Cross-resistance among next generation anti-androgen drugs through the AKR1C3/AR-V7 axis in advanced prostate cancer

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ABSTRACT

With the emergence of Enzalutamide and Darolutamide, there are currently four next generation anti-androgen drugs approved in the treatment of advanced prostate cancer patients. However, whether cross-resistance exists among these agents with similar mechanisms of action is not fully understood. In this study we found that enzalutamide and abiraterone resistant prostate cancer cells are further cross-resistant to apalutamide and darolutamide. Mechanistically, we have determined that the AKR1C3/AR-V7 axis confers this cross-resistance. Knockdown of AR-V7 in enzalutamide resistant cells re-sensitizes cells to apalutamide and darolutamide treatment. Furthermore, targeting AKR1C3 re-sensitizes resistant cells to apalutamide and darolutamide treatment through AR-V7 inhibition. Chronic apalutamide treatment in C4-2B cells activates the steroid hormone biosynthesis pathway and increases AKR1C3 expression which confers resistance to enzalutamide, abiraterone and darolutamide.

INTRODUCTION

Treatment strategies for castration-resistant prostate cancer (CRPC) have evolved over the past decade. The next generation anti-androgens, XTANDIR (Enzalutamide), ZYDAG® (Abiraterone acetate), ERELEADA™ (Apalutamide) and NUBO-020 (Darolutamide) extend survival times and improve quality of life in advanced prostate cancer patients. Despite these advances, resistance occurs frequently and there is currently no definitive cure for CRPC. In addition, more therapeutic options become available with additional possibilities of diverse sequential treatments. However, identifying the key factors responsible for the cross-resistance among different therapies is also essential for determining the optimal long-term sequential treatment schemes for patients with CRPC. Our previous studies identified that similar mechanisms of resistance to enzalutamide or abiraterone occur following treatment and cross-resistance exists between these therapies in advanced prostate cancer. In this study, we will investigate the role of the AKR1C3/AR-V7 axis in apalutamide and darolutamide resistance.

Enzalutamide/apalutamide-resistant prostate cancer cells are cross-resistant to apalutamide and darolutamide

Enzalutamide (Enzlamutamide) and Darolutamide (Darolutamide) are a second and third generation of second-generation androgen receptor (AR) antagonists. Enzalutamide and abiraterone- resistant prostate cancer cells are further cross-resistant to apalutamide and darolutamide. Mechanistically, we have determined that the AKR1C3/AR-V7 axis confers this cross-resistance. Knockdown of AR-V7 in enzalutamide resistant cells re-sensitizes cells to apalutamide and darolutamide treatment. Furthermore, targeting AKR1C3 re-sensitizes resistant cells to apalutamide and darolutamide treatment through AR-V7 inhibition. Chronic apalutamide treatment in C4-2B cells activating the steroid hormone biosynthesis pathway and increases AKR1C3 expression which confers resistance to enzalutamide, abiraterone and darolutamide.

AR-V7 confers apalutamide and darolutamide resistance

APAL and DARO are more potent and selective AR inhibitors relative to ENZ and ABIR.

Targeting AKR1C3 resensitizes resistant cells to apalutamide and darolutamide treatment through AR-V7 inhibition

TARGETING AKR1C3 RESSENSITIZES RESISTANT CELLS TO APALUTAMIDE AND DARO.

CONCLUSIONS

- Enzalutamide and abiraterone resistant prostate cancer cells are further cross-resistant to apalutamide and darolutamide.
- Apalutamide and darolutamide share similar resistance mechanisms with enzalutamide and abiraterone.
- The AKR1C3/AR-V7 complex confers cross-resistance to second generation AR-targeted therapies in advanced prostate cancer.

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Figure 1. Enzalutamide-resistant prostate cancer cells are cross-resistant to apalutamide and darolutamide. A. C4-2B parental, C4-2B ENZ and CWR22Rv1 cells were treated with abiraterone (AB) or enzalutamide (ENZ) or control (CTR). The survival rate was calculated. B. C4-2B parental, C4-2B ENZ and CWR22Rv1 cells were treated with abiraterone (AB) or darolutamide (DARO) or control (CTR). The survival rate was calculated. C. C4-2B parental, C4-2B ENZ and CWR22Rv1 cells were treated with abiraterone (AB) or apalutamide (APAL) or control (CTR). The survival rate was calculated. D. C4-2B parental, C4-2B ENZ and CWR22Rv1 cells were treated with abiraterone (AB) or enzalutamide (ENZ) or control (CTR). The survival rate was calculated. E. C4-2B parental, C4-2B ENZ and CWR22Rv1 cells were treated with abiraterone (AB) or apalutamide (APAL) or control (CTR). The survival rate was calculated. *p<0.05.

Figure 2. AR-V7 confers apalutamide and darolutamide resistance. (A) C4-2B parental, C4-2B APAL and CWR22Rv1 cells were treated with 20 µM apalutamide (APAL) or darolutamide (DARO) or control (CTR) for 3 days. Whole cell lysates were collected and subjected to Western blotting. (B) C4-2B parental, C4-2B APAL and CWR22Rv1 cells were treated with 20 µM apalutamide (APAL) or darolutamide (DARO) or control (CTR) for 3 days. Whole cell lysates were collected and subjected to Western blotting. (C) C4-2B parental, C4-2B APAL and CWR22Rv1 cells were treated with 20 µM apalutamide (APAL) or darolutamide (DARO) or control (CTR) for 3 days. Whole cell lysates were collected and subjected to Western blotting.

Figure 3. AR-V7 confers apalutamide and darolutamide resistance through AR-V7 regulation. A. C4-2B APAL and DARO cells were infected with lentiviral shRNA against AKR1C3 or lentiviral control shRNA and then treated with 20 µM apalutamide (APAL) or darolutamide (DARO) or control (CTR) for 3 days. Whole cell lysates were collected and subjected to Western blotting. (B) C4-2B APAL and DARO cells were infected with lentiviral shRNA against AKR1C3 or lentiviral control shRNA and then treated with 20 µM apalutamide (APAL) or darolutamide (DARO) or control (CTR) for 3 days. Whole cell lysates were collected and subjected to Western blotting.

Figure 4. Chronic apalutamide treatment in C4-2B cells upregulates the steroid biosynthesis pathway. C4-2B parental and C4-2B APAL cells were cultured in the absence or presence of apalutamide or control (CTR) for 3 days, then collected and subjected to Western blotting. (A) Whole cell lysates were subjected to Western blotting. (B) Whole cell lysates were subjected to Western blotting. (C) Whole cell lysates were subjected to Western blotting. *p<0.05.

Figure 5. Targeting AKR1C3 resensitizes resistant cells to apalutamide and enzalutamide. A. C4-2B parental, C4-2B APAL, C4-2B DARO and C4-2B ENZ cells were treated with 20 µM apalutamide (APAL) or enzalutamide (ENZ) or control (CTR) for 3 days. Whole cell lysates were collected and subjected to Western blotting. (B) C4-2B parental, C4-2B APAL, C4-2B DARO and C4-2B ENZ cells were treated with 20 µM apalutamide (APAL) or enzalutamide (ENZ) or control (CTR) for 3 days. Whole cell lysates were collected and subjected to Western blotting. (C) C4-2B parental, C4-2B APAL, C4-2B DARO and C4-2B ENZ cells were treated with 20 µM apalutamide (APAL) or enzalutamide (ENZ) or control (CTR) for 3 days. Whole cell lysates were collected and subjected to Western blotting.

Chronic apalutamide treatment in C4-2B cells upregulates the steroid biosynthesis pathway

Targeting AKR1C3 resensitizes resistant cells to apalutamide and enzalutamide

Following treatment, cells were treated with 20 µM apalutamide (APAL) or 5 µM darolutamide (DARO) or control (CTR) for 3 days. Whole cell lysates were collected and subjected to Western blotting.

Apalutamide and darolutamide share similar resistance mechanisms with enzalutamide and abiraterone.

The AKR1C3/AR-V7 complex confers cross-resistance to second generation AR-targeted therapies in advanced prostate cancer.

Merits

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