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Minority report: The minor spliceosome as a novel cancer vulnerability factor

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The minor spliceosome regulates the removal of a conserved subset of introns present in genes with regulatory functions. In this issue of *Molecular Cell*, Augspach et al.[1] report that elevated levels of U6atac snRNA, a key minor spliceosome component, contribute to prostate cancer cell growth and can be a novel therapeutic target.

Removal of introns from primary transcripts (pre-mRNA splicing) is an essential step in eukaryotic gene expression. While most human introns are removed by the conventional (U2-type) spliceosome, about 1 in 300 introns are removed by a distinct machinery known as the minor (U12-type) spliceosome.[2] The evolutionary origins and functional significance of the minor spliceosome and of its target "minor introns" have remained enigmatic. What is the purpose of having two machineries and two intron classes, considering that genes with minor introns typically contain only one such intron along with multiple U2-type introns? These genes are often involved in important cell regulatory functions, such as the control of DNA and RNA metabolism, MAP kinase signaling, and cytoskeletal organization. The removal of the minor intron from their primary transcripts appears to be rate limiting for mature mRNA and protein production (Figure 1).[2,3] Recently, Augspach et al.[1] report that the activity of the minor spliceosome, controlled through the levels of its U6atac snRNA component, can play many roles in cancer and can be envisioned as a novel pan-cancer vulnerability factor.

Splicing perturbations are a hallmark of cancer, frequently associated with altered expression or mutations in spliceosome components or splicing regulatory factors.[4] Previous work linked alterations of the minor spliceosome with myelodysplastic syndromes and leukemias, where mutations in the minor spliceosome protein ZRSR2 induce minor intron retention events, including one leading to increased self-renewal of hematopoietic stem cells.[5] Augspach et al.[1] expand the roles of the minor spliceosome in cancer in important new directions. They report that U6atac snRNA is upregulated in cancer samples from multiple origins, as well as in a variety of cancer cell lines. This included prostate cancer lines corresponding to different progression stages, and in organoids, correlating with increased activity of the minor spliceosome. siRNA-mediated knockdown of U6atac reduces minor spliceosome activity and affects expression or alternative splicing of multiple genes important for cell proliferation and cancer progression, altering cell cycle and reducing proliferation. Remarkably, this occurs without affecting the growth of non-cancerous cell lines, arguing that reducing the activity of the minor spliceosome offers a therapeutic window in oncology.

The rate-limiting function of U6atac snRNA levels on minor spliceosome activity and cancer cell growth, distinguishing between benign and tumor tissue, is also remarkable from a mechanistic perspective. Recognition of relatively conserved specific sequences at the exon/intron boundaries of minor introns (characteristically including AT and AC dinucleotides at their 5′ and 3′ ends, respectively, hence their denomination as "atac introns") is accomplished by U11/U12 snRNP ribonucleoprotein complexes, which contain U11 and U12 snRNAs harboring sequences complementary to the 5′ and 3′ splice sites, respectively. This is followed by assembly of the U4atac/U5/U6atac tri-snRNP. Similar to its major spliceosome counterpart, U6 snRNA, U6atac snRNA plays a key role in building the RNA-based catalytic center of the spliceosome, which carries out the two

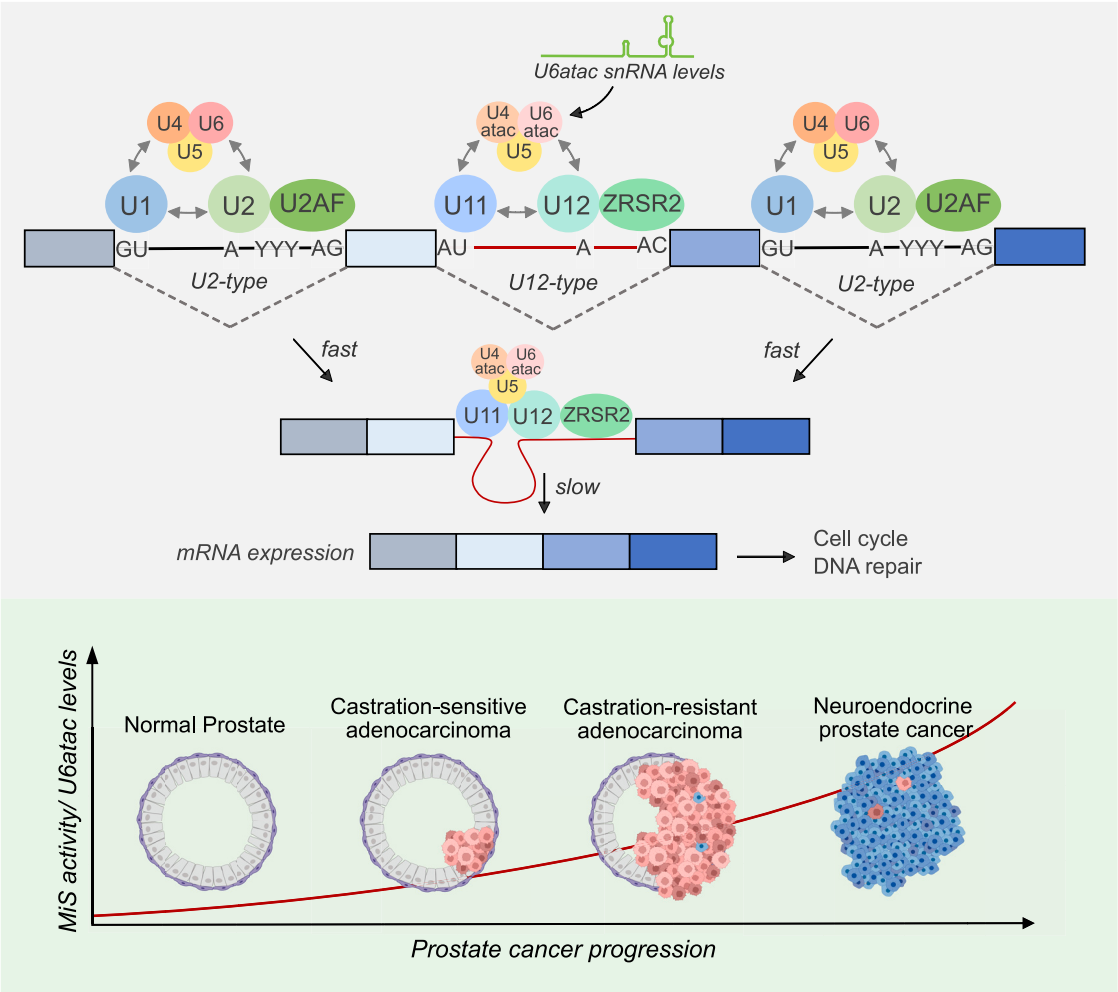

Figure 1. Increased activity of the minor spliceosome contributes to prostate cancer progression

The levels of U6atac snRNA are rate limiting for the activity of the minor spliceosome and the splicing of minor introns, determining the expression levels of ~714 minor intron-containing genes. Augspach et al.[1] show that prostate cancer progression to therapy-resistant subtypes depends on increasing levels of U6atac/minor spliceosome activity, which control the expression of crucial genes for cancer cell growth, survival, and lineage plasticity.

transphosphorylation reactions leading to intron excision.[2,6] A previous study made the important observation that U6atac snRNA is highly unstable and its low levels are rate limiting for minor intron removal (Figure 1).[7] Stress-activated p38MAPK signaling stabilizes U6atac levels, triggering the expression of many minor intron-containing mRNAs, including the tumor suppressor PTEN or cytokine production. Therefore, minor introns may have evolved as a conserved mechanism for coordinated control of critical biological processes.[7] Similarities can be drawn between this model of minor intron function and that of a class of U2-type introns known as "detained introns."[8] These are also introns whose removal is rate limiting for mRNA production and is triggered by specific signals during development or in response to environmental clues. Interestingly, transcripts harboring detained introns remain associated with the site of gene transcription until the intron is excised; localized accumulation of unspliced transcripts might also contribute to regulation of minor intron-containing genes.

Are the regulatory effects of U6atac abundance on cancer progression based on the expression of a large program of minor intron-containing genes, or are there a few targets with particularly critical functions? For example, a previous CRISPR-based screen converged on LZTR1, a regulator of Ras-related GTPases, as a key target to explain the impact of minor spliceosome factor ZRSR2 mutations in leukemia.[5] Prostate cancer is a common cancer successfully treated with androgen deprivation and androgen receptor signaling inhibitors. However, prolonged treatment often leads to relapse and development of resistance in the forms of castration-resistant prostate cancer adenocarcinoma (CRPC-adeno) and neuroendocrine prostate cancer (CRPC-NE), two subtypes with bad prognosis and limited treatment options. Previous work showed that tumor aggressiveness correlates with alterations in splicing programs involving U2-type introns,[9,10] including the generation of aberrant alternatively spliced

transcripts of the androgen receptor[10] and the activation of a neural microexon splicing program.[11] Augspach et al.[1] show that minor intron splicing increases with prostate tumor progression stages (Figure 1) and is directly regulated by androgen receptor signaling during therapy resistance by increased expression of U6atac. This explains the high activity of minor intron splicing in CRPC-adeno, while the lack of AR expression in CRPC-NE indicates that other pathways can enhance minor spliceosome function. Transdifferentiation of CRPC-adeno into CRPC-NE is driven by the loss of REST, a transcriptional repressor of neural genes. Interestingly, Augspach et al.[1] observed that minor spliceosome inhibition decreases the inclusion of a microexon flanked by U2-type introns in *REST* transcripts, producing a non-functional protein isoform, which may explain the decrease of neuroendocrine markers in CRPC-NE upon U6atac knockdown. These results point to REST splicing as an important mediator of the effects of U6atac upregulation on prostate cancer progression toward the neuroendocrine subtype and to crosstalk between major and minor spliceosome functions. On the other hand, minor intron-containing genes are enriched in oncogene protein-protein interaction networks, suggesting impact on multiple pathways relevant to cancer.

In summary, Augspach et al.[1] show that inhibition of minor spliceosome activity reduces the viability and lineage plasticity of prostate cancer cells (but not of non-cancerous cell lines), as well as the growth of advanced, therapy-resistant patient-derived prostate cancer organoids. These effects are synergistic with anti-cancer drug treatments currently used in the clinic. Minor spliceosome inhibition provokes G1/S cell-cycle arrest and a reduction of expression of genes in essential pathways for prostate cancer progression such as AR signaling and epithelial-mesenchymal transition (EMT). While work in animal models will be necessary to confirm these findings, the results of Augspach et al.[1] open the door to exploit the special dependence of cancer cells on minor spliceosome function for the design of novel therapies for prostate and other cancers.

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DECLARATION OF INTERESTS

J.V. is on the advisory board for *Molecular Cell* and of Remix Therapeutics, Stoke Therapeutics, and IntronX.

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