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 physiologic 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, which 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 luteinizing-hormone–releasing hormone (LHRH) agonists or antagonists, AR antagonists, and inhibitors of 5α-reductase enzymes (finasteride or dutasteride), and other steroid metabolism enzyme inhibitors (ketoconazole or the recently available abiraterone acetate). Clin Cancer Res; 17(18): 1–6. ©2011 AACR.

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 nmol/L; ref. 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 NH2- 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 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 shown 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 46 XY chromosomes (6). The 5α-reductase syndrome provides physiologic 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 observation suggests that the pubertal increase in T compensates for low DHT, or that a pubertal increase 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 shows an initial reliance of prostate...
The backdoor pathway of DHT synthesis involves the conversion of androstanediol to DHT by 17β-HSD6. Conversion of cholesterol to pregnenolone by the P450 side-chain cleavage enzyme (P450scc) 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 Δ4 are substrates for 5α-reductase type 1, 2, or 3 (5αR1, 2, or 3). Finasteride is a 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. T and DHT are the 2 biologically active androgens that activate AR. T is the major circulating active androgen formed in the testis. DHT is formed from T in the testis and can be synthesized in a so-called backdoor pathway (green) from progesterone and androsterone precursors, independent of DHEA, androstenedione, or T intermediates.
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, which render AR more sensitive to much lower levels of intratumoral androgen and to growth factor and cytokine activation independent of androgen. AR mutations, although rare in prostate cancer, can decrease specificity of AR steroid binding, which 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 nmol/L 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 1 patient with rapidly progressing prostate cancer, MAGE-11 mRNA levels were 3 orders of magnitude above the normal range, whereas AR mRNA in this patient sample was undetectable using quantitative reverse transcription-PCR (RT-PCR; ref. 28). These findings suggest that highly plastic prostate cancer cells use alternative mechanisms to eventually escape drug treatments that target AR.

Metabolism of Androstenediol to Dihydrotestosterone

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; ref. 29). Studies in the beagle dog (30) and tammar wallaby (31, 32) indicate an alternative backdoor pathway of DHT synthesis that uses androstenediol as precursor instead of T. Androstenediol 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 androstenediol with moderate affinity, but androstenediol must be converted to DHT to induce transactivation by wild-type AR. Enzymes that convert androstenediol to DHT include 17β-hydroxysteroid dehydrogenase 6 [17β-HSD6 or HSD17B6, also known as retinol dehydrogenase (RODH) 3α-HSD; ref. 39], 17β-hydroxysteroid dehydrogenase 10 (17β-HSD10 or HSD17B10; ref. 40), retinol dehydrogenase 5 (RDH5; ref. 39), dehydrogenase/reductase short-chain dehydrogenase/reductase family member 9 (DHR59; ref. 33, 41), and retinol dehydrogenase 4 (RODH4; ref. 42).

Recent studies suggest that benign human prostate and prostate cancer cells express predominantly 17β-HSD6 as the major enzyme that converts androstenediol to DHT, and it is DHT that accounts for AR transactivation in the presence of androstenediol (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 androstenediol to DHT, and AR activation in the presence of androstenediol.

Androstenediol is not a major adrenal androgen. Thus, to serve as a significant intracellular precursor for DHT synthesis, androstenediol 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 types 1 and 2 isoforms and the conversion of androstanediol to DHT (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 types 1 and 2 (8, 44), which 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 expressed 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, which culminates in the formation of DHT.

Continuing in this pathway, reductive 3α-HSD converts 17α-hydroxydihydroprogesterone to 17α-hydroxyallopregnanolone (Fig. 1). 17α-hydroxyalase/17,20 lyase (P450c17) acts on 17α-hydroxyallopregnanolone to form androsterone. Once androsterone is formed, 2 possible pathways involve 17β-HSD6 to catalyze the formation of DHT. 17β-HSD6 converts androsterone to androstenedione, which is converted to DHT by 17β-hydroxysteroid dehydrogenase 3 (17β-HSD3). Androsterone is converted to androstenediol by 17β-HSD3 (50), and androstenediol 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, seems 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 on the basis of 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 T 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-pregnan-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, de Bono, and colleagues to search for a potential drug target for treating prostate cancer. It seems to be a critical enzyme in prostate cancer cells, and is a potential drug target for treating prostate cancer.

Mohler et al.
drugs that target androgen biosynthesis to achieve temporary reduction in tumor growth.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

We are grateful for the assistance of Andrew T. Hnat in preparing Fig. 1.

References


33. Bauman DR, Stekelbroeck S, Williams MV, Peeth DM, Penning TM. Identification of the major oxidative 3alpha-hydroxysteroid

Grant Support

National Cancer Institute (NCI) PO1-CA77739. NCI Cancer Center Support Grant to Roswell Park Cancer Institute CA16156, and the University of North Carolina at Chapel Hill Lineberger Cancer Center CA43026. U.S. Public Health Service Grants HD16910 from the National Institute of Child Health and Human Development, and Department of Defense Prostate Cancer Research Program PC09380.

Received April 27, 2011; revised May 17, 2011; accepted May 17, 2011; published OnlineFirst June 24, 2011.


38. Cooper WC, Jin Y, Penning TM. Elucidation of a complete kinetic mechanism for a mammalian hydroxysteroid dehydrogenase (HSD) and identification of all enzyme forms on the reaction coordinate: the example of rat liver 3alpha-HSD (AKR1C9). J Biol Chem 2007;282:33484–93.


47. Yokoi H, Tsuruo Y, Miyamoto T, Ishimura K. 5alpha-reductase type 1 immunolocalized in the adenral gland of normal, gonadecto-
Potential Prostate Cancer Drug Target: Bioactivation of Androstanediol by Conversion to Dihydrotestosterone

James L. Mohler, Mark A. Titus and Elizabeth M. Wilson

Clin Cancer Res  Published OnlineFirst June 24, 2011.

Updated version
Access the most recent version of this article at: doi:10.1158/1078-0432.CCR-11-0644

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.