Published OnlineFirst October 28, 2016; DOI: 10.1158/1535-7163.MCT-16-0186

in Fig. 2E, Indocin significantly reduced testosterone levels in C4-2B MDVR and CWR22Rv1 cells.

To examine if exogenous expression of AKR1C3 increases testosterone level and upregulates AR transcriptional activity, LNCaP-neo and LNCaP-AKR1C3 cells were cultured in phenol red–free and serum-free conditions for 3 days and testosterone levels were measured by LC-MS. LNCaP-AKR1C3 cells express significantly higher levels of testosterone than parental LNCaP cells (Fig. 3A). AR transcriptional activity was examined in LNCaP-neo and LNCaP-AKR1C3 cells by real-time PCR, Western blot, and ChIP assay. As shown in Fig. 3B–D, LNCaP-AKR1C3 cells express higher PSA at both the mRNA and protein level and exhibit enhanced AR recruitment to the PSA promoter compared with LNCaP-neo cells. These data suggest that AKR1C3 not only functions as a steroidogenesis enzyme that induces androgen production but also influences AR transcriptional activity, possibly through AR overexpression and AR coactivation, and thus conferring resistance to abiraterone.

Abiraterone-resistant prostate cancer cells express higher levels of AKR1C3

To further confirm our hypothesis that AKR1C3 plays a pivotal role in abiraterone resistance, we generated an abiraterone acetate–resistant prostate cancer cell line by continuous culture of C4-2B cells in media containing abiraterone acetate. As shown in Fig. 4A, after 12 months of being cultured in media containing abiraterone acetate, C4-2B AbiR cells exhibited more resistance to abiraterone acetate treatment than C4-2B parental cells. Abiraterone acetate significantly suppressed cell growth in C4-2B parental cells in a dose-dependent manner, while abiraterone acetate had moderate effect on C4-2B AbiR cells. This result was also confirmed by clonogenic assay. Abiraterone acetate significantly inhibited the number of colonies in C4-2B parental cells in a dose-dependent manner compared with C4-2B AbiR cells (Fig. 4B). C4-2B AbiR cells express higher levels of AKR1C3 mRNA and protein than C4-2B parental cells (Fig. 4C). C4-2B AbiR cells also express higher levels of AR protein than C4-2B parental cells. Next, we examined if knockdown of AKR1C3 expression in C4-2B AbiR cells could resensitize these cells to abiraterone. Knockdown of AKR1C3 expression using two different AKR1C3 shRNA significantly resensitized C4-2B AbiR cells to abiraterone treatment (Fig. 4D). These data suggest chronic abiraterone treatment induces AKR1C3 overexpression, which in turn confers resistance to abiraterone.

Targeting AKR1C3 with Indocin enhances abiraterone treatment *in vitro* and *in vivo*

To examine if targeting AKR1C3 enhances abiraterone treatment, we treated CWR22Rv1 cells with Indocin, an AKR1C3 inhibitor (12), in conjunction with abiraterone. CWR22Rv1 cells were treated with 20 μmol/L Indocin with or without 10 μmol/L abiraterone for 3 days, and total cell numbers were counted. As shown in Fig. 5A, 10 μmol/L abiraterone had limited effects on cell growth, 20 μmol/L Indocin inhibited cell growth, and the combination treatment further inhibited cell growth. These results were also confirmed by clonogenic assay: Indocin

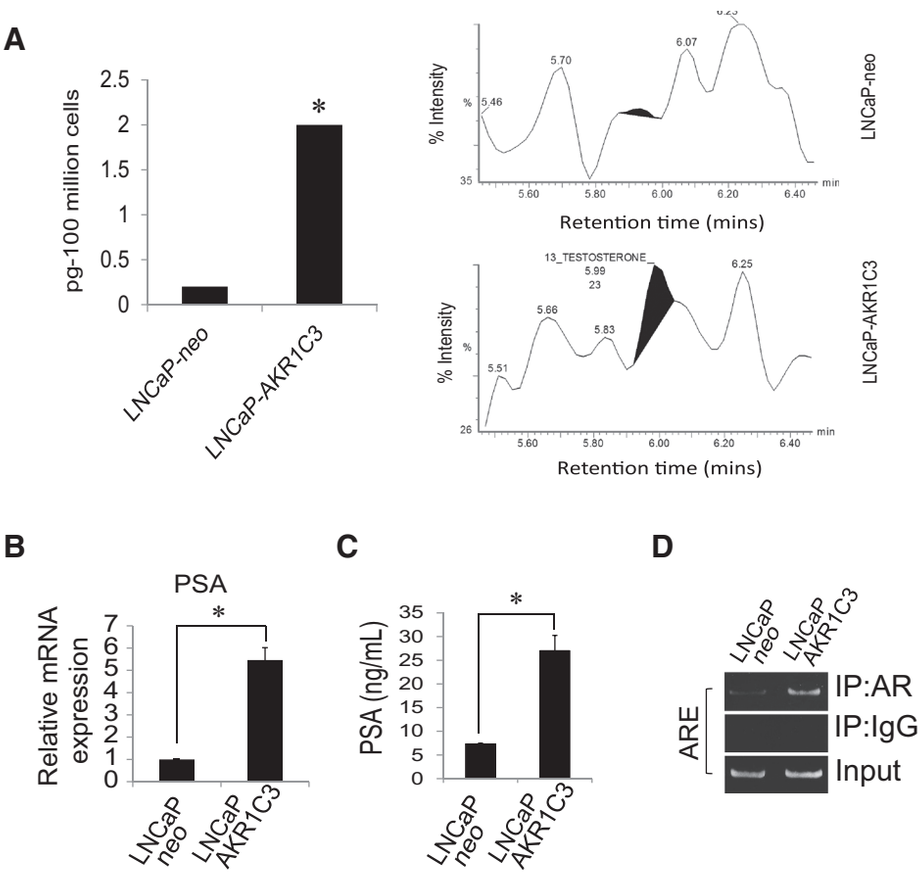

Figure 3.

Exogenous AKR1C3 promotes testosterone production and regulates AR transcriptional activity in prostate cancer. **A,** LNCaP-neo and LNCaP-AKR1C3 cells were cultured in serum-free, phenol red–free RPMI1640 medium for 3 days. One hundred million cells were collected per group, and testosterone level was examined by LC-MS. **B,** LNCaP-neo and LNCaP-AKR1C3 cells were cultured in CS-FBS conditions for 3 days, total RNA was extracted, and PSA mRNA levels were examined by qRT-PCR, **C,** The supernatants were collected and PSA level was examined by PSA ELISA. **D,** LNCaP-neo and LNCaP-AKR1C3 cells were cultured in CS-FBS conditions for 3 days, and whole-cell lysates were subjected to ChIP assay. *, $P < 0.05$.

Published OnlineFirst October 28, 2016; DOI: 10.1158/1535-7163.MCT-16-0186

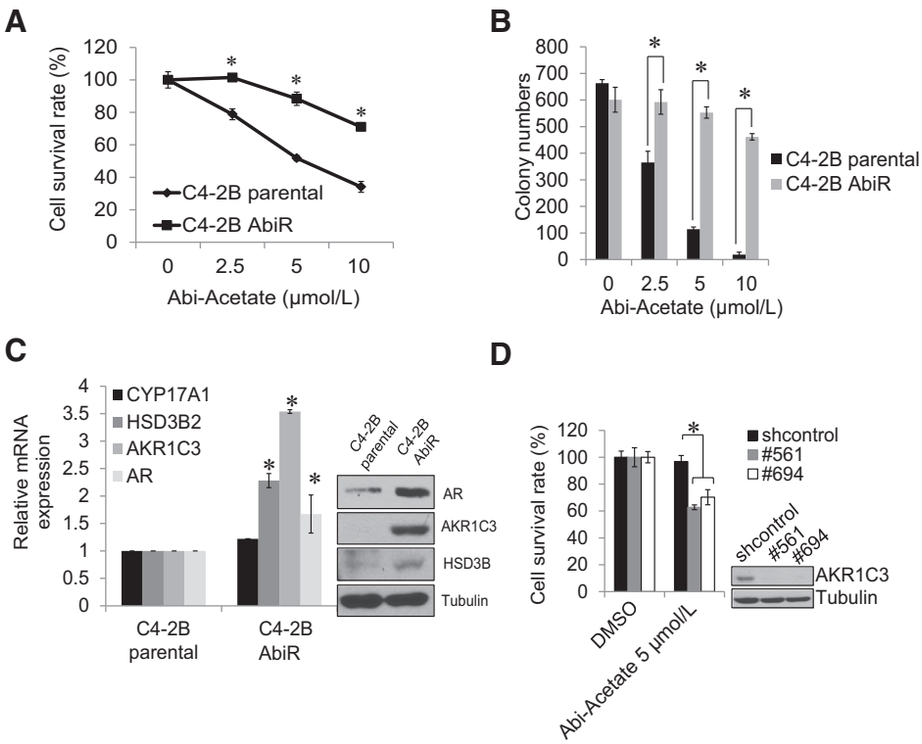

Figure 4.

Abiraterone-resistant prostate cancer cells express higher levels of AKR1C3. **A,** C4-2B parental cells and C4-2B AbiR cells were treated with different concentrations of abiraterone acetate in RPMI 1640 media containing 10% FBS, total cell numbers were counted, and cell survival rate was calculated on day 3. **B,** The clonogenic ability of C4-2B parental and C4-2B AbiR cells treated with 2.5 μmol/L, 5 μmol/L, or 10 μmol/L abiraterone acetate. The colonies were counted and results are presented as mean ± SD of two experiments performed in duplicate, **C,** C4-2B parental cells and C4-2B MDVR cells were cultured in RPMI 1640 media containing 10% FBS for 3 days, total RNA was extracted, and AKR1C3, CYP17A1, HSD3B2, and AR mRNA levels were analyzed by qRT-PCR. Whole-cell lysates were immunoblotted with the indicated antibodies. **D,** C4-2B AbiR cells were transiently transfected with AKR1C3 shRNA (#561 and #694). Following treatment with 5 μmol/L abiraterone acetate for 3 days, total cell numbers were counted and cell survival rate (%) was calculated. Knockdown effects were examined by Western blot. Abi: Abiraterone. *, $P < 0.05$.

combined with abiraterone further inhibited colony numbers and reduced colony size in CWR22Rv1 cells compared with either treatment on its own (Fig. 5B).

To test if inhibition of AKR1C3 activity enhances abiraterone treatment *in vivo*, a CWR22Rv1 xenograft model was developed. As shown in Fig. 5C and D, CWR22Rv1 tumors were resistant to abiraterone. Treatment with Indocin alone significantly inhibited tumor growth and combination of abiraterone and Indocin further inhibited tumor growth compared with either treatment alone. Indocin inhibited Ki67 expression while combination treatment further lowered Ki67 expression (Fig. 5E). These data demonstrate that inhibition of AKR1C3 activity by Indocin overcomes abiraterone resistance, providing preclinical data to support further clinical development of combination therapy of abiraterone and Indocin to treat advanced prostate cancer.

Discussion

Intratumoral androgen biosynthesis is well characterized as a mechanism of CRPC (30–33). Many enzymes are involved in androgen synthesis, including CYP17A1, AKR1C3, and HSD3B. CYP17A1 can be inhibited by abiraterone in clinical treatments (4, 34). However, serum levels of androgens are still high in some patients treated with abiraterone, suggesting that inhibition of androgen synthesis by abiraterone is incomplete, and sustained steroidogenesis contributes to resistance (10, 35, 36). In this study, we demonstrated that AKR1C3 is overexpressed in abiraterone-resistant prostate cancer cells. Overexpression of AKR1C3 increases intracrine androgens and AR expression and induces abiraterone resistance, while downregulation of AKR1C3 sensitizes resistant cells to abiraterone treatment. Furthermore, we demonstrated that Indocin (an AKR1C3 inhibitor) inhibits the levels of intracrine androgens, suppresses AR and AR variants expression, and overcomes abiraterone resistance and enhances abiraterone treatment both *in vitro* and *in vivo*.

Abiraterone inhibits CYP17A1, resulting in the reduction of testosterone levels in advanced prostate cancer patients. However, the serum level of DHEA-S is still in the high range, and may serve as an ample pool for intracrine androgen synthesis (29). Many enzymes involved in androgen synthesis, including AKR1C3 and HSD3B, are elevated in abiraterone-resistant C4-2B AbiR cells. AKR1C3 is one of the most important enzymes in androgen synthesis; it catalyzes the reduction of 5α-dihydrotestosterone to 5α-androstane-3α,17β-dior by its 3α-HSD activity and conversion of androstenedione to testosterone by its 17β-HSD activity (16). We showed that the levels of intracrine androgens are

Published OnlineFirst October 28, 2016; DOI: 10.1158/1535-7163.MCT-16-0186

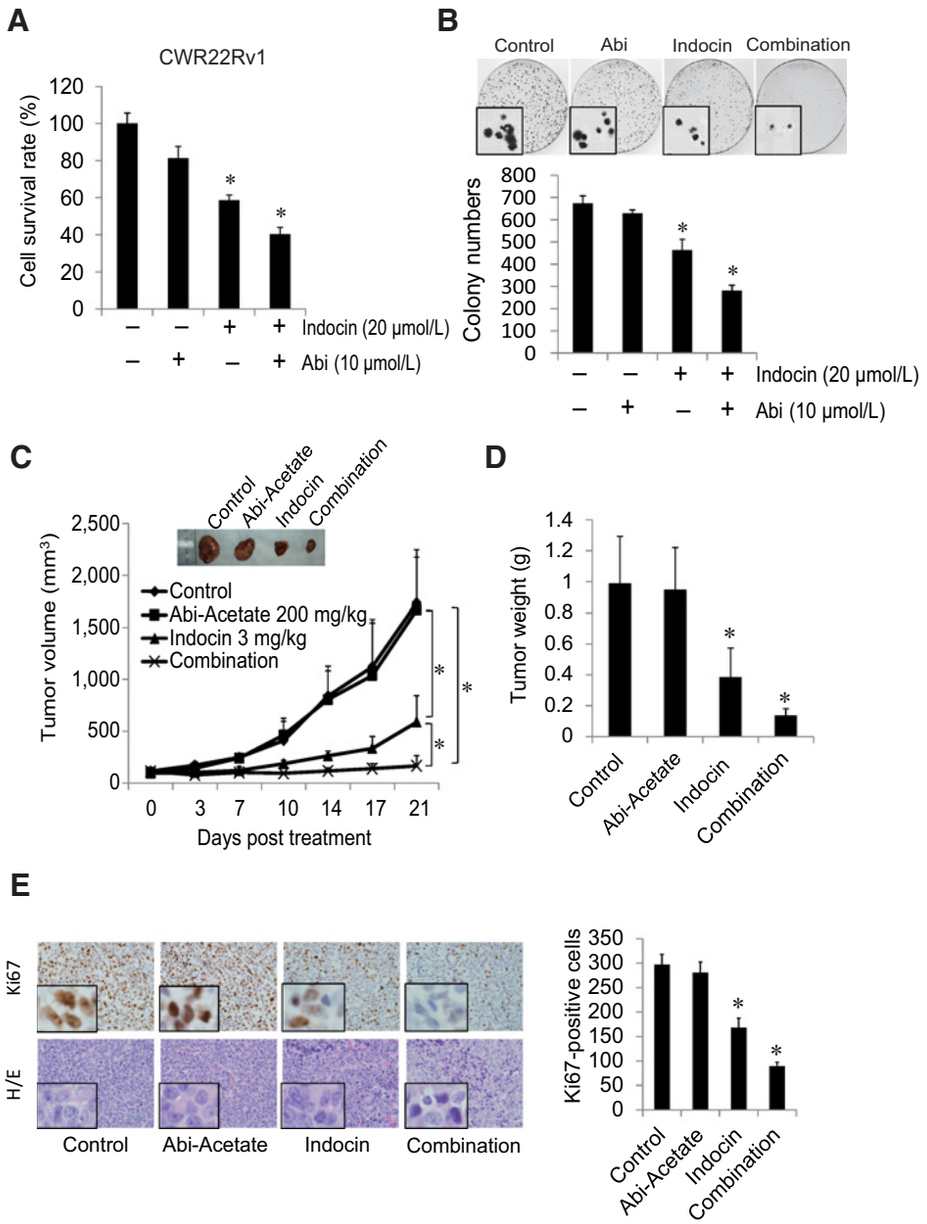

Figure 5.

Indocin enhances abiraterone treatment *in vitro* and *in vivo*. **A,** CWR22Rv1 cells were treated with 20 μmol/L Indocin with or without 10 μmol/L abiraterone for 2 days, and total cell numbers were counted and cell survival rate was calculated. **B,** CWR22Rv1 cells were treated with 20 μmol/L Indocin with or without 10 μmol/L abiraterone, and clonogenic assays were performed; colonies were counted and results are presented as mean ± SD of 2 experiments performed in duplicate, the representative pictures were taken under a microscope, **C,** Mice bearing CWR22Rv1 xenografts were treated with vehicle control, abiraterone acetate (200 mg/Kg p.o), Indocin (3 mg/Kg i.p), or their combination for 3 weeks. Tumor volumes were measured twice weekly. **D,** Each group of tumors was weighed. **E,** IHC staining of Ki67 in each group was performed and quantified. *, $P < 0.05$. Abi, Abiraterone; Indocin, Indomethacin.

elevated in AKR1C3-overexpressing LNCaP-AKR1C3 cells compared with their parental cells, suggesting that AKR1C3 activation results in an increase in androgen synthesis in these cells.

In addition to its function as a critical enzyme in intracrine androgen synthesis, AKR1C3 also catalyzes the formation of prostaglandin (PG) $F_{2\alpha}$ and 11β-$PGF_{2\alpha}$ from PGH2 and PGD2, respectively. The $PGF_{2\alpha}$ and 11β-$PGF_{2\alpha}$ can inactivate proliferator peroxisome activator receptor gamma (PPARγ) and displays antiproliferative effects (37). Recently, the AKR1C3 has been found to directly interact with E3 ubiquitin ligase siah2 and control its stabilization, and thus regulate the siah2-dependent AR signaling (38). Furthermore, AKR1C3 has been found to specifically interact with the AR and is recruited to the ARE on the promoter of androgen responsive genes. AKR1C3 coactivating the AR in prostate cancer tissue might magnify androgen effects (18). This makes the fact that AKR1C3 is pharmacologically targetable in prostate cancer highly important.

AR deregulation has been found in 80% of CRPC patients (39). AR overexpression can confer AR hypersensitivity to low levels of androgen and may contribute to drug resistance (40). In the present study, we found that AR is overexpressed in abiraterone-resistant C4-2B-AbiR and AKR1C3-overexpressing LNCaP-AKR1C3 cells. Knockdown of AKR1C3 expression down regulates full-length AR as well as AR variants expression in these abiraterone-resistant cell lines, suggesting that AKR1C3 regulates AR and AR variants expression. The effects of altered AKR1C3 expression on androgen signaling can be due to a number of potential mechanisms including changes in AR and AR variants expression (41–43), changes in cellular concentration of androgens (12), and/or AKR1C3 functions as a coregulator for AR (18).

Taken together, AKR1C3 is not only an enzyme that catalyzes testosterone synthesis but also affects AR expression and/or functions as an AR coactivator to regulate AR activity.

Both abiraterone and enzalutamide are FDA approved. However, when a patient becomes resistant to one drug, the subsequent response rate to the other drug is 20% or less (43–45). This suggests common resistance mechanisms exist for the observed cross resistance. In previous studies, we demonstrated that AKR1C3 activation increases intracrine androgens and confers resistance to enzalutamide (12). Here, we showed that AKR1C3 activation also induces resistance to abiraterone. Thus, AKR1C3 activation could represent a common resistance mechanism for cross resistance between enzalutamide and abiraterone. We showed that enzalutamide-resistant C4-2B MDVR cells are also resistant to abiraterone treatment, knocking down AKR1C3 expression blocked the recruitment of AR to the AREs and reduced the levels of intracrine androgens, thus resensitizing the cells to abiraterone treatment. These results further the understanding of cross-resistance between abiraterone and enzalutamide in prostate cancer, and provide the groundwork for the development of meaningful treatment strategies by targeting AKR1C3 to enhance next generation antiandrogen therapies.

Indocin, a NSAID used for reducing fever, pain and inflammation, has been shown to inhibit AKR1C3 activity (12, 46). Indocin blocks AKR1C3 function through binding with the AKR1C3 active site (20). Indocin exhibits a strong selectivity for AKR1C3 (8.2 μmol/L) over AKR1C1 and AKR1C2 (over 100 μmol/L; ref. 46). Recently, several derivatives of Indocin, as well as other compounds, were developed that exhibit higher potency to inhibit AKR1C3 activity (47, 48). These, in addition to baccharin (49) and other more specific AKR1C3 inhibitors such as (3-(4-(Trifluoromethyl)phenylamino)benzoic acid), have been identified and characterized (48). We showed that Indocin is able to inhibit the levels of intracrine androgens in C4-2B MDVR cells and CWR22Rv1 cells and suppress prostate cancer tumor growth and enhance enzalutamide and abiraterone treatment, suggesting that Indocin is more potent than SN33638, an inhibitor of AKR1C3, which has limited activity in the inhibition of testosterone production and cell proliferation (50). Indocin has been on the market and safely taken by patients, including prostate cancer patients, for decades. Together, this study and the previous report of Indocin synergizing with enzalutamide therapy pave the way for the development of Indocin as a potential treatment strategy to block AKR1C3 activation to overcome treatment resistance and enhance the therapeutic effect of enzalutamide and abiraterone.

In summary, we have identified AKR1C3 activation as a critical mechanism of resistance to abiraterone through increasing intracrine androgen synthesis and enhancing androgen signaling. Furthermore, this study provides a preclinical proof-of-principle for clinical trials investigating combination treatment of Indocin with abiraterone for advanced prostate cancer.

Disclosure of Potential Conflicts of Interest

C. Liu has ownership interest (including patents) in Patent application covering the use of indomethacin. C.P. Evans reports receiving commercial research grant from, has received honoraria from the speakers bureau of, and is a consultant/advisory board member for, Medivation/Astellas. A.C. Gao has ownership interest (including patents) in a patent application. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: C. Liu, C.P. Evans, A.C. Gao
Development of methodology: C. Liu, A.C. Gao
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): C. Liu, C.M. Armstrong, A. Lombard, A.C. Gao
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): C. Liu, A. Lombard, C.P. Evans, A.C. Gao
Writing, review, and/or revision of the manuscript: C. Liu, C.M. Armstrong, C.P. Evans, A.C. Gao
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): C. Liu, W. Lou, A.C. Gao
Study supervision: C. Liu, C.P. Evans, A.C. Gao

Grant Support

This work was supported in part by grants NIH/NCI CA140468, CA168601, CA179970 (A.C. Gao), DOD PC130062 (A.C. Gao), and US Department of Veterans Affairs, ORD VA Merits I01BX0002653 (A.C. Gao).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received March 30, 2016; revised September 8, 2016; accepted September 28, 2016; published OnlineFirst October 28, 2016.

References

1. Tannock IF, de Wit R, Berry WR, Horti J, Pluzanska A, Chi KN, et al. Docetaxel plus prednisone or mitoxantrone plus prednisone for advanced prostate cancer. N Engl J Med 2004;351:1502–12.
2. de Bono JS, Oudard S, Ozguroglu M, Hansen S, Machiels JP, Kocak I, et al. Prednisone plus cabazitaxel or mitoxantrone for metastatic castration-resistant prostate cancer progressing after docetaxel treatment: A randomised open-label trial. Lancet 2010;376:1147–54.
3. Scher HI, Fizazi K, Saad F, Taplin ME, Sternberg CN, Miller K, et al. Increased survival with enzalutamide in prostate cancer after chemotherapy. N Engl J Med 2012;367:1187–97.
4. de Bono JS, Logothetis CJ, Molina A, Fizazi K, North S, Chu L, et al. Abiraterone and increased survival in metastatic prostate cancer. N Engl J Med 2011;364:1995–2005.
5. Haidar S, Ehmer PB, Barassin S, Batzl-Hartmann C, Hartmann RW. Effects of novel 17alpha-hydroxylase/C17, 20-lyase (P450 17, CYP 17) inhibitors on androgen biosynthesis in vitro and in vivo. J Steroid Biochem Mol Biol 2003;84:555–62.
6. Antonarakis ES, Lu CX, Wang H, Luber B, Nakazawa M, Roeser JC, et al. AR-V7 and resistance to enzalutamide and abiraterone in prostate cancer. New Engl J Med 2014;371:1028–38.
7. Chen EJ, Sowalsky AG, Gao S, Cai C, Voznesensky O, Schaefer R, et al. Abiraterone treatment in castration-resistant prostate cancer selects for progesterone responsive mutant androgen receptors. Clin Cancer Res 2015;21:1273–80.
8. Romanel A, Gasi Tandefelt D, Conteduca V, Jayaram A, Casiraghi N, Wetterskog D, et al. Plasma AR and abiraterone-resistant prostate cancer. Sci Transl Med 2015;7:312re10.
9. Mostaghel EA, Marck BT, Plymate SR, Vessella RL, Balk S, Matsumoto AM, et al. Resistance to CYP17A1 inhibition with abiraterone in castration-resistant prostate cancer: Induction of steroidogenesis and androgen receptor splice variants. Clin Cancer Res 2011;17:5913–25.
10. Chang KH, Li R, Kuri B, Lotan Y, Roehrborn CG, Liu J, et al. A gain-of-function mutation in DHT synthesis in castration-resistant prostate cancer. Cell 2013;154:1074–84.
11. Tian Y, Zhao L, Zhang H, Liu X, Zhao L, Zhao X, et al. AKR1C3 overexpression may serve as a promising biomarker for prostate cancer progression. Diagn Pathol 2014;9:42.
12. Liu C, Lou W, Zhu Y, Yang JC, Nadiminty N, Gaikwad NW, et al. Intracrine androgens and AKR1C3 activation confer resistance to enzalutamide in prostate cancer. Cancer Res 2015;75:1413–22.

13. Labrie F, Luu-The V, Lin SX, Labrie C, Simard J, Breton R, et al. The key role of 17 beta-hydroxysteroid dehydrogenases in sex steroid biology. Steroids 1997;62:148–58.
14. Bauman DR, Steckelbroeck S, Williams MV, Peehl DM, Penning TM. Identification of the major oxidative 3alpha-hydroxysteroid dehydrogenase in human prostate that converts 5alpha-androstane-3alpha,17beta-diol to 5alpha-dihydrotestosterone: a potential therapeutic target for androgen-dependent disease. Mol Endocrinol 2006;20:444–58.
15. Penning TM, Burczynski ME, Jez JM, Hung CF, Lin HK, Ma H, et al. Human 3alpha-hydroxysteroid dehydrogenase isoforms (AKR1C1-AKR1C4) of the aldo-keto reductase superfamily: Functional plasticity and tissue distribution reveals roles in the inactivation and formation of male and female sex hormones. Biochem J 2000;351:67–77.
16. Stanbrough M, Bubley GJ, Ross K, Golub TR, Rubin MA, Penning TM, et al. Increased expression of genes converting adrenal androgens to testosterone in androgen-independent prostate cancer. Cancer Res 2006;66:2815–25.
17. Wako K, Kawasaki T, Yamana K, Suzuki K, Jiang S, Umezu H, et al. Expression of androgen receptor through androgen-converting enzymes is associated with biological aggressiveness in prostate cancer. J Clin Pathol 2008;61:448–54.
18. Yepuru M, Wu Z, Kulkarni A, Yin F, Barrett CM, Kim J, et al. Steroidogenic enzyme AKR1C3 is a novel androgen receptor-selective coactivator that promotes prostate cancer growth. Clin Cancer Res 2013;19:5613–25.
19. Liedtke AJ, Adeniji AO, Chen M, Byrns MC, Jin Y, Christianson DW, et al. Development of potent and selective indomethacin analogues for the inhibition of AKR1C3 (Type 5 17beta-hydroxysteroid dehydrogenase/prostaglandin F synthase) in castrate-resistant prostate cancer. J Med Chem 2013;56:2429–46.
20. Lovering AL, Ride JP, Bunce CM, Desmond JC, Cummings SM, White SA. Crystal structures of prostaglandin D(2) 11-ketoreductase (AKR1C3) in complex with the nonsteroidal anti-inflammatory drugs flufenamic acid and indomethacin. Cancer Res 2004;64:1802–10.
21. Brunelli C, Amici C, Angelini M, Fracassi C, Belardo G, Santoro MG. The non-steroidal anti-inflammatory drug indomethacin activates the eIF2alpha kinase PKR, causing a translational block in human colorectal cancer cells. Biochem J 2012;443:379–86.
22. Tse AK, Cao HH, Cheng CY, Kwan HY, Yu H, Fong WF, et al. Indomethacin sensitizes TRAIL-resistant melanoma cells to TRAIL-induced apoptosis through ROS-mediated upregulation of death receptor 5 and downregulation of survivin. J Invest Dermatol 2014;134:1397–407.
23. Liu C, Lou W, Zhu Y, Nadiminty N, Schwartz C, Evans CP, et al. Niclosamide inhibits androgen receptor variants expression and overcomes Enzalutamide resistance in castration resistant prostate cancer. Clin Cancer Res 2014;20:3198–210.
24. Liu C, Armstrong C, Zhu Y, Lou W, Gao AC. Niclosamide enhances abiraterone treatment via inhibition of androgen receptor variants in castration resistant prostate cancer. Oncotarget 2016;7:32210–20.
25. Gaikwad NW. Ultra performance liquid chromatography-tandem mass spectrometry method for profiling of steroid metabolome in human tissue. Anal Chem 2013;85:4951–60.
26. Liu C, Zhu Y, Lou W, Cui Y, Evans CP, Gao AC. Inhibition of constitutively active Stat3 reverses enzalutamide resistance in LNCaP derivative prostate cancer cells. Prostate 2014;74:201–9.
27. Liu C, Nadiminty N, Tummala R, Chun JY, Lou W, Zhu Y, et al. Andrographolide targets androgen receptor pathway in castration-resistant prostate cancer. Genes Cancer 2011;2:151–9.
28. Liu C, Lou W, Armstrong C, Zhu Y, Evans CP, Gao AC. Niclosamide suppresses cell migration and invasion in enzalutamide resistant prostate cancer cells via Stat3-AR axis inhibition. Prostate 2015;75:1341–53.
29. Tamae D, Mostaghel E, Montgomery B, Nelson PS, Balk SP, Kantoff PW, et al. The DHEA-sulfate depot following P450c17 inhibition supports the case for AKR1C3 inhibition in high risk localized and advanced castration resistant prostate cancer. Chem Biol Interact 2015;234:332–8.
30. Cai C, Chen S, Ng P, Bubley GJ, Nelson PS, Mostaghel EA, et al. Intratumoral de novo steroid synthesis activates androgen receptor in castration-resistant prostate cancer and is upregulated by treatment with CYP17A1 inhibitors. Cancer Res 2011;71:6503–13.
31. Ishizaki F, Nishiyama T, Kawasaki T, Miyashiro Y, Hara N, Takizawa I, et al. Androgen deprivation promotes intratumoral synthesis of dihydrotestosterone from androgen metabolites in prostate cancer. Sci Rep 2013;3:1528.
32. Locke JA, Guns ES, Lubik AA, Adomat HH, Hendy SC, Wood CA, et al. Androgen levels increase by intratumoral de novo steroidogenesis during progression of castration-resistant prostate cancer. Cancer Res 2008;68:6407–15.
33. Mohler JL, Titus MA, Bai S, Kennerley BJ, Lih FB, Tomer KB, et al. Activation of the androgen receptor by intratumoral bioconversion of androstanediol to dihydrotestosterone in prostate cancer. Cancer Res 2011;71:1486–96.
34. Ryan CJ, Smith MR, de Bono JS, Molina A, Logothetis CJ, de Souza P, et al. Abiraterone in metastatic prostate cancer without previous chemotherapy. N Engl J Med 2013;368:138–48.
35. Attard G, Reid AH, Auchus RJ, Hughes BA, Cassidy AM, Thompson E, et al. Clinical and biochemical consequences of CYP17A1 inhibition with abiraterone given with and without exogenous glucocorticoids in castrate men with advanced prostate cancer. J Clin Endocrinol Metab 2012;97:507–16.
36. Li Z, Bishop AC, Alyamani M, Garcia JA, Dreicer R, Bunch D, et al. Conversion of abiraterone to D4A drives anti-tumour activity in prostate cancer. Nature 2015;523:347–51.
37. Desmond JC, Mountford JC, Drayson MT, Walker EA, Hewison M, Ride JP, et al. The aldo-keto reductase AKR1C3 is a novel suppressor of cell differentiation that provides a plausible target for the non-cyclooxygenase-dependent antineoplastic actions of nonsteroidal anti-inflammatory drugs. Cancer Res 2003;63:505–12.
38. Fan L, Peng G, Hussain A, Fazli L, Guns E, Gleave M, et al. The steroidogenic enzyme AKR1C3 regulates stability of the ubiquitin ligase Siah2 in prostate cancer cells. J Biol Chem 2015;290:20865–79.
39. Linja MJ, Savinainen KJ, Saramaki OR, Tammela TL, Vessella RL, Visakorpi T. Amplification and overexpression of androgen receptor gene in hormone-refractory prostate cancer. Cancer Res 2001;61:3550–5.
40. Waltering KK, Helenius MA, Sahu B, Manni V, Linja MJ, Janne OA, et al. Increased expression of androgen receptor sensitizes prostate cancer cells to low levels of androgens. Cancer Res 2009;69:8141–9.
41. Cai C, He HH, Chen S, Coleman I, Wang H, Fang Z, et al. Androgen receptor gene expression in prostate cancer is directly suppressed by the androgen receptor through recruitment of lysine-specific demethylase 1. Cancer Cell 2011;20:457–71.
42. Cai C, Wang H, Xu Y, Chen S, Balk SP. Reactivation of androgen receptor-regulated TMPRSS2:ERG gene expression in castration-resistant prostate cancer. Cancer Res 2009;69:6027–32.
43. Mitsiades N, Sung CC, Schultz N, Danila DC, He B, Eedunuri VK, et al. Distinct patterns of dysregulated expression of enzymes involved in androgen synthesis and metabolism in metastatic prostate cancer tumors. Cancer Res 2012;72:6142–52.
44. Bianchini D, Lorente D, Rodriguez-Vida A, Omlin A, Pezaro C, Ferraldeschi R, et al. Antitumour activity of enzalutamide (MDV3100) in patients with metastatic castration-resistant prostate cancer (CRPC) pre-treated with docetaxel and abiraterone. Eur J Cancer 2014;50:78–84.
45. Azad AA, Eigl BJ, Murray RN, Kollmannsberger C, Chi KN. Efficacy of enzalutamide following abiraterone acetate in chemotherapy-naive metastatic castration-resistant prostate cancer patients. Eur Urol 2015;67:23–9.
46. Byrns MC, Jin Y, Penning TM. Inhibitors of type 5 17beta-hydroxysteroid dehydrogenase (AKR1C3): Overview and structural insights. J Steroid Biochem Mol Biol 2011;125:95–104.
47. Adeniji AO, Chen M, Penning TM. AKR1C3 as a target in castrate resistant prostate cancer. J Steroid Biochem Mol Biol 2013;137:136–49.
48. Adeniji AO, Twenter BM, Byrns MC, Jin Y, Chen M, Winkler JD, et al. Development of potent and selective inhibitors of aldo-keto reductase 1C3 (type 5 17beta-hydroxysteroid dehydrogenase) based on N-phenyl-aminobenzoates and their structure-activity relationships. J Med Chem 2012;55:2311–23.
49. Endo S, Matsunaga T, Kanamori A, Otsuji Y, Nagai H, Sundaram K, et al. Selective inhibition of human type-5 17beta-hydroxysteroid dehydrogenase (AKR1C3) by baccharin, a component of Brazilian propolis. J Nat Prod 2012;75:716–21.
50. Yin YD, Fu M, Brooke DG, Heinrich DM, Denny WA, Jamieson SM. The activity of SN33638, an inhibitor of AKR1C3, on testosterone and 17beta-estradiol production and function in castration-resistant prostate cancer and ER-positive breast cancer. Front Oncol 2014;4:159.

Published OnlineFirst October 28, 2016; DOI: 10.1158/1535-7163.MCT-16-0186

Molecular Cancer Therapeutics

Inhibition of AKR1C3 Activation Overcomes Resistance to Abiraterone in Advanced Prostate Cancer

Chengfei Liu, Cameron M. Armstrong, Wei Lou, et al.

Mol Cancer Ther 2017;16:35-44. Published OnlineFirst October 28, 2016.