Roles for the Backdoor Pathway of Androgen Metabolism in Prostate Cancer Response to Castration and Drug Treatment

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Abstract

Almost all men who present with advanced prostate cancer (CaP) and many men who fail potentially curative therapy are treated with androgen deprivation therapy (ADT). ADT is not curative and CaP recurs as the lethal phenotype. The goal of this review is to describe the evolution of adrenal androgen blockade, how new androgen measurement methods have furthered understanding of androgen metabolism, and how further understanding of the backdoor pathway of androgen metabolism may lead to interventions that extend survival even more.

Key words: Adrenal androgens, "Backdoor” Pathway, Prostate cancer, Dihydrotestosterone, CYP17A1.

Orchiectomy, adrenalectomy and early adrenal androgen inhibiting agents

Huggins and Hodges demonstrated that CaP was responsive to ADT by orchiectomy; however, orchiectomy alone did not prevent CaP recurrence [1]. A proposed mechanism for failure of ADT was androgen production by the adrenal glands that promoted CaP survival. Huggins and Scott reported that bilateral adrenalectomy after orchiectomy further decreased androgen levels, however, androgen levels were not abolished [2]. Adrenalectomy did not become standard treatment for CaP because patients died due to adrenal insufficiency. Patient survival after adrenalectomy improved after the development of corticosteroid replacement therapy [3, 4]. Despite the addition of corticosteroids, patient survival after adrenalectomy remained poor, either because therapy that addressed testicular and adrenal androgens had no effect or hormone therapy was successful but, the tumor recurred and adrenalectomy fell out of favor [5-8]. The development of pharmaceuticals that could reduce adrenal androgen synthesis led to re-examination of a role for adrenal androgens in advanced CaP.

Aminoglutethimide blocks cytochrome P450, family 11, subfamily A, polypeptide 1 (CYP11A1), which catalyzes the conversion of cholesterol to pregnenolone [9, 10]. Aminoglutethimide was shown to decrease adrenal androgen levels similar to those achieved after bilateral adrenalectomy [11, 12]. Aminoglutethimide was co-administered initially with cortisol [13] and then with hydrocortisone [14]. Aminoglutethimide depleted dehydroepiandrosterone-sulfate (DHEA-sulfate), levels and androstenedione (ASD) levels remained unchanged [15]. ASD in circulation provides CaP substrate to produce either testosterone (T) or androstenedione (5α-dione); both can be converted to dihydrotestosterone (DHT) through either 5α reduction or 17β-hydroxysteroid (17β-HSD) enzymatic activity, respectively [16, 17]. The failure of aminoglutethimide to lower ASD levels and drug toxicity as a result of high aminoglutethimide levels are two factors that lead clinicians from aminoglutethimide to ketoconazole for adrenal androgen synthesis blockade [15].
Ketoconazole is an antifungal [18] that can block androgen synthesis by inhibiting P450-dependent enzymatic activities, like 17,20-lyase activity [19]. At higher concentrations, ketoconazole inhibits 11β-hydroxylase, 20,22-desmolase and 17α-hydroxylase activities. However, 17,20-lyase inhibition is the most relevant clinically [20-22]. Ketoconazole was demonstrated to reduce T [23], ASD and DHEA to undetectable levels in most patients [20, 24, 25]. However, the decline in adrenal androgen levels was not associated with decreased PSA levels or CaP regression [26-28]. Ketoconazole’s lack of enzyme specificity, drug toxicities, patient side effects and the need for steroid supplementation warranted development of a more specific adrenal androgen synthesis inhibitor [29-31]. Although ketoconazole was identified as a better adrenal androgen synthesis inhibitor than aminoglutethimide, clinical interest for adrenal androgen specificity, drug toxicities, patient side effects and the need for steroid supplementation warranted development of a more specific adrenal androgen synthesis inhibitor [29-31]. Although ketoconazole was identified as a better adrenal androgen synthesis inhibitor than aminoglutethimide, clinical interest for adrenal androgen inhibition remained low until adrenal androgens were shown to clearly be involved in DHT synthesis.

Mass spectrometry, abiraterone and the backdoor pathway of androgen metabolism

In 1979, Geller hypothesized that CaP was capable of synthesizing levels of T and DHT sufficient for AR transactivation in medically or surgically castrate men [32]. The conversion of adrenal androgens to T and DHT was shown important for androgen receptor (AR) transactivation and this conversion was postulated to be performed by “peripheral body tissues” [27, 33, 34]. However, these data were acquired using radioimmunoassay and were met with skepticism due to lack of assay standardization and concern for ensuring androgen specificity [35]. However, this issue was resolved in 2004 when prostatic tissue levels of T and DHT in CR-CaP were measured using liquid chromatography tandem mass spectrometry (LC-MS/MS) [36]. LC-MS/MS is more reliable, accurate and allows higher throughput androgen analysis [35, 37, 38]. Tissues must be procured quickly, extracted carefully and LC-MS/MS measurement of tissue androgens remains difficult. LC-MS/MS methods must be adapted for each matrix (whole blood, plasma or serum and tissue from primary, solid organ metastasis or bone marrow metastasis) and cell culture media or cultured cells). Measurements need rigorous controlled and the development of internal standards [36, 39]. Limitations of sensitivity require cell samples of about 100,000 that precludes laser microdissection to separate well benign from malignant epithelium and epithelium from microenvironment and analysis of androgen levels in various subcellular components. The presence of intratumoral T and DHT in CaP and CR-CaP have been confirmed by others [40, 41] and Mostagel et al. detected T and DHT in bone marrow metastatic CaP [42]. These studies paved the way for determining how tissue levels of T and DHT are generated either from weak adrenal androgens [43] or from cholesterol, the starting substrate for androgen synthesis [41, 44] or through an alternate backdoor pathway that involves DHT generation without using T as a substrate (Figure 1).

Study of the conversion of adrenal androgens to T or DHT revealed that CYP17A1 is a key enzyme for androgen synthesis in the intratumoral de novo steroid synthesis pathways and CYP17A1 is a central enzyme involved in backdoor pathway metabolism. One potent inhibitor against CYP17A1 is the new FDA-approved drug, abiraterone [31, 45-48]. Abiraterone was investigated first in men with castration-recurrent, metastatic CaP who had failed chemotherapy [49] in whom extension of survival averaged 4 months [50]. Patients who had not undergone chemotherapy [51] had 57% reduction in risk of radiographic progression but the co-primary endpoint of survival had not been reached vs. 8.3 months in the placebo arm. In addition to CYP17A1 inhibition, abiraterone has been shown to inhibit 3β-hydroxysteroid dehydrogenase (3β-HSD), which has been reported to convert DHEA to ASD, which is then 5α-reduced by 5α-reductase 1 to 5α-dione and then DHT (Figure 1) in cell lines and LAPC4 xenografts in laboratory mice. Rodents lack adrenal CYP17A1 expression and consequently the effect of abiraterone must be modulated by inhibition of 3β-HSD [16, 52].

Clinical failure of abiraterone has been attributed to several mechanisms involving the backdoor pathway [53] and AR splice variants [54]. Abiraterone inhibition has been shown to elevate CYP17A1 expression levels and induce progesterone accumulation. The over abundant amount of progesterone can compete against abiraterone for CYP17A1 leading to generation of metabolites involved with backdoor metabolism and hence DHT production [53]. Cai et al. demonstrated that abiraterone caused up-regulation of CYP17A1 more than 10-fold in 2 of 5 VCaP xenografts studied that may provide a growth advantage and CYP17A1 inhibition may result in up-regulation of CYP11A1 and AKR1C3; both enzymes are capable of increasing intratumoral de novo androgen synthesis by activating different androgen metabolism pathways [53]. Mostaghel et al. studied two different xenografts derived from the LuCaP xenograft model, LuCaP 23CR and 35CR [54]. When treated with abiraterone, both xenograft cell lines showed increased CYP17A1 and 17β-HSD-3 (2 enzymes mediating conversion of adrenal androgen intermediates to T in the prostate and testis respectively [55, 56]), full length
AR, and AR splice variants expression. The major differences between the xenografts were the mechanism and time of recurrence. LuCaP 23CR recurred earlier and this earlier recurrence was independent of intracrine production of T and DHT. In contrast, LuCaP 35CR recurred later and had statistically significant increases in T and a trend toward significant increases in DHT. Greater complexity was observed in the expression of oxidative genes mediating back conversion of 5\textit{\alpha}androstane-3\textit{\alpha},17\textit{\beta}-diol (androstane-diol) to DHT. The authors suggested inhibitors of other critical components of the steroid metabolism pathway, such as 3\textit{\beta}-HSD1 or 5\textit{\alpha}-reductase type 1 or 2 could offset adaptive up-regulation of CYP17A1 [54].

Other investigators suggested that dose escalation may overcome the CYP17A1 overexpression [49] or that targeting AR splice variants in conjunction with treatment with abiraterone might be beneficial [54]. Mosteghel et al. lament the fact that “serum samples adequate to assess circulating androgen levels were not available for analysis in this study”, which may have provided insight into mechanisms of resistance to CYP17A1 inhibition with abiraterone [54]. Li et al. postulate that increasing abiraterone concentration also may inhibit 3\textit{\beta}-HSD simultaneously with counteracting the effects of over expression of CYP17A1 [52].

New CYP17A1 inhibitors are under development to address the clinical flaws associated with abiraterone. Two inhibitors in clinical trials currently are TOK-001 and TAK-700. TOK-001 is a novel CYP17A1 inhibitor that has completed a phase 1 clinical trial, ARMOR1 [57]. TOK-001 was tolerated well and demonstrated anti-tumor activity. Purushottamachar et al. provided evidence that TOK-001 not only inhibits CYP17A1 activity but has AR antagonistic capabilities similar to bicalutamide [47, 58]. TAK-700 was designed to inhibit specifically the 18,20-lyase activity of CYP17A1, which focused the drug to target specifically adrenal androgen synthesis. TAK-700 demonstrated specific CYP17A1 18,20-lyase activity, was well tolerated by patients and exhibited anti-tumoral effects in phase 1 and phase 2 clinical trials [59]. However, the phase 3 post-chemotherapy clinical trial, ELM-PC 5 (C21005), was stopped because patients showed disease progression in the TAK-700 + prednisone arm and the study would not be able to reach its primary end-point.

![Diagram of androgen metabolism pathways to DHT synthesis](http://www.ijsbs.com)

**Figure 1. Four androgen metabolism pathways to DHT synthesis.** Pathway 1 is the 5\textit{\alpha}-reduction of T to DHT (dashed lines). Pathway 2 uses adrenal androgens, DHEA or ASD, to synthesize T or 5\textit{\alpha}-dione that are converted to DHT (faint gray long dashes). Pathway 3 is the cholesterol pathway (small gray dashes). Pathway 4 is the backdoor pathway of DHT synthesis using androstanediol instead of T to generate DHT (outlined in bold). Abiraterone inhibits 17\textit{\alpha}-hydroxylase and 17,20-lyase of CYP17A1 (relevant to Pathways 2 and 4) whereas TAK-700 or TOK-001 inhibit only 17,20-lyase (relevant to Pathway 2).
Perspectives and challenges

Huggins introduced the first strategies for depleting serum testicular and adrenal androgen levels as a treatment for CaP in the 1940s and 1950s. However, 60-70 years after the initial works of Huggins and despite newer adrenal androgen inhibitors, which are more specific, safer and more effective at targeting adrenal androgen synthesis than their predecessors, CaP still recurs and patient survival remains disappointing. Hence, it appears that depletion of serum levels of testicular and adrenal androgens are not sufficient to deplete tumor tissue levels.

Androgen metabolism pathways are biologically and technically challenging to study. The biological downsides include enzyme or pathway redundancy, such that if one arm of the DHT synthesis pathway is inhibited, another arm or set of enzymes are activated to overcome the initial enzyme or pathway blockade. The literature is confused by the lack of standard nomenclature for androgen metabolism enzymes, the many enzymes that catalyze the same conversion and the presence of many isozymes for most androgen metabolism enzymes. Another challenge to studying androgen metabolism enzymes is enzymatic activity is difficult to measure. Enzyme activity cannot be determined reliably from message or protein levels. Enzymes are sensitive to pH and cofactor availability, and both are difficult to measure accurately in vivo. Enzyme assays are conducted in vitro using these uncertain pH cofactor levels that make in vitro assays even more artificial. One reliable method is to measure substrate to product conversion using LC-MS/MS but LC-MS/MS is technically challenging.

The available in vitro and in vivo model systems for studying androgen metabolism may reflect the human situation poorly. CaP cell lines used for modeling CaP are cultured in FBS that contains 0.03 nM T [60], which is close to serum levels of T in the castrated human male. These CaP cell lines, which include the commonly labeled androgen-sensitive LNCaP and LAPC4, have adapted to a castration-like environment to grow in culture and therefore may not be as androgen sensitive as necessary to model the clinical situation. Perhaps CaP cell lines should be trained using more physiologically relevant levels of androgens before subjecting these cells to an androgen-deprived environment using double charcoal stripped serum.

Studying androgen metabolism in vivo is complicated by the mouse host and the xenografts. Mice produce very low levels of adrenal androgens; the castration environment is dissimilar to the human high levels of circulating adrenal androgens [61-63]. Therefore, the mouse model to mimic human castration should be humanized by introducing and maintaining stable adrenal androgen levels prior to study. The CaP xenografts implanted into mice respond differently to castration. For example, the growth of LNCaP and LAPC4 xenografts slow after castration but neither xenograft regresses completely. These models may be less appropriate [64] because both xenografts respond to ADT with slowed growth and not tumor regression. The CWR22 tumor model is a more physiologically relevant model since the CWR22 model mimics the changes in human CaP after ADT. The CWR22 tumor model is an androgen-dependent human CaP xenograft that is propagated subcutaneously in nude mice. The CWR22 is a better xenograft model than LNCaP or LAPC4 because the CWR22 model regresses after ADT. After CWR22 tumor regresses, the tumor recurs 4-5 months after ADT [65-68]. Additionally, recurrent CWR22 has AR protein expression levels and AR-dependent or AR-independent gene expression profiles that are similar to androgen-stimulated CWR22 [69-75]. Our laboratory has used the CWR22 model to model AR transactivation by testicular androgens synthesized by intracrine metabolism [76, 77].

However, the studies by Cai and Mostaghel et al. support the notion that no matter how well we design or redesign our inhibitors, tumors are capable of rendering new agents ineffective. For example, both studies provide evidence that the androgen metabolism pathways can reconfigure themselves to overcome DHT inhibition and produce DHT by activating arms of the androgen metabolism pathways not affected by the inhibitors. In addition to androgen metabolism reconfiguration, the tumor overcomes abiraterone by increasing AR splice variant expression, rendering abiraterone inhibition ineffective. A better understanding of these pathways may allow their interruption using multi-functional inhibitors, such as TOK-001 or ARN-509 [57, 78] or optimal combination of single target agents that affect multiple androgen synthesis pathways at one time [79].

Abbreviations

17β-HSD: 17β-hydroxysteroid dehydrogenase; 5α-dione: 5α-androstanedione; 3β-HSD: 3β-hydroxysteroid dehydrogenase; ADT: androgen deprivation therapy; AND: androsterone; AR: androgen receptor; androstenediol: 5androstane-3a,17β-diol; ASD: androstenedione; CaP: prostate cancer; CR-CaP: castration recurrent prostate cancer; CYP11A1: cytochrome P450, family 11, subfamily A, polypeptide 1; CYP17A1: cytochrome p450, family 17, subfamily A polypeptide 1; DHEA: dehydroepiandrosterone; DHEA-sulfate: dehydroepiandrosterone-sulfate; DHT: dihydrotestosterone; LC-MS/MS: liquid chro-
matography tandem mass spectrometry; LHRH: luteinizing hormone releasing hormone; T: testosterone.

Competing Interests

The authors have declared that no competing interest exists.

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