Enhancing Androgen Receptor Antagonist-Mediated Interferon Responses in Prostate Cancer

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Background. Prostate cancer (PCa) is largely dependent on the androgen receptor (AR), a master transcriptional regulator in prostate epithelium. While AR has been extensively studied as a transcriptional activator, it also mediates transcriptional repression, though the underlying mechanisms remain incompletely understood. By integrating RNA-seq and ChIP-seq (AR and H3K27Ac) data in VCaP cells, we found that AR binding sites are generally not associated with genes upregulated by AR inhibition using enzalutamide (Enz), suggesting indirect or epigenetically mediated repression.

Methods. Cell culture and treatments, RNA-sequencing, AR and H3K27Ac ChIP-sequencing, DNA methylation and hydroxymethylation profiling, protein analysis by Western blotting, and bioinformatic analysis of both generated and publicly available datasets.

Results. Gene Set Enrichment Analysis (GSEA) revealed that genes upregulated by Enz were strongly enriched for interferon (IFN) response pathways. Notably, a substantial fraction of these transcripts originated from endogenous repetitive elements (EREs), particularly SINE/Alu and LINE/L1 elements. DNA methylation emerged as a key mechanism of basal AR-mediated repression of these elements. Treatment with the DNA methyltransferase inhibitor decitabine (DAC) upregulated ~55% of genes induced by Enz, while inhibition of H3K27 methylation via EZH2 inhibitor (GSK126) had a limited effect, underscoring the dominant role of DNA methylation in silencing EREs.

Given the immunologically "cold" nature of PCa, characterized by poor antigen presentation and minimal response to immune checkpoint blockade, we hypothesized that AR inhibition might enhance tumor immunogenicity via reactivation of EREs and induction of IFN signaling. Indeed, Enz treatment led to increased expression of HLA class I proteins across PCa cell lines. In parallel, analysis of TCGA dataset further supported the involvement of DNA methylation in repressing IFN pathway genes.

Methylation profiling showed no significant change in global 5-methylcytosine (5mC) levels post-Enz, but revealed a notable increase in 5-hydroxymethylcytosine (5hmC), particularly at EREs, indicating active DNA demethylation. This correlated with induction of ERE-derived double-stranded RNA (dsRNA), triggering type I IFN responses as evidenced by phosphorylation of EIF2α and activation of the NF-κB pathway, ultimately leading to increased HLA class I expression.

To identify strategies to enhance this effect, we targeted ADAR1, a dsRNA-editing enzyme that suppresses viral mimicry. ADAR1 depletion, in combination with Enz, significantly increased HLA class I expression. Additionally, inhibiting EIF2α downstream signaling with ISRIB further enhanced HLA levels. Finally, co-treatment with Enz and birinapant, a SMAC mimetic that activates both canonical and non-canonical NF-κB signaling, produced a marked increase in HLA class I expression.

Conclusions. These findings highlight a novel epigenetic mechanism by which AR may suppresses tumor immunogenicity and suggest novel strategies to enhance antigen presentation in prostate cancer.

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