Tumorigenesis and Neoplastic Progression

Evidence for Steroidogenic Potential in Human Prostate Cell Lines and Tissues

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Malignant prostate cancer (PCa) is usually treated with androgen deprivation therapies (ADTs). Recurrent PCa is resistant to ADT. This research investigated whether PCa can potentially produce androgens de novo, making them androgen self-sufficient. Steroidogenic enzymes required for androgen synthesis from cholesterol (CYP11A1, CYP17A1, HSD3β, HSD17B3) were investigated in human primary PCa (n = 90), lymph node metastases (LNMs; n = 8), and benign prostatic hyperplasia (BPH; n = 6) with the use of IHC. Six prostate cell lines were investigated for mRNA and protein for steroidogenic enzymes and for endogenous synthesis of testosterone and 5α-dihydrotestosterone. All enzymes were identified in PCa, LNMs, BPH, and cell lines. CYP11A1 (rate-limiting enzyme) was expressed in cancerous and noncancerous prostate glands. CYP11A1, CYP17A1, HSD3β, and HSD17B3 were identified, respectively, in 78%, 52%, 16%, and 82% of human BPH and PCa samples. Approximately 10% of primary PCa, LNMs, and BPH expressed all four enzymes simultaneously. CYP11A1 expression was stable, CYP17A1 increased, and HSD3β and HSD17B3 decreased with disease progression. CYP17A1 expression was significantly correlated with CYP11A1 (P = 0.0009), HSD3β (P = 0.0297), and HSD17B3 (P = 0.0090) in vitro, suggesting CYP17A1 has a key role in prostatic steroidogenesis similar to testis and adrenal roles. In vitro, all cell lines expressed mRNA for all enzymes. Protein was not always detectable; however, all cell lines synthesized androgen from cholesterol. The results indicate that monitoring steroidogenic metabolites in patients with PCa may provide useful information for therapy intervention. (Am J Pathol 2012, 181: 1078–1087; http://dx.doi.org/10.1016/j.ajpath.2012.06.009)

The term castrate-resistant prostate cancer (CRPC) implies that this type of prostate cancer (PCa) can proliferate and survive without the testes, the principal androgen-producing tissue. Androgens function via androgen receptor (AR), a ligand-activated nuclear transcription factor. Most CRPCs continue to express AR and androgen-regulated proteins such as prostate-specific antigen.1 PCa may survive independently of androgens because of AR gene amplification, somatic missense mutations of the AR, AR coregulator mutations, altered growth factor signaling and kinase activation of AR, and AR activation by adrenal androgens.2,3 In CRPC, the androgens testosterone and dihydrotestosterone (DHT) are recorded at levels sufficient for activation of AR in patients who have undergone chemical or physical castration.4,5 Recent results that show de novo androgen synthesis with the use of a PCa cell line (LNCaP) xenograft mouse model suggest PCa cells possess steroidogenic properties that enable survival in androgen-depleted environments.6 Therefore, CRPCs that express AR are likely to be androgen dependent, with the original source of androgen being replaced by adrenal or intratumoral androgen synthesis. These PCa cases are considered to be androgen self-sufficient.7

Cholesterol is central to steroid hormone synthesis for normal reproductive function and bodily homeostasis. Steroid hormones, including androgens, are produced typically within the testes, adrenal glands, ovaries, placenta, and the brain.8 The rate-limiting enzyme in the

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Steroidogenic pathway is CYP11A1, a member of the cytochrome P450 family and predominantly expressed in the gonads and adrenal cortex. CYP11A1 is responsible for side chain cleavage of cholesterol, converting it to pregnenolone. The following enzymes are required to convert pregnenolone to testosterone: CYP17A1 which contains both 17α-hydroxylase (hydroxyl addition to pregnenolone and progesterone) and 17,20-lyase (side-chain cleavage from 17-hydroxyprogesterone and 17-hydroxypregnenolone) activity and is expressed in the testes and adrenal glands. Ovaries, skin, and the prostate have been found to express HSD3β which converts dehydroepiandrosterone to androstenedione and is expressed predominantly in adrenal gland, testis, and ovary; HSD17β3 which catalyzes the conversion of androstenedione and dehydroepiandrosterone to testosterone and androstenediol, respectively, is expressed in the cytoplasm and nucleus of cells within the seminiferous tubules of the testes.

CYP11A1, CYP17A1, HSD3β, and HSD17β3 have received limited investigation in prostate tissues. The CYP17A1 protein was confirmed in prostate tissue by immunohistochemistry (IHC). HSD3β transcripts and protein have been reported in normal prostate and benign prostatic hyperplasia (BPH), but no isotype was described. HSD3β2 RNA, but not protein, has been reported in PCa. HSD17β3 was, until recently, found only in testis but has now been detected in some CRPCs. Protein and mRNA expression for HSD17β3 has been confirmed in mouse LNCaP xenografts and mRNA in human PCa. RNA transcripts for CYP11A1 were found in human PCa tissues, but the protein was not analyzed. Recent reports show that mRNA for these enzymes and other steroidogenic enzymes are expressed weakly and variably in PCa cell lines and mouse xenograft models, but no IHC analysis has been performed for these enzymes, as a group, in localized PCa. The present investigation tested the hypothesis that prostate cell lines express mRNA and proteins for CYP11A1, CYP17A1, HSD3β2, and HSD17β3 and are able to produce androgens from cholesterol. In addition, human primary PCa, lymph node metastases (LNM), and BPH were analyzed with IHC for expression and localization of these proteins.

Materials and Methods

All chemicals were obtained from Sigma-Aldrich (St. Louis, MO) unless otherwise noted. Approval for use of human tissues was obtained from the Human Research Ethics Committee of the Royal Prince Alfred Hospital and The University of Queensland.

Cell Lines

Prostate cell lines LNCaP, 22Rv1, PC3, DU145, RWPE1 (American Type Culture Collection, Manassas, VA), and ALVA41 (provided by K.A. Landers, Queensland Institute of Medical Research, Herston, Australia) were used. LNCaP, 22Rv1, PC3, DU145, and ALVA41 are PCa cell lines, whereas RWPE1 was immortalized from normal prostate cells. The cells were routinely maintained in RPMI 1640 media with 10% fetal bovine serum and penicillin/streptomycin (Invitrogen, Life Technologies, Mt. Waverley, Australia).

RNA and Protein Analyses

RNA was collected twice from each cell line, and each collection was separated by at least two passages. RNA was extracted from cells with the use of an RNeasy Mini Kit (Qiagen, Clifton Hill, Australia) and reverse transcribed with a Superscript First Strand Synthesis System (Invitrogen, Carlsbad CA). Quantitative RT-PCR primers for the candidate genes were as previously published.

The following primers were synthesized: CYP11A1 forward, 5'-CTGCATCTTCACTGTCCTGCC-3'; CYP11A1 reverse, 5'-GGTAGACCACTGAGAACCACTC-3'; CYP17A1 forward, 5'-TCCCCAGGTTGGTTTTTCTGAT-3'; CYP17A1 reverse, 5'-GGTCGAGGGCGCTGTAGTTAC-3'; HSD3β2 forward, 5'-CTGCTGCGCTTTTTTACACA-3'; HSD3β2 reverse, 5'-AGAAAGTGTGTGGGCCGACT-3'; HSD17B3 forward, 5'-CTGAAAGCTCACACACAAAGGTC-3'; HSD17B3 reverse, 5'-CTGCTGCTTTTGCTTCCTTACG-3'; GAPDH forward, 5'-AGCACCACTTTGGTCAGTAC-3'; and GAPDH reverse, 5'-TACAGTTGCAATGAC-3'.

Real-time PCR was performed in 15-μL volumes [cDNA, 2× QuantitTec SYBR Green PCR Master Mix (Qiagen Pty Ltd, Chadstone Centre, Australia), and 5 pmol of each primer] with the use of a 72-well Rotor-Gene 6000 Real-Time Rotary Analyzer (Corbett Life Science, Mortlake, Australia). Cycles consisted of a hot start (95°C for 15 minutes), denaturing step (20 seconds at 95°C), annealing step (20 seconds at 55°C), and an extension step (20 seconds at 72°C) for 35 to 40 cycles. The PCR finished with a melt curve between 65°C and 99°C. Standard curves for each gene were generated from control cDNA, then expression for each gene in each individual cell line was normalized to the expression of the housekeeping gene GAPDH in the same cell line. Data were analyzed by the Pfaffl method and are presented as mean ± SEM.

For Western blot analysis, cell lysates were collected in RIPA buffer [150 mmol/L NaCl, 25 mmol/L NaF, 0.5 mmol/L EDTA, 0.1% (w/v) SDS, 0.5% (w/v) sodium deoxycholate, 1% IGEPAL CA-630, in 50 mmol/L Tris-Cl buffer (pH 7.5)], containing protease and phosphatase inhibitors (2 μg/mL phenylmethylsulfonyl fluoride, 10 μg/mL leupeptin, 10 μg/mL aprotinin, 1 mmol/L sodium orthovanadate), and then sonicated briefly to disrupt cell membranes. Protein concentration was determined with a Pierce BCA Protein Assay Kit (Thermo Scientific, Rockford, IL). Equal amounts of protein (40 μg) were separated with 10% SDS-PAGE, transferred to polyvinylidene fluoride membranes, and probed with the selected antibodies. Polyclonal anti-human antibodies (Santa Cruz Biotechnology, Santa Cruz, CA) were as follows: CYP11A1 (sc-18043), CYP17A1 (sc-46084), HSD3β (sc-30820), and HSD17B3 (sc-66415). Duplicate Western blot analyses with blocking peptides (Santa Cruz Biotechnology) to inhibit specific antibody binding were...
done as antibody controls (data not shown). Horseradish peroxidase-conjugated secondary antibodies (Invitrogen, Thornten, Australia) were detected with chemiluminescence substrate (Pierce, Rockford, IL; Catalog numbers 1859674 and 1859675) and exposed to X-ray film.

HPLC and Radiolabeling Analysis

For treatments with 14C-labeled cholesterol, phenol red-free RPMI 1640 growth medium (no serum, no antibiotics) was used. Cells were grown to ≥80% confluence in 6-well tissue culture plates. Before treatment, cells were washed twice in PBS to remove residual serum components, then incubated for 24 hours in phenol red-free RPMI 1640 (no serum, no antibiotics). Then 14C-labeled cholesterol (1 µCi/well) in fresh phenol red-free RPMI 1640 (no serum, no antibiotics) was delivered to the cells and incubated for a further 7 days. Cells were lysed with 1 mol/L NaOH; lysates were collected, vortexed for 1 minute, and then incubated at room temperature for ≥90 minutes to allow base hydrolysis of steroid esters. Control lysates were spiked with 14C-labeled cholesterol immediately before diethyl ether extraction. Radiolabeled experiments were done in triplicate and repeated. Lysates from LNCaP, 22Rv1, PC3, DU145, ALVA41, and RWPE1 cells were extracted twice with diethyl ether. The organic fraction was evaporated under nitrogen gas. Precipitates were resuspended in 50% methanol/25% ethanol/25% water spiked with 10 µg/mL cholesterol. Waters high-performance liquid chromatography (HPLC) system, including an Altima C18, 5 µm, 150 mm × 4.6 mm (Alltech, Baulkham Hills, Australia) column and Millenium version 2.10 software, was used for chromatographic separation. Each chromatography run was at 1 mL/min and as follows: isocratic flow 40:60 acetonitrile (ACN)/H2O (0 to 20 minutes), linear gradient to 50:50 can/EtOH (20 to 30 minutes), isocratic flow 50:50 ACN/EtOH (30 to 41 minutes), linear gradient to 40:60 ACN/H2O (41 to 42 minutes), and isocratic flow 40:60 ACN/H2O (42 to 50 minutes). Fractions for testosterone, DHT, and cholesterol were collected as spiked molecules eluted from samples treated with 14C-cholesterol and untreated. Fractions were vacuum evaporated, and precipitates were reconstituted in liquid scintillant (PerkinElmer, Waltham, MA) before radio-counting (Wallac MicroBeta 1450 LSC Counter; PerkinElmer).

Immunohistochemistry

Samples were obtained from the Royal Prince Alfred Hospital and Aquesta Pathology (Toowong, Australia) and consisted of 6 BPH samples obtained by transurethral resection of the prostate, 90 samples of clinically localized PCa (79 from radical prostatectomy, 11 from transurethral resection of the prostate) of Gleason scores 5 to 10, and 8 samples of LNMs. Sections (4 µm) on Superfrost Plus histology slides were dewaxed and rehydrated to buffer. Primary antibodies were those used for Western blot analysis except for HSD3β, whereby sc-100466 was used. Antibody concentration was 4 µg/mL in Renaissance Antibody Diluent (Biocare Medical, Concord, CA). Secondary antibodies were from Dako (Dako Australia Pty Ltd, Botany, Australia). Positive control sections were from either human adrenal or testicular tissue. Negative controls were performed routinely without primary antibodies. Staining intensity and localization for CYP11A1, CYP17A1, HSD3β, and HSD17β3 were analyzed by N.C.B. and D.L. from de-identified slides. A positive result was recorded only if a sample had staining in PCa cells or glandular epithelium of BPH.

Statistical Analysis

When appropriate, data are presented as means ± SEMs. Linear regression was used to examine changes in steroidogenic enzyme expression correlating with increasing Gleason score and disease progression. Pearson’s χ2 test with Fisher’s exact test was used to ascertain relations in paired enzyme expression. P < 0.05 was considered significant. A one-tailed t-test was used for comparisons of samples treated with 14C-cholesterol, based on observations whereby changes in treated groups were only positive relative to the control.

Results

RNA and Protein of CYP11A1, CYP17A1, HSD3β2, and HSD17β3 in Prostate Cell Lines

To determine whether the prostate cell lines were capable of synthesizing androgens de novo, we quantified transcripts encoding the enzymes responsible for synthesis of testosterone from cholesterol (CYP11A1, CYP17A1, HSD3β2, and HSD17β3). All four enzymes analyzed were expressed in all six prostate cell lines (Figure 1). In the cell lines LNCaP, 22Rv1, DU145, and RWPE1, expression for each enzyme was generally low relative to GAPDH. However, the cell lines PC3 and ALVA41 showed strong expression of all four enzymes compared with GAPDH.

Western blot analysis was performed to examine protein expression of the selected steroidogenic enzymes in the six prostate cell lines. All four enzymes were expressed (Figure 2). The enzymes CYP17A1, HSD3β, and HSD17β3 were expressed in all six cell lines. CYP11A1 expression was found in PC3, DU145, ALVA41, and RWPE1 but not in LNCaP and 22Rv1. Human adrenal tissue was used as a positive control for CYP11A1, CYP17A1, and HSD3β. HSD17β3 is not expressed in human adrenal tissue. Fresh human testis tissue for a positive control of HSD17β3 protein was not available to us. However, positive staining for HSD17β3 in paraffin-embedded testis tissue was seen (Figure 3).
from 5 to 10. Only CYP11A1, CYP17A1, and HSD17β3 were present in LNMs, and only CYP11A1, HSD3β, and HSD17β3 were expressed in BPH. Each series of micrographs (Figure 3) has expression of each enzyme in positive tissue (adrenal gland or testis), BPH, and PCa. Negative controls for all antibodies were routinely clear of any stain (data not shown).

CYP11A1

In Figure 3, A–D, the positive control (Figure 3A; human adrenal tissue) had moderate-to-high expression in the glandular cortex. Low-to-high staining in BPH (Figure 3B) was predominantly cytoplasmic in the glandular luminal epithelium. Occasional epithelial basal cell cytoplasmic staining was also identified. There was little nuclear staining in these cells. In PCa, the staining intensity was variable (Figure 3, C and D), with moderate-to-low staining, mainly in the luminal epithelium. Staining in noncancerous regions was relatively consistent and of moderate intensity, particularly in the stroma, endothelial cytoplasm, smooth muscle, and nerve cells. CYP11A1 was expressed in 78% (100% BPH, 77% primary PCa, 75% LNMs) of the human
prostate and PCa tissue samples investigated (Table 1). The number of positively stained cells within each positive tissue section ranged from 5% to 90% within glandular structures and cancerous foci. All Gleason score groups, BPH, and LNM contained samples that were positive for CYP11A1.

In Figure 3, E–H, expression was low in the cytoplasm of human adrenal tissue (Figure 3E). BPH (Figure 3F) staining was predominantly cytoplasmic of luminal epithelial cells. There was little nuclear staining. CYP17A1 staining is evident in the cytoplasm of cells in the adrenal cortex, and no staining was present in the medulla (E). CYP17A1 staining is shown in glands with BPH (F) and PCa (G and H). Staining in BPH was predominantly cytoplasmic of luminal epithelial cells. The PCa staining was variable, with moderate-to-low level staining and mainly cytoplasmic of luminal epithelial cells. There was little nuclear staining. CYP17B3. Nuclear and cytoplasmic staining were seen in spermatogonia (along the basement membrane) and primary and secondary spermatocytes within the seminiferous tubules (I). The nucleus and cytoplasm of spermatids and spermatozoa cannot be differentiated, but these cells are positively stained (I). HSD17β staining is also shown in human BPH (J) and PCa (K and L). Staining in BPH was high and was predominantly cytoplasmic of luminal epithelial cells. There was occasional stromal cell staining. The cancer tissue staining was low to high and mainly cytoplasmic of luminal epithelial cells, but weak nuclear staining was also evident. Cytoplasmic staining in stromal and endothelial cells was generally low, but uniform across the sections. HSD3β was cytoplasmic in cells of the adrenal cortex glands, and no staining was present in the medulla (M). IHC staining is shown for HSD3β in human BPH (N) and PCa (O and P) tissues. Positive staining in BPH was moderate to high and cytoplasmic in the luminal epithelium. Staining in the cancer tissue was variable (minimal or absent through to moderate) and was mainly cytoplasmic in the epithelial cells. There was little nuclear staining.

Table 1. Summary of Patient Samples Positive by Immunohistochemical Analysis for Expression of CYP11A1, CYP17A1, HSD3β and HSD17β3 in BPH, Primary PCa, and LNM

<table>
<thead>
<tr>
<th>No. of samples</th>
<th>No. of positive samples (%)</th>
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</thead>
<tbody>
<tr>
<td>BPH</td>
<td></td>
</tr>
<tr>
<td>No. of samples</td>
<td>CYP11A1</td>
</tr>
<tr>
<td>6</td>
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<tr>
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<tr>
<td>LNM</td>
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</tr>
<tr>
<td>Total</td>
<td>104</td>
</tr>
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</table>

CYP11A1

In Figure 3, E–H, expression was low in the cytoplasm of human adrenal tissue (Figure 3E). BPH (Figure 3F) staining was predominantly cytoplasmic in luminal epithelial cells and was variable, with low-to-high staining. PCa

Table 1. Summary of Patient Samples Positive by Immunohistochemical Analysis for Expression of CYP11A1, CYP17A1, HSD3β and HSD17β3 in BPH, Primary PCa, and LNM
staining (Figure 3, G and H) was variable, with low-to-moderate staining mainly in the cytoplasm of the luminal epithelium. CYP17A1 was expressed in 52% (0% BPH, 53% primary PCa, 75% LNMs) of the human prostate samples investigated (Table 1). The number of positively stained cells within each positive tissue section ranged from 10% to 90% within glandular structures and cancer foci. PCa with a Gleason score of 6 frequently showed weak-to-intermediate cytoplasmic reactivity in most cells. Frequently in these cases, the noncancerous glands were also positive and showed a similar intensity of staining, in contrast to the lack of any staining in the BPH samples.

HSD17β3

In Figure 3, I–L, human testis was used as positive tissue (Figure 3I) and was localized to the cytoplasm and nucleus of cells within the seminiferous tubules as reported previously.24 Spermatogonia along the basement membrane, primary and secondary spermatocytes, spermatids, and spermatocytes all stained positively for HSD17β3. Cells positive for HSD17β3 were found in human BPH (Figure 3J) and PCa (Figure 3, K and L). Staining in BPH was variable and predominantly cytoplasmic in luminal epithelial cells. PCa staining was variable, with low-to-high staining mainly in the cytoplasm of luminal epithelial cells. Cytoplasmic stromal staining was generally low but uniform across the sections. There was little nuclear staining. HSD17β3 was expressed in 82% (100% BPH, 83% primary PCa, 50% LNMs) of the human prostate tissue samples (Table 1). The number of positively stained cells within each positive tissue section ranged from 5% to 95% within glandular structures and cancer foci. All Gleason score groups, the BPH group, and the LNM group contained samples positive for HSD17β3 expression. The cancerous glands displayed more uniform cytoplasmic staining compared with the noncancerous glands that had a paler, slightly granular, cytoplasmic appearance and occasionally luminal membrane accentuation.

HSD3β

In Figure 3, M–P, the human adrenal gland was used for positive control tissue (Figure 3M), where it was expressed in the adrenal cortex and was localized predominantly to the cytoplasm. HSD3β in human BPH (Figure 3N) and PCa (Figure 3, O and P) is shown. BPH staining was moderate to high and cytoplasmic in luminal epithelium. Staining in cancer tissue was variable (absent to moderate) and was mainly cytoplasmic in the luminal epithelium. There was little nuclear staining. HSD3β was expressed in 16% (33% BPH, 16% primary PCa, 0% LNM) of the human prostate tissue samples investigated (Table 1). The number of positively stained cells within each positive tissue section ranged from 5% to 20% of glandular structures and cancer foci. Gleason scores were from 6 to 10. The BPH group contained positive samples. IHC was predominantly negative in noncancerous glands, but occasional glands showed intermediate-to-strong membranous reactivity along the luminal border and some weak cytoplasmic reactivity. This reaction was not seen in all cases, and, in the cases where it was positive, the percentage of positive glands varied from focal (eg, 2% to 5% of glands) to 50%. Ganglion cells and skeletal muscle often showed weak-to-intermediate cytoplasmic reactivity.

Only ~10% (10 PCa, 0 BPH, 0 LNM) of the prostate samples (n = 104) expressed all four enzymes simultaneously, and all were primary PCa samples. These 10 samples were from Gleason scores 6 to 10.

To determine whether the number of positively stained tissues for each steroidogenic enzyme was related to PCa progression, each enzyme was analyzed against BPH, increasing Gleason score, and LNMs. A contingency plot and linear regression analysis, considering each individual result within each group, for CYP11A1, CYP17A1, HSD3β, and HSD17β3 expression (%) are presented in Figure 4. Slopes for CYP17A1 (positive), HSD3β (negative), and HSD17β3 (negative) were significantly non-zero (P = 0.0030, P = 0.0331, and P = 0.0393, respectively), indicating that the number of positive samples for these enzymes was changing with disease progression. No significant change was observed for CYP11A1 (P = 0.2055), indicating this enzyme remained stable during disease progression.

Pearson’s χ2 test with Fisher’s exact test was used to ascertain relations of paired enzyme expression. The Fisher’s exact test is recommended when sample sizes are relatively small. This allows significance to be calculated exactly rather than assuming an approximate distribution as produced by a χ2 test. Paired enzyme comparisons generated six contingency tables (Figure 5). A significant (P < 0.05) relation for the occurrence of enzyme expression was found between CYP17A1 (P = 0.0009) and CYP11A1, CYP17A1 and HSD3β (P = 0.0297), and CY17A1 and HSD17β3 (P = 0.0090). CYP17A1 appeared to be the central link in all three relations, suggesting it plays an important role not only in steroidogenesis but, possibly, in regulating expression of other steroidogenic enzymes.

Androgen Synthesis by Prostate Cell Lines

The cell lines, LNCaP, 22Rv1, PC3, DU145, ALVA41, and RWPE1, were selected to assess their ability to synthesize the androgens testosterone and DHT from 14C-labeled cholesterol in a serum-free environment. Extracts from cell lysates were separated by HPLC, then specific fractions were analyzed for radioactivity. Data presented here (Figure 6) showed that the six cell lines produce endogenous testosterone and DHT. Radiation counts for LNCaP, 22Rv1, and RWPE1 cell lines were approximately 1.5 to two times the control samples. Radiation counts for PC3, DU145, and ALVA41 were approximately two to four times the control samples.
Discussion

Prostate cancer (PCa) that progresses to a castrate-resistant phenotype has a poor prognosis. Most of these cancers express active AR, often at elevated levels, which is cause for concern because of its positive effect on cancer growth and progression. Many theories of nonandrogenic or no-ligand AR activation have been devised to explain how CRPC may be bypassing androgen ablation therapies. Evidence from the present work and from other recent reports indicates an alternate theory that AR activation may continue in CRPC by androgens sourced de novo from PCa cells in a paracrine or autocrine manner. CRPC may have the potential to produce androgens from either adrenal steroid precursors or intratumoral cholesterol. Chemical castration from luteinizing hormone-releasing hormone agonists reduces testicular androgen but does not affect synthesis of adrenal androgens. Consequently, CRPC may synthesize the androgens testosterone and dihydrotestosterone, providing that CYP17A1, HSD17β3, and HSD3β are expressed within the cancer. Alternatively, if intratumoral androgen synthesis originates from cholesterol, then CYP11A1 also needs to be present. These four enzymes (CYP11A1, CYP17A1, HSD3β, and HSD17β3) are known to play important roles in the conversion of cholesterol to testosterone. All four were found in human PCa in the present project. True et al. found HSD17β3-positive samples and expression levels increased with PCa progression when Gleason patterns 3, 4, and 5 were...
compared. In the present study, the number of positive samples of HSD17β/H9252 decreased with disease progression; however, the present samples were analyzed according to Gleason score and not Gleason pattern. This variation in methodologic approach likely contributed to the disparate results. Only 10% of the tissue examined expressed all four enzymes simultaneously. This may be attributed to the small sample size but is likely because of the low presence of HSD3β (16%). In contrast, the other three enzymes were present in ~50% to 80% of the tissues analyzed. This result suggests that the majority of PCa tissues studied can potentially produce testosterone and DHT from adrenal precursors such as progesterone and 17β-hydroxy progesterone, but only a minority of samples may produce testosterone and DHT from intratumoral cholesterol.

For any tissue to be classified as steroidogenic, it must express the CYP11A1 enzyme. In the present study, CYP11A1 expression was confirmed in human BPH, primary PCa, LNM, and prostate cell lines, suggesting prostate glandular epithelium may possess steroidogenic potential even before PCa develops. CYP17A1 has received much interest during the past few years, mainly because of promising clinical trial results for a specific inhibitor (abiraterone acetate) of this enzyme.27 Use of abiraterone acetate for treatment of CRPC was approved by the Food and Drug Administration in April 2011 (http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm253055.htm; accessed September 9, 2011).27,28 Abiraterone acetate irreversibly binds CYP17A1, preventing it from converting pregnenolone or progesterone to dehydroepiandrosterone and androstenedione, respectively. In the present investigation, CYP17A1 protein was confirmed in 52% of the prostate samples studied. Notably, CYP17A1 expression in PCa was significantly associated with the expression of the other enzymes investigated. This finding suggests that CYP17A1 provides a critical link to these steroidogenic enzymes and may play a key role in prostatic steroidogenesis similar to its classical role in testes and adrenals.

The use of androgen deprivation therapies for treatment of PCa may positively select cells or foci expressing this group of enzymes among a heterogenic cell population, thus contributing to cancer progression or recurrence. However, endogenous de novo androgen synthesis cannot be determined from paraffin-embedded tissue. Endogenous de novo androgen synthesis had been shown in vitro with the use of CRPC tumors sourced from LNCaP mouse xenografts but was considered negligible compared with the circulating adrenal androgens from normal human prostate and PCa.6,21 Data generated in the present study from analyzing androgenesis in LNCaP, 22Rv1, PC3, DU145, ALVA41, and RWPE1 cell lines shows that these cells can produce testosterone and DHT endogenously. A recent report that used two of these cell lines (LNCaP and 22Rv1) supports this result.29 In vitro protein and mRNA expression of the four enzymes also supports the potential for endogenous androgenesis within prostate cells. HSD17β mRNA and protein had not previously been identified in PC3 and DU145 cell lines, but were identified in these cell lines in the present investigation. CYP17A1 mRNA but not protein was detected for LNCaP and 22Rv1 cells, and only low mRNA was detected for HSD17β, but note that all cell lines were capable of androgenesis, indicating functional protein of all four enzymes, or all necessary enzymes for a
particular cell type. Discrepancies between mRNA and protein levels may be accounted for by posttranscriptional regulation of protein, or differences in mRNA and protein turnover in different cells. The correlation between mRNA and protein expressions is often only weakly or moderately positive with correlation coefficients ranging from 0.2 to 0.6.30

In summary, PCa is a multifocal and heterogeneous disease. It has been proposed that most cases of PCa, regardless of disease stage, have an inherent ability to adapt and survive independently of androgens.2 However, regardless of androgen source, most CRPCs rely on AR activation by androgen. A new consideration is that some CRPC phenotypes are androgen self-sufficient, through synthesis of androgens from sources other than the testes. Therapies targeted at steroidogenic enzymes, such as abiraterone acetate against CYP17A1, have proven beneficial for interrupting intratumoral and adrenal steroidogenesis.27,28 We have shown here that many PCa cases express the key steroidogenic enzymes and that PCa cells in culture are capable of synthesizing testosterone and DHT from cholesterol. For patients with PCa, monitoring all steroidogenic metabolites from cholesterol to testosterone may prove beneficial so that individual therapies that disrupt steroidogenesis may be administered.

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