

SLC45A3-ELK4 Is a Novel and Frequent Erythroblast Transformation–Specific Fusion Transcript in Prostate Cancer

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Abstract

Chromosomal rearrangements account for all erythroblast transformation–specific (ETS) family member gene fusions that have been reported in prostate cancer and have clinical, diagnostic, and prognostic implications. Androgen-regulated genes account for the majority of the 5' genomic regulatory promoter elements fused with ETS genes. *TMPRSS2-ERG*, *TMPRSS2-ETV1*, and *SLC45A3-ERG* rearrangements account for roughly 90% of ETS fusion prostate cancer. *ELK4*, another ETS family member, is androgen regulated, involved in promoting cell growth, and highly expressed in a subset of prostate cancer, yet the mechanism of *ELK4* overexpression is unknown. In this study, we identified a novel ETS family fusion transcript, *SLC45A3-ELK4*, and found it to be expressed in both benign prostate tissue and prostate cancer. We found high levels of *SLC45A3-ELK4* mRNA restricted to a subset of prostate cancer samples. *SLC45A3-ELK4* transcript can be detected at high levels in urine samples from men at risk for prostate cancer. Characterization of the fusion mRNA revealed a major variant in which *SLC45A3* exon 1 is fused to *ELK4* exon 2. Based on quantitative PCR analyses of DNA, unlike other ETS fusions described in prostate cancer, the expression of *SLC45A3-ELK4* mRNA is not exclusive to cases harboring a chromosomal rearrangement. Treatment of LNCaP cancer cells with a synthetic androgen (R1881) revealed that *SLC45A3-ELK4*, and not endogenous *ELK4*, mRNA expression is androgen regulated. Altogether, our findings show that *SLC45A3-ELK4* mRNA expression is heterogeneous, highly induced in a subset of prostate cancers, androgen regulated, and most commonly occurs through a mechanism other than chromosomal rearrangement (e.g., trans-splicing). [Cancer Res 2009;69(7):2734–8]

Introduction

Emerging data suggest that erythroblast transformation specific (ETS)–rearranged prostate cancer, similar to other translocation tumors, represents a distinct subclass of prostate cancer based on studies demonstrating varying morphologic features (1), survival

(2, 3), and a specific expression profile (4, 5). Androgen-regulated genes account for the majority of the 5' genomic regulatory promoters elements fused with ETS genes in prostate cancer (6). The promoter of the androgen-regulated *transmembrane protease, serine 2* (*TMPRSS2*) gene is fused to the coding region of members of the ETS family of transcription factors, most commonly v-ets erythroblastosis virus E26 oncogene homologue (avian; *ERG*; ref. 7). *Solute carrier family 45, member 3* (*SLC45A3*), also referred to as prostein, is a prostate-specific, androgen-regulated gene that has been shown to be a 5' partner with *ETV1* and *ETV5* (6, 8) and more recently with the coding sequence of *ERG* (9).

Interestingly, *ELK4* (ETS-domain protein SRF accessory protein 1), a member of the ETS family of transcription factors, has recently been described as a novel androgen receptor target in LNCaP cells promoting cell growth and is highly expressed in a subset of prostate cancer samples compared with benign prostate tissues (10). Herein, we report the expression of novel *SLC45A3-ELK4* transcripts in prostate cancer. We provide data characterizing different *SLC45A3-ELK4* mRNA variants and evidence that this transcript does not primarily arise from a chromosomal rearrangement as seen for other ETS fusion events in prostate cancer.

Materials and Methods

Sample preparation. Tissue samples were collected as part of an institutional review board–approved protocol and RNA was extracted (see Supplementary Methods).

Conventional reverse transcription-PCR sequencing. Reverse transcription-PCR (RT-PCR) was performed using primers to *SLC45A3* exon 1 (5-CCGCGGAGTAACCTGGAGATTT-3) and *ELK4* exon 2 (5-TGCCCATCAT-TAGAGGTCCAACAG-3; see Supplementary Methods for details).

Quantitative RT-PCR using Taqman technology. We used TaqMan Gene Expression Assays (see Supplementary Methods for details).

Chromosome 1q32, *SLC45A3* to *ELK4* region assessment was performed using quantitative PCR (qPCR) and primers specific to 13 regions on chromosome 1 (see Supplementary Methods for details).

Functional studies were performed using R1881 on LNCaP cells (see Supplementary Methods for details).

Results

***SLC45A3-ELK4* mRNA is expressed in prostate cancer, benign prostate tissue, and the LNCaP cancer line.** We developed a Taqman assay targeting *SLC45A3* exon 1 and *ELK4* exon 2 (Fig. 1). We initially screened RNA from 31 prostate cancer samples, 6 benign prostate tissue samples, and 11 cell lines including malignant prostate (LNCaP, PC-3, 22Rv1, VCaP, NCI-H660, DU-145), nonprostate (ACHN, Caki-1, A-498, HK-2), and

Note: Supplementary data for this article are available at Cancer Research Online (<http://cancerres.aacrjournals.org/>).

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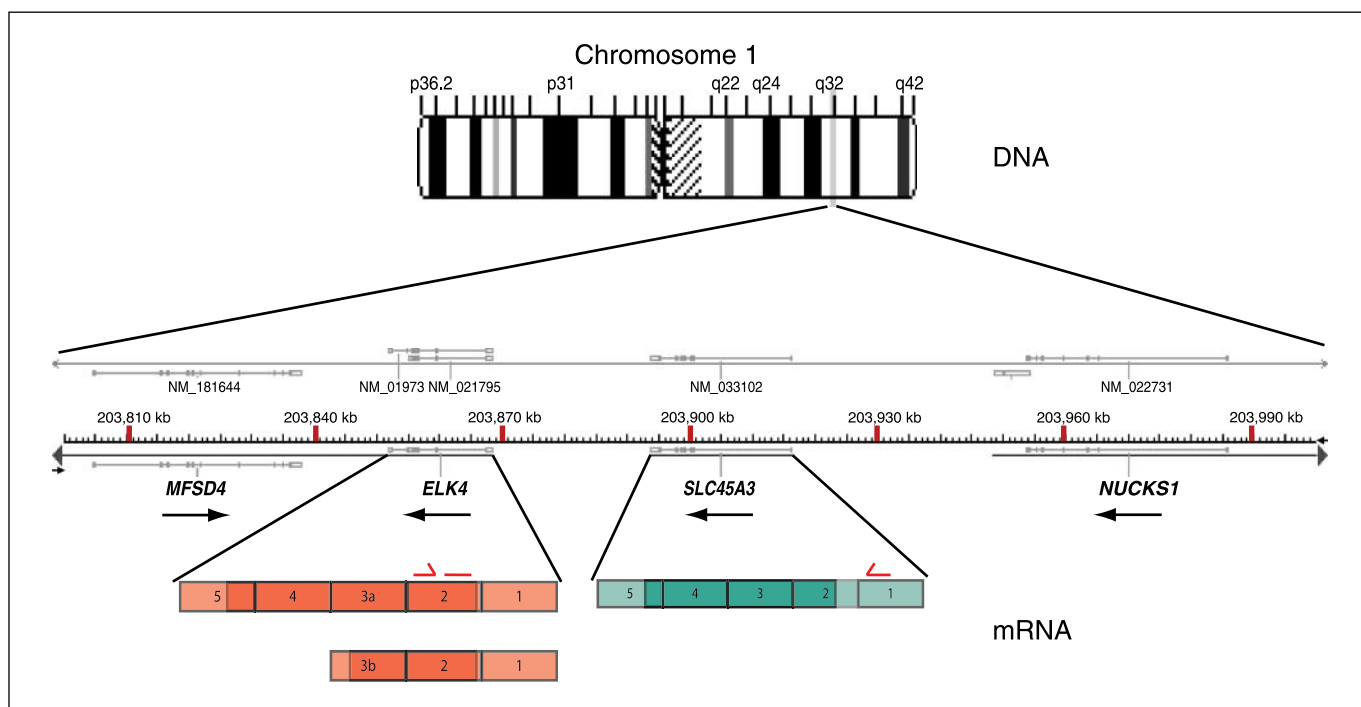


Figure 1. Schematic of chromosome 1q32.1 (Chr.1q32.1) demonstrating the orientation and relative distance of *SLC45A3* and *ELK4*. Red arrows and bar, the *SLC45A3-ELK4* Taqman assay primers and probe, respectively.

nonmalignant prostate (RWPE-1) epithelial cell lines. All samples yielded detectable albeit low *SLC45A3-ELK4* mRNA transcript expression levels (Fig. 2A). Three prostate cancer samples showed high *SLC45A3-ELK4* expression with levels >10-fold over the median level calculated from benign prostate tissue. Levels of endogenous *ELK4* mRNA varied widely in all prostate samples tested. Although we found a good overall correlation between endogenous *ELK4* mRNA and *SLC45A3-ELK4* mRNA levels ($r = 0.86$), several samples yielded significantly different expression values between the two transcripts. We also found relative increased levels of *SLC45A3-ELK4* in PC-3 and LNCaP cells and the human epithelial-like kidney adenocarcinoma cell line ACHN.

***SLC45A3-ELK4* mRNA variants in prostate cancer.** We performed conventional RT-PCR followed by cDNA sequencing to characterize the composition of the *SLC45A3-ELK4* transcripts of amplified products obtained from 35 prostate cancer samples, 6 benign samples, 6 prostate cancer cell lines, and 1 benign cell line (Supplementary Fig. S1). Given the lower sensitivity of this approach, only the majority of the samples yielded a major product that consisted of *SLC45A3* exon 1 fused to *ELK4* exon 2 (Fig. 2B; see Supplementary Information for junction sequence). Three less common products were detected consisting of a portion of *SLC45A3* exon 2 fused to *ELK4* exon 2. Interestingly, we found one amplified product that consisted of 84 bp of intergenic sequence separating *SLC45A3* exons 1 and 2 to *ELK4* exon 2. Using the unbiased approach, 5' RNA ligase-mediated rapid amplification of cDNA ends (RACE), we confirmed another *SLC45A3-ELK4* mRNA variant consisting of *SLC45A3* exons 1-3 fused to the same 84-bp sequence described above followed by *ELK4* exon 2 in sample 1701_A.

***SLC45A3-ELK4* mRNA can be detected using a noninvasive assay.** We screened 14 prebiopsy, postdigital exam urine specimens from men who were at risk of having prostate cancer using our *SLC45A3-ELK4* Taqman assay. According to pathology reports of

the biopsied prostate tissue, 8 of the 14 specimens were diagnosed with prostate cancer (Fig. 2C). Detectable levels of *SLC45A3-ELK4* transcript were measured in six of eight corresponding urine specimens and two of the six specimens from men whose biopsies did not reveal prostate cancer (sensitivity of 75% and a specificity of 67%). Interestingly, as seen in the prostate tissue, high levels (>10-fold) were detected in only a few of the prostate cancer-associated samples.

Chromosome rearrangement does not account for *SLC45A3-ELK4* expression. The development of a standard fluorescence *in situ* hybridization (FISH) break-apart assay requires using bacterial artificial chromosomes that usually span 100 to 150 kb. The distance from *SLC45A3* to *ELK4* is 25 kb and thus was not suitable for detecting a possible deletion between these genes (Supplementary Fig. S2). To explore for genomic loss within the region separating *SLC45A3* and *ELK4*, we analyzed 13 loci on chromosome covering this region (Fig. 3). The resulting amplicon raw data was normalized to a region on chromosome 1 (within *ARHGEF*) that is not altered from HapMap SNP data (11). Deletion or partial deletion of this region was observed in several samples with both high (420_D and 1024_D) and low (38_T, 436_D and 25_T) *SLC45A3-ELK4* transcript levels. The majority of samples were assessed as copy number neutral or showed genomic gain in this region. This included one sample (427_A) with high levels of *SLC45A3-ELK4* mRNA but copy number neutral and one sample (1701_A) that had low *SLC45A3-ELK4* mRNA and high DNA amplification in this region. Taken together, we did not observe a consistent loss of genomic DNA in cases with *SLC45A3-ELK4* expression.

***SLC45A3-ELK4* is androgen regulated.** To address the confounding results from Makkonen and colleagues (10), who recently reported that *ELK4* is a novel androgen receptor target in LNCaP cells, we repeated their experiment using our assay for the *SLC45A3-ELK4* transcript in addition to an assay for *ELK4* that

does not target the fusion transcript. As anticipated, 12 hours following treatment with a synthetic androgen (R1881, 1 nmol/L), we observed a 25-fold induction of SLC45A3-ELK4 but no change in ELK4 (Fig. 4). This induction was abrogated in the presence of the androgen antagonist Flutamide. As a control, we also measured the levels of KLK3 (PSA) mRNA and observed a similar profile.

Discussion

This is the first description of novel multiple ETS family fusion (SLC45A3-ELK4) transcripts, high levels of which are restricted to a subset of prostate cancer samples. Characterization of the fusion mRNA revealed a major variant in which SLC45A3 exon 1 is fused to ELK4 exon 2. Other minor variants include other downstream exons of both genes and more interestingly, an 84-bp chromosome sequence separating the two genes. Chromosome 1q32.1 has been

cited as a region that is involved in chromosome loss in prostate cancer (12). Interestingly, exon 1 of SLC45A3 is located roughly 50 kb telomeric on 1q32.1 from ELK4 exon 2 and is transcribed in the same direction. We observed a chromosome deletion of the interstitial region separating TMPRSS2 and ERG in 60% of TMPRSS2-ERG fusion prostate cancers (13). However, our data suggest that this is a less common event and that the expression of SLC45A3-ELK4 fusion transcript may more commonly occur through another mechanism. This is consistent with a recent report analyzing ETS genes and known 5' fusion partners using FISH (9).

Chimeric mRNA resulting from trans-splicing has been observed between pre-mRNAs from the same gene (homotypic trans-splicing; ref. 14) and pre-mRNAs from different genes (intergenic trans-splicing; ref. 15) as well as reported from computational analyses (16). We have also noted numerous trans-splicing events

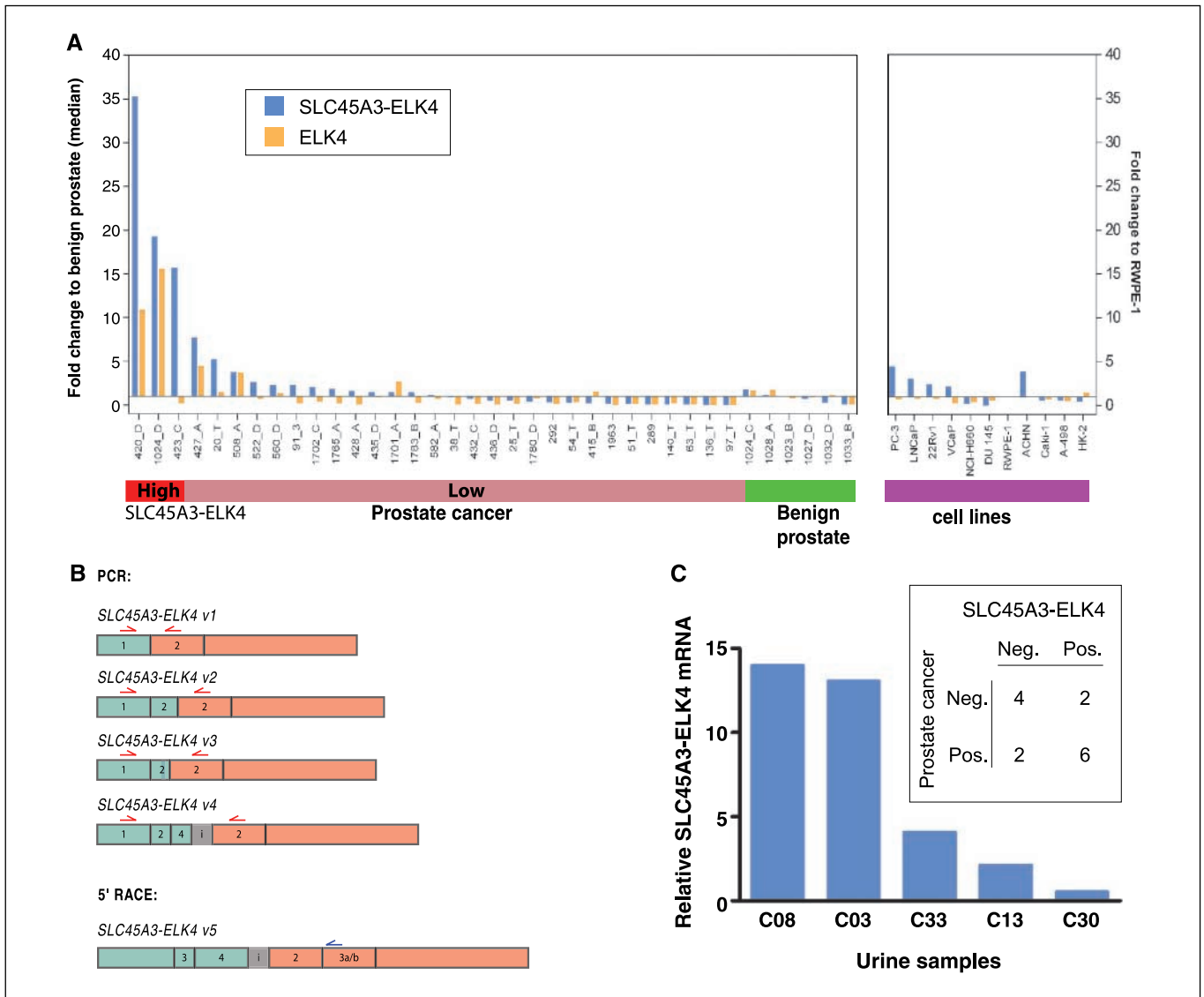


Figure 2. A, Taqman expression data of SLC45A3-ELK4 and ELK4 mRNA levels in 31 prostate cancer samples (red bar) relative to the median of values obtained from the 6 benign samples (green bar) in which cases yielding higher than 10-fold relative SLC45A3-ELK4 mRNA levels are indicated in dark red; and 10 cell lines (nine cancer and one benign, HK-2) relative to RWPE-1 (purple). B, schematic of the sequencing results obtained from PCR (primers are indicated in red) and 5' RACE (primer is indicated in blue) that correspond to the different SLC45A3-ELK4 mRNA variants (v; see Supplementary Information for junction sequence). C, Taqman assay results from RNA extracted from five samples (C08, C03, C33, and C30 corresponding to cancer-positive biopsies and C13 corresponding to cancer negative biopsy). Inset, contingency table of the 14 samples that yielded adequate TCFL1 values.

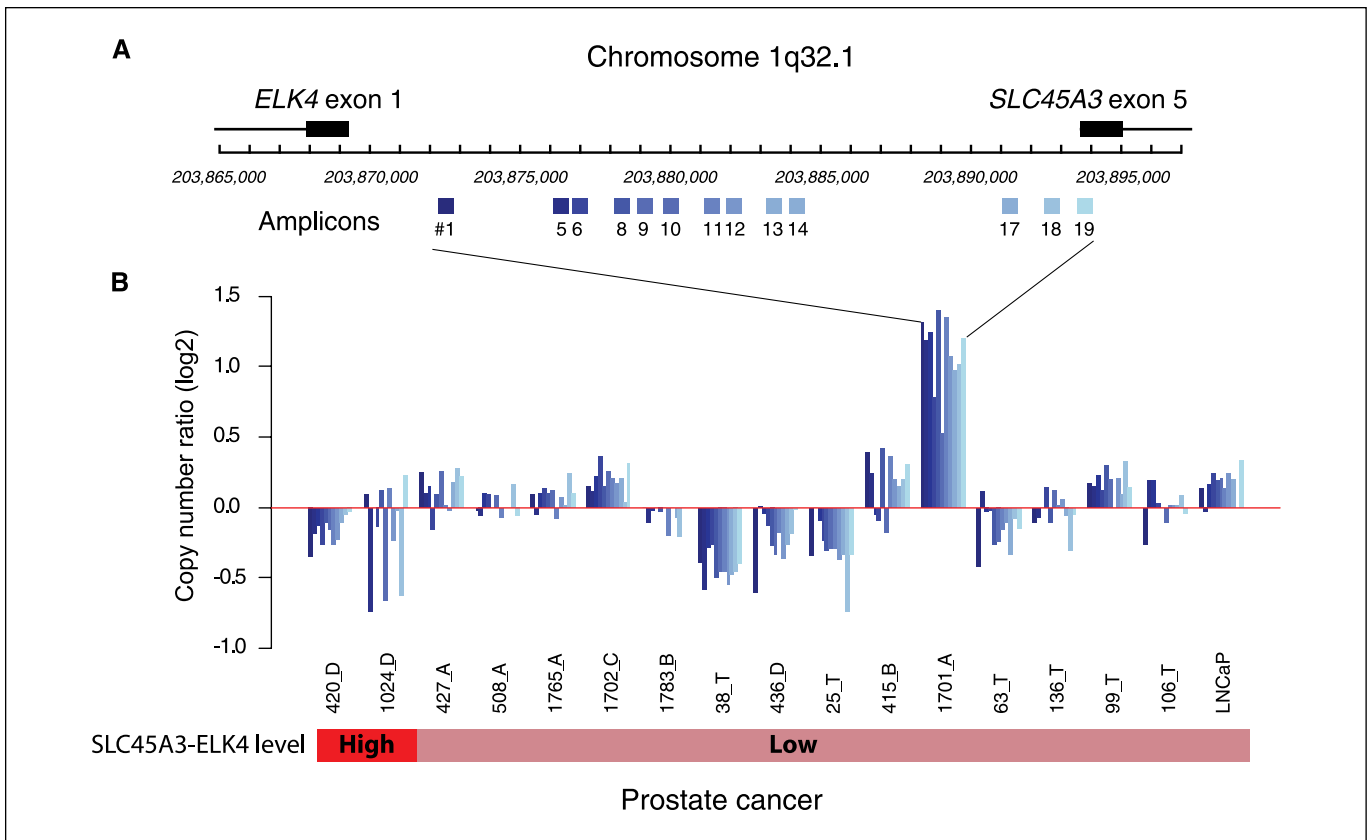


Figure 3. A, schematic of the region Chr.1q32 demonstrating the position of the primer pairs (blue boxes). *SLC45A3* exon 5 and *ELK4* exon 1 positions are indicated. B, qPCR results obtained for 16 prostate cancer samples (ordered from left to right as a function of *SLC45A3-ELK4* mRNA levels) and from LNCaP cells. Colored bars, samples with over 10-fold higher (red) or benign-like (light red) *SLC45A3-ELK4* mRNA levels. All qPCR experiments were run in triplicate. Bars, the average calibrated values.

in RNA-sequencing data generated thus far on prostate cancer samples.⁸

In prostate cancer cases with known *TMPRSS2-ERG* or *SLC45A3-ERG* fusions, we do not see a mutually exclusive expression of *SLC45A3-ELK4* as observed with the other prostate cancer fusions (7). Interestingly, the three samples that yielded high *SLC45A3-*

ELK4 transcript levels were negative for *ERG* rearrangement by FISH.

Makkonen and colleagues reported an induction of *ELK4* mRNA variants upon androgen stimulation, which was most pronounced in metastatic, hormone-refractory prostate cancer. Our current study confirms that *ELK4* is overexpressed in prostate cancer but only in a subset of tumors and correlated with high *SLC45A3-ELK4* mRNA. Only *SLC45A3-ELK4* mRNA, and not endogenous *ELK4* mRNA, is up-regulated upon treatment of LNCaP cells with R1881.

⁸ M.A. Rubin, unpublished observations.

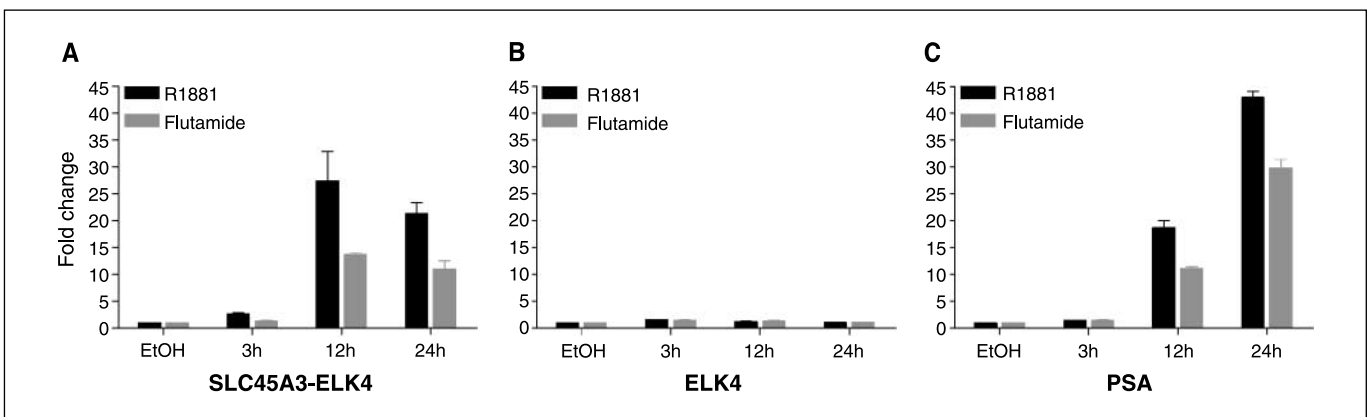


Figure 4. Median fold induction of *SLC45A3-ELK4* (A), *ELK4* (B), and *KLK3* (PSA, C) mRNA in LNCaP cells treated with 1 nmol/L R1881 in the absence or presence of 10 μ mol/L flutamide at the indicated time points. All experiments were run in triplicate; error bars, SE.

This finding suggests that Makkonen and colleagues were measuring SLC45A3-ELK4 expression and not the wild-type *ELK4*. This has direct implications to the putative oncogenic properties of ELK4. Recent work from our group identified an estrogen-mediated activation of *TMPRSS2-ERG* transcription (4). Non-androgen-regulated mechanisms for *SLC45A3* will also need to be explored in future work. Based on our data, we found increased levels of SLC45A3-ELK4 in the human epithelial-like kidney adenocarcinoma cell line ACHN. This suggests that this transcript is not prostate specific. Large-scale screening of multiple cancer and noncancer tissue types is needed to determine the specificity and the extent of SLC45A3-ELK4 expression.

Finally, the SLC45A3-ELK4 fusion transcript may also have potential clinical applications. Similar to *TMPRSS2-ERG* and *PCA3* (17, 18), SLC45A3-ELK4 is detected at higher levels in prostate cancer than in benign prostate tissue. We showed a proof-of-principle that the SLC45A3-ELK4 transcript can be detected at high levels on urine samples from patients with prostate cancer and therefore might be a useful biomarker. Clearly, larger cohorts of urine samples will need to be analyzed to be able to determine if detection of this transcript will provide added diagnostic utility.

In summary, we describe for the first time novel fusion events between the androgen-regulated 5' prime promoter *SLC45A3* and the ETS gene *ELK4*. We were unable to confirm if some of these

fusions are due to genomic rearrangement or if trans-splicing explains this phenomenon. A combination of the two mechanisms is also possible. The SLC45A3-ELK4 transcripts can be detected in a range of prostate cancers and benign prostate tissues; however, due to higher levels in cancer, we also propose SLC45A3-ELK4 transcripts as a putative prostate cancer biomarker. The biological implications of this fusion transcript are yet to be determined.

Addendum

While the Article was in review, another article reported the presence of the SLC45A3-ELK4 transcripts in prostate cancer tumor cell lines and metastatic samples (19).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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References

- Mosquera JM, Perner S, Demichelis F, et al. Morphological features of *TMPRSS2-ERG* gene fusion prostate cancer. *J Pathol* 2007;212:91-101.
- Attard G, Clark J, Ambrosine L, et al. Duplication of the fusion of *TMPRSS2* to *ERG* sequences identifies fatal human prostate cancer. *Oncogene* 2008;27:253-63.
- Demichelis F, Fall K, Perner S, et al. *TMPRSS2:ERG* gene fusion associated with lethal prostate cancer in a watchful waiting cohort. *Oncogene* 2007;26:4596-9.
- Setlur SR, Mertz KD, Hoshida Y, et al. Estrogen-dependent signaling in a molecularly distinct subclass of aggressive prostate cancer. *J Natl Cancer Inst* 2008;100:815-25.
- Tomlins SA, Rhodes DR, Yu J, et al. The role of *SPINK1* in ETS rearrangement-negative prostate cancers. *Cancer Cell* 2008;13:519-28.
- Tomlins SA, Laxman B, Dhanasekaran SM, et al. Distinct classes of chromosomal rearrangements create oncogenic ETS gene fusions in prostate cancer. *Nature* 2007;448:595-9.
- Tomlins SA, Rhodes DR, Perner S, et al. Recurrent fusion of *TMPRSS2* and ETS transcription factor genes in prostate cancer. *Science* 2005;310:644-8.
- Helgeson BE, Tomlins SA, Shah N, et al. Characterization of *TMPRSS2:ETV5* and *SLC45A3:ETV5* gene fusions in prostate cancer. *Cancer Res* 2008;68:73-80.
- Han B, Mehra R, Dhanasekaran SM, et al. A fluorescence *in situ* hybridization screen for E26 transformation-specific aberrations: identification of *DDX5-4* fusion protein in prostate cancer. *Cancer Res* 2008;68:7629-37.
- Makkonen H, Jaaskelainen T, Pitkanen-Arsiola T, et al. Identification of ETS-like transcription factor 4 as a novel androgen receptor target in prostate cancer cells. *Oncogene* 2008;27:4865-76.
- McCarroll SA, Kuruvilla FG, Korn JM, et al. Integrated detection and population-genetic analysis of SNPs and copy number variation. *Nat Genet* 2008;40:1166-74.
- Wolf M, Mousset S, Hautaniemi S, et al. High-resolution analysis of gene copy number alterations in human prostate cancer using CGH on cDNA microarrays: impact of copy number on gene expression. *Neoplasia* 2004;6:240-7.
- Perner S, Demichelis F, Beroukhi R, et al. *TMPRSS2:ERG* fusion-associated deletions provide insight into the heterogeneity of prostate cancer. *Cancer Res* 2006;66:8337-41.
- Takahara T, Tasic B, Maniatis T, Akanuma H, Yanagisawa S. Delay in synthesis of the 3' splice site promotes trans-splicing of the preceding 5' splice site. *Mol Cell* 2005;18:245-51.
- Pradet-Balade B, Medema JP, Lopez-Fraga M, et al. An endogenous hybrid mRNA encodes TWE-PRIL, a functional cell surface TWEAK-APRIL fusion protein. *EMBO J* 2002;21:5711-20.
- Akiva P, Toporik A, Edelheit S, et al. Transcription-mediated gene fusion in the human genome. *Genome Res* 2006;16:30-6.
- Hessels D, Smit FP, Verhaegh GW, Witjes JA, Cornel EB, Schalken JA. Detection of *TMPRSS2-ERG* fusion transcripts and prostate cancer antigen 3 in urinary sediments may improve diagnosis of prostate cancer. *Clin Cancer Res* 2007;13:5103-8.
- Laxman B, Morris DS, Yu J, et al. A first-generation multiplex biomarker analysis of urine for the early detection of prostate cancer. *Cancer Res* 2008;68:645-9.
- Maher CA, Kumar-Sinha C, Cao X, et al. Transcriptome sequencing to detect gene fusions in cancer. *Nature Epub* 2009 Jan 11.