Pharmacologic Basis for the Enhanced Efficacy of Dutasteride against Prostatic Cancers

Yi Xu, Susan L. Dalrymple, Robyn E. Becker, Samuel R. Denmeade, and John T. Isaacs

Abstract

**Purpose:** Prostatic dihydrotestosterone (DHT) concentration is regulated by precursors from systemic circulation and prostatic enzymes of androgen metabolism, particularly 5α-reductases (i.e., SRD5A1 and SRD5A2). Therefore, the levels of expression SRD5A1 and SRD5A2 and the antiprostatic cancer growth response to finasteride, a selective SRD5A2 inhibitor, versus the dual SRD5A1 and SRD5A2 inhibitor, dutasteride, were compared.

**Experimental Design:** Real-time PCR and enzymatic assays were used to determine the levels of SRD5A1 and SRD5A2 in normal versus malignant rat and human prostatic tissues. Rats bearing the Dunning R-3327H rat prostate cancer and nude mice bearing LNCaP or PC-3 human prostate cancer xenografts were used as model systems. Tissue levels of testosterone and DHT were determined using liquid chromatography-mass spectrometry.

**Results:** Prostate cancer cells express undetectable to low levels of SRD5A2 but elevated levels of SRD5A1 activity compared with nonmalignant prostatic tissue. Daily oral treatment of rats with the SRD5A2 selective inhibitor, finasteride, reduces prostate weight and DHT content but did not inhibit R-3327H rat prostate cancer growth or DHT content in intact (i.e., noncastrated) male rats. In contrast, daily oral treatment with even a low 1 mg/kg/d dose of the dual SRD5A1 and SRD5A2 inhibitor, dutasteride, reduces both normal prostate and tumor DHT content and weight in intact rats while elevating tissue testosterone. Daily oral treatment with finasteride significantly (P < 0.05) inhibits growth of LNCaP human prostate cancer xenografts in intact male nude mice, but this inhibition is not as great as that by equimolar oral dosing with dutasteride. This anticaner efficacy is not equivalent, however, to that produced by castration. Only combination of dutasteride and castration produces a greater tumor inhibition (P < 0.05) than castration monotherapy against androgen-responsive LNCaP cancers. In contrast, no response was induced by dutasteride in nude mice bearing androgen-independent PC-3 human prostatic cancer xenografts.

**Conclusions:** These results document that testosterone is not as potent as DHT but does stimulate prostate cancer growth, thus combining castration with dutasteride enhances therapeutic efficacy.

Dihydrotestosterone (DHT) is the major intracellular growth factor for normal and neoplastic prostatic epithelial cells due to its high-affinity binding to androgen receptors (AR; ref. 1). The intracellular DHT concentration determines the prostatic cell content via its ability to regulate the proportion of ligand-occupied AR (2, 3). Once a critical threshold of AR is occupied by DHT, signal transduction pathways are activated which control the growth and functional activities of the prostatic epithelium (1, 3). The intracellular DHT concentration is thus paramount and regulated by both the supply of testosterone and other precursors from the systemic circulation and the complex interplay between intracellular prostatic enzymes of androgen metabolism, particularly the 5α-reductase (i.e., SRD5A1) family of reductive enzymes that irreversibly convert testosterone into DHT (4). The SRD5A1 family includes two isoforms each encoded by a distinct gene (5). SRD5A1 (i.e., 5α-reductase type I) is expressed widely and is the major isoform expressed in tissues, such as liver and skin (5). Distinguishing characteristics of this type I isoform are its neutral (i.e., pH 7.0) optima and high (i.e., micromolar) K_m for testosterone (5). SRD5A2 (i.e., the type II isoform) is more restrictive in its expression, being the major isoform expressed by male sex accessory tissues, such as the prostate (5). Distinguishing characteristics of this type I isoform are its acidic (i.e., pH 5.0) optima and low (i.e., nmol/L) K_m for testosterone (5). Whereas the major isoform expressed in the prostate stroma is SRD5A2, normal and malignant prostate epithelial cells express SRD5A1 (5). Thus, DHT concentration in normal and neoplastic prostatic tissue is effected by varying inputs from both SRD5A1 and SRD5A2 isoforms.

A greater degree of suppression of the intracellular DHT concentration is required to inhibit the growth of malignant versus nonmalignant prostatic epithelial cells (2, 3, 6, 7). Thus,
Table 1. Comparison of the serum half-life of various steroidal 5α-reductase inhibitors in rats versus humans and their species-specific inhibitory potency against SRD5A1 and SRD5A2 isoforms

<table>
<thead>
<tr>
<th>Inhibitor (refs.)</th>
<th>Species</th>
<th>Serum half-life</th>
<th>Inhibitor potency (IC50), nmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>SRD5A1 isoform</td>
</tr>
<tr>
<td>Episteride (8–11)</td>
<td>Rat</td>
<td>3 h</td>
<td>20 ± 7</td>
</tr>
<tr>
<td></td>
<td>Human</td>
<td>27 h</td>
<td>350 ± 50</td>
</tr>
<tr>
<td>Finasteride (12–14)</td>
<td>Rat</td>
<td>2 h</td>
<td>5.4 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>Human</td>
<td>6 h</td>
<td>360 ± 40</td>
</tr>
<tr>
<td>Dutasteride (14, 15)</td>
<td>Rat</td>
<td>31 h</td>
<td>0.3 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>Human</td>
<td>&gt;3 d</td>
<td>6 ± 1</td>
</tr>
</tbody>
</table>
finasteride at a dose that reduced normal VP cell and DHT content (7, 32). These results document that H tumors express SRD5A activity not inhibited by type 2 specific inhibitors. The 5α-reductase inhibitor, dutasteride, is identical to finasteride, except in position 17 (Table 1). In dutasteride, the carbamoyl group at position 17 is coupled to a bis(trifluoromethyl)-phenyl moiety instead of a t-butyl moiety. This modification shifts the serum half-life and increases the inhibition potency of dutasteride as a reversible SRD5A1 inhibitor and a time-dependent, irreversible SRD5A2 inhibitor (ref. 23; Table 1). Due to its longer serum half-life and lower IC₅₀ for both SRD5A1 and SRD5A2 (Table 1), dutasteride at clinical oral doses of 5 mg/d, which produces steady-state serum drug levels of ≥1 μmol/L (20), is a dual 5α-reductase inhibitor. Therefore, a series of models were used to determine whether there is an enhanced efficacy when dutasteride is used to inhibit both SRD5A1 and SRD5A2 for the treatment of prostate cancer.

**Materials and Methods**

**Reagents.** The 5α-reductase inhibitor dutasteride [17β-N-(2,5-bis (trifluoromethyl)phenyl-carbamoyl)-4-aza-5α-androstan-1-en-3-one] was provided by GlaxoSmithKline (Research Triangle Park, NC), and finasteride [17β-N-tert-butylcarbamoyl]-4-aza-5α-androstan-1-en-3-one] was obtained from Kemprotec Ltd. (Middlesbrough, United Kingdom). For oral dosing, animals received daily gavage with 200 μl polyethylene glycol 400/1% Tween 80 (Sigma; St. Louis, MO) as vehicle containing indicated dose of drug.

**Tissues, tumors, and cell lines.** Nonmalignant human prostate tissue was obtained from radical prostatectomy specimens from seven patients undergoing surgery for localized prostate cancer under an institutional review board–approved protocol. The rat tumors used (i.e., H and G sublines) are members of the Dunning R-3327 system of institutional review board–approved protocol. The rat tumors used patients undergoing surgery for localized prostate cancer under an institutional review board–approved protocol. The rat tumors used to various agents was determined using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay as described (35).

**Real-time Taqman reverse transcription-PCR quantitation of SRD5A1 and SRD5A2 expression.** The Taqman primers and probes for human and rat SRD5A1 and SRD5A2 genes were purchased from Applied Biosystems (Foster City, CA). Using the appropriate species-specific primers and copy number standards, SRD5A1 and SRD5A2 mRNA levels in the rat or human tissues were determined and expressed in copy number per microgram of total RNA.

**5α-Reductase enzymatic assays.** The determination of SRD5A1 and SRD5A2 isoform-specific enzymatic activities was achieved by subtracting the SRD5A1 isoform-specific activity from the total 5α-reductase activity for each tissue. The SRD5A1 isoform-specific activity was determined by irreversibly inhibiting the SRD5A2 activity selectively within tissue homogenates using a preincubation step with NADPH in the presence of finasteride at a concentration of 5 nmol/L for rodent tissues or 100 nmol/L for human tissues. This assay is based on the studies of Azzolina et al., which documented that within 1 hour of incubation with 0.5 nmol/L NADPH and these concentrations of finasteride, SRD5A2 isoform is completely and irreversibly inhibited (36). In addition, this group documented that the subsequent dilution of finasteride to 0.5 nmol/L for rat tissues and 10 nmol/L for human tissue does not allow reactivation of the inhibited SRD5A2 isoform but is too low a concentration to inhibit the SRD5A1 isoform (36). Based on these results, 1:1 wet weight/volume whole (unfractionated) cell homogenates were produced with an all-glass tissue homogenizer (Kontes, Inc., Vineland, NJ) using 0.1 mol/L sodium phosphate (pH 6.6) containing 0.5 mmol/L NADPH (i.e., H buffer). Two separate 100 μl aliquots were processed: (a) with no finasteride pretreatment for the determination of the total 5α-reductase (i.e., SRD5A1 plus SRD5A2) activity and (b) with a 1-hour finasteride (i.e., 5 nmol/L for rat tissues or 100 nmol/L for human tissues) and 0.5 mmol/L NADPH preincubation at 37°C for the selective determination of SRD5A1 only. Both of the aliquots were then diluted with the addition of 400 μl H buffer. A 100 μl aliquot for each of these diluted mixtures was then incubated at 37°C with 100 μl H buffer containing 0.5 mmol/L NADPH and 0.1 μmol/L [3H]testosterone (Amersham; Piscataway, NJ; specific activity, 50 Ci/mmol) to give a final concentration of 0.5 mmol/L NADPH and 50 nmol/L testosterone. At 30 and 60 minutes, 30 μl of the mixtures were processed by TLC to determine the picomoles of 5α-reduced products found per hour per 10⁶ cells as described previously (7).

**Animal studies.** All animals used in these studies were maintained in accordance with the NIH Guide for the Care and Use of Laboratory Animals and the specific protocols used were approved by the Johns Hopkins Medical Institutions Animal Care and Use Committee. Adult male inbred Copenhagen rats (175-200 g body weight) were used for serial passage of the R-3327H and R-3327G rat prostatic cancer lines and 4- to 6-week-old male athymic nude BALB/c nu/nu mice were used for passage of the PC-82, LNCaP, and PC-3 human prostate cancer variants as described previously (7). All animals were obtained from Harlan Sprague-Dawley (Indianapolis, IN).

For the testing of the in vivo antitumor growth response of Dunning R-3327H rat prostatic cancers to 5α-reductase inhibitors, Copenhagen male rats were inoculated s.c. in the flank with 20 mg minced H tumor in 0.5 mL Matrigel (Collaborative Biomedical, Bedford, MA). When the H tumors reached 1 to 2 cm³ in size, randomization was done into groups of 10 tumor-bearing animals each and the groups were given various treatments. Tumor volumes were recorded each 5 days using microcalipers to determine the volume of the tumors as described previously (7). After 55 days of treatment, blood was drawn and VP and H tumors were removed and weighed.

For testing against the LNCaP and PC-3 human prostate cancer, male nude mice were inoculated in the flank with 200 μl Matrigel containing 2 × 10⁶ viable LNCaP or PC-3 cells harvested from exponentially growing in vitro cultures. When the tumors reached a starting size of 100 mm³, randomization was done into groups of 10 tumor-bearing animals each and the groups were given various treatments. After the indicated time of treatment, the tumor weights were determined for each animal.

**Serum and tissue DHT and testosterone determination.** The blood and tissues were extracted and extracts were assayed for determination of their testosterone and DHT levels using a validated tandem mass spectrometry method by PPD-Pharmaco (Richmond, VA).

**Statistical analysis.** Data (mean ± SE) were analyzed by one-way ANOVA. P < 0.05 is considered significant.

**Results**

**Expression of SRD5A1 and SRD5A2 in normal and malignant prostate tissues.** SRD5A2 mRNA expression is 2-fold higher than SRD5A1 in the normal rat and 4-fold higher in human prostate tissue (Fig. 1A). SRD5A1 mRNA is detectable in early-passage cultures of nonimmortalized normal human prostatic epithelial (i.e., PREC) cells and in three different immortalized
normal human prostatic cell lines (i.e., hTERT, RWPE-1, and HPR-AR) at levels comparable with that expressed by normal human prostatic tissue (Fig. 1B). Although SRD5A1 mRNA is expressed in all malignant human (i.e., PC-3, CWR22Rv1, MDA-PC-2B, C4-2B, DU145, LAPC-4, PC-82, or LNCaP; Fig. 1B) and rat (i.e., H and G; Fig. 1C) prostate cancer variants, expression is significantly elevated in several of these (i.e., MDA-PC-2B and LAPC-4 human and H rat prostate cancer). In contrast, normal rat and human prostatic tissues and H and G rat prostate cancer tissues contain detectable levels of SRD5A2, whereas none of the human normal or malignant cell lines do. This is because these tissue samples, unlike the cell lines, contain supporting host stromal cells and such stromal cells express SRD5A2 (5). As further documentation that the SRD5A2 mRNA level detectable from tissue sample is derived from contaminating stromal not epithelial cells, reverse transcription-PCR analysis was done on a pure population of G rat prostate cancer cell line exponentially growing in cell culture. When these in vitro cultured G cells were analyzed for SRD5A1 and SRD5A2 expression, the level of SRD5A1 mRNA remains high, whereas SRD5A2 is undetectable.

SRD5A1 versus SRD5A2 isofrom-specific enzymatic activity. These mRNA results document that the SRD5A1 is the major, if not exclusive, isofrom expressed by malignant rat and human prostate cancer cells. Previous studies showed that SRD5A1 mRNA levels are correlated with 5α-reductase activity (37, 38). In these previous studies, the 5α-reductase enzymatic assays were done at both acidic and neutral pH in an attempt to evaluate the activity associated with SRD5A1 versus SRD5A2 isoforms. Unfortunately, Smith et al. (39) documented that the pH profile of the SRD5A1 versus SRD5A2 is both too broad and varies depending on the concentration of testosterone used in the assay [i.e., at 20 nmol/L testosterone concentration, SRD5A2 has a pH 5.0 (optima) that is shifted to an optima at pH 6.5 at 1 μmol/L testosterone concentration] to allow accurate determination based on pH alone. An accurate determination of the isofrom-specific activities is possible if a preincubation treatment with the appropriate concentration of finasteride is used to selectively, and irreversibly, inhibit SRD5A2 activity so that the SRD5A1 isofrom-specific activity can be accurately determined. By subtracting this SRD5A1 isofrom-specific activity from the total (i.e., SRD5A1 + SRD5A2) activity assayed without finasteride treatment, the SRD5A2 isofrom-specific activity can be determined.

Using this method, the SRD5A1 or SRD5A2 activity was determined from a series of normal or malignant rat and human prostatic tissues harvested directly from their host without cell culturing. These results documented that the level of SRD5A2 enzymatic activity is >10-fold higher than SRD5A1 enzymatic activity in the normal rat VP (Table 2). These enzymatic activity differences are thus higher than predicted by the 2-fold difference in mRNA expression (Fig. 1A). In contrast, the SRD5A2 activity in the H and G rat prostate cancer tissues derived from contaminating tumor stromal cells is >90% reduced compared with normal rat prostate (Table 2). Again, this is a greater reduction in activity than predicted by mRNA levels (Fig. 1C). For the H rat prostate cancer, this reduction in SRD5A2 activity is coupled to a >5-fold increase in SRD5A1 activity (Table 2), which is consistent with the comparative mRNA levels (Fig. 1C). Such an enhancement in SRD5A1 activity was not observed for the G rat prostate cancer (Table 2).

With regard to human tissues, the SRD5A2 activity is >10-fold higher than SRD5A1 activity in nonmalignant human prostate tissue (Table 2), which is higher than predicted based on mRNA expression data (Fig. 1A). Both xenografted PC-82 and LNCaP tumor tissue express a low level of SRD5A2 activity.

### Table 2. Level of enzymatic activity of SRD5A1 and SRD5A2 isoforms in various tissues harvested directly from hosts with no cell culturing

<table>
<thead>
<tr>
<th>Tissue (n = 3-5)</th>
<th>SRD5A1</th>
<th>SRD5A2</th>
</tr>
</thead>
<tbody>
<tr>
<td>RatVP</td>
<td>30 ± 5</td>
<td>391 ± 21</td>
</tr>
<tr>
<td>R-3327H rat prostate cancer</td>
<td>164 ± 21 *</td>
<td>31 ± 9 *</td>
</tr>
<tr>
<td>R-3327G rat prostate cancer</td>
<td>35 ± 3 *</td>
<td>15 ± 4 *</td>
</tr>
<tr>
<td>Human prostate tissue</td>
<td>48 ± 7</td>
<td>545 ± 75</td>
</tr>
<tr>
<td>PC-82 human prostate cancer</td>
<td>20 ± 6 *</td>
<td>10 ± 5 *</td>
</tr>
<tr>
<td>LNCaP human prostate cancer</td>
<td>104 ± 34</td>
<td>18 ± 2 *</td>
</tr>
</tbody>
</table>

*P < 0.05 versus rat prostate.

*P < 0.05 versus human prostate.
(Table 2), derived from contaminating tumor stromal cells. SRD5A1 activity was low in the PC-82 but elevated 2-fold in the LNCaP prostate cancer tissue compared with nonmalignant prostate tissue (Table 2).

Pharmacologic basis for selective efficacy of finasteride and dutasteride for normal rat prostate versus H rat prostate cancer. Because SRD5A1 enzymatic activity is high in the R-3327H rat prostate cancer tissue and not in the normal rat prostate, R-3322G rat prostate, or PC-82 human prostate cancer (Table 2), this could explain why finasteride suppress the tissue DHT concentration in these latter tissues but not in the R-3327H rat prostate cancers. This suggests that a dual 5α-reductase inhibitor with a longer serum half-life and/or higher potency is needed to suppress the DHT concentration in R-3327H rat prostatic cancers. Therefore, rats s.c. bearing 1 to 2 cm³ R-3327H rat prostatic cancers were randomized into receiving daily oral b.i.d. treatment with varying doses of either finasteride or dutasteride and the effect on tumor volume was monitored for 55 days of treatment. The choice of dose range used is based on pharmacokinetic modeling that the nadir level of serum finasteride produced by multiday oral dosing is 0.5 nmol/L at 0.7 mg/kg/d (i.e., 1.89 nmol/kg/d), 5.4 nmol/L at 7 mg/kg/d (i.e., 18.9 nmol/kg/d), and 54 nmol/L at 70 mg/kg/d (i.e., 189 nmol/kg/d; ref. 22). Due to its >10-fold longer half-life, similar pharmacokinetic modeling predicts the equimolar dosing with dutasteride should produce nadir serum values of 125 nmol/L at 1 mg/kg/d (i.e., 1.89 nmol/kg/d), 1.25 μmol/L at 10 mg/kg/d (i.e., 18.9 nmol/kg/d), and 12.5 μmol/L at 100 mg/kg/d (i.e., 189 nmol/kg/d; ref. 22). Experimental confirmation of the accuracy of these modeling data is provided by the observation that oral dosing of rats with 1 mg/kg/d produced peak serum dutasteride levels of 263 nmol/L (40).

As controls, groups of H rat prostate cancer-bearing animals were dosed b.i.d. orally with vehicle or surgically castrated and then given oral dosing b.i.d. of vehicle. These studies document that the R-3327H rat prostate cancer essentially stops growth following surgical castration (Fig. 2). This response to castration is correlated with a >90% decrease in serum testosterone and H tumor tissue concentration of both testosterone and DHT (Table 3). Likewise, the concentration of testosterone and DHT decreases by >90% within the VP of such castrated animals. This is correlated with a 70% reduction in the weight of the VP of the castrated animals. Treatment with finasteride, even at doses of 70 mg/kg/d (189 nmol/kg/d), is unable to decrease the growth of the H rat prostate cancers (Fig. 2) due to its inability to lower H tumor DHT concentration (Table 3). In contrast to the situation for the H tumor, the weight and DHT concentration of VP, which expresses only a low level of SRD5A1 (Table 2), is significantly decreased in these tumor-bearing rats treated with finasteride (Table 3). In fact, there is a dose-response relationship between amount of finasteride and decreases in both prostatic weight and prostatic DHT concentration (Table 3). When tumor-bearing animals are treated with dutasteride, not only did the VP weight decrease, but also there is also a significant decrease in H tumor growth (Fig. 2) and the tumor weight at the end of 55 days of treatment (Table 3). This antitumor effect is associated with a dose-response reduction in DHT content coupled with a >2-fold increase in serum testosterone and tissue testosterone levels in both H tumor and VP (Table 3). Once the DHT content in the H tumor is reduced by 75% to 93 nmol/L by the lowest dosing of dutasteride, however, there is no further anticancer growth inhibition produced by the higher doses, although the highest dose reduces DHT content by an additional 90% (Table 3). These results document that although the highest dutasteride dose reduces H tumor DHT to the essential same nadir produced by castration it does not produce the same magnitude of prostate cancer growth inhibition. This is consistent with an elevated tumor testosterone level stimulating prostate cancer growth.

In vivo response of LNCaP human prostate cancer cells to 5α-reductase inhibition alone and in combination with androgen ablation. Based on the ability of testosterone to stimulate prostate cancer growth, the efficacy of dual SRD5A inhibition, theoretically, should be enhanced by simultaneously lowering the tumor testosterone levels by androgen ablation. As a model system to evaluate this possibility, the LNCaP cell line was used because its expresses only a low level of SRD5A1 (Table 2), is significantly decreased in these tumor-bearing rats treated with finasteride (Table 3). In fact, there is a dose-response relationship between amount of finasteride and decreases in both prostatic weight and prostatic DHT concentration (Table 3). When tumor-bearing animals are treated with dutasteride, not only did the VP weight decrease, but also there is also a significant decrease in H tumor growth (Fig. 2) and the tumor weight at the end of 55 days of treatment (Table 3). This antitumor effect is associated with a dose-response reduction in DHT content coupled with a >2-fold increase in serum testosterone and tissue testosterone levels in both H tumor and VP (Table 3). Once the DHT content in the H tumor is reduced by 75% to 93 nmol/L by the lowest dosing of dutasteride, however, there is no further anticancer growth inhibition produced by the higher doses, although the highest dose reduces DHT content by an additional 90% (Table 3). These results document that although the highest dutasteride dose reduces H tumor DHT to the essential same nadir produced by castration it does not produce the same magnitude of prostate cancer growth inhibition. This is consistent with an elevated tumor testosterone level stimulating prostate cancer growth.

Specificity of antiprostatic cancer responsiveness to dutasteride. Lazier et al. reported that dutasteride at concentrations of ≥50 μmol/L kills both AR-positive androgen-sensitive LNCaP and AR-negative androgen-independent PC-3 cells in culture in a manner not reversed by DHT supplementation (41). Because
the 100 mg/kg/d oral dose of dutasteride used for the LNCaP model produces serum dutasteride levels of ≈12.5 μmol/L (22), this raises the issue of whether at this dose the efficacy of dutasteride alone or in combination with androgen ablation involves its specific ability to inhibit SRD5A and/or a general toxic effect on cell survival. To resolve this, intact male nude mice were inoculated with PC-3 cells and allowed to go untreated until the cancers reached 100 mm³ and then randomized into four groups of 10 tumor-bearing mice each. One group each was (a) given b.i.d. daily dosing with vehicle only (i.e., intact controls), (b) given b.i.d. daily dosing with 100 mg/kg/d dutasteride, (c) castrated and given b.i.d. dosing with vehicle (i.e., castrate controls), or (d) castrated and given b.i.d. dosing with dutasteride. After 14 days of treatment, there was no difference in the size of the PC-3 cancers in any of the four groups (i.e., cancers were 395 ± 20 mm³ in intact controls, 375 ± 45 mm³ in castrate controls, 410 ± 39 mm³ in intact mice given dutasteride, and 400 ± 31 mm³ in castrated mice given dutasteride). These results are consistent with a requirement for the prostate cancer being AR positive and sensitive to its signaling for it to be responsive to 100 ng/kg/d dutasteride.

**Discussion**

The present studies showed that prostate cancer cells express only a low level of SRD5A2 mRNA and enzymatic activity but often express enhanced levels of SRD5A1 activity compared with nonmalignant prostate tissue. These observations have significant implications for the use of 5α-reductase inhibitors for prostate cancer. 5α-Reductase inhibitors do not suppress the systemic levels of testosterone and their long-term use does not produce muscle, bone, or libido loss, although such treatments lower prostatic DHT inducing regression of nonmalignant prostatic tissue (17, 19). These clinical observations document that regulation of nonmalignant prostatic epithelial survival and proliferation is primarily driven by prostatic DHT and by not testosterone levels. This is because SRD5A2 enzymes in the prostate stroma produce an adequate level of DHT to occupy a sufficient number of AR within the nuclei of prostate stromal cells to induce their production of a critical level of specific paracrine peptide growth factors (termed andromedins; refs. 1, 4). Once formed in the stromal compartment, these paracrine andromedins diffuse into the epithelial compartment where they bind to their cognate receptors in the basal and luminal cells regulating their proliferation and survival, respectively (1, 4). If sufficient inhibition of stromal SRD5A2 is produced to prevent adequate production of DHT and thus stromal andromedins,
then regression of the prostatic epithelium is induced (7). This stromal-based paracrine AR axis in nonmalignant prostate tissue explains why a SRD5A2 selective inhibitor, such as finasteride, can produce nonmalignant prostatic regression and can be used for treatment of benign prostatic hyperplasia, although it does not inhibit SRD5A1 expressed within prostatic epithelial cells.

During prostatic carcinogenesis, molecular changes occur producing a gain of function in the AR axis from a paracrine to an autocrine pathway in which occupancy of the AR by DHT within the nuclei of malignant cells directly controls the autocrine production of growth factors for survival and proliferation of these malignant cells (1, 42). Coupled with these changes, there is enhanced expression of the SRD5A1 isoform by prostate cancer cells, enhanced SRD5A1 expression is consistent with why previous clinical trials with the SRD5A2-specific inhibitor, finasteride, for the treatment of metastatic prostatic cancer have had limited success either when used as monotherapy (21, 22) or when combined with antiandrogens (43). In the present studies, the dual SRD5A1 and SRD5A2 inhibitor, dutasteride, has been documented to have enhanced in vivo efficacy compared with a SRD5A2-specific inhibitor against both rat and human prostate cancers. This efficacy is not equivalent as monotherapy, however, to that produced by standard androgen ablation alone. These results document that although testosterone is not as potent as DHT in driving malignant growth it still does stimulate such growth. The present experimental studies document that a combination of dual SRD5A1 and SRD5A2 inhibition with standard androgen ablation produces at least an additive antitumor growth-inhibitory effect compared with either monotherapy alone. The rationale for such an enhancement is that standard androgen ablation decreases testicular testosterone production but does not completely eliminate prostate cancer tissue testosterone content (44). Thus, in the androgen-depressed environment induced by standard androgen ablation, local 5α-reduction within metastatic prostate cancer cells amplifies the remaining androgen signal. Such androgenic amplification can be inhibited by simultaneous treatment with a dual 5α-reductase inhibitor, such as dutasteride. This dutasteride-androgen ablation combinational approach while limiting the amplification of androgen does not eliminate all AR-dependent signaling. This suggests that to block remaining AR growth signaling a novel (i.e., "bulky") antiandrogen may need to be added to the dutasteride-androgen ablation combination (45). Presently, we are developing such novel monofunctional and bifunctional "bulky" antiandrogens for such combinatorial AR-targeted therapy.

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