Connecting androgen receptor signaling and the DNA damage response: Development of new therapies for advanced prostate cancer

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ABSTRACT

Androgen receptor-mediated cell signaling involves complex molecular pathways that are interconnected with DNA damage response, including replication stress-driven DNA repair. Understanding the relationships between androgen receptor signaling and DNA damage response at the molecular level will likely lead to novel and effective combination therapy for advanced prostate cancer.

Introduction

Almost all men with prostate cancer (PCa) who initially respond to androgen deprivation therapy (ADT) eventually develop resistance to ADT and progress to metastatic castration-resistant prostate cancer (mCRPC). Enzalutamide (ENZ), an androgen receptor (AR)-signaling inhibitor, maximally suppresses androgen signaling in PCa cells, in part, through regulation of gene expression. In clinical studies, ENZ was shown to prolong survival of the patients by 4.8 months. Unfortunately, ENZ resistance invariably arises and the disease continues to progress. The development of effective combination therapy strategies for mCRPC is clearly needed.

Recent studies have demonstrated that approximately 25% of mCRPC harbors genomic alterations in DNA damage response (DDR) genes. Importantly, recent publications have shown that AR signaling upregulates expression of specific DDR genes, and that these genes are associated with PCa metastasis.

We recently showed that ENZ and AZD7762 (checkpoint kinase 1/2 (CHK1/2) inhibitor) combination treatment generates significant therapeutic effects in preclinical models of CRPC. In this study we initially confirmed AR-mediated transcriptional activation of cell division cycle 6 (CDC6), and showed that CDC6 was upregulated in metastatic PCa tissues and was positively correlated with AR expression. In addition to its well-documented function in the assembly of pre-replication complexes, CDC6 facilitates ATR serine/threonine kinase (ATR) binding to chromatin and activation of ATR-CHK1 signaling and cell cycle checkpoint in Xenopus. We were hopeful we could demonstrate that CDC6 was regulated by AR and linked to ATR-CHK1 signaling in human PCa cells. We reasoned that inhibiting ATR-CHK1 signaling at multiple points in the signaling cascade, i.e., by ENZ and AZD7762, would result in maximum suppression of G2/M checkpoint activation and lead to marked PCa cell death. We demonstrated that AR or CDC6 knockdown together with the CHK1/2 inhibitor AZD7762 suppressed topoisomerase (DNA) II-binding protein 1 (TOPBP1)-ATR-CHK1 signaling, and led to marked apoptosis in both VCaP cells which harbor a p53 mutation, and in C4–2B cells which are p53 wild-type. This result was important since our results showed that neither AR knockdown nor AZD7762 single agent treatment promoted apoptosis in C4–2B cells. It was anticipated that AR knockdown alone may have minimal proapoptotic activity in C4–2B cells, which are androgen-independent, despite a functional AR. It was also expected that that AZD7762 would have reduced activity in C4–2B cells compared with that in VCaP cells, since it is known that fully functional tumor protein p53 (TP53, best known as p53) can counteract the effects of CHK1 inhibitors under some conditions. We demonstrated the capacity of p53 to block C4–2B cells in G0–G1, confirming that AR knockdown and AZD7762 combination treatment effects occurred within the context of functional p53. These studies show that the AR-CDC6-ATR-CHK1-signaling axis can be successfully targeted regardless of p53 status in CRPC. We also documented a novel effect of AZD7762 in both VCaP and C4–2B cells, i.e., AR or CDC6 knockdown together with AZD7762 markedly reduced TOPBP1 protein levels, and that TOPBP1 knockdown significantly increased the sensitivity of VCaP and C4–2B cells to AZD7762. These results revealed an additional target of AZD7762, i.e., TOPBP1, for future consideration.

To translate our gene-based signaling studies to a clinical context we completed additional experiments using ENZ together with AZD7762 in signaling analysis and efficacy studies using multiple cell line and patient-derived PCa
Figure 1. The CDC6-mediated DNA damage response as a target for novel therapeutic options against aggressive prostate cancer. Recent studies have shown that targeting AR–CDC6–ATR–CHK1 signaling with enzalutamide and AZD7762 combination treatment leads to superior growth suppression in castration-resistant prostate cancer models. These studies provide a template for future research that aims to identify novel AR and DDR-targeting therapies for advanced and drug-resistant prostate cancer. AR, androgen receptor; CDC6, cell division cycle 6; ATR, ATR serine/threonine kinase; CHK1, checkpoint kinase 1.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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