

Decoding enhancer regulation in advanced prostate cancer

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Background: Metastatic castration resistant prostate cancer (mCRPC) is a lethal disease with limited treatment options. This is a disease state that is characterized by large-scale changes in gene expression, which are driven at least in part through widespread remodeling of the chromatin landscape leading to aberrant enhancer function. As one example, a distal enhancer of the androgen receptor (AR) is amplified in ~80% of patients with mCRPC, supports high levels of AR expression, and is sufficient to confer resistance to enzalutamide. As such, targeting enhancer dysregulation has significant therapeutic potential in mCRPC, yet the specific factors that regulate enhancer activity have yet to be fully defined. Here we investigate mechanisms of enhancer activation through high-throughput tethering and screening approaches to define key enhancer regulatory proteins.

Methods: To identify protein cofactors sufficient for enhancer activation, we employed a novel high-throughput pooled screening platform developed in our laboratory called COMET (Combinatorial Effector Targeting). This method relies on a large library of >100K protein domain combinations that can be targeted to an endogenous locus, and screened for transcriptional activity. The advantage of this approach is that it (1) captures the direct effect of proteins on enhancer activity through targeted tethering, and (2) enables tethering pairs of proteins as a powerful tool for defining complex regulatory logic. We targeted the COMET library to a validated distal enhancer, and performed a FACS-based screen and long-read sequencing to isolate domain combinations sufficient for enhancer activation.

Results: Our enhancer COMET screen identified 496 significant protein domain combination hits associated with enhancer activation. These include combinations containing known transcriptional activators, highlighting the validity of this approach. In addition, the screen identified multiple combinations containing various domains of FOXA1, a transcription factor and oncogenic driver that is frequently mutated in prostate cancer and is critical for AR binding and target gene activation. We are pursuing additional mechanistic studies to examine how FOXA1 regulates enhancer activity.

Conclusions: Here we nominate candidate enhancer regulatory proteins in high-throughput, with the goal of identifying new routes for manipulating enhancer activity for therapeutic benefit.

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