Characterization of TMPRSS2:ETV5 and SLC45A3:ETV5 Gene Fusions in Prostate Cancer

Beth E. Helgeson,1 Scott A. Tomlins,1 Nameeta Shah,1 Bharathi Laxman,1 Qi Cao,1 John R. Prensner,1 Xuhong Cao,1 Nirmish Singla,1 James E. Montie,2,3 Sooryanarayana Varambally,1,3 Rohit Mehra,1,3 and Arul M. Chinnaiyan1,2,3

1Michigan Center for Translational Pathology, Department of Pathology, 2Department of Urology, and 3Comprehensive Cancer Center, University of Michigan Medical School, Ann Arbor, Michigan

Abstract

Recurrent gene fusions involving oncogenic ETS transcription factors (including ERG, ETV1, and ETV4) have been identified in a large fraction of prostate cancers. The most common fusions contain the 5′ untranslated region of TMPRSS2 fused to ERG. Recently, we identified additional 5′ partners in ETV1 fusions, including TMPRSS2, SLC45A3, HERV-K_22q11.23, C150RF21, and HNRPA2B1. Here, we identify ETV5 as the fourth ETS family member involved in recurrent gene rearrangements in prostate cancer. Characterization of two cases with ETV5 outlier expression by RNA ligase–mediated rapid amplification of cDNA ends identified one case with a TMPRSS2:ETV5 fusion and one case with a SLC45A3:ETV5 fusion. We confirmed the presence of these fusions by quantitative PCR and fluorescence in situ hybridization. In vitro recapitulation of ETV5 overexpression induced invasion in RWPE cells, a benign immortalized prostatic epithelial cell line. Expression profiling and an integrative molecular concepts analysis of RWPE-ETV5 cells also revealed the induction of an invasive transcriptional program, consistent with ERG and ETV1 overexpression in RWPE cells, emphasizing the functional redundancy of ETS rearrangements. Together, our results suggest that the family of 5′ partners previously identified in ETV1 gene fusions can fuse with other ETS family members, suggesting numerous rare gene fusion permutations in prostate cancer. [Cancer Res 2008;68(1):73–80]

Introduction

Whereas gene fusions are common in hematologic and mesenchymal malignancies, until recently, they have not been well defined in common epithelial tumors. To nominate candidate oncogenes from DNA microarray data, we developed a bioinformatics approach [cancer outlier profile analysis (COPA)] to identify genes with marked overexpression in a subset of cancers (1). COPA identified ERG and ETV1 as prominent “outliers” across multiple prostate cancer data sets. Through several molecular techniques, we identified fusions of the 5′ untranslated region (5′-UTR) of TMPRSS2 (21q22) to ERG (21q22) or ETV1 (7p21) in cases that overexpressed the respective ETS family member (1). Subsequently, rare fusions of TMPRSS2 and the ETS family member ETV4 have also been identified (2, 3). Multiple studies have shown that TMPRSS2:ERG fusions are the most predominant subtype of ETS gene fusions (~50% of prostate cancers), and fusions involving ETV1 or ETV4 are rare (~1–10% of prostate cancers; refs. 1–7).

More recently, we discovered additional 5′ fusion partners involved in ETV1 gene fusions, including the 5′-UTRs from SLC45A3, HERV-K_22q11.3, C150RF21, and HNRPA2B1 (4). Importantly, because these 5′ partners are differentially regulated by androgen (androgen-induced, androgen-repressed and androgen insensitive), they define distinct classes of ETS gene rearrangements. To date, these additional 5′ partners have only been identified in ETV1 fusions, and it is unknown if they can fuse with ERG (in rare TMPRSS2:ERG negative cases with ERG outlier expression) or additional ETS family members.

Here we report the discovery of TMPRSS2:ETV5 and SLC45A3:ETV5 gene fusions, identifying a fourth ETS family member, ETV5 (3q27), involved in recurrent gene rearrangements in prostate cancer. This study also shows that the family of 5′ fusion partners we previously identified can fuse to additional ETS family members, suggesting numerous rare ETS gene fusion combinations.

Materials and Methods

Samples and cell lines. Prostate tissues were from the radical prostatectomy series and the Rapid Autopsy Program (8), which are both part of the University of Michigan Prostate Cancer Specialized Program of Research Excellence Tissue Core. Samples were collected with informed consent and prior institutional review board approval. The benign immortalized prostate cell line RWPE was obtained from the American Type Culture Collection. Total RNA was isolated from all samples with Trizol (Invitrogen).

In silico analysis of ETV5 outlier expression. The normalized expression values for ERG, ETV1, ETV4, and ETV5 from four prostate cancer profiling studies [Glinsky et al. (9), Lapointe et al. (10), Vanaja et al. (11), and Yang et al. (GSE8218)] and this study (X. Cao et al.) in the Oncomine database (12) were downloaded, and heat maps were generated using Cluster 3.0 and Java Treeview.

Quantitative PCR. Quantitative PCR was done using SYBR Green dye on an Applied Biosystems 7300 Real-time PCR system (Applied Biosystems) as described (1, 2, 4). Oligonucleotide primers were synthesized by Integrated DNA Technologies and are listed in Supplementary Table S1.

RNA ligase–mediated rapid amplification of cDNA ends. RNA ligase–mediated rapid amplification of cDNA ends (RLM-RACE) was done using the GeneRacer RLM-RACE kit (Invitrogen) according to the manufacturer’s instructions as described (1, 2, 4). To obtain the 5′ end from PCA_ETV5_1, first-strand cDNA was amplified using the GeneRacer 5′ primer and ETV5 exon6_racer-r. For amplification from PCA_ETV5_2, ETV5 exon11-r was used.
Reverse-transcription PCR. To confirm the expression of TMPRSS2:ETV5 fusion transcripts in PCa_ETV5_1, we carried out reverse transcription PCR (RT-PCR) as described (1). cDNA was PCR amplified with Platinum Taq High Fidelity using primers for TMPRSS2:ETV5a/TMPRSS2:ETV5b and TMPRSS2:ETV5c (Supplementary Table S1), and products were resolved by electrophoresis, cloned into pCR-TOPO, and sequenced as described (1).

Fluorescence in situ hybridization. Interphase fluorescence in situ hybridization (FISH) on formalin-fixed paraffin-embedded tissue sections was done as described (2, 4). Bacterial artificial chromosomes (listed in Supplementary Table S2) were obtained from the BACPAC Resource Center.

In vitro ETV5 overexpression. Full-length human ETV5 cDNA (BC007333) in the Gateway compatible vector pDONR-Dual was obtained from the Harvard Institute of Proteomics (HsCD00003658). Adenoviral and lentiviral constructs were generated by recombination with pAD/CMV/V5 (Invitrogen) and pLent6i/CMV/V5 (Invitrogen), respectively, using LR Clonase II (Invitrogen). Control pAD/LACZ clones were obtained from Invitrogen and Control pLent6i/GUS clones were generated by recombination using a control entry clone (pENTR-GUS, Invitrogen). The University of Michigan Vector Core generated the viruses. The benign immortalized prostate cell line RWPE was infected with adenoviruses expressing ETV5 or LACZ and used 48 h after infection. RWPE cells were also infected with lentiviruses expressing ETV5 or GUS, and stable clones were generated by selection with blasticidin (Invitrogen). ETV5 expression was confirmed by immunoblotting with a mouse monoclonal anti-ETV5 antibody (Abnova) at 15000 dilution. Mouse monoclonal anti–glyceraldehyde-3-phosphate dehydrogenase (GAPDH) antibody (Abcam) was applied at 1:30,000 dilution for loading control.

Invasion assays. Invasion assays were done as described (4). Equal numbers of the indicated cells were seeded onto basement membrane matrix (extracellular matrix, Chemicon) present in the insert of a 24-well culture plate, with fetal bovine serum added to the lower chamber as a matrix (extracellular matrix, Chemicon) present in the insert of a 24-well culture plate, with fetal bovine serum added to the lower chamber as a

expression harbored chromosomal rearrangements (1, 2, 4, 15), suggesting that rearrangements similarly cause ETV5 outlier expression. Previously, we used an exon-walking quantitative PCR strategy that showed loss of outlier expression at the 5’ end of the ETS transcript in cases that harbored gene fusions (1, 2). Using 10 primer pairs across the 13 exons in the ETV5 transcript, we found outlier expression of ETV5 exons 2 to 13 in PCa_ETV5_1 and ETV5 exons 8 to 13 in PCa_ETV5_2 (Fig. 1C). Thus, to identify a potential 5’ partner, we carried out RLM-RACE, which identified three products in PCa_ETV5_1 and one product in PCa_ETV5_2. Sequencing of the PCa_ETV5_1 products showed three different TMPRSS2:ETV5 fusions (TMPRSS2:ETV5a–TMPRSS2:ETV5c; Fig. 3A). The TMPRSS2:ETV5a transcript contained exon 1 of TMPRSS2 fused to exon 2 of ETV5 and TMPRSS2:ETV5b contained exons 1 to 3 of TMPRSS2 fused to exon 2 of ETV5. In the TMPRSS2:ETV5c transcript, sequencing revealed a distinct 1st exon and exons 2 to 3 of TMPRSS2 fused to exon 2 of ETV5. This 1st exon is ~1.5 kb downstream of the reference sequence 1st exon and overlaps with a reported EST (DA460066; Supplementary Fig. S1).

Sequence of the PCa_ETV5_2 RLM-RACE product revealed exon 1 of SLC45A3 fused to exon 8 of ETV5 (Fig. 3A). Recently, we identified SLC45A3 as a 5’ fusion partner in ETV1 gene rearrangements (4). Our discovery of the SLC45A3:ETV5 fusion confirms SLC45A3 as another recurrent 5’ partner in ETS gene fusions and suggests that the other 5’ fusion partners may be involved in additional rare ETS rearrangements. Together, RLM-RACE of both cases confirmed the exon-walking quantitative PCR expression patterns and shows the existence of ETV5 gene fusions in prostate cancers with ETV5 outlier expression.

To confirm the presence of the fusion transcripts found in PCa_ETV5_1 and PCa_ETV5_2, we carried out quantitative PCR. As shown in Fig. 3B, quantitative PCR detected TMPRSS2:ETV5b/c exclusively in PCa_ETV5_1 and SLC45A3:ETV5 in PCa_ETV5_2. To confirm the expression of individual TMPRSS2:ETV5 isoforms in PCa_ETV5_1, we carried out RT-PCR using primers specific for TMPRSS2:ETV5a/TMPRSS2:ETV5b and TMPRSS2:ETV5c. Sequencing of amplified products using TMPRSS2:ETV5a/TMPRSS2:ETV5b primers confirmed the expression of both TMPRSS2:ETV5a and TMPRSS2:ETV5b (Supplementary Fig. S2). PCR using primers for TMPRSS2:ETV5c produced two products, which were identified as

Results and Discussion

In this study, we applied CPA to an expression profiling data set generated as part of an integrative molecular study of prostate cancer progression.6 Consistent with previous profiling studies, in this data set, CPA identified ERG as the second ranking outlier at the 90th percentile and ETV1 as the 61st outlier at the 95th percentile. Intriguingly, CPA ranked another ETS family member, ETV5, as the 4th ranking outlier at the 95th percentile, with 2 of 12 (17%) localized cancers showing outlier expression (noted as PCa_ETV5_1 and PCa_ETV5_2; Fig. 1A). Whereas one of the two

6 http://www.oncomine.org
X. Cao et al., in preparation.
TMPRSS2:ETV5c and TMPRSS2:ETV5d, another isoform containing the novel 1st exon of TMPRSS2 (found in TMPRSS2:ETV5c) fused to exon 2 of ETV5 (Fig. 3A and Supplementary Fig. S2).

Next, to confirm these fusions at the genomic level, we carried out FISH using split signal assays around the 5’ partners (TMPRSS2 and SLC45A3) as well as fusion assays (5’ TMPRSS2 or SLC45A3 and 3’ ETV5; Fig. 3C). In Pca_ETV5_1, multiple hybridizations failed to produce interpretable signals for TMPRSS2, ETV5, or ERG probes (positive control) because the only tumor cells in the tissue section were present in a small focus at the extreme edge. Review of all blocks from the case failed to yield more informative sections. However, in Pca_ETV5_2, we confirmed rearrangements in SLC45A3 and ETV5 and fusion of the 5’ SLC45A3 and 3’ ETV5 signals (Fig. 3C). Together, quantitative PCR and RT-PCR validated the presence of TMPRSS2:ETV5 and SLC45A3:ETV5 in Pca_ETV5_1 and Pca_ETV5_2, respectively, and FISH confirmed the fusion of the SLC45A3 and ETV5 genomic loci in Pca_ETV5_2.

In previous studies, we and others have shown that ERG and ETV1 mediate invasiveness in prostate cancer cell lines harboring ETS rearrangements and benign prostate cell lines ectopically overexpressing ETS family members (4, 16). Thus, we recapitulated ETV5 overexpression in vitro to determine its role in prostate cancer. We generated adenoviruses and lentiviruses expressing ETV5 and infected the immortalized benign prostate cell line RWPE to generate transient and stable RWPE-ETV5 cells, respectively (Fig. 4A). Transient and stable ETV5 overexpression in RWPE cells had no effect on proliferation (data not shown); however, we observed increased invasion through a modified basement membrane assay in RWPE-ETV5 cells compared with control infected RWPE cells [transient, 4.7-fold (P = 0.0006); stable, 4.8-fold (P = 0.0008); Fig. 4A].

Figure 1. ETV5 exon expression in prostate cancers with outlier expression. A, applying the bioinformatics algorithm COPA to our prostate cancer expression profiling data set identified the ETS family members ERG, ETV1, and ETV5 as high-ranking outliers. The expression of each gene in benign prostate (green), clinically localized prostate cancer (red), and metastatic prostate cancer (black) in normalized expression units is indicated. For each gene, the COPA ranking at the indicated percentile from this study is indicated. ETV5 outliers (Pca_ETV5_1 and Pca_ETV5_2) are indicated. B, ETV5 expression was measured by quantitative PCR in our expression profiling data set (n = 29) plus an additional 44 clinically localized prostate cancers. In total, we assayed 10 benign prostate tissue samples (green), 56 localized prostate cancers (Pca; red), and seven metastatic prostate cancers (Met; black). Relative quantities of the ETV5 transcript in each sample were normalized to the average of GAPDH and HMBS. The relative amount of ETV5 was calibrated to the median of all samples. ETV5 outliers identified in A are indicated. C, quantitative PCR was used to determine the relative expression of ETV5 exons in prostate cancers with ERG overexpression (Pca_ERG_1 and Pca_ERG_2), a prostate cancer that was negative for ETS overexpression (Pca_no_ETS), and Pca_ETV5_1 and Pca_ETV5_2. Exon(s) measured by the respective primer pair are indicated below the graph. Relative quantities of each exon were normalized to the average of GAPDH. The relative amount of ETV5 exons in each sample was calibrated to the median of the five prostate cancers. A schematic of the noncoding (light purple) and coding (dark purple; start codon in exon 2) regions of ETV5 are shown; the numbers above the exons (indicated by boxes) indicate the last base of each exon. The gray and white bars below the schematic represent the overexpressed exons in Pca_ETV5_1 and Pca_ETV5_2, respectively.

6 S.A. Tomlins et al., submitted for publication.
To investigate the transcriptional program regulated by ETV5 overexpression, we profiled transient RWPE-ETV5 cells and analyzed the expression signatures using the Oncomine Concepts Map, a tool for analyzing associations between >20,000 biologically related gene sets by disproportionate overlap (13, 14). Previously, Oncomine Concepts Map analysis has identified enrichment of concepts related to invasion in our "overexpressed in RWPE-ERG or RWPE-ETV1" signatures, consistent with the phenotypes of these cells (4). We identified 420 features overexpressed in transient RWPE-ETV5 compared with RWPE-LACZ cells.

Whereas we observed enrichment of our "overexpressed in RWPE-ERG or RWPE-ETV1" concepts in our "overexpressed in RWPE-ETV5" signature (Fig. 4B), we unexpectedly observed more significant enrichment with our "underexpressed in RWPE-ERG or RWPE-ETV1" signatures. For example, our underexpressed and overexpressed in RWPE-ERG (transient) signatures were both enriched in our overexpressed in RWPE-ETV5 signature (OR, 7.97 and 2.45; \( P = 1.3 \times 10^{-25} \) and \( 5 \times 10^{-4} \), respectively). Because distinct subsets of genes overexpressed in RWPE-ETV5 cells were both overexpressed and underexpressed in RWPE-ERG and RWPE-ETV1 cells, this suggests that ETV5, ERG, and ETV1 differentially regulate a common set of target genes when overexpressed in benign prostate cells.

Importantly, Oncomine Concepts Map analysis identified a network of invasion related concepts that shared enrichment with our overexpressed in RWPE-ETV5, RWPE-ERG, and RWPE-ETV1

---

**Figure 2.** Expression of ERG, ETV1, ETV4, and ETV5 in prostate cancer profiling studies. The expression of ERG, ETV1, ETV4, and ETV5 in five prostate cancer profiling studies from the Oncomine database was used to generate heat maps. Rows, genes; columns, benign prostate tissue samples (green), localized prostate cancers (red), and metastatic prostate cancers (black). Yellow and blue, relative overexpression and underexpression, respectively, according to the color scale. Gray cells, features that did not pass filtering. The number of ETV5 outliers in each study is indicated after the last name of the first author.
Figure 3. Fusion of TMPRSS2 and SLC45A3 to ETV5 in prostate cancers. A, RLM-RACE was used to characterize the complete ETV5 transcript in PCa_ETV5_1 and PCa_ETV5_2. Sequencing revealed three distinct TMPRSS2:ETV5 transcripts in PCa_ETV5_1 and one SLC45A3:ETV5 transcript in PCa_ETV5_2. An additional TMPRSS2:ETV isoform (TMPRSS2:ETV5d) identified by RT-PCR is also shown. Structures for ETV5 (purple), TMPRSS2 (red), and SLC45A3 (red) reference sequences are shown. The numbers above the exons (indicated by boxes) indicate the last base of each exon. Untranslated regions are shown in corresponding lighter shades. Identified fusions are colored and numbered from the original reference sequences. B, the expression of TMPRSS2:ETV5 and SLC45A3:ETV5 fusion transcripts was confirmed by quantitative PCR. C, FISH was done to confirm ETV5 and SLC45A3 rearrangements and SLC45A3:ETV5 fusion in PCa_ETV5_2. Schematics of bacterial artificial chromosomes located 5’ and 3’ to ETV5 and SLC45A3 that were used as probes for interphase FISH are shown. Chromosomal coordinates are from the March 2006 build of the human genome using the University of California Santa Cruz Genome Browser. Bacterial artificial chromosomes are indicated as numbered rectangles. Genes are shown with the direction of transcription indicated by the arrowhead and exons indicated by bars. FISH was done using bacterial artificial chromosomes as indicated with the corresponding fluorescent label on PCa_ETV5_2. Sections were assayed for split 5’/3’ signals of ETV5 (left) and SLC45A3 (middle) and the fusion of 5’ SLC45A3 and 3’ ETV5 signals (right). Red and green arrows, split signals; yellow arrows, fused signals.
Figure 4. Overexpression of ETV5 in benign immortalized prostate cells induces invasion. A, the benign immortalized prostate cell line RWPE was infected with an adenovirus expressing ETV5 or LACZ control (left) or a lentivirus expressing ETV5 or GUS control (right) and cells were assayed for invasion through a modified basement membrane assay [mean (n = 3) + SE]. Immunoblotting confirmation of ETV5 overexpression and photomicrographs of invaded cells as indicated are shown. Representative of three independent experiments. B, transient overexpressing RWPE-ETV5 and RWPE-LACZ cells were profiled on Agilent Whole Genome microarrays and expression signatures were loaded into the Oncomine Concept Map. Molecular concept map analysis of the overexpressed in RWPE-ETV5 compared with RWPE-LACZ signature (ringed yellow node). Each node represents a molecular concept or a set of biologically related genes. The node size is proportional to the number of genes in the concept. The concept color indicates the concept type according to the legend. Each edge represents a significant enrichment (P < 5e−4). Enrichments involving concepts representing overexpressed and underexpressed in RWPE cells expressing ERG or ETV1 signatures are shown in orange and cyan, respectively. Inset, an overlay map identifying genes present (red cells) across multiple concepts in the overexpressed in RWPE-ETV5 enrichment network (indicated by number). C, RWPE-ETV5 cells were treated with the PLAU inhibitor amiloride, the PLAU and PLAT inhibitor PAI-1, or MMP inhibitors (including the pan-MMP inhibitor GM-6001) as indicated and assayed for invasion as in A.


References


Characterization of TMPRSS2:ETV5 and SLC45A3:ETV5 Gene Fusions in Prostate Cancer

Beth E. Helgeson, Scott A. Tomlins, Nameeta Shah, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/68/1/73

Supplementary Material
Access the most recent supplemental material at:
http://cancerres.aacrjournals.org/content/suppl/2008/01/10/68.1.73.DC1

Cited articles
This article cites 22 articles, 9 of which you can access for free at:
http://cancerres.aacrjournals.org/content/68/1/73.full#ref-list-1

Citing articles
This article has been cited by 30 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/68/1/73.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link
http://cancerres.aacrjournals.org/content/68/1/73.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.