Mechanism of AR enhancer activation in castration-resistant prostate cancer

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Background: Amplification of the androgen receptor (AR) locus is the most frequent alteration in metastatic castration-resistant prostate cancer (CRPC). Recent studies have demonstrated that a distal enhancer of the AR is co-amplified with the AR gene body, driving increased AR transcription and resistance to androgen deprivation therapy. However, the mechanism of AR enhancer activation in advanced disease is unknown. Elucidating the mechanism of AR enhancer activation will provide critical insights into the development of castration-resistance and opportunities for therapeutic intervention.

Methods: We used CRISPR-Cas9 screening in an AR transcription reporter cell line to identify trans-acting factors required for enhancer activity. To establish direct activation of the AR enhancer by candidate factors, we evaluated binding to the enhancer and their impact on epigenetic features characteristic of active enhancers. In a complimentary approach, we conducted a CRISPR-Cas9 base editor screen to mutagenize the endogenous AR enhancer to map cis-regulatory elements at the nucleotide level required for enhancer mediated transcription.

Results: We discovered that the transcription factors HOXB13, GATA2, and TFAP2C bind to the AR enhancer and regulate AR transcription in prostate cancer cell lines, patient-derived xenograft models, and CRPC models treated with AR signaling inhibitors. Transcription factor binding modulates features associated with active regulatory elements including H3K27 acetylation, chromatin accessibility, and enhancer-promoter looping as determined by ChIP-seq, ATAC-seq, and 4C-seq, respectively. Interestingly, the AR enhancer belongs to a set of regulatory elements that require HOXB13 to maintain binding of the pioneer factor FOXA1, further delineating the role of HOXB13 in CRPC. The CRISPR-Cas9 base editor screen revealed that a single point mutation is sufficient to decrease enhancer H3K27ac, chromatin accessibility, and AR transcription.

Conclusions: HOXB13, GATA2, and TFAP2C regulate AR transcription in CRPC through activation of its distal enhancer. In parallel CRISPR base editing screening, we identified cis-regulatory elements in the AR enhancer required for enhancer mediated transcription. This work provides a framework to functionally interrogate disease-related noncoding elements and identify potential therapeutic targets.

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