Investigating the distinct phase separation properties of androgen receptor isoforms in prostate cancer

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Background
The androgen receptor (AR) is a key target in prostate cancer (PCa) treatment. Resistance mechanisms often involve AR alterations such as gene and enhancer amplification or the emergence of splice variants like AR-V7, which lacks the ligand-binding domain (LBD), the main target for AR pathway inhibitors (ARPI). We recently reported that full-length AR (AR-FL) forms nuclear condensates in response to androgens to execute its oncogenic programs (**PMID: 36535377**). AR-V7, however, exhibits distinct phase behavior in androgen-sensitive models. This study aims to investigate AR-FL and AR-V7 condensates across different PCa models and in vitro, compare their phase behavior, and identify the condensate-dependent transcriptome.

Methods
We used confocal fluorescence microscopy to study condensates formed by endogenous AR-FL and AR-V7 using immunofluorescence with specific antibodies. For exogenous AR-FL and AR-V7, we used non-dimerizing EGFP tags to visualize the condensates. To assess the role of chromatin accessibility, we used PROTAC AU15330 to degrade the ATPases SMARCA2/4 of the SWI/SNF complex and evaluate their role in AR-V7 condensate formation. We also evaluated the interdependency between AR-FL and AR-V7 condensates by using ARV-110, a degrader of AR-FL, in PCa models. To assess the role of AR-V7 condensates in transcription, we assessed their colocalization with RNA Pol II and MED1 using immunofluorescence and proximity ligation assays (PLA) and assessing target gene expression via qPCR. To examine the role of AR-V7 condensates in transcription, we assessed their colocalization with RNA Pol II and MED1 through immunofluorescence and proximity ligation assays (PLA), and we analyzed target gene expression via qPCR. Additionally, we identified an AR-V7 mutant that disrupts condensate formation without affecting nuclear translocation or DNA binding, and used it for RNA-seq to study the condensate-dependent transcriptome. Finally, we expressed and purified recombinant AR-FL and AR-V7 proteins and characterized their phase behavior *in vitro*.

Results
AR-V7-mEGFP forms nuclear condensates more prominently in PCa models that express splice variants, independent of androgen presence or AR-FL expression. These AR-V7 condensates exhibit dynamic phase behavior and require chromatin accessibility for their formation. In both PCa models and *in vitro*, AR-V7 requires concentrations 3 to 5 times higher than AR-FL to initiate condensate formation. Additionally, AR-V7 condensates are transcriptionally active, as they colocalize with the transcriptional players MED1 and RNA Pol II, and manipulating their formation affects the expression of target genes. Using a mutant that disrupts AR-V7 condensates, AR-V7-5YS, identified the KRAS signaling pathway as dependent on these condensates.

Conclusions
AR-V7 forms phase-separated condensates that are crucial for transcriptional regulation in PCa, distinguishing its role from that of AR-FL. By identifying AR-V7 condensate-dependent programs, we can gain insights into androgen-independent pathways that may be explored for future drug targeting, offering potential new therapeutic strategies for managing aggressive PCa subtypes.

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Conflicts of Interest Disclosure Statement
The authors declare no conflicts of interest.