MOLECULAR & CELLULAR ONCOLOGY
2017, VOL. 4, NO. 4, e1321167 (2 pages)
https://doi.org/10.1080/23723556.2017.1321167

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AUTHOR'S VIEW

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.

ARTICLE HISTORY
Received 11 April 2017
Revised 12 April 2017
Accepted 14 April 2017

KEYWORDS
ATR-CHK1; combination therapy; enzalutamide; mCRPC

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).[1] Enzalutamide (ENZ), an androgen receptor (AR)-signaling inhibitor, maximally suppresses androgen signaling in PCa cells, in part, through regulation of gene expression.[2] In clinical studies, ENZ was shown to prolong survival of the patients by 4.8 months.[3] 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.[4] Importantly, recent publications have shown that AR signaling upregulates expression of specific DDR genes,[5-7] and that these genes are associated with PCa metastasis.[7]

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.[8] 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.[9] 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 proapopotic 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

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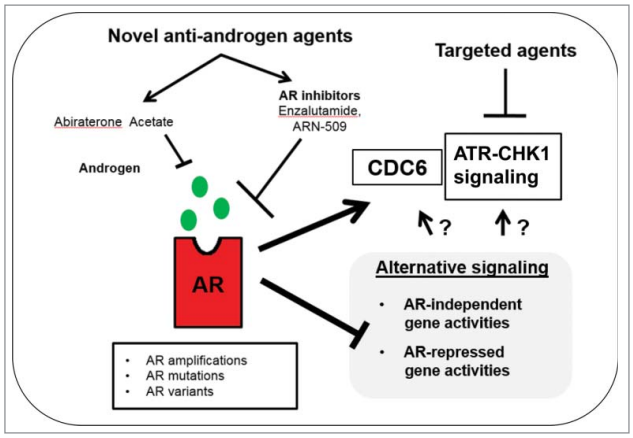

Figure 1. The CDC6-mediated DNA damage response as a target for novel therapeutic options against aggressive prostate cancer. Recent studies have shown that targeting AR-CDC6-ATR-CHK1 signaling with enzalutamide and AZD7762 combination treatment leads to superior growth suppression in castration-resistant 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.

xenograft models. The results of in vitro studies showed that ENZ and AZD7762 combination treatment suppressed TOPBP1-ATR-CHK1 signaling, and the results were comparable to those from *AR* or *CDC6* knockdown together with AZD7762. Importantly, we further showed that overexpression of *CDC6* blocked the proapoptotic activities of ENZ and AZD7762 combination treatment, further validating the role of CDC6 as an indirect therapy target. The results of in vivo xenograft studies showed that ENZ and AZD7762 combination treatment can result in synergistic tumor growth suppression, and established a foundation for clinical trials that target ATR-CHK1 signaling in combination with AR inhibition.

In a broader context, the results of this study establish new AR-DDR molecular connections that can be considered for therapy targeting, i.e., the direct AR target gene, CDC6, and downstream ATR-CHK1 signaling. During PCa progression AR functions as a driver of growth and survival through multiple mechanisms, including upstream alterations in androgen synthesis, *AR* gene alterations, and aberrant AR signaling. These activities are strongly selected for during the development and persistence of CRPC. The recent findings that AR targets include multiple DDR genes,[5-7] and that DDR genes are upregulated in PCa metastasis establish the concept that AR-driven DDR is a survival pathway in CRPC.[7] Our recent publication that demonstrates AR-CDC6-ATR-CHK1 signaling as an important survival pathway and drug target in CRPC strengthens this concept and extends the AR-DDR connection.[8] We showed that CDC6 "connects" AR and ATR-CHK1 signaling, meaning that both *AR* knockdown and *CDC6* knockdown, as well as the AR inhibitor ENZ show similar patterns of suppression of ATR-CHK1 signaling, and complement the proapoptotic activities of AZD7762 (Fig. 1). Interestingly, recent studies show that CDC6 can contribute to cisplatin resistance in bladder cancer cells.[10] It is interesting to speculate that CDC6 may also play a role in resistance to ENZ. A possible mechanism of adaptive drug resistance could potentially involve AR target gene derepression, similar to that which we described previously for upregulation of DDR genes in AR-independent PCa.[7] These AR-repressed oncogenic activities may be de-repressed by androgen-signaling inhibitors and lead to resistance mechanisms that involve CDC6 (Fig. 1).

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Funding

We acknowledge the support of NCI MD Anderson Prostate Cancer SPORE Grant P50 CA140388, NCI Cancer Center Support Grant P30 CA16672, and the MD Anderson Moon Shot Program.

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