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Novel 5′ Fusion Partners of *ETV1* and *ETV4* in Prostate Cancer[1,2]

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Abstract

Gene fusions involving the erythroblast transformation-specific (ETS) transcription factors *ERG*, *ETV1*, *ETV4*, *ETV5*, and *FLI1* are a common feature of prostate carcinomas (PCas). The most common upstream fusion partner described is the androgen-regulated prostate-specific gene *TMPRSS2*, most frequently with *ERG*, but additional 5′ fusion partners have been described. We performed 5′ rapid amplification of cDNA ends in 18 PCas with *ETV1*, *ETV4*, or *ETV5* outlier expression to identify the 5′ fusion partners. We also evaluated the exon-level expression profile of these ETS genes in 14 cases. We identified and confirmed by fluorescent *in situ* hybridization (FISH) and reverse transcription-polymerase chain reaction the two novel chimeric genes *OR51E2-ETV1* and *UBTF-ETV4* in two PCas. *OR51E2* encodes a G-protein–coupled receptor that is overexpressed in PCas, whereas *UBTF* is a ubiquitously expressed gene encoding an HMG-box DNA-binding protein involved in ribosome biogenesis. We additionally describe two novel gene fusion combinations of previously described genes, namely, *SLC45A3-ETV4* and *HERVK17-ETV4*. Finally, we found one PCa with *TMPRSS2-ETV1*, one with *C15orf21-ETV1*, one with *EST14-ETV1*, and two with *14q133-q21.1–ETV1*. In nine PCas (eight *ETV1* and one *ETV5*), exhibiting ETS outlier expression and genomic rearrangement detected by FISH, no 5′ fusion partner was found. Our findings contribute significantly to characterize the heterogeneous group of ETS gene fusions and indicate that all genes described as 5′ fusion partners with one ETS gene can most likely be rearranged with any of the other ETS genes involved in prostate carcinogenesis.

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Abbreviations: PCa, prostate carcinoma; FISH, fluorescence *in situ* hybridization; 5′RACE, 5′ rapid amplification of cDNA ends

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[2]This article refers to supplementary materials, which are designated by Tables W1 to W3 and Figures W1 and W2 and are available online at www.neoplasia.com.
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Introduction

Gene fusions involving the erythroblast transformation-specific (ETS) transcription factor family of genes are a recurrent feature of prostate adenocarcinomas (PCas). These gene aberrations, caused by chromosomal structural abnormalities, originate fusion transcripts that lead to overexpression of N-truncated ETS proteins or, more rarely, to full-length ETS proteins or chimeric fusion proteins [1–3]. Fusion of the androgen-regulated promoter region of the *TMPRSS2* gene with *ERG* is the most common ETS rearrangement, being present in about 50% of PCa and in 20% of high-grade prostatic intraepithelial neoplasia lesions [1,4]. Other rarer fusion events can occur involving the PEA3 subfamily of ETS members, namely, *ETV1*, *ETV4*, and *ETV5* [1,5,6] or the ERG subfamily member *FLI1* [3].

Besides the prostate-specific and androgen-induced *TMPRSS2*, several ETS fusion partners have been described, namely, *HERPUD1*, *NDRG1*, *SLC45A3*, *ACSL3*, *HERV-K_22*, *HERVK17*, *CANT1*, *DDX5*, *KLK2*, *FOXP1*, *EST14*, *HNRPA2B1*, *C15orf21*, and the chromosomal region 14q13.3-14q21.1 [2,7,8], presenting heterogeneous tissue specificities and androgen responsiveness. Fusion partners like the *SLC45A3* gene or the endogenous retroviral element (*HERVK17*) display similar tissue specificity as *TMPRSS2* and are equally androgen-induced. Contrarily, the fusion partner *C15orf21*, despite being overexpressed in PCas, is repressed by androgens. However, there are ubiquitously expressed 5′ fusion partners, such as the *HNRPA2B1* gene, displaying no evidence of androgen regulation. Finally, ETS family genes may be rearranged with prostate-specific enhancers in chromosomal regions such as 14q13.3-14q21.1.

We have previously performed a comprehensive characterization of ETS rearrangements on a series of 200 clinically localized PCas and found rearrangements involving *ERG*, *ETV1*, *ETV4*, *ETV5*, and *FLI1* in 52%, 7%, 1.5%, 0.5%, and 0.5%, respectively [3]. In the present work, we focused on the 18 PCas that showed outlier expression levels of *ETV1*, *ETV4*, or *ETV5* and a genomic rearrangement of the corresponding ETS locus. The combined use of exon-level expression profiles from exon microarrays, 5′ rapid amplification of cDNA ends (5′RACE), and fluorescence *in situ* hybridization (FISH) with bacterial artificial chromosome (BAC)–specific probes allowed us to identify novel 5′ fusion partners for *ETV1* and *ETV4*, as well as to describe novel combinations of genes known to be involved in PCa gene fusions.

Materials and Methods

PCa Samples

We studied a set of 18 PCas with outlier mRNA expression levels of *ETV1* (n = 14), *ETV4* (n = 3), and *ETV5* (n = 1) and with a genomic rearrangement previously demonstrated by FISH but with yet unknown fusion partners [3]. These samples were selected from a cohort of 200 patients with clinically localized PCa consecutively diagnosed and treated with radical prostatectomy that were previously typed for ETS rearrangements [3]. This study was approved by the Institutional Review Board, and informed consent was obtained from all subjects.

Gene Expression Microarrays

RNA was extracted from tissue samples using TRIzol (Invitrogen by Life Technologies, Carlsbad, CA), as previously described [3], and 1 μg of RNA was processed into cDNA and hybridized to Affymetrix GeneChip Human Exon 1.0 ST arrays, following the manufacturer's recommendations. The Affymetrix Expression Console v1.1 software was used to obtain exon-level robust multi-array average (RMA)-normalized expression values for the core probe sets only.

5′Rapid Amplification of cDNA Ends

The 5′RACE was performed using the SMARTer RACE cDNA amplification kit and protocol (Clontech Laboratories, Inc, Saint-Germain-en-Laye, France). Briefly, first-strand cDNA was reverse transcribed from 1 μg of total RNA using the SMARTScribe Reverse Transcriptase with the 5′RACE cDNA synthesis primer (5′-CDS) and the SMARTer IIA oligo from the kit. An aliquot of the cDNA was then amplified using a forward gene-specific primer (GSP) and a universal primer mix. Polymerase chain reaction (PCR) conditions used were as described by the manufacturer. Nested PCRs using the nested universal primer as the reverse primer and a nested gene-specific primer (NGSP) were performed to increase the specificity and product yields of 5′RACE. Primers used on 5′RACE and nested PCR are listed in Table W1. Nested PCR products were analyzed on a 2% agarose gel (SeaKem LE Agarose; Lonza, Basel, Switzerland) and the bands were purified using the QIAquick Gel Extraction Kit (Qiagen, Hilden, Germany).

Cloning and Sequencing

Purified 5′ nested race PCR products were cloned into pCR4-TOPO plasmids using the TOPO TA Cloning Kit for sequencing (Invitrogen). Colonies were picked and the plasmids were purified using the Qiagen Plasmid Miniprep Kit (Qiagen) and subsequently sequenced using the M13 forward and T3 primers using BigDye Terminator V3.1 sequencing chemistry on a 3730 DNA Analyzer (Applied Biosystems by Life Technologies, Foster City, CA) according to the manufacturer's recommendations. Genomic alignment of the resulting sequences was performed using BLAST (http://www.ncbi.nlm.nih.gov/blast/) and BLAT (http://genome.ucsc.edu/). All exons identified were numbered according to the longest matching transcripts of the Ensembl database (http://www.ensembl.org/).

Reverse Transcription–PCR

The reverse transcription (RT)–PCR assay for detection of prostate fusion transcripts was performed with the following primer combinations: TMPRSS2_F and ETV1_R (*TMPRSS2-ETV1*), C15orf21_S and ETV1_AS1 (*C15orf21-ETV1*), SLC45A3_S and ETV4_AS (*SLC45A3-ETV4*), UBTF-S and ETV4_AS (*UBTF-ETV4*), OR51E2_S and ETV1_AS2 (*OR51E2-ETV1*), EST14_S and ETV1_AS2 (*EST14-ETV1*), and HERVK17_S and ETV4_AS (*HERVK17-ETV4*; Table W2). PCRs were performed with the Qiagen OneStep RT-PCR Kit (Qiagen) according to the manufacturer's instructions. Amplified products were analyzed on a 2% agarose gel and further validated by sequencing.

Fluorescence In Situ *Hybridization*

To validate the 5′RACE findings, we performed FISH using BAC clones targeting the 5′ fusion partner and the ETS gene on tissue sections from paraffin blocks of the index tumor (Table W3). BAC clones were selected using the University of California, Santa Cruz (UCSC) Human Genome Browser and obtained from the BACPAC Resources Centre (Oakland, CA). BAC DNA was extracted, amplified, labeled, and prepared for hybridization as previously reported [9]. Adequate mapping and probe specificity of all BAC clones was confirmed by hybridization onto human metaphase spreads of normal