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SLC45A3-ELK4 Chimera in Prostate Cancer: Spotlight on Cis-Splicing

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Summary

Using a series of detailed experiments, Zhang *et al* establish that the prostate cancer RNA chimera SLC45A3-ELK4 is generated by cis-splicing between the two adjacent genes and does not involve DNA rearrangements or trans-splicing. The chimera expression is induced by androgen treatment likely by overcoming the read-through block imposed by the intergenic CCCTC-insulators bound by CTCF repressor protein. The chimeric transcript, but not wild type ELK4, is shown to augment prostate cancer cell proliferation.

Gene fusions represent one of the most common genomic aberrations in cancers. Characterization of the oncogenic gene fusion BCR-ABL1 in chronic myeloid leukemia culminating in the successful treatment of the disease with a molecularly targeted therapy represents a compelling “bench to bedside” paradigm(1) that has inspired the discovery and characterization of similar oncogenic “driver” fusions in other hematological malignancies, soft tissue tumors, and rare epithelial carcinoma(2, 3). More recently, driven by the development of sensitive genomic techniques such as microarrays and high throughput sequencing in cytogenetically complex tissues of common solid cancers like prostate, lung, and breast etc., many recurrent gene fusions involving genes such as ALK, RET, BRAF, ERG, and NOTCH have been discovered that provide novel diagnostic, prognostic and therapeutic leads, that are targetable by available or experimental therapeutics and thus have implications for personalized medicine (4–7). This is driving an ongoing quest for the discovery of recurrent gene fusions across major types of cancers using the high throughput sequencing approaches.

Chromosomal rearrangements (including recombination, insertion, deletion, inversion, tandem duplication or amplification) are regarded as the principal mechanism of the formation of gene fusions that may involve genes on the same chromosome (intra-chromosomal fusions) or different chromosomes (inter-chromosomal fusions). Next generation sequencing of cancer samples has revealed that most individual tumors harbor

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multiple gene fusions, that include potential oncogenic ‘drivers’ as well as random by-products of genomic aberrations, generically described as ‘passenger’ fusions. While the discovery of gene fusions resulting from chromosomal aberrations forms the primary objective of chimera analyses, RNA-seq is unraveling yet another category of gene fusions that typically involve adjacent genes (in the same coding orientation) which express chimeric transcripts without involving concomitant chromosomal rearrangements. These RNA chimeras, variously described as ‘read-throughs’, TIC (transcription induced chimeras) or CoTIS (co-transcription of adjacent genes coupled with intergenic splicing), are widely observed in virtually all samples analyzed, including in benign samples across different tissue types. Some of these RNA chimeras however appear restricted to individual tissue types, and a few of these have been observed to be highly expressed in cancers, that may indicate potential functional relevance with respect to cellular differentiation and disease development (8–10). One such RNA chimera involves two adjacent genes, 60 Kb apart on Chr1q32.1, the androgen driven, prostate specific gene, solute carrier family 45, member 3 (SLC45A3) and the gene encoding ETS-family oncogenic transcription factor, ELK4, frequently observed at high levels in prostate cancer (9–11).

In this issue of Cancer Discovery, Zhang and colleagues (12) describe studies pertaining to a structural and functional characterization of SLC45A3-ELK4 chimera in prostate cancers. First, analyzing the likely mechanisms underlying the expression of the SLC45A3-ELK4 chimera, 3 scenarios - chromosomal rearrangement, trans-splicing and cis-splicing - were considered. While previous analyses of the chromosomal region between SLC45A3 and ELK4 have ruled out chromosomal deletion (10, 11), here using hybridization probes encompassing the two genes as well as the intergenic region, Southern blot analysis was carried out to rule out the absence of a potential balanced translocation in the fusion positive LNCaP cells, effectively negating the possibility of a DNA based aberration underlying this chimera. Next, to forestall the potential artifacts associated with PCR, such as false chimera generated by template switching, the authors provide a direct evidence of the endogenous chimeric transcript in the index cases using RNase protection assay. This was followed by several experiments to differentiate between the two distinct modes of chimera formation at the RNA level that is, cis- versus trans-splicing. An *in vitro* trans-splicing assay was carried out using nuclear extracts of chimera positive LNCaP cells and green monkey kidney cells CV-1 that harbor distinct polymorphisms in its ELK4 sequence. Real time-PCR was carried out using a common forward primer located on SLC45A3 and two distinct human and monkey ELK4 specific reverse primers. Absence of any trans-spliced RNA product composed of human SLC45A3 and monkey ELK4 led to the conclusion that trans-splicing is not involved. As a direct evidence of cis-splicing, the RNA corresponding to the intergenic region was detected by RT-PCR.

Next, the authors examined role of CTCF binding to the recently described two ‘insulator’ sequences present in the intergenic region between SLC45A3 and ELK4(12), in controlling the expression of the chimera (Fig. 1A). Insulator sequences typically lie within intergenic regions and prevent runaway transcription across neighboring genes, through association with CCCTC-binding factor (CTCF) (13). The role of CTCF has been described in the regulation of alternative splicing (14), as well as through association of DNA methylation at CTCF binding sites, in the generation of alternative splice variants (15). Here, the authors show that CTCF binds to the two insulators and this binding is attenuated upon androgen treatment. SLC45A3 being strongly induced by androgen, it likely forces a run off transcription overcoming CTCF-insulator block. This provides the mechanism for selective expression of SLC45A3-ELK4 in prostate cancer. Further, CTCF knockdown induced SLC45A3-ELK4 expression whereas it abrogated the expression of the trans-spliced chimeric transcript JAZF1-JJAZ1 (16), that further rules out the possibility of trans-splicing and provides additional support for a cis-splicing mechanism.

Analyzing potential phenotypic effects of the fusion transcript overexpression, knockdown of SLC45A3-ELK4 but intriguingly, not by wild type ELK4 (or SLC45A3), resulted in reduced proliferation. Probing this further, cell cycle analysis of LNCaP cells transfected with fusion specific siRNA (again, not with ELK4 specific siRNA) displayed G1 arrest, even as no cell death was observed. The G1 arrest was attributed to upregulation of cyclin dependent kinase inhibitor *CDKN1A* (*p21*) in LNCaP cells transfected with siRNA against *SLC45A3-ELK4*, but not with siELK4. Microarray analysis also identified upregulation of cell cycle inhibitor *p21* and *S100A10* etc., in LNCaP cells transfected with si-SLC45A3-ELK4 but not by siELK4. Surprisingly, authors found no effect on cell invasion by SLC45A3-ELK4, unlike previous reports on other ETS family oncogenes like ERG and ETV1, where an effect on invasion but not proliferation was observed (17, 18). The study also reports the expression of SLC45A3-ELK4 in androgen independent prostate cancer cell line PC3 as well as non-prostate cancer types and even benign cells, as has been observed previously (9), suggesting that SLC45A3-ELK4 expression may also involve AR independent pathways.

Analyzing clinical correlates of SLC45A3-ELK4 expression, a significant association was found with prostate cancers with Gleason score 7 or more. Notably, prostate cancer metastases were found to show the highest levels of chimera expression. If validated in larger sample cohorts, this chimeric RNA could potentially be an attractive diagnostic/prognostic marker.

With increasing depth of coverage in transcriptome sequencing, chimeric RNA sequences are routinely observed in all samples, presumably driven by strong expression of the 5′ genes. The tissue specific expression of SLC45A3-ELK4 is certainly attributable to androgen driven, prostate specific expression of SLC45A3. Follow up studies will need to identify chimera derived protein(s) and characterize its difference(s) from wild type protein as, two distinct fusion isoforms have been described (9, 10) (Fig. 1B), SLC45A3_exon1-ELK4_exon2 encoding full length open reading frame for ELK4 protein and SLC45A3_exon4-ELK4_exon2 that encodes a putative chimeric protein with amino acid stretches corresponding to both SLC45A3 and ELK4. The latter product could potentially account for the chimera specific increase in cell proliferation in prostate cancer cells reported in the present study.

A majority of prostate cancers are characterized by the presence of recurrent gene fusions primarily involving androgen regulated upstream genes, TMPRSS2 and SLC45A3, fused to one of the genes of the ETS family of oncogenic transcription factors (19) (Fig. 1C). SLC45A3-ELK4 represents a novel class of fusions in this group generated through cis-splicing and adds to the repertoire of prostate cancer specific chimera that may serve as disease specific biomarkers.

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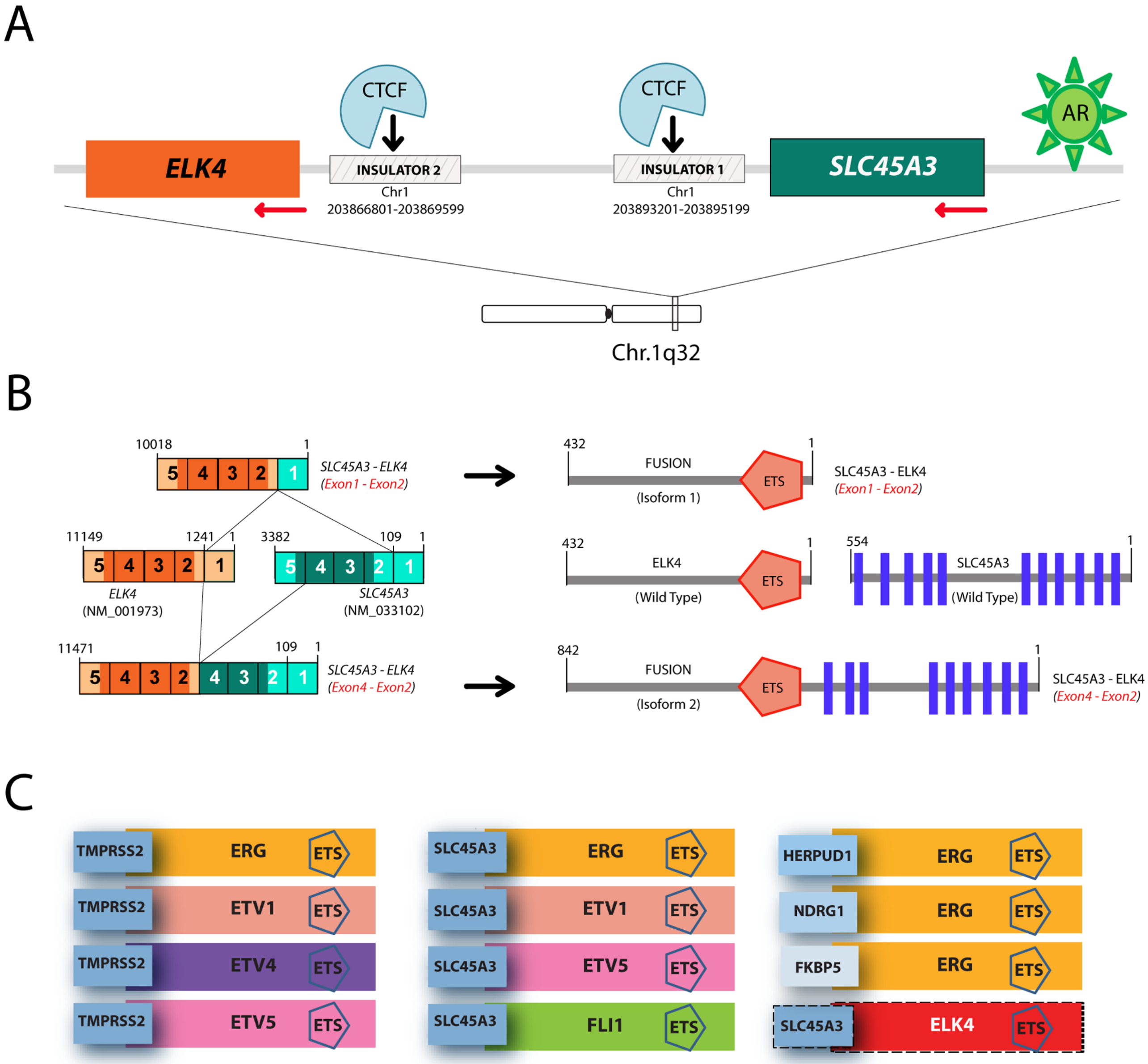

Figure 1.

A. Schematic representation of the genomic coordinates of SLC45A3 and ELK4 on chr1q32 (reverse strand) and the location of the two intergenic insulator sequences. Androgen Receptor driven transcription initiated at SLC45A3 promoter, overrides the repressive effect of CTCF bound insulator sequences. **B.** Architecture of potential fusion isoforms generated by cis-splicing between SLC45A3 (green) and ELK4 (orange), as revealed by transcriptome sequencing. The lighter colors indicate 5′ and 3′ un-translated regions (Left Panel). Predicted protein products resulting from the chimeric isoforms are shown (Right Panel). The SLC45A3_exon1-ELK4_exon2 (Left Panel, Top) putatively encodes full length ELK4 (Right Panel, Top), and SLC45A3_exon4-ELK4_exon2 (Left Panel, Bottom) encodes a fusion protein as indicated (Right Panel, Bottom). **C.** Schematic representation of known ETS family gene fusions driven by androgen responsive promoters in prostate cancer. As indicated, TMPRSS2 and SLC45A3 are the most frequent 5′ partners. SLC45A3-ELK4 fusion represents a unique ‘RNA only’ chimera (not represented by a DNA based aberration) indicated by the hatched box.