Kingdom (UK) and China, wherein 41.3% of the UK patients but only 7.5% of Chinese cohort were found to harbour ERG rearrangements detected by FISH (Mao et al., 2010). The recent, contradicting report that 90% of the Chinese RP specimens harboured ETS rearrangements further emphasize the multifactorial nature of the variability in the frequency of this gene fusion (Sun et al., 2010). TMPRSS2:ERG gene fusions are reported in 10-21% of high-grade prostatic intraepithelial neoplastic (HGPIN) lesions, but are identified almost exclusively adjacent to fusion-positive cancer (Cerveira et al., 2006; Perner et al., 2007; Carver et al., 2009b; Han et al., 2009; Zhang et al., 2010; Mosquera et al., 2008). Benign prostatic hyperplasia (BPH) and normal epithelium are negative for ERG rearrangements and fusion transcripts, with the exception of the report by Clark and colleagues (Rajput et al., 2007; Wang et al., 2006; Cerveira et al., 2006; Clark et al., 2007; Perner et al., 2007; Dai et al., 2008; Han et al., 2009; Mosquera et al., 2009; Zhang et al., 2010; Sun et al., 2010; Lu et al., 2009). This study found that eight of 17 (47%) normal epithelial samples adjacent to fusion-positive CaP were also positive for TMPRSS2:ERG rearrangements and found a 6% fusion-positive rate in BPH samples (Clark et al., 2007). Hormone refractory and/or metastatic CaP exhibits less variability in the occurrence of TMPRSS2:ERG rearrangements with reported frequencies ranging from 29-59% (Perner et al., 2007; Mehra et al., 2008; Gopalan et al., 2009; Han et al., 2009; Boormans et al., 2010; Saramaki et al., 2008; Attard et al., 2009; Stott et al., 2010). Additionally, minute prostatic adenocarcinoma, a form
of CaP that is considered less clinically significant, harbours ETS fusions in approximately half of reported cases, underscoring the necessity of examining all prostatic malignancies for aggressive features such as the fusion gene (Albadine et al., 2009).

Investigators have also evaluated this rearrangement in the peripheral and transitional zones of the prostate. Nearly half of the peripheral tumours (43.3%) derived from RP samples were ERG rearrangement positive, whereas all 30 corresponding transitional tumours displayed a normal ERG locus by FISH (Guo et al., 2009). In contrast, other studies found TMPRSS2:ERG (13.3%) and ERG rearrangements (11.9%) are present in transitional zone tumours, despite being identified at a lower rate compared to peripheral zone lesions (Bismar and Trpkov, 2010; Falzarano et al., 2010).

These data illustrate the enormous variability in the frequency of TMPRSS2:ERG fusion positivity with respect to the cohort, disease stage, origin of sample as well as the method of detection. Interestingly, a comprehensive study of patient samples from 54 tumour types, including sarcomas and haematological malignancies, for ETS gene fusions and ERG rearrangements by FISH found these alterations to be exclusive to CaP samples (Scheble et al., 2010). The same result was obtained when RT-PCR was performed to detect TMPRSS2:ERG and TMPRSS2:ETV1 fusion transcripts in gastric and colorectal carcinomas (Yoo et al., 2007). These findings provide strong evidence that TMPRSS2:ETS gene fusions are specific to CaP.

IV. **TMPRSS2:ETS gene fusions: genes and protein structure**

IV. i. **TMPRSS2 and the androgen receptor**

TMPRSS2, a 70 KDa serine protease family member is associated with physiological and pathological processes such as digestion, tissue remodelling, blood coagulation, fertility, inflammatory responses, tumour cell invasion and apoptosis. The normal function of this protein is not yet known but is composed of a type II transmembrane domain, receptor class A low density lipoprotein domain, scavenger receptor cysteine-rich domain, protease domain, and cytoplasmatic domain (Paoloni-Giacobino et al., 1997). The 32 KDa serine domain, protease domain, and cytoplasmic domain

IV. ii. **ETS transcription factors**

Twenty-seven human ETS transcription factor family members have been identified, all of which share a conserved DNA binding domain that recognizes unique sequences containing GGA(ART) (Nye et al., 1992). ERG (21q22.2) is the ETS transcription factor most commonly known to participate in CaP gene fusions. The ERG gene contains 11 exons, with the transcriptional start site in exon 3. The ERG protein can interact with ETS members as well as other transcription factors, such as Jun and Fos, through its protein-protein interacting domain, SAM-PNT (Carriere et al., 1998; Verger et al., 2001; Basuayux et al., 1997). The conserved ETS DNA binding domain permits binding to purine rich DNA sequences (Reddy et al., 1991; Reddy et al., 1987), and in this manner, exert its effects in numerous cellular processes including membrane remodelling, angiogenesis, differentiation, proliferation, and tumourigenesis (Carver et al., 2009a; Kruse et al., 2009; Oikawa et al., 2003; Randi et al., 2009; Ellett et al., 2009; Birdsey et al., 2008; McLoughlin et al., 2001; Sato et al., 2001). Emerging evidence suggests that formation of the fusion gene may promote prostatic tumourigenesis, progression, and invasive disease and is associated with CaP-related mortality (Demichelis et al., 2007; Klezovitch et al., 2008; Wang et al., 2008; Hawsenworth et al., 2010). Functionally, ERG overexpression in CaP is highly implicated in promoting motility and invasiveness (Perner et al., 2007; Singh et al., 2002; Trojanowska et al., 2000; Tomlins et al., 2008a; Sreekumar et al., 2009; Schulz et al., 2010). In CaP, ERG expression has been associated with elevated levels of histone deacetylase 1 (HDAC1) and subsequent down regulation of HDAC1 target genes, upregulation of WNT pathway proteins, and inhibition of apoptotic signalling (Iljin et al., 2006). HDAC1 upregulation is common in CaP, but was found to be uniformly increased in ERG rearranged tumours (Iljin et al., 2006; Bjorkman et al., 2008). Activation of the WNT pathway leads to transcription of numerous genes involved in tumourigenesis, including AR, MYC, JUN, cyclinD1, BMP4 and
MMP7 (Terry et al., 2006; Schweizer et al., 2008). Highlighting the importance of WNT pathway activation in fusion-positive CaP is the resultant increase in AR transcription and expression levels. Consequently transcription of the fusion gene is increased, further amplifying ERG expression. Moreover, beta-catenin and the AR interact in an androgen-dependent manner to regulate AR target genes, whereas in androgen-insensitive tumours, both beta-catenin and AR target genes are expressed (Schweizer et al., 2008). ERG overexpression has recently been shown to activate C-MYC and result in its overexpression. These experiments revealed that C-MYC is activated by ERG and together their co-overexpression results in the suppression of prostate-epithelial differentiation genes and shorter time to biochemical recurrence (Hawksworth et al., 2010; Sun et al., 2008). ERG can also induce ICAM2 expression, resulting in AKT activation via PDK1 and subsequent inhibition of BAD leading to suppression of apoptotic signals (McLaughlin et al., 2001). In addition, ERG can bind BRCA1, a co-activator of the AR, and together were shown to regulate IGFR expression (Chai et al., 2001; Schayek et al., 2009). IGFR expression eventually leads to activation of AKT upon IGFR ligand-binding. ERG regulates MMPs thus influencing extracellular matrix (ECM) remodelling and the invasive potential of the cell (Ellett et al., 2009; Hawksworth et al., 2010; Singh et al., 2002; Schulz et al., 2010). Recently, Carver and colleagues have identified ETS binding sites in the promoter regions of CXCR4 and ADAMTS1, two genes involved in cellular motility and invasion (Carver et al., 2009a). Furthermore, it was shown that ERG directly upregulates the expression level of CXCR4 and ADAMTS1 (Carver et al., 2009b). Together, these studies provide a compelling evidence for a central, functional role of the fusion gene in the biology of prostatic carcinoma.

V. Fusion variants

The TMPRSS2:ERG fusion gene constitutes the majority (>85%) of ETS rearrangements in CaP, likely owing to their close proximity (2.7 Mb) and identical orientation on chromosome 21. Although, the remainder of this review will predominately focus on the TMPRSS2:ERG rearrangement, numerous alternative ETS members also can fuse to TMPRSS2, albeit at a much lower frequency. Similarly, variability in 5' partners have also been identified (Table 1). An intensive study examining ETV1 rearrangements found that only 9 of 23 samples had previously identified 5' partners, demonstrating the dramatic variability in fusion partners pairing with ETS transcription factors other than ERG (Attard et al., 2008b). The combined frequency of the remaining fusion variants accounts for approximately 10% of cases.

Five prime partners are divided into classes based on their tissue specificity and sensitivity to androgens (Tomlins et al., 2007). Class I is reserved for TMPRSS2; Class II represents other prostate-specific androgen inducible 5' UTR or endogenous retroviral elements; Class III represents the prostate-specific but androgen repressed partners; Class IV represents the non-tissue-specific promoters that are ubiquitously expressed, (i.e. house-keeping genes-this class often forms a chimeric or fusion protein, unlike the previous divisions of gene fusions); and finally Class V consists of ETV1-specific rearrangements, including the localization of the entire ETV1 locus to prostate specific locus, 14q13.3-14q21.1 (Tomlins et al., 2007; Attard et al., 2008b). To date only a single study has identified fusion genes in CaP devoid of ETS transcription factor participation (Palanisamy et al., 2010).

VI. Detection and classification

VI. i. FISH

Two strategies for FISH experiments are frequently used to detect the TMPRSS2:ERG fusion gene in CaP. The three-colour break-apart strategy, developed by Yoshimoto et al. (2006), uses differentially labelled bacterial artificial chromosome (BAC) clones as probes for the 3' (RP11-476D17) and 5' (RP11-95I21) segments of ERG with an additional BAC probe specific for the 5' region of TMPRSS2 (RP11-535H11) or for the transcriptional regulatory sequences (telomeric) of TMPRSS2 (RP11-35C4, RP11-891L10; RP11-260O11; Figure 1) (Yoshimoto et al., 2006). Using this probe configuration enables not only the detection of ERG rearrangement, but also allows confirmation that ERG's coding sequences are juxtaposed to the transcriptional regulatory region of TMPRSS2. Moreover, the mechanism of rearrangement can also be deduced by this approach (Yoshimoto et al., 2006; Yoshimoto et al., 2007). Characterization of rearrangement method is essential as differential clinical impacts are observed with the various mechanisms resulting in ETS gene fusions.

<table>
<thead>
<tr>
<th>5' partner</th>
<th>Class</th>
<th>3' partner</th>
<th>Initial reference</th>
</tr>
</thead>
<tbody>
<tr>
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<td>I</td>
<td>ERG</td>
<td>Tomlins et al. (2005)</td>
</tr>
<tr>
<td>TMPRSS2</td>
<td>I</td>
<td>ETV1</td>
<td>Tomlins et al. (2005)</td>
</tr>
<tr>
<td>HERV-K_22q11.23</td>
<td>II</td>
<td>ETV1</td>
<td>Tomlins et al. (2007)</td>
</tr>
<tr>
<td>Gene</td>
<td>Class</td>
<td>ETV</td>
<td>Authors</td>
</tr>
<tr>
<td>------------</td>
<td>-------</td>
<td>--------</td>
<td>------------------------------</td>
</tr>
<tr>
<td>SLC45A3</td>
<td>II</td>
<td>ETV1</td>
<td>Tomlins et al. (2007)</td>
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<tr>
<td>C15orf21</td>
<td>III</td>
<td>ETV1</td>
<td>Tomlins et al. (2007)</td>
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<td>IV</td>
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<td>Tomlins et al. (2007)</td>
</tr>
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<td>II</td>
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<td>Attard et al. (2008b)</td>
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<tr>
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<td>II</td>
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<td>ETV5</td>
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<td>BRAF*</td>
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<tr>
<td>ESRP1</td>
<td>ETS neg</td>
<td>RAF1*</td>
<td>Palanisamy et al. (2010)</td>
</tr>
</tbody>
</table>

*Only 3’ partners identified to date that are not a member of the ETS transcription factor family.

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**Figure 1: In house BAC probe configuration for three-colour break-apart TMPRSS2:ERG gene fusion FISH**

This schematic ideogram depicts the positions of differentially labelled bacterial artificial chromosome (BAC) clones specific for 3’ and 5’ regions of the ERG gene (RP11-476D17 in spectrum orange and RP11-95I21 in spectrum green, respectively), within the 21q22.2-3 region. Telomeric to this, the TMPRSS2 gene locus is represented by RP11-335H11 (spectrum red) which spans the gene, or by three BAC clones downstream (telomeric) of the TMPRSS2 gene (RP11-35C4, RP11-891L10 and RP11-260O11 in spectrum aqua). This probe design permits the accurate identification of TMPRSS2:ERG gene fusions as well as ERG rearrangements independent of fusion with TMPRSS2 fusion.

Work by Attard and colleagues classified the TMPRSS2:ERG rearrangement mechanisms according to the pattern of interphase FISH signals (Attard et al., 2008a). Class N describes the normal ERG locus, therefore co-localization of the two ERG probe signals in close proximity to the TMPRSS2 signal (less than one signal diameter) (Figure 2A). The majority of fusion-positive cases present with a heterozygous deletion at cytoband 21q22.2-3, termed Class Edel (Figure 2B). The deletion typically spans ERG, exons 1-3 and a partial deletion of exon 4, the known intervening genes (based on RefSeq Genes: NCRNA00114, ETS2, PSMG1, BRWD1, NCRNA00257, HMGN1, WRB, LCA5L, SH3BGR, C21orf88, B3GALT5, IGSF5, PCP4, DSCAM, C21orf130, MIR3197, BACE2, PLAC4, FAM3B, MX2, and MX1) and the coding exons of TMPRSS2. The three-colour FISH of an Edel rearrangement displays co-localization of the 3’ ERG and TMPRSS2 signals, and absence of the 5’ ERG signal. Less
frequently a genomic rearrangement leading to insertion of those sequences elsewhere in the genome to an unknown chromosome location can occur resulting in the separation of the 5’ ERG signals from the co-localization of the 3’ ERG and TMPRSS2 signals, thus described as ERG split or Class Esplit (Figure 2C). In both types of TMPRSS2:ERG rearrangements the unaffected chromosome 21 generally display a Class N signal configuration. Finally, additional copies of TMPRSS2:ERG gene fusions is identified as Class 2+Edel (Attard et al., 2008a).
Figure 2: Classification of TMPRSS2:ERG gene fusion by interphase FISH.
Prostate cancer patient samples were hybridized with the three-colour probe set, described in Figure 1, and counterstained with DAPI. The nuclei of interest (in dashed boxes) are magnified in the insets. A) Class N, where no ERG rearrangement has occurred. Co-localization of 3' and 5' ERG probes (often visualized as a yellow signal), with the 5' TMPRSS2 BAC probe signals (red) indicates a normal Chr21q22.2-3 locus. Frequently, the signals for the TMPRSS2 probe, situated 2.7 Mb away from the ERG locus, may be separated from the ERG signals by up to one probe signal width. B) Class Edel is characterized by the co-localization of 3' ERG probe to the TMPRSS2 probe signals, and the absence of the 5' ERG signal. This represents rearrangement with the loss of the intervening sequence. The unaffected Chr21 displays Class N configuration. C) Class Esplit is characterized by the co-localization of the 3' ERG and TMPRSS2 signals, with the retention of the 5' ERG signal elsewhere in the nucleus. The unaffected Chr21 displays Class N configuration.

The second strategy employs a two-colour break-apart FISH design to identify rearrangements in specific ETS genes (Mehra et al., 2007; Zhang et al., 2010). By this method, confirmation of fusion between two genes and identification of the specific 5' partner is not possible because the probes are specific for a single gene, and therefore, this strategy is an indirect method for the detection of fusion genes in CaP.

VI.ii. Other methods of detection
RT-PCR is another technique frequently employed to determine the fusion status of prostatic tissue samples. However, this approach is limited to the detection of the hybrid transcripts, and is unable to obtain important cytogenetic information such as the genomic mechanism that generated the gene fusion (Edel vs Esplit). To date, as many as 17 fusion transcripts and splice variations have been characterized, the most common fusion transcript is composed of exon 1 of TMPRSS2 fused to exon 4 of ERG (T1:E4 (Wang et al., 2006; Jhavar et al., 2008)). The majority of the remaining transcripts result in truncation of the ERG protein product by 39-99 amino acids, while the few that initiate translation from the native start codon in exon 3 produce full length ERG (Tomlins et al., 2005; Soller et al., 2006; Wang et al., 2006; Clark et al., 2007; Clark et al., 2008a). One of the transcripts produces a genuine TMPRSS2:ERG fusion protein and eight contain premature stop codons and are unlikely to result in ERG overexpression (Tomlins et al., 2005; Soller et al., 2006; Wang et al., 2006; Clark et al., 2007; Clark et al., 2008a). These studies further revealed that elaborate heterogeneity exists in hybrid transcripts present, between foci and within individual tumour foci of the same patient. Variability in the translation start site consequentially affects the size of the mRNA transcript (373-885 bp) and therefore the protein product potentially modifying the functional capacity of the gene fusion product (Wang et al., 2006). Distinct transcript variants display differential prognostic influence based on the resultant biological activities (Hermans et al., 2009; Wang et al., 2008). A significant challenge remains in relating the clinical
prognosis to the fusion gene and will be addressed in the subsequent section. Indirect methods suggestive of gene fusions in CaP are being employed to broadly determine ETS rearrangement status with perhaps the anticipation of implementing a high-throughput method of detection. Analysis of array comparative genomic hybridization (aCGH) data, specifically the 21q22.2-3 region may permit identification of Class Edel TMPRSS2:ERG gene fusions (Watson et al., 2009; Ishkanian et al., 2009). Class Esplit retains the intervening sequences within the nuclei, in a copy neutral manner and consequently aCGH cannot identify this rearrangement. Overexpression of ETS transcription factors by gene expression microarrays may also imply an ETS rearrangement has occurred. Due to inconclusive results, FISH or RT-PCR assays are necessary to validate microarray findings pertaining to the ETS gene fusions. A novel multiplexing technology recently developed uses nanostructured microelectrodes integrated onto a chip and has the capability to detect disease-specific biomarkers, including differentiation of various fusion transcripts (Fang et al., 2009). More recently, immunohistochemistry (IHC) is also being explored as a means to detect the fusion by ERG protein overexpression, in the absence of confirming fusion at the genomic or transcript level (Furusato et al., 2010; Park et al., 2010). Of the numerous direct (three-colour FISH, RT-PCR) and indirect (two-colour FISH, microarrays, IHC) methods employed to determine fusion status to date, the three-colour FISH yields the most cytogenetic and genomic information. In addition, to identifying the fusion status and class, the inherent cell-by-cell analysis addresses the heterogeneous nature of the disease, and provides a biological context for such information.

VII. Fusion gene formation and chromosomal instability

There is increasing research interest and effort focusing on the genomic events and attributes that lead to the formation of ETS gene fusions. A complex TMPRSS2:ERG rearrangement found in a single patient was meticulously examined and described by Yoshimoto et al. (2007). This patient had a microdeletion of the sequences from 5’ ERG to and including the TMPRSS2 coding sequences with a concurrent translocation of the region immediately telomeric of 5’ untranslated TMPRSS2 sequences. A detailed multicolour FISH assay mapped the region between ERG and TMPRSS2, revealing the complexity of chromosomal rearrangements that can lead to the formation of fusion genes in CaP. This case demonstrates the valuable information available through the use of complex multicolour FISH assays. The molecular mechanisms that underlie this recurrent translocation are just beginning to be understood. For example, fine mapping of the deletion breakpoints located within the ERG and TMPRSS2 loci, followed by sequencing, revealed the presence of consensus sequences homologous to the human Alu-Sq and Alu-Sp subfamily (Liu et al., 2006). The presence of these consensus sequences within intronic regions correlated with the presence of the fusion gene and may be a factor contributing to the deletion at 21q22.2-3, resulting in the fusion gene. More recently, genotyping of familial CaP revealed that the fusion gene associates with polymorphisms in DNA repair genes, specifically POLI and ESCO1 (Luedeke et al., 2009). Using FISH, the AR was shown to induce chromosomal proximity of TMPRSS2 and ERG by binding to the promoter region of TMPRSS2 (Mani et al., 2009). Subsequently, LnCaP cells were irradiated to induce double-strand breaks inducing the formation of the TMPRSS2:ERG gene fusion upon dihydrotestosterone stimulation in the previously fusion-negative cell line (Mani et al., 2009). The DNA-bound AR is also implicated in chromatin architecture modifications that can cause double-strand breaks, commonly repaired by non homologous end joining machinery, and may result in the formation of gene fusions (Lin et al., 2009). Also recently, androgen signalling was shown to recruit topoisomerase II beta with the AR to the breakpoints resulting TMPRSS2:ERG fusion genes (Haffner et al., 2009). Undoubtedly, specific nucleotide sequences within the 21q22.2-3 region are of great importance and further elucidation as to their role in the formation of gene fusions is essential. The TMPRSS2:ERG gene fusion is an unique model to query sequence level polymorphisms that may lead to the formation of intra-chromosomal rearrangements largely due to the close proximity and orientation of the involved genes as well as the high rate of recurrence. Chromosomal instability, defined as the formation of novel chromosome alterations and rearrangements at an elevated rate, compared to normal cells may also be a factor contributing to the formation of ETS gene fusions in CaP. It is well established that deletion of the tumour suppressor PTEN (10q23.31), a common genomic aberration in CaP, results in an elevated level of chromosomal instability through activation of AKT. One of sequelae of this change is the phosphorylation and inhibition of the cell cycle check point kinase 1 (Chk1), an important kinase preventing cell cycle progression in response to DNA damage (Pac et al., 2005; Sanchez et al., 1997). Furthermore, nuclear PTEN interacts with kinetochore proteins and induces the expression of RAD51, a protein required to reduce the incidence of spontaneous double-strand breaks (Shen et al., 2007). PTEN deficiency ultimately alters multiple cell cycle checkpoints, which could potentially delay DNA damage repair and/or chromosome segregation (Gupta et al., 2009). Overall, PTEN has a variety of roles in maintaining chromosomal stability and integrity, and the recurrent PTEN loss in CaP may represent an important trigger in the events leading to the formation of TMPRSS2:ETS gene fusions.
VIII. Heterogeneity of multifocal disease

CaP, one of the most heterogeneous epithelial carcinomas, is also notoriously multifocal in nature. As a multifocal disease, CaP permits the investigation and comparison of recurrent genomic events within distinct foci of individual patients. In 32 RP specimens with spatially distinct foci, ERG rearrangement status was examined using two-colour FISH (Barry et al., 2007). Nineteen samples displayed interfocal homogeneity, with 80% of the samples negative for ERG rearrangement. The remaining 13 samples exhibited interfocal heterogeneity but intrafocal homogeneity, with two samples harbouring three separate foci each with a different rearrangement status: Class N, Edel and Esplit. Similarly, whole mount examination of RP specimens for fusion transcripts yielded multiple fusion transcripts in individual CaP foci with an identical hybrid transcript profile in the adjacent HGPIN (Furusato et al., 2008). Two other studies examining fusion transcript variants found different transcript hybrids in discrete regions of a single prostate (Wang et al., 2006; Clark et al., 2007). Multiple TMPRSS2:ETS gene fusion-positive foci may arise independently and exhibit interfocal heterogeneity. Investigation of ERG rearrangement in multifocal CaP and corresponding metastases provides a glimpse of the potential biological impact of this aberration in the context of disease progression (Perner et al., 2009). This study reported that the metastatic lesion was always positive for ERG rearrangement through the same mechanism (Edel vs Esplit) as that present in at least one of the prostatic tumour foci (not necessarily the index focus). The authors suggest that this rearrangement may be a factor contributing to the development of metastases, regardless of clinicopathological criteria of the individual foci.

IX. Prognostic significance

There exists appreciable controversy with respect to the prognostic significance of the TMPRSS2:ETS fusion gene in CaP, with studies suggesting that the fusion gene has a favourable (Winnes et al., 2007; Petrovics et al., 2005; Winnes et al., 2007; Saramaki et al., 2008), unfavourable (Yoshimoto et al., 2008; Mehra et al., 2007a; Perner et al., 2006; Wang et al., 2006; Nam et al., 2007a; Nam et al., 2007b; Attard et al., 2008a; Mehra et al., 2008; Reid et al., 2010; Demichelis et al., 2007; Rostad et al., 2009; Lapointe et al., 2007a) or no association (Mehra et al., 2008; Yoshimoto et al., 2006; Neill et al., 2007; Fletcher et al., 2008; Dai et al., 2008; Furusato et al., 2008; Darnell et al., 2009; Gopalan et al., 2009; Rubio-Briones et al., 2010; Fitzgerald et al., 2008; Lee et al., 2010; Sun et al., 2010; Rouzier et al., 2008) with clinical outcomes. When no association between clinicopathological criteria and the presence of the fusion gene was found, it was often attributable to small sample sizes. The most compelling evidence suggests a trend towards unfavourable factors outcome in CaP progression. Notably, an interesting comparison of two studies looking at conservatively managed men had opposing results from an association with CaP-specific death (Demichelis et al., 2007) to no reduction in CaP-specific survival for patients harbouring this rearrangement (Fitzgerald et al., 2008).

The role of deletion (Edel) or retention (Esplit) of intervening sequences in tumour biology has also been scrutinized with relatively uniform results suggesting that Edel is not only the more prevalent mechanism but is also associated with more aggressive disease (Mwamukonda et al., 2010; Attard et al., 2008a; Mehra et al., 2008). Edel was associated with worse prostate-specific survival and shorter time to biochemical recurrence than Class Esplit (Yoshimoto et al., 2006; Attard et al., 2008a). While, Class 2+Edel was associated with lethal disease and significantly worse clinical outcome, particularly when combined with prostate-specific clinicopathological criteria (Yoshimoto et al., 2006; Attard et al., 2008a). Another study also demonstrated that Edel rearrangements have a more aggressive tendency since all fusion-positive androgen-independent metastases had lost 5’ ERG sequences (Mehra et al., 2008) and Edel was associated with clinically aggressive features of progression (Perner et al., 2006). These findings are consistent with elevated ERG expression and led investigators to speculate that the loss of 5’ ERG is associated with aggressive CaP.

It must be considered that the commonly deleted 2.7 Mb between ERG and TMPRSS2 could contain important tumour suppressor genes (Yoshimoto et al., 2006) and its loss in the Edel rearrangement leads to haploinsufficiency of a tumour suppressor gene that underlies the aggressive clinical course. One gene candidate for this effect within this region, HMGN1, a nucleosome binding protein has previously been associated with CaP progression (Birger et al., 2005). In agreement with this view, the Esplit rearrangement has not yet been shown to associate with any particular clinical outcome to date. However, given the low frequency of this event (~10% of TMPRSS2:ERG rearrangements), Esplit rearrangements cannot be excluded as a measure of prognosis until further studies are completed (Attard et al., 2008a).

On the other hand, one investigation demonstrated no statistically significant association with clinicopathological criteria and Edel or Esplit, but evidence did suggest 2+Edel is a factor contributing to CaP-specific mortality (Fitzgerald et al., 2008). Conversely, TMPRSS2:ERG rearrangement alone were associated with lower histological grade, but no other clinical features or CaP-specific death (Gopalan et al., 2009). However, the same group also found that copy number increases (CNI) of the ERG locus with or without TMPRSS2:ERG gene fusions was associated with high grade and advanced stage, while cancers with CNI and 2+Edel rearrangements tended to be more
clinically aggressive (Fine et al., 2010). While the above results are conflicting to the previous studies that showed no association with extra copies of the unrearranged ERG locus (Attard et al., 2008a), the difference may be due to the deletion of the intervening genes potentially generating a more aggressive tumourigenic phenotype.

SPINK1 (5q32) is a gene identified in a more recent COPA meta-analysis as being overexpressed in 10% of CaP, all of which are exclusively ETS fusion negative (Tomlins et al., 2008b). For this reason, SPINK1 expression and the TMPRSS2:ERG fusion gene were evaluated to determine their relative significance as prognostic biomarkers in biopsy samples from hormonally treated men (Leinonen et al., 2010). In this cohort, the fusion gene was associated with Ki-67 staining, age at diagnosis and tumour area, but not with any prostate-specific clinicopathological criteria (Leinonen et al., 2010). On the other hand, cases that overexpressed SPINK1 had a significantly shorter time to biochemical recurrence, but no association with any other criteria.

X. Clinical utility

Promptly following the emergence of adverse clinical correlations of fusion-positive CaP Mosquera and colleagues evaluated a series of CaP cases to determine if there was a morphological phenotype associated with this genomic alteration (Mosquera et al., 2007). Blinded to ERG rearrangement status, the group identified five histologic criteria that were significantly related to ERG rearrangement positive samples: blue-tinged mucin, cribriform growth, macronucleoli, intraductal tumor spread, and signet-ring cell features. Only 24% of ERG rearranged samples did not identify with any of the above mentioned morphological features and 93% of samples with three features were positive for ERG rearrangement. The authors speculate that the morphological characteristics shared by ERG rearranged tumours could result from ETS dysregulated pathways and could be utilized in the routine assessment performed by pathologists. More recently, the same group published a similar set of morphological features with the addition of collagenous micronodules (Mosquera et al., 2009). A significant association between perineural invasion, blue-tinged mucin, and intraductal tumor spread with a positive gene fusion status has been documented (Nigwekar et al., 2008), while another study confirmed the association that ETS fusion-positive CaP samples were more likely mucin-positive than mucin-negative (Tu et al., 2007). These results should be taken, however, in the context of somewhat contradictory reports, such that the TMPRSS2:ERG gene fusion correlates with low Gleason grade and is inversely related to high-grade morphological features. These studies hold promise that morphological markers routinely examined by pathologists could be coupled with the current clinicopathological criteria for a more comprehensive evaluation of biopsy or RP specimens providing well-informed judgments on diagnosis, prognosis and treatment decisions.

In an attempt to optimize the clinical utility of this biomarker, investigators are turning to less invasive survey modes, such as urine specimens and circulating tumour cells (CTCs). Post-digital rectal exam (DRE) urine analyzed by qRT-PCR found 17.2% positive rate for ERG overexpression. Subsequent examination of the corresponding biopsy samples from the same cohort revealed a 40% positive rate (Rice et al., 2010). The clinical yield of this assay can be improved, as indicated by another study which found a 69% positive rate following prostatic massage versus only 24% when no prostatic message was performed before collecting the urine samples (Rostad et al., 2009). Two additional studies report similar results (42% and 59%) of fusion-positive transcripts in post-DRE urine (Hessels et al., 2007; Laxman et al., 2006). Expressed prostatic secretion has also been a successful specimen for the non-invasive detection of fusion transcripts (Clark et al., 2008b). These studies demonstrate the potential for ETS gene fusion detection using non-invasive approaches following physical evaluation of the prostate by DRE, adding valuable information to disease stratification prior to radical treatments. A handful of studies have also used FISH to detect ERG rearrangement in CTCs in effort to monitor disease recurrence or treatment efficacy (Mao et al., 2008; Attard et al., 2009; Stott et al., 2010).

XI. Role of ETS in prostate tumorigenesis: Driver?

Discrepancies regarding the fusion gene are not limited to its diagnostic potential and prognostic significance. Controversy also exists in the sequence of genomic and molecular alterations in CaP initiation and progression. Several groups speculate that the formation of the TMPRSS2:ETS fusion gene is required for CaP initiation in ETS positive CaP tumours, with other aberrations occurring later in the course of disease advancement and metastasis (Perner et al., 2007; Tomlins et al., 2007; Attard et al., 2009). Recently, Attard and colleagues demonstrated that CTCs from individual castration-resistant CaP patients were either clonally positive or negative for ERG rearrangement, however, loss of PTEN and increase in AR gene copy number was heterogeneous in the CTCs derived from a single patient (Attard et al., 2009). These results suggest that the formation of the fusion gene occurred before PTEN loss and gain of the AR locus. However, this study was performed on a very small cohort of castration-resistant CaP and may not necessarily reflect the sequence of accumulating genomic alterations in CaP.

TMPRSS2:ETS fusion status in HGPIN occurs at a low frequency, and almost exclusively only when juxtaposed to fusion-positive CaP. These HGPIN lesions, however, do not exhibit the chromosomal copy
number changes seen in 42% of the paired CaP as assessed by CGH (Cerveira et al., 2006). These results propose that formation of the fusion gene may precede chromosomal-level alterations and is consistent with the literature indicating that few gross chromosome- or arm-level chromosomal copy alterations are present in localized CaP. Another study investigating a range of prostate tissues comprising benign, precursor, malignant and metastatic samples found that the majority of patient samples examined showed homogeneity with respect to fusion status and mechanism (Edel vs Esplit). The presence of fusion-positive HGPIN was interpreted as additional evidence that this entity is a true-precursor to CaP, a notion that has eluded indisputable proof (Perner et al., 2007). In contrast, a recent study that examined both PTEN genomic loss and fusion status by FISH observed PTEN loss in benign and HGPIN lesions, while ERG rearrangement was identified in HGPIN, albeit at a lower frequency than PTEN loss, and absent in BPH lesions (Bismar et al., 2010). Maintaining this model of progression and accumulation of genomic changes in CaP it is possible that heterozygous loss of PTEN may be a 'driver lesion', in a subset of CaP, and PTEN haploinsufficiency may facilitate the selective formation of the fusion gene (Figure 3). TMPRSS2:ERG gene fusion may occur as a secondary alteration and may function as an 'enhancer' permitting the cell to achieve a higher level of aggressiveness and invasiveness. When this sequence of events was emulated in transgenic mice, ERG overexpression resulted in marked acceleration of preneoplastic lesions to invasive CaP on a Pten deleted background, but ERG overexpression alone simply displayed slight atypical histology compared to control mice (Carver et al., 2009a; Carver et al., 2009b). In vitro experiments using cell lines demonstrated ERG overexpression provided enhanced motility without affecting proliferation, in agreement with Tomlins et al. (2007); Carver et al. (2009b). These findings corroborate the view that effectors of ERG overexpression affect cellular processes which are complimentary to unregulated factors downstream of AKT as a result of PTEN deficiency, but on their own are not sufficient to provoke the transition from benign to neoplastic. The implication is not that PTEN loss is the driver in CaP, but that ETS gene fusions are enhancer alterations significantly affecting cellular processes further progressing prostatic tumourigenesis when the background is primed first by a driver event capable of initiating sufficient dysregulation leading to the development of a preneoplastic lesion. However, because PTEN loss and the presence of the fusion gene are significantly associated events in CaP (Yoshimoto et al., 2008; Carver et al., 2009a; Han et al., 2009; King et al., 2009; Bismar et al., 2010) it is likely that PTEN inactivation may be an important driver lesion for fusion-positive CaP. Together the driver event and ETS overexpression lead to a significantly aggressive and invasive lesion. Therefore, elucidation of the cellular pathways affected by ETS overexpression is fundamental to the comprehension of the aggressive nature observed in the majority of fusion-positive CaP, and to developing novel therapeutic strategies to specifically target this subset of CaP.

XII. Concluding remarks

Difficulty in assigning prognostic significance, diagnostic and therapeutic utility to ETS gene fusions is a result of a myriad of factors including, the heterogeneity of the disease, the mechanism of rearrangement (Edel vs Esplit), the technique used to assess the presence of the fusion as well as the cohort examined. Nevertheless, continued controversy between positive and negative clinical associations of the gene fusion dictates further studies are required using larger cohorts to determine the absolute potential of this genomic aberration as a biomarker for CaP diagnostic utility, prognostic significance, and stratification of patients to aid in treatment decisions. Furthermore, comprehension of the pathways affected by ETS overexpression will aid in the potential of implementing ETS specific therapies to target this aggressive subtype of CaP and benefit many patients.
Figure 3: Model of prostate cancer progression showing ETS gene fusions as an enhancer lesion.

Cooperation of unregulated pathways downstream of PTEN with effectors of ERG overexpression is likely a crucial event in the progression of an invasive and aggressive prostatic adenocarcinoma. Heterozygous genomic deletion of PTEN in benign prostatic precursors may represent an early event, and act as a driver lesion leading to proliferation, survival and genomic instability—all initial requisites of cancer. As a consequence of such heightened genomic instability, PTEN haploinsufficiency may facilitate the selective formation of the fusion gene with consequent acquisition of additional invasive properties. The presence of both rearrangements within a lesion is associated with accelerated disease progression and poor prognosis, indicating that synergistic molecular interactions exist between their complementary pathways. Continuing instability generates genotypic heterogeneity and diversity, such that subclones bearing PTEN homozygous deletions and amplified AR loci have further selective advantage for aggressive tumour progression, androgen escape and metastases.
References


Nye JA, Petersen JM, Gunther CV, Jonsen MD, Graves BJ. Interaction of murine ets-1 with GGA-bridge sites establishes the ETS domain as a new DNA-binding motif. Genes Dev. 1992 Jun;6(6):975-90

Basuayux JP, Ferreira E, Stéhelin D, Buttichi G. The Ets transcription factors interact with each other and with the c-Fos/c-Jun complex via distinct protein domains in a DNA-dependent and -independent manner. J Biol Chem. 1997 Oct 17;272(42):26188-95


Balk SP, Knudsen KE, AR, the cell cycle, and prostate cancer. Nuc Recept Signal. 2008 Feb 1;6:001


Haskard DO, Dejana E, Mason JC, Randi AM. Transcription factor Erg regulates angiogenesis and endothelial apoptosis through VE-cadherin. Blood. 2008 Apr 1;111(7):3498-506


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