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Molecular Subtyping of Primary Prostate Cancer Reveals Specific and Shared Target Genes of Different ETS Rearrangements[1,2]

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Abstract

This work aimed to evaluate whether ETS transcription factors frequently involved in rearrangements in prostate carcinomas (PCa), namely ERG and ETV1, regulate specific or shared target genes. We performed differential expression analysis on nine normal prostate tissues and 50 PCa enriched for different ETS rearrangements using exon-level expression microarrays, followed by *in vitro* validation using cell line models. We found specific deregulation of 57 genes in *ERG*-positive PCa and 15 genes in *ETV1*-positive PCa, whereas deregulation of 27 genes was shared in both tumor subtypes. We further showed that the expression of seven tumor-associated *ERG* target genes (*PLA1A*, *CACNA1D*, *ATP8A2*, *HLA-DMB*, *PDE3B*, *TDRD1*, and *TMBIM1*) and two tumor-associated *ETV1* target genes (*FKBP10* and *GLYATL2*) was significantly affected by specific ETS silencing in VCaP and LNCaP cell line models, respectively,

Abbreviations: AR, androgen receptor; DAC, 5-aza-2′deoxycytidine; NPT, normal prostate tissue; PCA, principal components analysis; PCa, prostate carcinoma; qMSP, quantitative methylation-specific PCR; qRT-PCR, quantitative real-time polymerase chain reaction; shRNA, short hairpin RNA; siRNA, small interfering RNA; TSA, Trichostatin A

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[2]This article refers to supplementary materials, which are designated by Tables W1 to W5 and Figure W1 and are available online at www.neoplasia.com.
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whereas the expression of three candidate *ERG* and *ETV1* shared targets (*GRPR*, *KCNH8*, and *TMEM45B*) was significantly affected by silencing of either ETS. Interestingly, we demonstrate that the expression of *TDRD1*, the topmost overexpressed gene of our list of *ERG*-specific candidate targets, is inversely correlated with the methylation levels of a CpG island found at −66 bp of the transcription start site in PCa and that *TDRD1* expression is regulated by direct binding of ERG to the CpG island in VCaP cells. We conclude that ETS transcription factors regulate specific and shared target genes and that *TDRD1*, *FKBP10*, and *GRPR* are promising therapeutic targets and can serve as diagnostic markers for molecular subtypes of PCa harboring specific fusion gene rearrangements.

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Introduction

Genomic rearrangements involving five members of the ETS family of transcription factors have been found in prostate carcinomas (PCa). Rearrangements of *ERG* and *ETV1* were first described by Tomlins et al. [1] and are found in approximately 50% and 5% to 10% of PCa, respectively [2,3]. Rearrangements of *ETV4* and *ETV5* were later identified in a small proportion of PCa, representing less than 5% of all rearranged cases [4–7]. Recently, we identified *FLI1* as the fifth member of the ETS family of transcription factors involved in gene fusions in PCa, being fused to the *SLC45A3* gene [8].

The products of specific chimeric genes could be ideal therapy targets, but the nuclear localization of the aberrant ETS proteins makes them a difficult therapy target *in vivo* [9]. Therefore, it is important to characterize in detail the downstream molecular targets of each of the aberrant transcription factors, not only to understand the deregulated signaling pathways but also because some of them may turn out to be more amenable to targeted therapy. *In vitro* studies revealed that *ERG* activates plasminogen and Wnt pathways to promote degradation of the extracellular matrix and decrease cell adhesion, but very few genes have been validated as direct *ERG* targets [10–12]. Because *ETV1* rearrangements are considerably less frequent than those of *ERG*, reports focusing on the oncogenic effectors of *ETV1* overexpression are scarce and not based in the expression profile observed in *ETV1* rearrangement-positive tumors, with some *in vitro* and *in vivo* models linking overexpression of *ETV1* with the invasion potential of cancer cells by activation of matrix metalloproteinases and integrins [13–15].

Despite the apparently overlapping oncogenic potential of *ERG* and *ETV1* gene fusions, it has not been established whether different ETS transcription factors have shared or specific downstream targets. We addressed this issue by using exon-level expression arrays in a series of 50 PCa enriched for different ETS rearrangements and validated the findings using *in vitro* cell line models.

Materials and Methods

Prostate Tissue Samples

We used a series of 50 tumor samples selected from a consecutive series of 200 clinically localized PCa that were previously typed for ETS rearrangements [8]. The 50 prostatectomy samples were selected to represent the various molecular subtypes of PCa, namely 21 samples with *ERG* rearrangement, 13 samples with *ETV1* rearrangement, 2 samples with other ETS rearrangements (one with *ETV4* and one with *ETV5* rearrangements), and 14 samples without known ETS rearrangement. For control purposes, nine normal prostate tissues (NPTs) were collected from cystoprostatectomy specimens of bladder cancer patients. This study was approved by the institutional review board, and informed consent was obtained from all subjects.

Prostate Cell Lines

VCaP and PNT2 cells were acquired from the European Collection of Cell Cultures (Sigma-Aldrich, St Louis, MO). LNCaP, PC3, and DU145 cells were acquired from the German Resource Centre for Biological Material (DSMZ, Braunschweig, Germany). 22Rv1 cells were kindly provided by Dr David Sidransky from the Johns Hopkins University School of Medicine. The virus packaging Retro-Pack PT67 cell line was acquired from Clontech Laboratories, Inc (Saint-Germain-en-Laye, France). All prostate cell lines were cultured under the recommended conditions, being karyotyped by G banding for validation purposes and tested for *Mycoplasma* spp. contamination (PCR Mycoplasma Detection Set; Clontech Laboratories). After transfection, cells were grown in medium supplemented with G418 (300 μg/ml; GIBCO by Life Technologies, Carlsbad, CA) or puromycin (5 μg/ml, Clontech Laboratories), as appropriate.

Gene Expression Microarrays

RNA was extracted from tissue samples using TRIzol (Invitrogen by Life Technologies, Carlsbad, CA), as previously described [8], and 1 μg of RNA was processed into complementary DNA (cDNA) and hybridized to GeneChip Human Exon 1.0 ST arrays, following the manufacturer's recommendations. The Affymetrix Expression Console v1.1 software was used to obtain gene-level RMA-normalized expression values for the core probe sets only. We used analysis of variance in Partek Genomics Suite 6.4 (Partek, Inc, St Louis, MO) to identify differentially expressed genes among the different sample groups. The two PCa with *ETV4* and *ETV5* rearrangements were not included in this analysis. Specific *ERG* target genes were identified from genes differentially expressed between each of the three group comparisons: NPT *versus ERG*-positive PCa, ETS-negative PCa *versus ERG*-positive PCa and *ETV1*-positive PCa *versus ERG*-positive PCa. To select specific *ETV1* target genes, the same approach was applied comparing NPT *versus ETV1*-positive PCa, ETS-negative PCa *versus ETV1*-positive PCa and *ERG*-positive PCa *versus ETV1*-positive PCa. Targets common to *ERG* and *ETV1* rearrangements were identified from the differentially expressed genes in each of the four group comparisons: NPT *versus ERG*-positive PCa, NPT *versus ETV1*-positive PCa, ETS-negative PCa *versus ERG*-positive PCa and ETS-negative PCa *versus ETV1*-positive PCa. Only differentially expressed genes with a false discovery rate less