

## Functional genetic and genomic approaches to studying androgen receptor splicing and therapy resistance in castration-resistant prostate cancer

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**Background:** There is currently no curative therapy for castration-resistant prostate cancer (CRPC), which is the second leading cause of cancer mortality among men in the United States. Although the second-generation antiandrogens, enzalutamide and abiraterone, have led to improved survival in CRPC, intrinsic and acquired resistance to these agents remain significant issues. Diverse mechanisms may contribute to drug resistance in advanced prostate cancer, including the expression of constitutively active androgen receptor splice isoforms (e.g. AR-V7) and complex genomic rearrangements in the androgen receptor gene and elsewhere in the genome. Elucidating the mechanisms by which some prostate cancers fail to respond to second-generation antiandrogens is critical to designing future therapies.

**Methods:** To identify trans-acting factors that may contribute to androgen receptor splicing in an unbiased and systematic fashion, we used CRISPR/Cas9 technology to generate an endogenous GFP reporter of AR-V7 expression in the 22Rv1 cell line, a CRPC cell line that strongly high expresses the AR-V7 variant. Reporter cells were infected with a genome-scale loss-of-function CRISPR library (76,441 sgRNAs targeting 19,114 genes) and a flow-based screen was performed to identify factors regulating AR/AR-V7 expression. In complementary studies, we are leveraging long-range whole genome sequencing of CRPC metastatic tissue, xenografts, and cell lines using the 10X Genomics platform to identify genomic features ("cis-factors") that may drive persistent androgen receptor signaling in advanced prostate cancer.

**Results:** Preliminary results have identified a number of genes as AR/AR-V7 regulators in 22Rv1 cells, and these genes are enriched for factors involved in androgen signaling and for RNA-binding factors. Individual validation of candidate hits is in progress, including investigating the effects of each on full-length AR vs. truncated AR isoform expression, the contribution of each candidate to androgen-independence in various cell contexts, and the role of each candidate in mediating resistance to enzalutamide. In exploratory sequencing studies, we have also identified multiple complex alterations in CRPC cell lines, xenografts, and patient samples that may act together with dysregulation of trans-acting factors to drive castration resistance.

**Conclusions:** Complementary functional genetic and genomic sequencing approaches may be useful in identifying the complex landscape of features contributing to castration resistance in advanced prostate cancer.

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