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SLC45A3-ELK4 is a Novel and Frequent ETS 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* over-expression 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 harbouring a chromosomal rearrangement. Treatment of LNCaP cancer cells with a synthetic androgen

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While this manuscript was in review, a paper reported the presence of the SLC45A3-ELK4 transcripts in prostate cancer tumor cell lines and metastatic samples (Maher CA, et al. Nature 2009).

(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).

Keywords

prostate cancer; ETS genes; splicing; SLC45A3; ELK4

INTRODUCTION

Emerging data suggests that 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 homolog (avian) (*ERG*) (7). *SLC45A3 (solute carrier family 45, member 3),* 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 in comparison to 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 supplemental methods).

Conventional RT-PCR Sequencing

RT-PCR was performed using primers to *SLC45A3* exon 1 (5-CCGCGGAGTAACCTGGAGATTT-3) and *ELK4* exon 2 (5-TGCCCATCATTAGAGGTCCAACAG-3, see Supplemental methods for details).

Quantitative RT-PCR using Taqman technology

We used TaqMan Gene Expression Assays (See Supplemental methods for details).

Chromosome 1q32, SLC45A3 to ELK4 region assessment was performed using qPCR and primers specific 13 regions on chrom.1 (See Supplemental methods for details).

Functional studies were performed using R1881 on LNCaP cells (See Supplemental 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 (Figure 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), non-prostate (ACHN, Caki-1, A-498, HK-2) and non-malignant prostate (RWPE-1) epithelial cell lines. All samples yielded detectable, albeit low SLC45A3-ELK4 mRNA transcript expression levels (Figure 2a). Three prostate cancer samples demonstrated high SCL45A3-ELK4 expression with levels greater than 10-fold over the median level calculated from benign prostate tissue. Levels of endogenous *ELK4* mRNA varied widely in all prostate samples tested. While 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 2 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 RTPCR 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 (Supplemental Figure 1). 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 (Figure 2b, see Supplemental 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 base pairs of intergenic sequence separating SLC45A3 exons 1, 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 non-invasive assay

We screened 14 pre-biopsy, post-digital exam urine specimens from men who were at risk for having prostate cancer using our SLC45A3-ELK4 Taqman assay. According to pathology reports of the biopsied prostate tissue, 8 out of the 14 were diagnosed with

prostate cancer (Figure 2c). Detectable levels of *SLC45A3-ELK4* transcript were measured in 6 out of 8 corresponding urine specimens and 2 out of the 6 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 FISH break-apart assay requires using BACs which usually span 100–150 kb. The distance from *SLC45A3* to *ELK4* is 25 kb and thus was not suitable for detecting a possible deletion between these genes (Supplemental Figure 2). To explore for genomic loss within the region separating *SLC45A3* and *ELK4*, we analyzed 13 loci on chromosome covering this region (Figure 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 demonstrated genomic gain in this region. This included 1 sample (427_A) with high levels of SLC45A3-ELK4 mRNA but copy number neutral and 1 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-ELK expression.

SLC45A3-ELK4 is androgen-regulated

To address the confounding results from Makkonnen et al (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, twelve hours following treatment with a synthetic androgen (R1881, 1nM), we observed a 25-fold induction of SLC45A3-ELK4 but no change in ELK4 (Figure 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 suggests 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 premRNAs from the same gene (homotypic trans-splicing) (14) and pre-mRNAs from different genes (intergenic trans-splicing) (15) as well as reported from computational analyses (16). We have also noted numerous trans-splicing events in RNA-sequencing data generated thus far on prostate cancer samples (MA Rubin, unpublished observations).

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 3 samples that yielded high SLC45A3-ELK4 transcript levels were negative for *ERG* rearrangement by FISH.

Makkonen et al. 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 over-expressed 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. This finding suggests that Makkonen et al. were measuring in 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 non-cancer 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 demonstrated a proof-of-principle that SLC45A3-ELK4 transcript can be detected at high levels urine samples from patients with prostate cancer and therefore might be a useful biomarker. Clearly, larger cohorts of urine samples will need to 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 but due to higher levels in cancer, we also propose SLC45A3-ELK4 transcripts as a putative prostate cancer biomarker. The biologic implications of this fusion transcript are yet to be determined.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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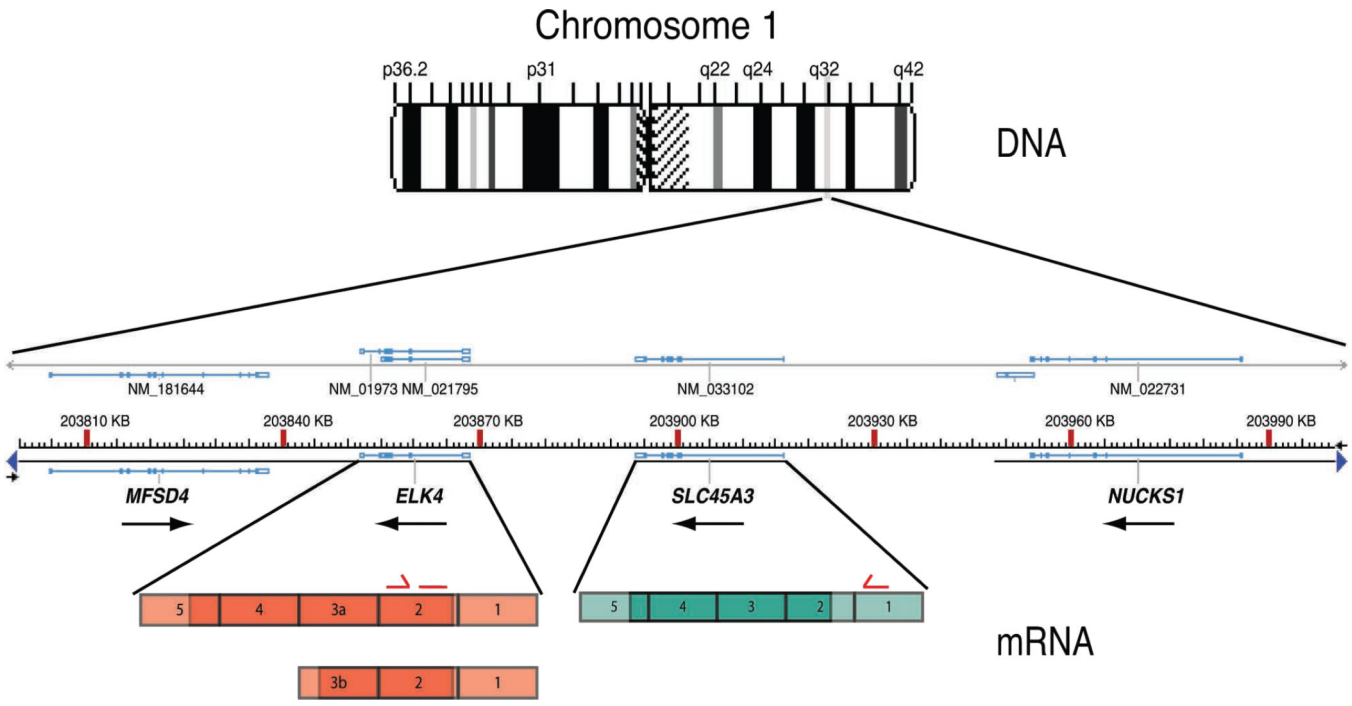

Figure 1.

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

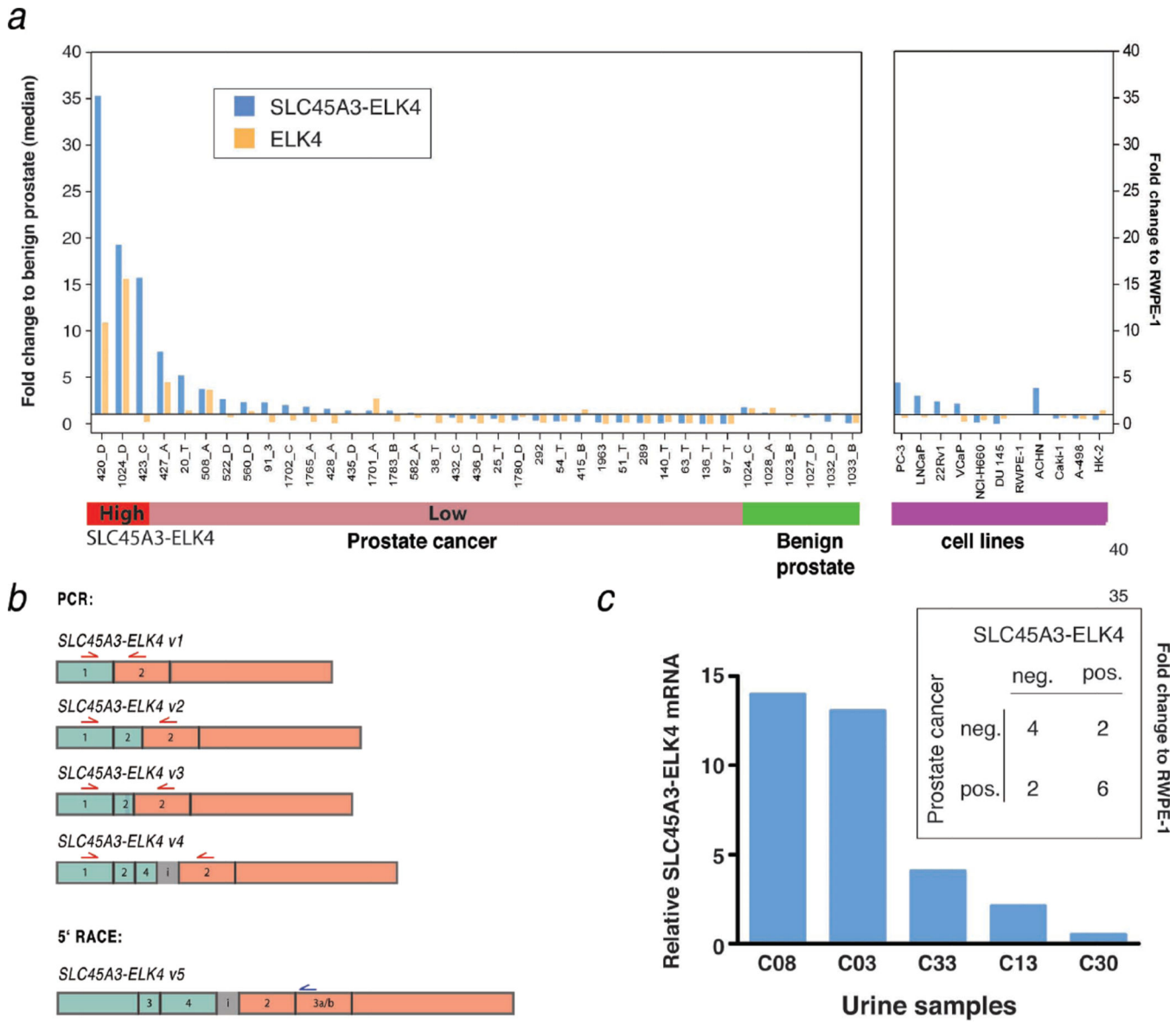

Figure 2.

Taqman expression data of SLC45A3-ELK4 and ELK4 mRNA levels in 31 prostate cancer samples (red bar) relative median of the 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 (9 cancer and 1 benign, HK-2) relative to RWPE-1 (purple) (**a**). Schematic of the sequencing results obtained from PCR (primers are indicated in red) and 5'RACE (primer indicate in blue) that correspond to the different SLC45A3-ELK4 mRNA variants (v, see Supplemental Information for junction sequence, **b**). Taqman assay results from RNA extracted from 5 samples (C08, C03, C33 and C30 corresponding to cancer positive biopsies and C13 corresponded to cancer negative biopsy (**c**)). Contingency table of the 14 samples that yielded adequate TCFL1 values (inset).

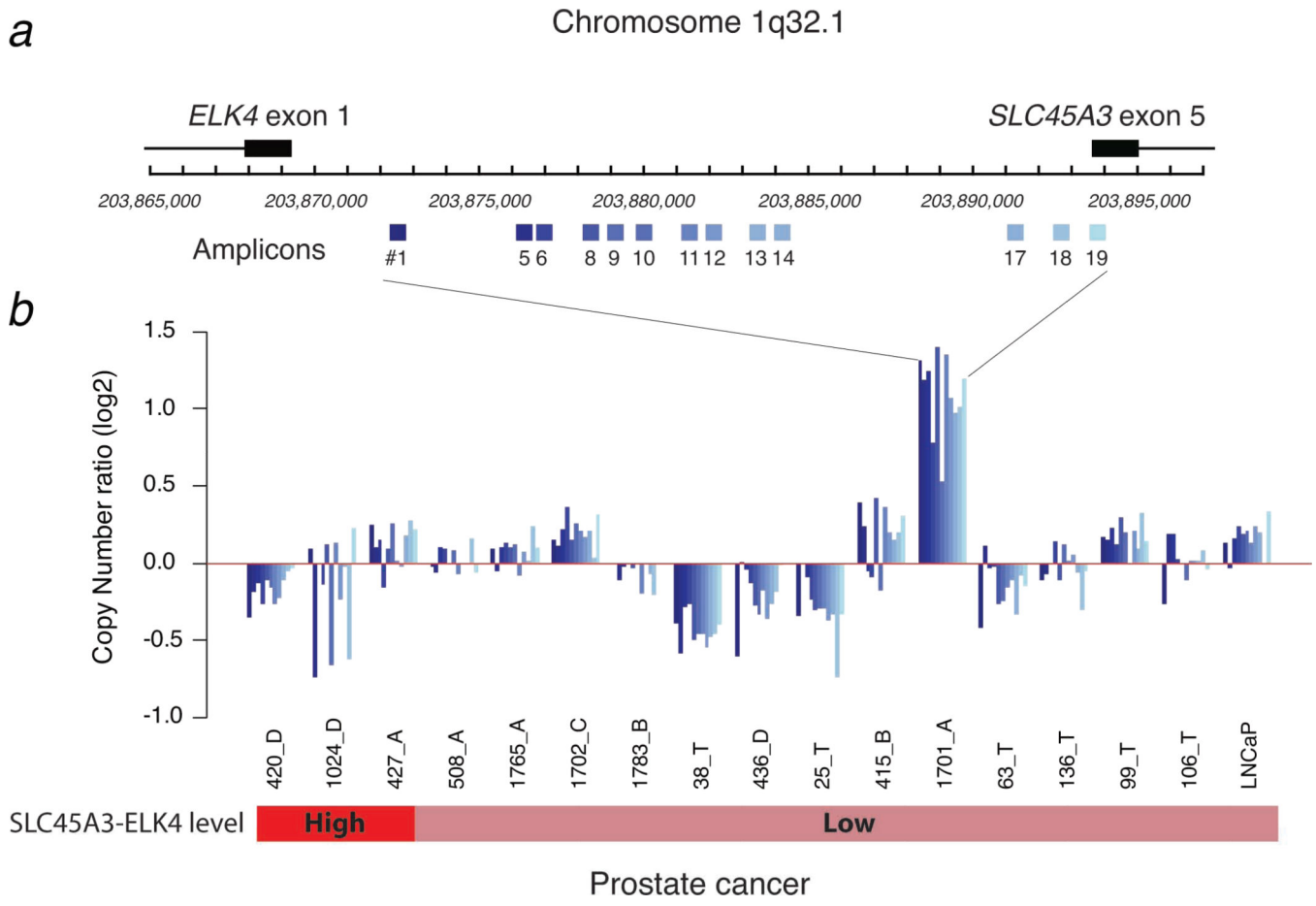

Figure 3.

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

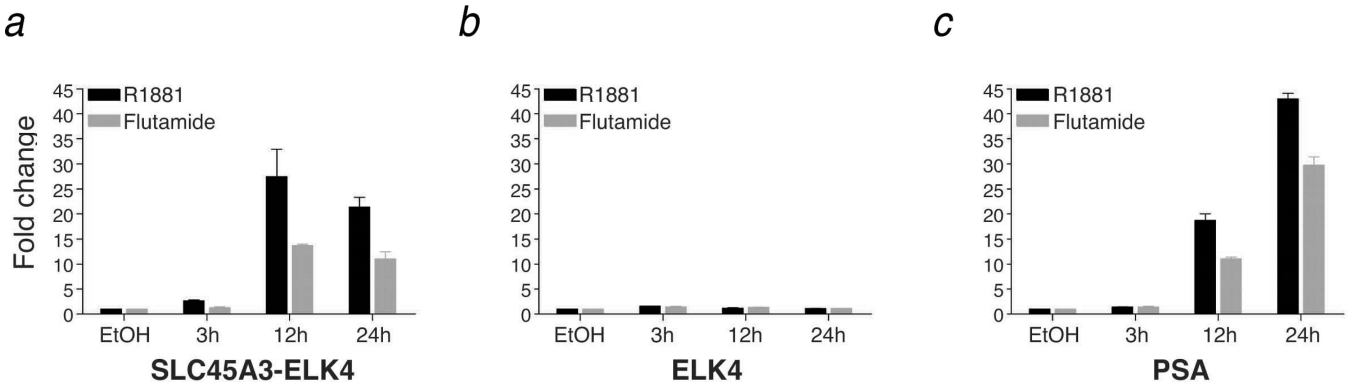

Figure 4.

Median fold induction of SLC45A3-ELK4 **(a),** ELK4 **(b)** and KLK3 (PSA, **c**) mRNA in LNCaP cells treated with 1nM R1881 in the absence or presence of 10 μM flutamide at the indicated time points. All experiments were run in triplicate (SEM indicated by the error bars).