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A novel mechanism to drive castration-resistant prostate cancer

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Abstract

Androgen receptor signaling is critical for prostate adenocarcinoma, even after androgen deprivation therapy. Persistence of intratumoral androgens has been found in castration-resistant prostate cancer and attributed to increased *in situ* synthesis. Recently, Sharifi and colleagues reported an additional mechanism that can enhance local androgenic exposure: down-regulation of an androgen-inactivating enzyme.

Keywords

castration-resistant prostate cancer; androgen receptor (AR) signaling; *HSD17B4* variant 2; testosterone; DHT

For the past 75 years, androgen deprivation therapy (ADT), in the form of gonadal androgen suppression via orchiectomy or GnRH analogs, has been the backbone of treatment for advanced and metastatic prostate adenocarcinoma (PC). Despite a high rate of initial responses to ADT, eventually the disease progresses to “castration-resistant” PC (CRPC). Very frequently in that state, androgen receptor (AR) signaling within the PC cell is reactivated due to a wide variety of mechanisms, which include overexpression of AR and its coactivators; persistence of intratumoral androgens; and ligand-independent mechanisms, such as constitutively-active AR variants that lack the LBD (for more details, see [1]), highlighting the importance of the AR axis in this disease.

Several studies have documented substantial androgen levels in CRPC tissues (sometimes even higher than levels present within primary PCs from untreated eugonadal men) despite suppressed circulating testosterone levels (summarized in [1]). This has been largely attributed to increased local expression of enzymes involved in *de novo* androgen synthesis and “intracrine” conversion of adrenal precursors [1, 2], including SRD5A1, SRD5A3 (5 α -reductases type 1 and 3, respectively, that convert testosterone to DHT), and *AKR1C3*. The latter converts the 17-keto steroids DHEA and androstenedione (that are weak androgens

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mainly produced in the adrenals) to the 17 β -OH steroids, including testosterone and, via 5 α -reductase, DHT. Moreover, a nucleotide polymorphism (1245A>C) in HSD3B1 results in a protein variant with increased steady-state levels and promotes androgen synthesis from extragonadal precursors [3].

Androgenic exposure in a tissue represents the net flux of the competing processes of androgen synthesis (both peripherally and locally) and import on one hand versus inactivation and export/clearance on the other (Figure). Thus, suppressed local androgen inactivation can be another mechanism to promote AR signaling in CRPC. The fraction of testosterone and androstenedione that is normally aromatized to estrogens in men (~0.3%) is too small to make a significant contribution to androgen inactivation, and DHT is not even a substrate for aromatase (because of its reduced A ring). This leaves two major pathways accounting for most of testosterone and DHT turnover. The first involves oxidation to 17-keto steroids, a reversible reaction catalyzed by member(s) of the 17 β -Hydroxysteroid dehydrogenase (17 β HSD) family. Of the 14 identified 17 β HSDs, types 2, 4, 8, 10, 11, and 14 are thought to be oxidative enzymes, and types 1, 3, 5, and 7 are reductive enzymes that favor the inverse reaction [4, 5]. The 17-keto steroids are converted to androsterone and its 5 β -epimer etiocholanolone, which are then conjugated via glucuronidation or sulfation and excreted in the urine. The other pathway involves hydroxylation to polar compounds (diols, triols) by the members of the CYP3A family, followed by glucuronidation, catalyzed by the UDP-glucuronosyltransferase (UGT) enzymes UGT2B7, UGT2B15, and UGT2B17 (only the latter two are expressed in the prostate), and excretion in the urine [6]. Decreased inactivation of androgens via the latter pathway has been proposed to occur in advanced PC, evidenced by reduced expression of CYP3A4, CYP3A5, and CYP3A7 in that setting [2, 7]. Furthermore, a UGT2B17 germline deletion polymorphism is common in various populations [8] and has attracted significant interest, yet its possible link to PC risk and biology remains controversial [9]. In any case, the suppression of the CYP3A/UGT2B pathway in CRPC would make androgen inactivation more dependent on the 17-keto steroid pathway.

Recently, Sharifi and colleagues reported an additional mechanism that may enhance local androgenic exposure in CRPC via decreased ligand inactivation [5]. They found that only one of the five *HSD17B4* splice isoforms (isoform 2) harbors enzymatic activity and that it is specifically downregulated in CRPC. After genetically suppressing isoform 2, they further demonstrated that this splice variant normally suppresses AR signaling by inactivating androgens via oxidation at 17 β -OH. This is the inverse step of the reaction catalyzed by AKR1C3 (also known as 17 β HSD5), which leads to synthesis of potent androgens (Figure). Both the frequent upregulation of the reductive enzyme (AKR1C3) and the loss of the oxidative enzyme (*HSD17B4* variant 2) in CRPC favor the 17-keto $\rightarrow$ 17 β -OH direction of this reversible reaction, which would be predicted to result in higher “intracrine” levels of active androgens [5]. In support, 17 β HSD4 silencing in a CRPC xenograft model shifted the equilibrium to a higher ratio of total active androgens compared with their respective inactive 17-keto-steroids, and enhanced tumor progression.

These important observations have several exciting clinical implications and raise follow-up questions: Does the loss of *HSD17B4* variant 2 have clinical prognostic significance in PC

and is it indeed associated with higher tissue androgen levels and AR transcriptional output in CRPC patients? If so, are those CRPCs more likely to respond to a second-generation AR antagonist (e.g. enzalutamide) compared to CRPCs that are driven by ligand-independent mechanisms? Such a finding would propose *HSD17B4* variant 2 as a useful predictive biomarker to guide further use of second-line endocrine therapies in CRPC. It also remains to be examined how the alternative splicing leading to this isoform is dysregulated in CRPC. Is it related to the alternative splicing events responsible for constitutively-active AR variants [1] ? In that case, could the CRPC cell's splicing machinery serve as a common target to pharmacologically restore *HSD17B4* variant 2 expression while suppressing AR variant levels, thus blocking both ligand-dependent and ligand-independent, respectively, AR signaling in CRPC? Are there any other (e.g. epigenetic) approaches to restore the expression of *HSD17B4* variant 2 in CRPC and accelerate DHT turnover *in situ*? Can the CYP3A/UGT2B pathway compensate for suppressed oxidation of 17 β -OH to 17-keto steroids in patients with decreased *HSD17B4* variant 2, or is there concomitant inactivation of both androgen turnover pathways? And finally, we should always consider the possibility that other, non-steroidal substrates may mediate (some of) the effects of these enzymes in PC pathophysiology and cell proliferation. E.g. *HSD17B4* has been reported to have roles in peroxisomal β -oxidation and lipid homeostasis [10].

Taken together, the study of Ko et al. [5] advances our understanding of the complex “intracrine” pathophysiology of CRPC by bringing to light another important adaptation/escape mechanism of PC cells to ADT.

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References

1. Mitsiades N. A road map to comprehensive androgen receptor axis targeting for castrationresistant prostate cancer. *Cancer Res.* 2013; 73(15):4599–605. [PubMed: 23887973]
2. Mitsiades N, et al. Distinct patterns of dysregulated expression of enzymes involved in androgen synthesis and metabolism in metastatic prostate cancer tumors. *Cancer Res.* 2012; 72(23):6142–52. [PubMed: 22971343]
3. Hettel D, Sharifi N. HSD3B1 status as a biomarker of androgen deprivation resistance and implications for prostate cancer. *Nat Rev Urol.* 2017
4. Lukacik P, et al. Structure and function of human 17beta-hydroxysteroid dehydrogenases. *Mol Cell Endocrinol.* 2006; 248(1-2):61–71. [PubMed: 16414178]
5. Ko HK, et al. Loss of an Androgen-Inactivating and Isoform-Specific HSD17B4 Splice Form Enables Emergence of Castration-Resistant Prostate Cancer. *Cell Rep.* 2018; 22(3):809–819. [PubMed: 29346776]
6. Belanger A, et al. Inactivation of androgens by UDP-glucuronosyltransferase enzymes in humans. *Trends Endocrinol Metab.* 2003; 14(10):473–9. [PubMed: 14643063]

7. Leskela S, et al. Cytochrome P450 3A5 is highly expressed in normal prostate cells but absent in prostate cancer. *Endocr Relat Cancer*. 2007; 14(3):645–54. [PubMed: 17914095]
8. Nadeau G, et al. Deletions of the androgen-metabolizing UGT2B genes have an effect on circulating steroid levels and biochemical recurrence after radical prostatectomy in localized prostate cancer. *J Clin Endocrinol Metab*. 2011; 96(9):E1550–7. [PubMed: 21733997]
9. Deng X, et al. Meta-analysis reveals a lack of association between UGT2B17 deletion polymorphism and tumor susceptibility. *PLoS One*. 2014; 9(5):e96812. [PubMed: 24802609]
10. Lieber DS, et al. Next generation sequencing with copy number variant detection expands the phenotypic spectrum of HSD17B4-deficiency. *BMC Med Genet*. 2014; 15:30. [PubMed: 24602372]

Figure. Pathways of steroidogenesis and their role as a source of residual intratumoral androgens in CRPC

The target sites of clinically relevant inhibitors are also shown. Cholesterol is the precursor for all steroidogenesis. CYP11A1 (also known as cholesterol side-chain cleavage enzyme, P450_{scc}) catalyzes the first and rate-limiting enzymatic step in steroidogenesis. Subsequently, CYP17A1 carries two enzymatic activities: 17-hydroxylase and 17,20 lyase. As the target of the FDA-approved agent abiraterone, CYP17A1 has risen to significant prominence in the PC field. The adrenal 17-keto weak androgens dehydroepiandrosterone (DHEA), DHEA sulfate and androstenedione can be converted to more potent 17 β -OH androgens (testosterone and DHT) via several 17 β HSDs, including 17 β HSD5 (*AKR1C3*). *AKR1C3* expression is elevated in advanced PC and also is induced by androgen deprivation [2]. Most PCs probably lack adequate CYP17A1 expression, and, as result, they remain dependent on the contribution of adrenal precursors (as a two-site, “adrenal-PC” steroidogenic unit) [1]. Yet, a small subset of PCs overexpress *CYP11A1*, *CYP17A1*, *HSD3B1*, *HSD3B2*, and *STAR*, and thus may be self-sufficient for de novo steroidogenesis [2].

The two major pathways of testosterone and DHT turnover are: a) Oxidation to 17-keto steroids by member(s) of the 17 β -Hydroxysteroid dehydrogenase (17 β HSD) family, followed by conversion to androsterone and its 5 β -epimer etiocholanolone (Phase I), which

are then conjugated via glucuronidation or sulfation (Phase II) and excreted in the urine; and b) Hydroxylation to polar compounds (diols, triols) by the members of the CYP3A family (Phase I), followed by glucuronidation catalyzed by the UDP-glucuronosyltransferase (UGT) enzymes of the UGT2B family (Phase II), and excretion in the urine. Only a small fraction of testosterone and androstenedione is aromatized to estrogens in men. In advanced PC, *CYP3A4*, *CYP3A5* and *CYP3A7* expression is decreased. Ko et al. [5] have now found that *HSD17B4 variant 2*, which catalyzes androgen oxidation at 17 β -OH and essentially reverses the effect of AKR1C3, is specifically downregulated in CRPC. This would be predicted to decrease DHT inactivation, increase *in situ* androgen levels and enhance AR activation in CRPC. Modified with permission from [1].