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Resisting resistance: Establishing new treatment options for CRPC patients

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ABSTRACT

Overcoming drug resistance in castrate resistant prostate cancer requires improved understanding of mechanisms by which resistance occurs, as well as new treatment options. A recent study published in Nature (Li *et al*. 2015) examines the mechanism of abiraterone activity, and reveals the bioactivity of a breakdown product with exciting repercussions for therapy regimes.

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Despite intense efforts toward early detection, increased screening and new agents for treatment, prostate cancer remains the second leading cause of cancer related deaths in American men. Androgen deprivation therapy has been successfully used to reduce tumor burden and alleviate symptoms, however, all patients eventually develop resistance and relapse. Identification of the mechanisms of resistance is of vital importance to overcome this barrier to treatment. Tumor progression after androgen deprivation therapy was originally thought to be androgen independent, but it has since been shown that the dynamics of androgen receptor (AR) sensitivity and signaling contributes heavily to resistance, as well as promoting disease progression, and advanced tumors remain hormone driven despite deprivation therapy.[1]

Resistant tumors gain the metabolic ability to convert precursor steroids to 5α-dihydrotestosterone (DHT), thus promoting androgen receptor signaling. As DHT synthesis requires enzymatic reactions to occur, patients may be treated with agents that block enzymatic activity. One such steroidal anti-androgen is abiraterone, a potent inhibitor of androgen biosynthesis, which acts via irreversible inhibition of the enzyme 17 α -hydroxylase/17,20-lyase (CYP17A1). Inhibition of CYP17A1 leads to the reduction of two 2 reactions; the hydroxylation of pregnenolone and progesterone to 17 α -hydroxy derivatives, due to 17 α hydroxylase activity, and loss of formation of dehydropiandrosterone (DHEA) and androstenedione by its 17,20-lyase activity. The outcomes are decreased levels of circulating DHEA, testosterone and DHT.[1]

Abiraterone activity is well documented in the clinic, showing antitumor activity and improved progression-free survival in both chemotherapy-naïve and chemotherapy-treated patients.[2] In a recent Nature paper, Li and colleagues discuss the impact of a breakdown product of abiraterone on prostate cancer.[4] Being a steroidal compound, abiraterone is susceptible to conversion by steroid metabolizing enzymes, leading to breakdown products with potential uses in the treatment of androgen-dependent disorders, and potentially more bioactivity, as first hypothesized in the early 90s.[3] Here, the authors investigated the bioactivity of Δ^4-abiraterone (D4A).

They showed that abiraterone is converted to the more physiologically active D4A in both mice and patients with prostate cancer,[4] confirming previously documented reports of D4A activity.[5,6] D4A was detectable by mass spectrometry in low levels (less than 20% of total abiraterone and D4A) in treated mice and patient serum, but was not detectable *in vitro* in the LAPC4 prostate cancer cell line. However, activity could be increased by over expression of the steroidal isoenzyme 3βHSD, suggesting that 3βHSD is a requirement for D4A synthesis.

3βHSD is also required for the conversion of DHEA to Δ^4-androstenedione (AD), a precursor to DHT. The authors assessed if D4A could inhibit this and other steroidal enzymes targeted by abiraterone. D4A inhibited 3βHSD in two 2 different prostate cancer cell lines, LNCaP and VCaP, with ten 10 times more potency than abiraterone, while CYP17A1 was inhibited to a similar extent as treatment with the parent drug in HEK293 cells. The *in vitro* results were also observed *in vivo*. In comparison to abiraterone, D4A showed improved activity in two 2 different 3βHSD-missense xenograft mouse models, blocking the conversion of DHEA to AD, and improving progression free survival at a concentration 10 times lower than abiraterone. Given that D4A inhibits 3βHSD activity with such potency, it may be of scientific interest and clinical benefit to screen patients for genetic variants within *HSD3B1* in order to assess patient ability to metabolize abiraterone to D4A. One such variant allele is 1245C, which decreases degradation of the enzyme leading to elevated DHT levels *in vitro*. *HSD3B1(1245C)* has recently been shown to be associated with prostate cancer progression in the clinic.[7] If 3βHSD is the main enzyme promoting D4A synthesis, then identifying patients with this germline mutation may assist in predicting response to abiraterone treatment.

Another steroidal enzyme also implicated in DHT synthesis, steroid-5α–reductase (SRD5A),[1] was also shown to be blocked by D4A, while abiraterone and the anti-androgen enzalutamide

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showed no inhibitory activity even at high concentrations (10μM, 100μM and 100μM respectively). The relative importance of this finding lies in the multiple 5α-reduced androgens produced by SRD5A conversion of AD. Inhibition of SRD5A consequentially gives reduced levels of 5α-diol, 5α-dion, androsterone and ultimately DHT.[1]

As well as being a steroidal inhibitor, abiraterone is a mild AR antagonist. In a competition assay used to determine if the binding affinity could be improved upon with D4A treatment, binding affinity in wild-type AR expressing cells (LAPC4) for abiraterone was considerably lower (IC_{50} > 500 nM) than enzalutamide (IC_{50} = 23 nM), but even more so than D4A (IC_{50} = 7.9 nM). Given that abiraterone binding to the AR is further hindered by mutations within the ligand binding domain, the impact of this on D4A activity was assessed. In cells expressing mutated AR (LNCaP) D4A again exhibited higher affinity (IC_{50}=5.3 nM) than either abiraterone (IC_{50} = 418 nM) or enzalutamide (IC_{50} = 24 nM). At the molecular level, upon enzymatic conversion the steroid A and B rings of abiraterone become identical to testosterone, giving a 3-keto structure, allowing for the improved antagonistic interactions with AR seen in these assays.

In addition to improved functionality, D4A effects the expression of genes up-regulated by androgens in prostate cancer. *PSA, TMPRSS2* and *FKBP5* were all suppressed *in vitro* by D4A to a greater extent than with abiraterone in both wild type and mutant AR expressing cell lines. Furthermore, D4A was able to significantly reduce endogenous PSA expression *in vitro*, the extent of which was comparable to enzalutamide.

The ability to treat patients with one potent steroidal agent, targeting both the AR and essential enzymes for DHT synthesis, may lead to improved clinical outcome with reduced side effects. However, patients treated with abiraterone exhibit only low levels of circulating D4A, the results of which are highly variable.[4] If improvements in detection assays can be made then circulating levels of D4A may be used as a biomarker to predict patient response to abiraterone. Additionally, several challenges remain prudent to improving patient therapy including; the multiple methods of continued AR signaling despite hormone deprivation therapy, the heterogeneity of CRPC, and mechanisms of resistance. Given the multitude of functions exhibited by D4A and the improved potency with which it produces response, treatment of CRPC with D4A appears to be a viable option. Whether it can be used as a single agent, or in combination therapy to improve patient outcome and overcome resistance remains to be seen. A thorough understanding of the mechanisms with which androgens orchestrate resistance can only aid to advance the design of targeted therapies, as well as identify druggable targets. While this is not the first paper to document the breakdown of abiraterone and the biological activity of D4A, Li *et al.*, highlight that the activity profile is more potent than abiraterone or enzalutamide, and show that D4A availability is reliant on 3βHSD activity, emphasizing the possible clinical uses of a previously overlooked metabolite. With enhanced understanding of the pharmacology of approved therapies, the potential to identify more potent agents for the treatment of CRPC is a possibility.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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