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<b>14. ABSTRACT</b> There has been a major focus on the androgen receptor (AR) pathway as the principal therapeutic target for CRPC including recently approved therapies such as next-generation antiandrogen enzalutamide and abiraterone. Despite these advances that provide temporary respite, almost all patients will go on to die from progressive and resistant prostate cancer. Therefore, there is an urgent need to identify resistant pathways that perpetuate disease progression. We provided preliminary data demonstrating that p52 increases AR variant V7 (AR-V7) expression and enhances prostate cancer cell resistance to next-generation antiandrogen enzalutamide treatment. We hypothesize that overexpression of p52 signaling activates resistance pathways to enzalutamide and co-targeting p52 will overcome treatment resistance. In this project, we will examine the potential mechanisms underlying p52-mediated treatment resistance (Aim 1). Aim 2 will validate the efficacy of co-targeting p52 to overcome treatment resistance to enzalutamide. We hope to identify the mechanisms of adaptive/resistant pathways that are responsible for enzalutamide resistance, and provide a rationale for therapeutic co-targeting to overcome enzalutamide resistance.					
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## Introduction

**Background:** Accumulating evidence suggests that abnormal activation of androgen receptor (AR) including AR variants such as AR-V7 contributes to castration-resistant prostate cancer (CRPC) growth. There has been a major focus on the androgen receptor (AR) pathway as the principal therapeutic target for CRPC including recently approved therapies such as next-generation antiandrogen enzalutamide and abiraterone. Despite these advances that provide temporary respite, almost all patients will go on to die from progressive and resistant prostate cancer. Therefore, there is an urgent need to identify resistant pathways that perpetuate disease progression during an effective AR blockade. NF- $\kappa$ B functions as a master transcription factor in regulating the expression of genes implicated in cell survival and chemo resistance. Numerous studies demonstrate that non-canonical NF- $\kappa$ B2/p52 (p52) is overexpressed in prostate cancer and overexpression of p52 facilitates CRPC progression through activating AR signaling and protecting cells from apoptotic death. We provided preliminary data demonstrating that p52 increases AR variant V7 (AR-V7) expression and enhances prostate cancer cell resistance to next-generation antiandrogen enzalutamide treatment.

**Hypothesis:** We hypothesize that overexpression of p52 signaling activates resistance pathways to enzalutamide and co-targeting p52 will overcome treatment resistance.

**Specific aims:** 1. Determine the potential mechanisms of p52-mediated treatment resistance in prostate cancer cells. 2. Co-targeting p52 to overcome treatment resistance to enzalutamide.

## Keywords

NF- $\kappa$ B2/p52, Androgen receptor, Variants, enzalutamide, resistance

## Accomplishments

We have made significant progress in **Major Task 2 and 3: Determining the potential mechanisms underlying p52-mediated AR-V7 production.** We discovered NF- $\kappa$ B2/p52:c-Myc:hnRNPA1 pathway regulates expression of androgen receptor splice variants and enzalutamide sensitivity in prostate cancer. Castration resistant prostate cancer (CRPC) remains dependent on androgen receptor (AR) signaling. Alternative splicing of the AR to generate constitutively active, ligand-independent variants is one of the principal mechanisms that promote the development of resistance to next-generation anti-androgens such as enzalutamide. We demonstrate that the splicing factor heterogeneous nuclear RNA-binding protein A1 (hnRNPA1) plays a pivotal role in the generation of AR splice variants such as AR-V7. HnRNPA1 is overexpressed in prostate tumors compared to benign prostates and its expression is regulated by NF- $\kappa$ B2/p52 and c-Myc. CRPC cells resistant to enzalutamide exhibit higher levels of NF- $\kappa$ B2/p52, c-Myc, hnRNPA1, and AR-V7. Levels of hnRNPA1 and of AR-V7 are positively correlated with each other in PCa. The regulatory circuit involving NF- $\kappa$ B2/p52, c-Myc and hnRNPA1 plays a central role in the generation of AR splice variants. Downregulation of hnRNPA1 and consequently of AR-V7 resensitizes enzalutamide-resistant cells to enzalutamide, indicating that enhanced expression of hnRNPA1 may confer resistance to AR-targeted therapies by promoting the generation of splice variants. These findings may provide a rationale for co-targeting these pathways to achieve better efficacy through AR blockade

We have made some progress for major **task 4 and 5: Examine the effect of inhibition of p52 signaling to improve enzalutamide treatment.** Having demonstrated that p52-mediated AR-V7 activation is through upregulation of hnRNPA1, which resulting in conferring resistance to enzalutamide, we next tried to inhibit hnRNPA1 expression and test if downregulation of hnRNPA1 could resensitize the resistant cells to enzalutamide treatment. We discovered that quercetin, a naturally occurring polyphenolic compound, reduces the expression of hnRNPA1, and consequently, that of AR-V7 (Fig 1). The suppression of AR-V7 by quercetin resensitizes enzalutamide-resistant prostate cancer cells to treatment with enzalutamide. Our results indicate that quercetin downregulates hnRNPA1 expression, downregulates the expression of AR-V7, antagonizes androgen receptor signaling, and resensitizes enzalutamide-resistant prostate cancer cells to enzalutamide treatment in vivo in mouse xenografts. These findings demonstrate that suppressing the alternative splicing of the androgen receptor may have important implications in overcoming the resistance to next-generation anti-androgen therapy (*Mol Cancer Ther Jul 20, 2017. PMID: 28729398*).

Figure 1

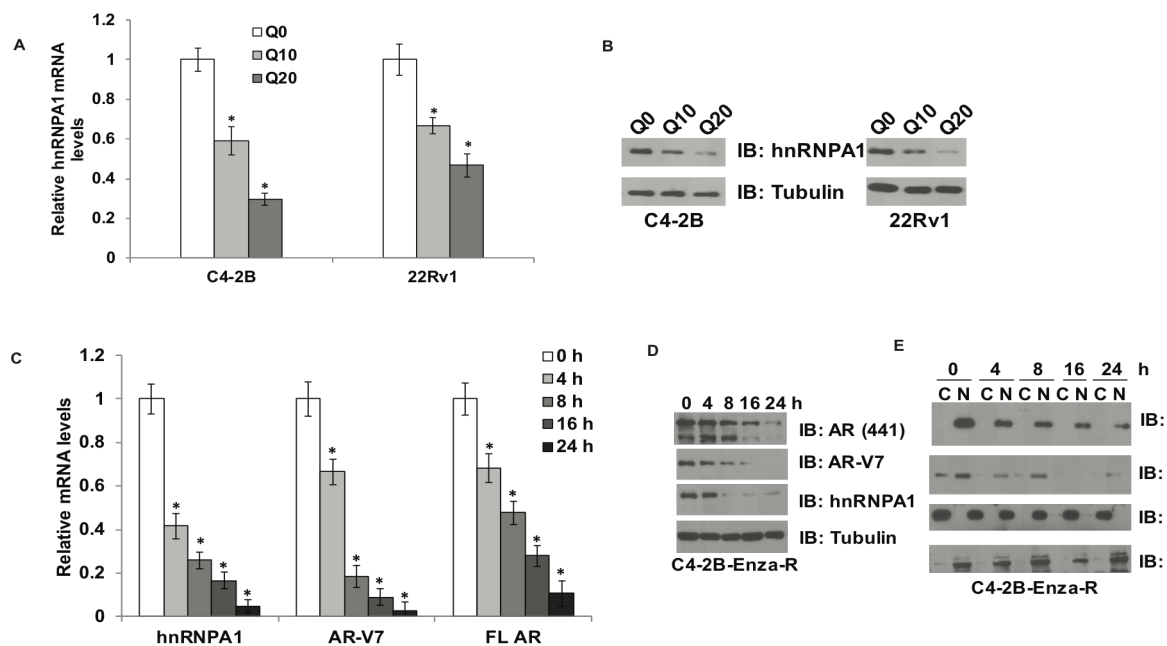


Figure 1: Quercetin downregulates hnRNPA1, FL AR, and AR-V7. C4-2B and CWR22Rv1 cells were treated with 10 or 20 μM quercetin and mRNA (A) and protein (B) levels of hnRNPA1 were analyzed using qRT-PCR and western blotting, respectively. Quercetin induced a dose dependent decrease in both mRNA and protein levels of hnRNPA1 in both cell lines tested. C4-2B-Enza-R cells were treated with 20 μM quercetin for 0, 4, 8, 16, or 24 h and mRNA (C) and protein (D) levels of hnRNPA1, FL AR, and AR-V7 were analyzed using qRT-PCR and western blotting, respectively. Quercetin induced a time-dependent decrease in hnRNPA1, FL AR, and AR-V7 levels. E) C4-2B-Enza-R cells were treated with 20 μM quercetin for 0, 4, 8, 16, or 24 h and cytoplasmic and nuclear extracts were prepared. Protein levels of hnRNPA1 and AR-V7 were analyzed in the extracts using western blotting. Quercetin induced a time-dependent decrease in the total levels of both hnRNPA1 and AR-V7. Reduction in the nuclear transport of hnRNPA1 accompanied by

cytoplasmic retention was not observed as reported earlier. Results are presented as means  $\pm$  SD of 3 independent experiments performed with triplicates. \* denotes  $p \leq 0.05$ .

We plan to target p52 in combination with enzalutamide in enzalutamide resistant C4-2BMDVR cells in vitro and in vivo. We will test if quercetin will downregulate p52 signaling resulting in overcoming p52-mediated resistance to enzalutamide. We will also design specific siRNAs targeting p52 and generate bioengineered p52 siRNAs for in vitro and in vivo testing.

#### **Key outcomes:**

- We demonstrated that p52 enhances enzalutamide resistance.
- We demonstrated that p52 induces ARv7 expression.
- We demonstrated that p52 induced enzalutamide resistance is mediated by ARv7.
- We demonstrated that p52 regulates AR-V7 expression via Lin28/let7c
- We demonstrated that enzalutamide resistant cells express higher levels of p52, ARv7, Lin28.
- We demonstrated NF-kappaB2/p52:c-Myc:hnRNPA1 pathway regulates expression of androgen receptor splice variants and enzalutamide sensitivity in prostate cancer.
- We discovered that quercetin, a naturally occurring polyphenolic compound, reduces the expression of hnRNPA1, and consequently, that of AR-V7. Treatment of enzalutamide resistant prostate cancer cells with quercetin resensitizes the resistant cells to enzalutamide treatment.

#### **Impact**

This proposes studies will not only uncover a novel pathway involved in resistant CRPC development, but may also provide proof-of-concept experiments for future development of therapies targeting resistant pathways that are responsible for acquired treatment resistance, and to increase the magnitude and duration of the benefits of second-generation antiandrogen.

#### **Changes/problems**

N/A

#### **Products**

##### Publications:

1. Nadiminty N., Tummala R, Liu C, Lou W, Evans CP, Gao AC. NF-kB/p52:c-myc:hnRNPA1 pathway regulates expression of androgen receptor splice variants and enzalutamide sensitivity in prostate cancer. *Mol Cancer Ther.* 14:1884-95, 2015. PMID: 26056150.
2. Tummala R, Lou W, Gao AC, Nadiminty N. Quercetin targets hnRNPA1 to overcome enzalutamide resistance in prostate cancer. *Mol Cancer Ther* Jul 20, 2017. PMID: 28729398.

**Participants & other collaborating organizations**

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**Special reporting requirements**

N/A

**Appendices**

N/A