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INVITED RESEARCH HIGHLIGHT

Prostate Cancer

What is the next generation therapeutic strategy for castration-resistant prostate cancer

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Prostate cancer (PCa) is one of the most common cancers in the world. Since androgen receptor (AR) signal plays key roles in the PCa progression, targeting androgens via the current androgen deprivation therapy (ADT) is the main therapeutic strategy for advanced PCa. However, most patients who receive ADT, including the second generation anti-androgens enzalutamide (also known as MDV3100) may finally develop the castration (or anti-androgen) resistance after 12–24 months treatment. In the manuscript by Asangani *et al.*, the authors demonstrated that targeting the amino-terminal bromodomains of BRD4 could preferentially suppress human castration-resistant prostate cancer (CRPC) cell lines. While further studies are required to understand the full impact of their findings, the innovative approach provides a potential novel epigenetic approach for the concerted blockade of oncogenic drivers in CRPC.

Prostate cancer is the most common cancer and the second leading cause of cancer-related deaths among men in the United States.[1] It is well known that the prostate is an androgen-dependent organ, and AR signaling plays critical roles in PCa progression. After the discovery of Huggins and Hodges,[2] the main therapy for the advanced PCa is ADT, which includes surgical castration, chemical castration (such as luteinizing hormone-releasing hormone analogues), targeting androgen biosynthesis (such as abiraterone, ABI) and anti-androgens (such as flutamide and bicalutamide). While current ADTs suppress PCa for the initial 12–24 months, most ADTs eventually fail while the patients develop CRPC. Enzalutamide is a newly developed second generation anti-androgen and was proven better efficacy than flutamide and bicalutamide to suppress PCa, through blocking AR nuclear translocation and AREs binding.[3] Although enzalutamide might lead to survival extension of up to 4.8 months, like other anti-androgens, it still failed to prevent CRPC development,[3] and enzalutamide resistance PCa finally occurred.[4] Interestingly, the authors of this manuscript, Asangani *et al.*[5] demonstrated that enzalutamide might promote PCa metastasis to femurs and liver, which had also been reported by another group.[6]

In this paper, Asangani *et al.*[5] described an alternative strategy for CRPC treatment, which targets the co-activators or mediators of AR transcriptional signaling. Bromodomain containing 4 (BRD4) is a conserved member of the bromodomain and extraterminal (BET) family of chromatin readers (including BRD2, BRD3 and BRDT), which has a critical role in RNA polymerase II (RNA Pol II) mediated transcription via facilitating recruitment of the positive transcription elongation factor P-TEFb. Similar to other BET-family proteins, BRD4 contains two conserved bromodomains, BD1 and BD2. JQ1 and I-BET762 are two selective small molecule inhibitors that can competitively bind to the bromodomain pocket and result in the displacement of BRD4 from active chromatin and the subsequent removal of RNA Pol II from target genes. BRD4 was shown to interact with sequence-specific DNA-binding transcription factors in a gene-specific manner and exhibit anti-proliferative effects in a range of malignancies.

In order to find out BRD4 roles in PCa, Asangani *et al.* picked up five PCa cell lines and one benign prostate cell line with high expression of BET proteins. When Asangani *et al.* treated these six cell lines with JQ1, they found only three AR-signaling positive cells were sensitive to JQ1. To confirm these results, they used siRNAs to knockdown BRD2, 3 and 4 and got similar results, showing significant inhibition of cell proliferation and invasion. Further experiments were conducted to detect whether JQ1 has effects on AR target genes. In AR positive VCaP, LNCaP and 22RV1 cell lines, JQ1 decreases the expression of PSA and ERG in a dose-dependent manner, on the other hand, bortezomib did not reverse the JQ1-mediated PSA and ERG protein loss. What's more, gene set enrichment analysis using the AR gene signature revealed significant repression of these genes in AR-positive cells. These results indicated that BRDs might function through regulating AR at transcription level.

As BRD4 is known to engage sequence-specific DNA-binding proteins, the authors proposed that AR may interact directly with BRD4. By gel filtration chromatography and immunoprecipitation experiments, they found that AR and BRD4 predominantly co-eluted in a high-molecular-weight complex and had an endogenous association between AR and BRD4. Further mechanism studies demonstrated that the mainly BD1 of BRD4 bind directly to the N-terminal domain (NTD) of AR. And JQ1 disrupted the interaction of BD1–AR NTD, which resulted in significant inhibition of cell proliferation and invasion in AR-positive PCa cells.

To compare the efficiencies of BRD4 inhibitor and anti-androgens (bicalutamide and enzalutamide), the authors performed ChIP-seq. They found while bicalutamide slightly attenuated DHT-mediated recruitment of AR to target loci, enzalutamide and JQ1 dramatically attenuated the recruitment. In limiting evaluation to AR and BRD4 co-recruitment, DHT-mediated AR recruitment

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to these loci was inhibited by enzalutamide and to a lesser extent by JQ1. Meanwhile, JQ1 almost completely inhibit DHT-induced BRD4 recruitment to the AR–BRD4 shared loci. Furthermore, gene expression analysis showed JQ1 had more efficient repression of DHT-induced AR-target genes than enzalutamide or bicalutamide did. With *in vivo* experiments, the authors compared the efficacy of JQ1 and enzalutamide to suppress tumor growth and metastasis. They found JQ1 led to a more significant reduction of tumor volume/weight than enzalutamide. Impressing, they also demonstrated that enzalutamide, but not JQ1 treatment leaded to spontaneous metastasis in VCaP xenograft model.

In summarize, following the enthusiasm of the efficacy of the second generation anti-androgen as enzalutamide, the researchers have to keep searching the next generation therapeutic strategy for CRPC. According to the knowledge we have, besides the common recognized castration resistance mechanism of the maintenance of AR signaling, including AR amplification, abnormal activation, mutation and alternative splicing, there are several other mechanisms which bypass AR signaling, such as cancer stem cell involvement and neuroendocrine cell enrichment. Different strategies have to be applied according to various mechanisms.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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