The expression of androgen receptor (AR) variants is a frequent, yet poorly-understood mechanism of clinical resistance to AR-targeted therapy for castration-resistant prostate cancer (CRPC). Among the multiple AR variants expressed in CRPC, AR-V7 is considered the most clinically-relevant AR variant due to broad expression in CRPC, correlations of AR-V7 expression with clinical resistance, and growth inhibition when AR-V7 is knocked down in CRPC models. Therefore, efforts are under way to develop strategies for monitoring and inhibiting AR-V7 in castration-resistant prostate cancer (CRPC).

The aim of this study was to understand whether other AR variants are co-expressed with AR-V7 and promote resistance to AR-targeted therapies. To test this, we utilized RNA-seq to characterize AR expression in CRPC models. RNA-seq revealed the frequent coexpression of AR-V9 and AR-V7 in multiple CRPC models and metastases. Furthermore, long-read single-molecule real-time (SMRT) sequencing of AR isoforms revealed that AR-V7 and AR-V9 share a common 3' terminal cryptic exon. To test this, we knocked down AR-V7 in prostate cancer cell lines and confirmed that AR-V9 mRNA and protein expression were also impacted. In reporter assays with AR-responsive promoters, AR-V9 functioned as a constitutive activator of androgen/AR signaling. Similarly, infection of AR-V9 lentivirus into AR-negative DU145 cells reduced androgen-independent cell proliferation. In conclusion, these data implicate co-expression of AR-V9 with AR-V7 as an important component of constitutive AR signaling and therapeutic resistance in CRPC.

**ACKNOWLEDGEMENTS**

- This work was supported by a grant from the National Institutes of Health (R01 CA174777 to S.M.D.).
- We would like to thank all the people from Dehm lab for the help and support during the work.