



AUTHOR'S VIEWS



The spliceosome as a new therapeutic vulnerability in aggressive prostate cancer

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ABSTRACT

Alternative splicing (AS) analysis across the entire spectrum of human prostate cancer evolution reveals the unexpected findings that intron retention is a hallmark of stemness and tumor aggressiveness, and androgen receptor controls a splicing program distinct from its transcriptional regulation. Importantly, twisted activity of the spliceosome causing abnormal AS landscape represents a therapeutic vulnerability in aggressive prostate cancer.

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Prostate cancer (PCa) is a heterogeneous malignancy and still causes a significant mortality worldwide.^{1,2} Androgen deprivation therapy (ADT) is the main therapeutic regimen for advanced PCa patients. However, most treated patients invariably develop the castration-resistant PCa (CRPC), currently a lethal form of the disease. Within the CRPC category, although the majority of them histologically present as adenocarcinomas (Ad) (i.e., CRPC-Ad), but a significant fraction (up to 25%) of them evolve to an aggressive, androgen receptor negative (AR⁻) indifferent CRPC with neuroendocrine (NE) features called CRPC-NE.³ Generally, CRPC is less dependent on androgen/AR signaling and relatively stem-like. Mechanisms underlying CRPC development and maintenance remain poorly understood.

Splicing dysregulation is one of the molecular hallmarks of cancer.¹ Recent pan-cancer studies have revealed that mis-splicing is prevalent in human cancers and many specific alternative splicing (AS) events (Figure 1a) contribute to tumor development in a context-dependent manner.^{4,5} In particular, studies on AR and AR variants have implicated splicing dysregulation in PCa resistance to ADT.¹ Notably, although the global AS landscape of PCa has been recently reported by using The Cancer Genome Atlas (TCGA) data,⁴ these studies mainly focused on primary PCa (pri-PCa; versus normal tissues) and generally overlooked CRPC. The potential functions and clinical relevance of global splicing abnormality in PCa remain unclear. In order to understand the molecular basis underlying splicing misregulation and thus, to develop potential therapeutic strategies against 'lethal' CRPC, we characterized the first comprehensive AS landscape in the spectrum of human PCa evolution using RNA-sequencing datasets containing collectively >570 clinical PCa samples.⁶

By using two AS mapping algorithms, we revealed that the severity of global splicing dysregulation correlates with disease progression (Figure 1b).⁶ Biologically, the mis-splicing impacts genes enriched in cancer-related pathways in a tumor-stage dependent manner. For instance, gene ontology (GO) terms linked to 'muscle and ion transport', 'lipid metabolism', and

'cell polarity' were pri-PCa specific whereas GO terms 'DNA damage', 'immunity', and 'nuclear pore' were enriched in CRPC, consistent with recent reports.^{3,7} Interestingly, and as expected, terms 'stem cell (SC) and development' and 'neuron and cell projection' were greatly enriched in CRPC-NE, in line with its stem-like and neural-like properties. Functionally, aberrant splicing regulates PCa biology, at least partially, by switching isoform expression of key cancer-related genes. For example, multiple delta like non-canonical Notch ligand 1 (*DLK1*) splice variants (ENST00000341267.8, ENST00000331224.10, ENST00000556051.1) were found up-regulated in CRPC-NE (vs. CRPC-Ad), and these isoforms expressed highly in *DLK1*⁺ cells, an SC population in human prostate,⁸ suggesting a functional link of *DLK* isoforms with stemness. Experimentally, knocking down clinically relevant isoforms of synaptotagmin 7 (*SYT7*) in two PCa lines inhibited cell proliferation and stemness,⁶ suggesting a causal role of mis-splicing in PCa pathogenesis. Interestingly, we found that the majority of AS events minimally changed the bulk gene expression, indicative of splicing dysregulation as an independent driver (vs. gene expression regulation) in oncogenesis.

RNA splicing can be dysregulated in cancer in many ways. In hematological cancer, recurrent point mutations in core spliceosome genes (e.g., splicing factor 3b subunit 1 (*SF3B1*), U2 small nuclear RNA auxiliary factor 1 (*U2AF1*)) have recognized as drivers for mis-splicing.⁵ Our genomic interrogation of 274 splicing-regulatory genes (SRGs) revealed copy number variants (CNV) as the main driver of AS alterations in PCa, which alter the expression of affected SRGs and illustrate cancer type-specific differences in mechanisms of splicing dysregulation. Regardless of mutational status, ~68% of SRGs were mis-expressed in various PCa stages. Many top altered SRGs are often co-mutated with either oncogenes (e.g., *MYC* proto-oncogene (*MYC*)) or tumor suppressors (e.g., RB transcriptional corepressor 1 (*RBI*)), warranting further dissection of their roles in *MYC*- or loss-of-*RBI*-driven tumorigenesis. Alternatively, mutations in splice sites may also cause AS abnormality.⁹ However, such mutations constitute the

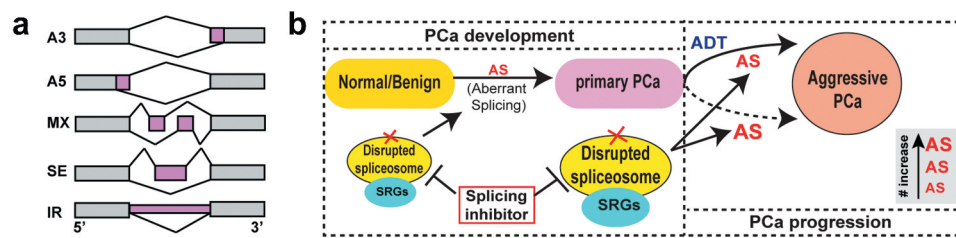


Figure 1. The spliceosome represents a therapeutic vulnerability in aggressive prostate cancer (PCa). (a) Five main types of alternative splicing (AS) patterns analyzed in our study. A3, alternative 3' splice sites; A5, alternative 5' splice sites; MX, mutually exclusive exons; SE, exon skipping; IR, intron retention. (b) The spliceosome machinery is disrupted due to mis-expression of splicing-regulatory genes (SRGs) in PCa evolution and contributes to PCa progression via modulating aberrant splicing events. The degree of global AS abnormalities aggravates along with disease progression. In addition to treatment responsive emergence (solid arrow) of aggressive PCa (i.e., castration-resistant PCa (CRPC)), some primary PCa with small-cell PCa (SCPC) and/or neuroendocrine PCa (NEPC) features intrinsically resist androgen deprivation therapy (ADT) (dashed arrow), and will progress to advanced stages. Significantly, the tumor AS landscape differs before and after ADT, suggesting a treatment-induced reshaping of global AS pattern that might have contributed to therapy resistance. Collectively, targeting aberrant splicing via spliceosome modulators (e.g., E7107) offers a new way to combat CRPC.

minority (~0.6%) of all somatic mutations in PCa,¹⁰ indicating that SRG dysregulation is the main mechanism underpinning mis-splicing. Importantly, we identified many unreported SRGs that can be linked, individually or in combination, to clinical features of advanced PCa, indicating a biomarker value.

One of our most significant findings is the link between the elevated intron retention (IR) level and PCa aggressiveness and stemness, which is evidenced by an increase in number of IR events detected in the spectrum of PCa evolution, in normal SC populations in various tissues, and in prostate cancer SCs.⁶ Consistently, IR in PCa impacts genes involved in stemness and cancer-promoting functions. As the least studied AS type, IR usually triggers nonsense-mediated RNA decay (NMD) to down-regulate gene expression. However, our data revealed, oppositely, that IR generally enhances gene expression and thus likely functions in PCa biology. In support, comparison of IR-affected genes in CRPC with that in human embryonic SC (hESC) and CD4⁺ T cells demonstrated that nearly 33% and 50% of the CRPC-IR genes also displayed IR in hESC and stem-like resting CD4⁺ T cells, respectively, directly suggesting a functional link of IR with stemness. Importantly, many IR-bearing transcripts exist in PCa cell lines. AR plays a central role in prostate tumorigenesis. We observed a generally negative association of AR activity with IR level in clinical datasets, but surprisingly, AR does not specifically regulate IR in androgen-sensitive PCa cells.

Of clinical relevance, aggressive PCa displays a preferential dependency on aberrant spliceosome activity and, E7107, the spliceosome modulator, effectively abolishes the growth of both *Myc*-driven autochthonous murine PCa and preclinical CRPC models *in vivo*.⁶ These findings offer a new therapeutic strategy for treating CRPC by targeting splicing misregulation (Figure 1b). Interestingly, our mechanistic study pointed out a potential action of E7107 to promote differentiation and reprogram PCa cells from an (aggressive) androgen-insensitive state to an (indolent) androgen-sensitive state. Animal studies revealed certain toxicities of E7107, highlighting the need to define intricate treatment window and doses for E7107. As we look forward to the future, we envision a potential treatment regimen in which CRPC is the first subject to a short-term splicing inhibition (to avoid toxicity and also to reprogram aggressive PCa cells) followed by anti-androgens treatment such as Enzalutamide to illuminate AR⁺

cells. Further characterization of the origins and consequences of aberrant AS in PCa will help enhance our understanding of PCa pathogenesis and accelerate novel drug development.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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