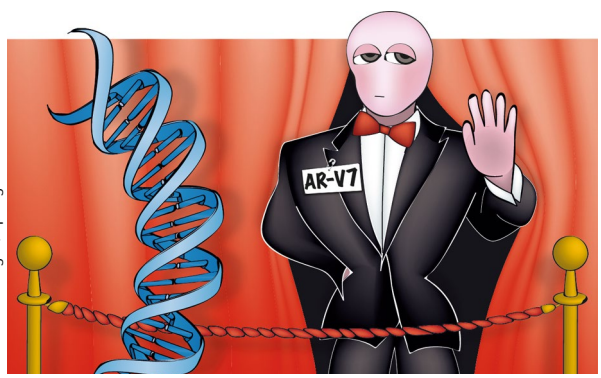
PROSTATE CANCER

AR-V7 — repress to impress

> AR-V7 heterodimerizes with the full-length AR … to repress transcription of a growth-suppressive gene subset

Expression of androgen receptor splice variant 7 (AR-V7), a hormone-independent and constitutively active transcription factor, has been implicated in the emergence of castration-resistant prostate cancer (CRPC), but the precise genomic mechanisms are unclear. Using transcriptome and cistrome analyses, a new study reports that AR-V7 heterodimerizes with the full-length AR (AR-FL) to repress transcription of a growth-suppressive gene subset in preclinical models.

The investigators initially performed differential gene expression analysis of RNA sequencing data from the LNCaP95 and 22Rv1 CRPC cell lines (which both express AR-V7 and AR-FL) following knockdown of AR-FL or AR-V7 to functionally delineate the transcriptional roles of each isoform. Importantly, AR-V7 depletion led to a markedly higher number of upregulated than downregulated genes, whereas the proportion of downregulated to upregulated genes was only slightly higher following AR-FL silencing, suggesting that AR-V7 preferentially represses a gene subset. Despite sharing some canonical AR targets, substantial differences were noted between the AR-FL and AR-V7 transcriptomes, inferring that these isoforms have divergent transcriptional roles.



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Chromatin immunoprecipitation followed by sequencing (ChIP–seq) was then performed in LNCaP95 cells to identify direct transcriptional targets of each isoform. Intriguingly, detailed cistrome analysis revealed a major overlap between AR-V7 and AR-FL binding sites, suggesting these isoforms co-occupy the same genomic loci. Indeed, sequential ChIP experiments at select target genes and FRET assays inferred that AR-V7 and AR-FL functionally interact. Further ChIP–seq experiments in LNCaP95 and 22Rv1 cells showed co-dependent binding of AR-FL and AR-V7 at androgen-response element (ARE)-containing chromatin sites with high levels of both isoforms. These results suggest that AR-FL and AR-V7 can modify their respective DNA-binding affinities through heterodimerization and co-localization.

As AR-FL and AR-V7 bind co-dependently to chromatin, despite having divergent transcriptional effects, Cato et al. hypothesized that other factors contribute to their divergent functionality. Affinities of the AR isoforms for co-regulator peptides were measured using a peptide-binding assay employing a pan-AR antibody. Results showed that knockdown of AR-FL, but not AR-V7, abrogated most AR–co-regulator binding interactions and increased AR binding to several co-repressors, notably nuclear receptor corepressor 1 (NCOR1) and NCOR2. Subsequent co-immunoprecipitation experiments revealed increased binding of AR-V7 to NCOR1 and NCOR2 upon AR-FL depletion, suggesting that the repressive function of AR-V7 is facilitated by the preferential interaction with NCOR family transcriptional corepressors. As NCOR1 and NCOR2 control histone deacetylase recruitment and, therefore, negatively regulate histone H3K27 acetylation (H3K27ac; an epigenetic mark of active transcription), ChIP–seq was used to measure H3K27ac levels following knockdown of AR-V7 or AR-FL. These experiments indicated that AR-V7-mediated transcriptional repression is a consequence of NCOR corepressor-mediated negative regulation of H3K27ac at a subset of AR binding sites.

Finally, the clinical relevance of these findings was evaluated. Gene-set enrichment (GSEA)-based comparisons between AR-V7 and AR-FL gene signatures (which included activated and repressed genes in response to isoform knockdown in LNCaP95 cells) were conducted and gene expression data from CRPC biopsy tissues analysed. These investigations showed that both AR-FL-dependent gene activation and AR-V7-dependent gene repression are key features of CRPC tumours. A subsequent genome-wide CRISPR knockout screen of 57 highly enriched genes from the GSEA identified four AR-V7-repressed genes — *SLC30A7*, *B4GALT1*, *HIF1A* and *SNX14* — that had a negative effect on LNCaP95 proliferation. Low expression of these four genes was positively correlated with recurrence, metastasis and disease-specific mortality in two patient cohorts.

"These data clearly define the importance of targeting AR-V7, either through inhibition of post-transcriptional splicing or protein degradation," concludes investigator Stephen Plymate.

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ORIGINAL ARTICLE Cato, L. et al. ARv7 represses tumor-suppressor genes in castration-resistant prostate cancer. *Cancer Cell* https://doi.org/10.1016/j.ccell.2019.01.008 (2019)
FURTHER READING Lu, J. et al. Are androgen receptor variants a substitute for the full-length receptor? *Nat. Rev. Urol.* **12**, 137–144 (2015)