Leveraging minor intron classification to target their splicing to treat prostate cancer

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Abstract. Introns make splicing essential for eukaryotic gene expression and are classified as major and minor spliced by their namesake major and minor spliceosome, respectively. Our recent manuscript broke this paradigm by re-classifying introns into i) minor, ii) minor-like, iii) minor-hybrid, iv) major-hybrid, v) major-like, vi) major, and vii) non-canonical introns (*NAR,* Jun 29: gkae550, '24, funded by PCF). We used 31 million introns from 263 genomes across six eukaryotic supergroups as a snapshot of intron variations across evolution accessible at *midb.pnb.uconn.edu*. We have identified these novel intron classes in prostate cancer (PCa) relevant genes including E2F transcription factors, REST, BRCA and others. The identification of the specific sequence elements that make introns responsive to minor versus major spliceosome can now be leveraged to design specific anti-sense oligos (ASOs) that can block splicing of genes crucial for PCa progression. We have previously shown that minor spliceosome specific snRNAs are upregulated across PCa metastasis, which was in line with elevated minor intron splicing efficiency. U6atac snRNAs, which is normally maintained at low levels through turnover is significantly upregulated in advanced PCa. We have shown that siRNA against U6atac effectively inhibits the minor spliceosome that blocks proliferation and survival of PCa cells. We have now developed an ASO against U6atac to effectively inhibit the minor spliceosome. We have also systematically inhibited 15 different splicing factors in C4-2 cells followed by RNAseq to identify complement of splicing factors responsible for splicing mechanism of these various intron classes. The objective here was to identify the splicing factors that can be targeted to alter splicing of specific intron types in genes relevant in prostate cancer progression. Moreover, we are now developing modified anti-sense oligos to specifically block splicing of minor introns in E2Fs and other minor intron-containing genes.

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