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Potential prostate cancer drug target: Bioactivation of androstanediol by conversion to dihydrotestosterone

James L. Mohler[1,2,3], **Mark A. Titus**[1], and **Elizabeth M. Wilson**[2,3,4]

[1]Department of Urology, Roswell Park Cancer Institute, Buffalo, NY

[2]Department of Urology, University at Buffalo, State University of New York, Buffalo, NY

[3]Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, NC

[4]Laboratories for Reproductive Biology, Department of Pediatrics and Department of Biochemistry and Biophysics, University of North Carolina, Chapel Hill, NC

Abstract

High affinity binding of dihydrotestosterone (DHT) to the androgen receptor (AR) initiates androgen-dependent gene activation required for normal male sex development *in utero*, and contributes to prostate cancer development and progression in men. Under normal physiological conditions, DHT is synthesized predominantly by 5α-reduction of testosterone, the major circulating androgen produced by the testis. During androgen deprivation therapy, intratumoral androgen production is sufficient for AR activation and prostate cancer growth even though circulating testicular androgen levels are low. Recent studies indicate that the metabolism of 5α-androstane-3α,17β-diol by 17β-hydroxysteroid dehydrogenase 6 in benign prostate and prostate cancer cells is a major biosynthetic pathway for intratumoral synthesis of DHT that binds AR and initiates transactivation to promote prostate cancer growth during androgen deprivation therapy. Drugs that target the so-called backdoor pathway of DHT synthesis provide an opportunity to enhance clinical response to LHRH agonists or antagonists, AR antagonists, inhibitors of 5α-reductase enzymes, finasteride or dutasteride, and steroid metabolism enzyme inhibitors, ketoconazole or the recently available abiraterone acetate.

BACKGROUND

Normal male sex development and growth depend on high affinity binding of dihydrotestosterone (DHT, 5α-androstan-17β-ol-3-one), the 5α-reduced product of testosterone (T, 4-androsten-17β-ol-3-one), to the androgen receptor (AR), an essential ligand-dependent transcription factor that regulates androgen-dependent gene transcription. AR binds T and DHT with similar high equilibrium binding affinity (equilibrium dissociation constant, Kd ~0.3 nM) (1), and T and DHT are the only naturally occurring steroids that activate wild-type AR. AR is not activated directly by binding either 5α-androstane-3α,17β-diol (androstanediol) or dehydroepiandrosterone (DHEA). Androgen specificity for AR transcriptional signaling is achieved through the highly structured AR ligand binding domain, and the selective ability of T and DHT to induce the AR NH_2- and carboxyl-terminal interaction that stabilizes AR and increases transcriptional activity (2, 3). The well established differences in potency between T and DHT (4) result not from differences in AR equilibrium androgen binding affinity, but from the greater hydrophobicity of DHT that strengthens human AR intermolecular interactions, slows the

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No potential conflicts of interest are disclosed.

dissociation rate of bound androgen, and stabilizes the ligand-bound AR to render DHT a more active androgen than T (1, 5).

The requirement for DHT in normal prostate growth and development of the external genitalia is demonstrated by the 5α-reductase syndrome. An inactivating mutation in the type 2 isoform of the 5α-reductase enzyme that converts T to DHT results in a small prostate gland and predominantly female or ambiguous external phenotype in affected newborn males with 46XY chromosomes (6). The 5α-reductase syndrome provides physiological evidence that circulating DHT arises from T, and that DHT is required for normal male sex development. On the other hand, untreated 5α-reductase syndrome patients begin to virilize at puberty. This suggests that the pubertal rise in T compensates for low DHT, or that a pubertal rise in the type 1 isoform of 5α-reductase (7, 8) acts on other steroid precursors such as 17α-hydroxyprogesterone to form 5α-pregnane-17α-ol-3,20-dione and eventually DHT (Fig. 1).

AR is a critical transcriptional regulator required to establish the normal male sex phenotype, and for the development and progression of prostate cancer (9–11). Regression of prostate cancer after medical or surgical androgen deprivation therapy is followed invariably by the development of castration-recurrent or castration-resistant prostate cancer (CRPC), which demonstrates an initial reliance of prostate cancer growth on AR mediated gene transcription in response to DHT. AR levels are often increased in CRPC consistent with the continued expression of AR-stimulated genes (12). Additional growth stimulating mechanisms in CRPC include increased levels of AR coregulators, transcription factors and/or phosphorylation that render AR more sensitive to much lower levels of intratumoral androgen, and growth factor and cytokine activation independent of androgen. AR mutations, though rare in prostate cancer, can decrease specificity of AR steroid binding that results in a more promiscuous transcriptional activator that responds to a broader range of ligands (13–16).

Recent studies highlight the potential importance of intratumoral androgen biosynthesis in prostate cancer (17–22). Mass spectrometry measurements of CRPC tissue extracts indicated ~2 nM DHT sufficient to activate AR (17, 18). In agreement with these findings, finasteride, a 5α-reductase type 2 inhibitor, or dutasteride, a dual 5α-reductase types 1 and 2 inhibitor (Fig. 1), were ineffective in preventing prostate cancer development (23, 24) or in treating aggressive prostate cancer (25, 26). One interpretation of these clinical studies is that alternative androgen biosynthetic pathways provide sufficient DHT to activate AR in the abnormal cellular environment of prostate cancer. CRPC also is characterized by increased expression of a family of related p160 coactivators, named for their approximate 160 kDa molecular weight, and other coregulators that enhance AR sensitivity to low level androgens (27, 28). One example is the AR coregulator, melanoma antigen-A11 (MAGE-11), whose mRNA levels increase in ~30% of CRPC. In one patient with rapidly progressing prostate cancer, MAGE-11 mRNA levels were three orders of magnitude above the normal range, whereas AR mRNA in this patient sample was undetectable using quantitative reverse transcription-polymerase chain reaction (RT-PCR) (28). These findings suggest that highly plastic prostate cancer cells utilize alternative mechanisms to eventually escape drug treatments that target AR.

METABOLISM OF ANDROSTANEDIOL TO DHT

The classical pathway for DHT synthesis is conversion in the testis of the major adrenal androgen androstenedione to T, followed by irreversible 5α-reduction of T to DHT by 5α-reductase type 2 in prostate and other, but not all, androgen target tissues (Fig. 1) (29). Studies in the beagle dog (30) and tammar wallaby (31, 32) indicate an alternative backdoor

pathway of DHT synthesis that utilizes androstanediol as precursor instead of T. Androstanediol is the major degradation product of DHT from the reductive 3α-hydroxysteroid dehydrogenase (HSD) activity of 3α-HSD aldo-keto reductases 1C (AKR1C) (Fig. 1), enzymes with both 3- and 17-ketosteroid reductase activity (33–38). AR binds androstanediol with moderate affinity, but androstanediol must be converted to DHT to induce transactivation by wild-type AR. Enzymes that convert androstanediol to DHT include 17β-hydroxysteroid dehydrogenase 6 (17β-HSD6 or HSD17B6, known also as retinol dehydrogenase (RODH) 3α-HSD) (39), 17β-hydroxysteroid dehydrogenase 10 (17β-HSD10 or HSD17B10) (40), retinol dehydrogenase 5 (RDH5) (39), dehydrogenase/reductase short-chain dehydrogenase/reductase family member 9 (DHRS9) (33, 41) and retinol dehydrogenase 4 (RODH4) (42).

Recent studies suggest that benign human prostate and prostate cancer cells express predominantly 17β-HSD6 as the major enzyme that converts androstanediol to DHT, and it is DHT that accounts for AR transactivation in the presence of androstanediol (43). Precise measurements of relative enzyme activity of several hydroxysteroid dehydrogenases active in this reaction are complicated by the requirement for optimal pH and nicotinamide adenine dinucleotide cofactors. However, 17β-HSD6 mRNA levels determined using RT-PCR and protein levels on immunoblots suggest a direct link between 17β-HSD6 expression, bioconversion of androstanediol to DHT, and AR activation in the presence of androstanediol.

Androstanediol is not a major adrenal androgen. Thus, to serve as a significant intracellular precursor for DHT synthesis, androstanediol must be synthesized from steroid precursors earlier in the biosynthetic pathway. The backdoor pathway of DHT biosynthesis in benign prostate and prostate cancer cells depends on intracellular 5α-reductase type 1 and 2 isoforms, and the conversion of androsterone to androstanediol or androstanedione (Fig. 1). In both pathways, 17α-hydroxyprogesterone serves as a principal intermediate independent of DHEA, androstenedione or T. Progesterone and 17α-hydroxyprogesterone were shown to be excellent substrates for 5α-reductase type 1 and 2 (8, 44) that form dihydroprogesterone and 17α-hydroxydihydroprogesterone, respectively (Fig. 1). 5α-reductase type 1 predominates in this reaction, and expression of 5α-reductase type 1 increases during prostate cancer progression (45, 46) and possibly in prostate cancer tissue during androgen deprivation therapy (47). A recently described ubiquitously expressed 5α-reductase type 3 isoform is present at higher levels in prostate cancer (48, 49). Although 17α-hydroxyprogesterone is not considered an important sex steroid precursor in normal human physiology (8), increased levels of 5α-reductase types 1 and 3 may facilitate conversion in this pathway that culminates in the formation of DHT.

Continuing in this pathway, reductive 3αHSD converts 17α-hydroxydihydroprogesterone to 17α-hydroxyallopregnanolone (Fig. 1). 17α-hydroxylase/17,20 lyase (P450c17) acts on 17α-hydroxyallopregnanolone to form androsterone. Once androsterone is formed, two possible pathways involve 17β-HSD6 to catalyze the formation of DHT. 17β-HSD6 converts androsterone to androstanedione, which is converted to DHT by 17βHSD3. Androsterone is converted to androstanediol by 17βHSD3 (50), and androstanediol is converted to DHT by 17β-HSD6. The complementary DNA for 17β-HSD6 was originally cloned from prostate (39). 17β-HSD6 is central to the backdoor pathway of DHT synthesis independent of circulating T, appears to be a critical enzyme in prostate cancer cells, and is a potential drug target for treating prostate cancer.

CLINICAL-TRANSLATIONAL ADVANCES

Accumulating evidence that intratumoral androgen production drives prostate cancer growth by activating AR has driven efforts to identify new drugs that target enzymes responsible for androgen biosynthesis. Clinical trials using the 5α-reductase inhibitors finasteride and dutasteride have achieved success in the treatment of benign prostate enlargement but less so for prostate cancer. Critical evaluation of new opportunities to interrupt androgen metabolism has been difficult for several reasons. Steroid metabolic enzymes often exist as multiple isozymes, enzyme function cannot be predicted based on enzyme protein or mRNA levels alone, and *in vitro* assay conditions may differ from the microenvironment of prostate cancer cells. Enzymes, substrates and products may be at low levels and enzymatic action may differ among tissue compartments so that whole tissue assays are misleading. In addition, fundamental knowledge gaps exist in androgen transport across membranes, such as how androgens transit the vascular endothelium.

The human *CYP17A1* gene codes for P450c17, an enzyme with dual functions in the intracrine metabolism of testosterone and DHT from progesterone via the cholesterol pathway (Fig. 1). 17α-hydroxylase and 17,20-lyase activities of P450c17 act on pregnenolone and progesterone precursors to produce DHT via 5α-pregnane-3α,17α-diol-20-one through the backdoor pathway. Ketoconazole is a nonspecific weak inhibitor of P450c17 with limited antitumor properties and excessive toxicity. Recent appreciation of intracrine metabolism of active androgens from adrenal precursors, and the efficacy but high toxicity of ketoconazole led Attard, deBono and colleagues to search for a “better ketoconazole.”

Abiraterone acetate is an irreversible inhibitor of the 17α-hydroxylase and lyase activities of P450c17 (51). Abiraterone acetate suppresses 17α-hydroxylation of pregnenolone and progesterone, and the 17,20-lyase activity of P450c17 that converts 17α-hydroxypregnenolone to DHEA, and 17α-hydroxyprogesterone to androstenedione (Fig. 1) (8). Because 17α-hydroxyprogesterone is a precursor of androsterone and androstanediol, abiraterone acetate would be expected to inhibit the backdoor pathway of DHT synthesis. Although serum androgen, estrogen and PSA levels were suppressed by abiraterone acetate, overall effects were often transient, and prolonged use was associated with a compensatory increase in ACTH and mineralocorticoid excess (52). The duration of response to abiraterone acetate monotherapy and abiraterone-corticosteroid combination therapy ranges from 9 to 14 months, with the response associated with high levels of adrenal androgens prior to initiation of therapy (53). These findings were consistent with a report that higher circulating adrenal androgen was associated with longer survival in men with CRPC (54).

Failure to achieve long term benefit from 5α-reductase or P450c17 inhibitors in the treatment of prostate cancer may result from incomplete enzyme inhibition, a compensatory rise in intratumoral T, alternative pathways of DHT synthesis, or increased AR sensitivity to low androgen in association with increased levels of AR coregulators and AR phosphorylation (55, 56). When androstanediol was provided as substrate, 17β-HSD6 was the major enzyme that converted androstanediol to DHT in normal human prostate and prostate cancer cells. Enzymatic conversion of androstanediol to DHT represents a new potential drug target to block prostate cancer progression that raises several questions. First, the extent to which 17β-HSD6 is responsible for androstanediol conversion to DHT in prostate cells remains unclear. Other enzymes such as DHRS9 and RDH5 may be active. In support of 17β-HSD6 as a drug target, AR transactivation in the presence of androstanediol correlated directly with 17β-HSD6 mRNA and protein in different cell types. However, 17β-HSD6 mRNA levels declined with time after castration in the androgen-dependent CWR22 human prostate cancer xenograft model and in human specimens, whereas DHRS9 and

RDH5 mRNA levels were relatively unchanged after castration. 17β-HSD6 could maintain DHT levels sufficient to activate AR, or rely on the activity of other enzymes. A key to defining 17β-HSD6 as a drug target in the treatment of prostate cancer will be to establish the relative contribution of other enzymes that maintain AR activation by androgen precursors.

A second consideration is the redundancy of androgen biosynthetic pathways that suggests prostate cancer cells deprived of testicular androgens recruit multiple mechanisms for the intracrine synthesis of DHT, the most potent naturally occurring AR ligand. A greater clinical response may be achieved through the use of inhibitors directed at different points along the pathway, with a longer lasting clinical response in advanced prostate cancer when a putative 17β-HSD6 inhibitor is combined with the dual 5α-reductase inhibitor dutasteride, LHRH agonist or AR antagonist. Such a multi-pronged approach could provide greater clinical benefit when administered earlier in the course of disease progression prior to the onset of CRPC.

Recent evidence has suggested the existence of constitutively active AR splice variants that lack the ligand binding domain and contribute to prostate cancer progression independent of intratumoral androgen (57–60). However, AR is highly susceptible to degradation in the low androgen environment of prostate cancer cells, such that partial AR degradation could account for some reported smaller forms (61). Although higher levels of AR splice variants were reported in certain prostate cancer cell lines (58) that are often subject to complex chromosome abnormalities, AR variants were in much lower abundance in prostate cancer specimens relative to full-length AR (57, 60). Overall the findings support the potential clinical usefulness of drugs that target androgen biosynthesis to achieve temporary reduction in tumor growth.

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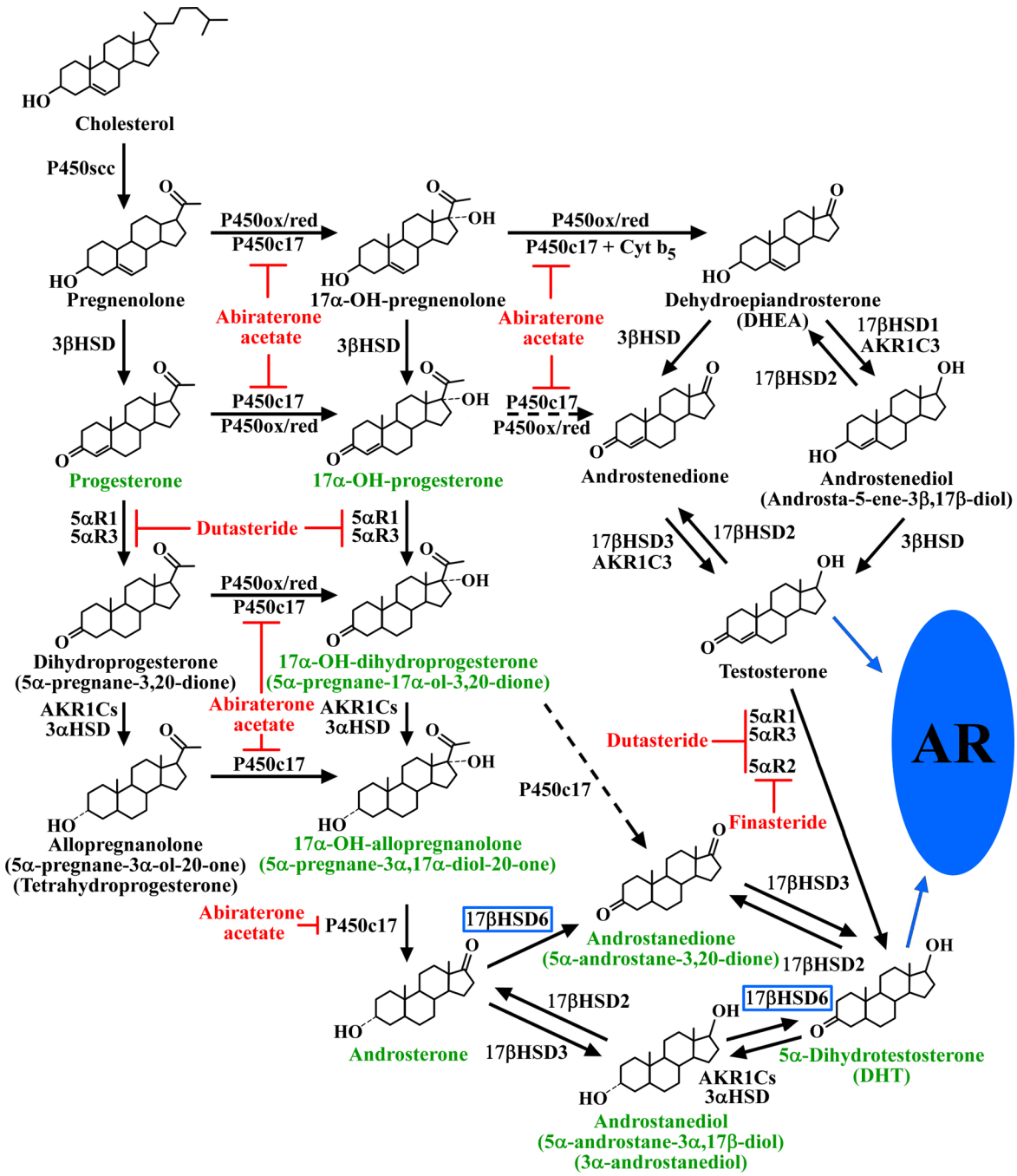

Figure 1. Backdoor pathway of DHT synthesis involves the conversion of androstanediol to DHT by 17β-HSD6

Conversion of cholesterol to prenenolone by the P450 side chain cleavage enzyme (P450ssc) is the first committed step in steroid biosynthesis. The 17α-hydroxylase/17,20-lyase (P450c17) catalyzes multiple 17α-hydroxylase and 17,20-lyase reactions in the steroidogenic pathway that require P450 oxidoreductase (P450ox/red) electron transfer. P450c17 coded by the *CYP17A1* gene is the target for inhibition by abiraterone acetate. Progesterone, 17α-hydroxyprogesterone (17α-OH-progesterone) and testosterone are substrates for 5α-reductase type 1, 2 or 3 (5αR1, 2 or 3). Finasteride is an 5αR2 inhibitor. Dutasteride is an inhibitor of 5αR1, 5αR2 and 5αR3. Isozymes of 3β-hydroxysteroid

dehydrogenase (3βHSD), 17β-hydroxysteroid dehydrogenase (17βHSD) and aldo-keto reductase (AKR1C3) are often reversible enzymes with oxidative and reductive activities that require nicotinamide adenine dinucleotide cofactors. Testosterone and DHT are the two biologically active androgens that activate AR. Testosterone is the major circulating active androgen formed in the testis. DHT is formed from testosterone in the testis, and can be synthesized in a so-called backdoor pathway (green) from progesterone and androsterone precursors independent of DHEA, androstenedione or testosterone intermediates.