Androgen Receptor Variant 7 (AR-V7) Condensates Promote Growth and Proliferation in Castration-Resistant Prostate Cancer

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Backgrounds: Biomolecular condensates are membraneless structures that compartmentalize the intracellular environment and facilitate key processes such as epigenetic regulation, stress responses, DNA repair, and transcription. Dysregulation of these condensates has been linked to diseases, including cancer. We previously reported that the full-length androgen receptor (AR-FL) forms nuclear condensates in response to androgens to transcribe its target genes in prostate cancer (PCa) cells (**PMID: 36535377**). AR-FL is a primary therapeutic target in PCa, but resistance can emerge through AR amplification or the expression of splice variants such as AR-V7, leading to castration-resistant PCa (CRPC). This study investigates the ability of AR-V7 to form condensates in androgen-independent PCa models and to activate oncogenic programs linked to cell proliferation and migration (**PMID: 39868336**).

Methods: We used confocal microscopy to study condensates formed by endogenous and exogenous AR-V7 tagged with non-dimerizing EGFP. Using a phase-altering AR-V7 mutant (AR-V7-5YtoS) that showed reduced condensate formation without impairing nuclear translocation or DNA binding, we identified AR-V7 condensate-dependent transcriptome using RNA-seq. We used Gene Set Enrichment Analysis (GSEA) to identify pathways enriched in condensate-dependent genes and g:Profiler to define growth-related genes. We validated the role of AR-V7 condensates in promoting the proliferation and migration of PCa cells using the Incucyte live-cell imaging system. We also knocked-down the identified growth-related genes with small interfering RNAs (siRNAs) and assessed their contribution to cell proliferation. We evaluated cell cycle distribution by Propidium Iodide staining and flow cytometry. Finally, to determine what defines condensate-dependent genes, we examined colocalization of recombinant AR-V7 droplets with various DNA sequences containing different numbers and arrangements of Androgen Response Elements (AREs) by confocal microscopy.

Results: We demonstrate that AR-V7 forms nuclear condensates independently of androgen stimulation and AR-FL in CRPC models. Using the phase-altering AR-V7-5YtoS mutant, we show that AR-V7 regulates gene expression through both condensate-dependent and condensate-independent mechanisms. Condensate-dependent genes display exponential activation in response to increasing AR-V7 levels and activate cell proliferation, migration, and G1/S cell cycle transition. Knocking down condensate-dependent genes using siRNAs, significantly impaired cell growth. Importantly, we found that condensate-dependent genes have a greater number of AR binding sites in their promoters than condensate-independent genes. Using recombinant AR-V7 protein, we also observed that AR-V7 droplets colocalize more strongly with DNA containing a higher numbers of AREs *in vitro* and preferentially associate with chimeric FOXA1-AREs over half-site or palindromic AREs.

Conclusions: Our findings reveal the role of AR-V7 condensates in activating growth-related oncogenic programs in CRPC and highlight condensate formation as a potential vulnerability for therapeutic development.

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