Prostate Short-Chain Dehydrogenase Reductase 1 (PSDR1): A New Member of the Short-Chain Steroid Dehydrogenase/Reductase Family Highly Expressed in Normal and Neoplastic Prostate Epithelium

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ABSTRACT

Genes regulated by androgenic hormones are of critical importance for the normal physiological function of the human prostate gland, and they contribute to the development and progression of prostate cancer cells. We used cDNA microarrays comprised of prostate-derived cDNAs to profile transcripts regulated by androgens in prostate cancer cells. This study identified a novel gene that we have designated prostate short-chain dehydrogenase/reductase 1 (PSDR1), that exhibits increased expression on exposure to androgens in the LNCaP prostate cancer cell line. Northern analysis demonstrated that PSDR1 is highly expressed in the prostate gland relative to other normal human tissues. The PSDR1 cDNA and putative protein exhibit homology to the family of short-chain dehydrogenase/reductase enzymes and thus identify a new member of this family. Cloning and analysis of the putative PSDR1 promoter region identified a potential androgen-response element. We used a radiation-hybrid panel to map the PSDR1 gene to chromosome 14q23-24.3. In situ hybridization localizes PSDR1 expression to normal and neoplastic prostate epithelium. These results identify a new gene involved in the androgen receptor-regulated gene network of the human prostate that may play a role in the pathogenesis of prostate carcinoma.

INTRODUCTION

Prostate adenocarcinoma is responsible for more than 39,000 deaths annually in the United States (1). Circulating androgens and the intracellular AR are critical mediators of prostate cancer growth and the progression to lethal disease. Landmark discoveries by Huggins and Hodges in 1941 (2) demonstrated that most prostate cancers are initially androgen-dependent, a finding that initiated the era of effective endocrine-based therapy for this malignancy. To date, surgical or chemical castration remains the mainstay of therapy for advanced prostate cancer. A reduction in serum testosterone leads to marked regression of prostate tumor cells (10). This concept has been extended to clinical medicine in which several trials suggest a benefit for an approach using intermittent rather than continuous androgen suppression in patient cohorts with hormone-responsive disease (11, 12).

The pivotal role of androgens in the biology and treatment of prostate cancer has led to intensive investigations designed to identify the molecular mediators of androgen action (13, 14). Among the genes shown to be regulated by androgens in prostate cells are several that encode enzymes belonging to the two major lipogenic pathways: fatty acid synthesis and cholesterol synthesis (15, 16). The regulation of cholesterol metabolism by androgens is especially intriguing because cholesterol is an essential precursor for the biosynthesis of androgens (17). Other molecules involved in androgen metabolism and androgen action are themselves androgen regulated. For example, 17-β-HSD, an enzyme that converts androsterone to testosterone, is androgen regulated (18), as is the expression of the AR itself (7, 19). It appears that multiple autoregulatory levels of androgen action may be operative in androgen-responsive tissues.

Our objective in this study was to identify genes that exhibit transcriptional regulation by androgens in human prostate cells. We hypothesized that such genes could be direct mediators of androgen action and that the characterization of these genes and their cognate proteins would provide insights into the mechanisms of androgen-dependent and androgen-independent cellular growth. We used cDNA microarrays comprised of cDNAs derived from human prostate tissues to quantify transcripts expressed in the androgen-sensitive LNCaP prostate tumor cell line under conditions of androgen starvation or androgen stimulation. Here we report the cDNA cloning, chromosomal mapping, genomic structure, and expression profile of a novel gene, PSDR1, that exhibits homology to the family of SDR enzymes. PSDR1 is the first SDR shown to be predominantly expressed in normal and neoplastic prostate tissue. We hypothesize that PSDR1 may play a role in steroid hormone metabolism in prostate cells and thus may be an ideal target for modulating hormone-mediated prostate cancer growth.

MATERIALS AND METHODS

Microarray Fabrication. A nonredundant set of 1500 prostate-derived cDNA clones were identified from the Prostate Expression DataBase (PEDB), a public sequence repository of EST data derived from human prostate cDNA.

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The abbreviations used are: AR, androgen receptor; PSA, prostate-specific antigen; ARE, androgen response element; DHT, dihydrotestosterone; EST, expressed sequence tag; dbEST, database of ESTs; CS-FCS, charcoal-stripped FCS; PRE, progesterone responsive element; HSD, hydroxysteroid dehydrogenase; RACE, rapid amplification of cDNA ends; SDR, short-chain dehydrogenase reductase; PSDR1, prostate SDR 1; tk, human glandular kallikrein.

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libraries (20). Individual clone inserts were amplified by the PCR using 2 μl of bacterial transformant culture as template with primers BL.m13F (5′-GTAAACAGCGCCGATGTAATG-3′) and BL.m13R (5′-ACACAG-GAAACAGCTATGACCAGT-3′) as described previously (21). PCR products were purified through Sephacyr S500 (Pharmacia), mixed 1:1 with DMSO (Amersham), and spotted in duplicate onto coated type IV glass microscope slides (Amersham) using a Molecular Dynamics GenII robotic spotting tool. After spotting, the glass slides were air-dried and UV-cross-linked with 500 mJ of energy and then baked at 95°C for 30 min.

Probe Preparation and Microarray Hybridization. Total RNA was isolated from LNCaP cells after 72 hrs of androgen depletion or supplementation using TRIzol (Life Technologies) according to the manufacturer’s directions. Fluorescence-labeled probes were made from 30 μg total RNA in a reaction volume of 20 μl containing 1 μl of anchored oligo(dT)18 primer (Amersham), 0.05 mM Cy3-dCTP or Cy5-dCTP (Amersham); 0.05 mM dCTP; 0.1 mM each dGTP, dATP, dTTP; and 200 units of Superscript II reverse transcriptase (Life Technologies). Reactants were incubated at 42°C for 120 min followed by heating to 94°C for 3 min. Unlabeled RNA was hydrolyzed by the addition of 1 μl of 5N NaOH and heating to 37°C for 10 min. One μl of 5 M HCl and 5 μl of 1 M Tris-HCl (pH 7.5) were added to neutralize the base. Unincorporated nucleotides and salts were removed by chromatography (Qiagen), and the cDNA was eluted in 30 μl of dH2O. One μg of dA/dT 12–18 (Pharmacia) and 1 μg of human Cot1 DNA (Life Technologies) were added to the probe, heat denatured at 94°C for 5 min, combined with an equal volume of 2× microarray hybridization solution (Amersham), and prehybridized at 50°C for 1 h. The mixture was then placed onto a microarray slide with a coverslip and hybridized in a humid chamber at 52°C for 16 hours. The slides were washed once with 1× SSC, 0.2% SDS at room temperature for 5 min, then twice with 0.1× SSC, 0.2% SDS at room temperature for 10 min. After washing, the slides were rinsed in distilled water to remove trace salts and dried.

Image Acquisition and Data Analyses. Fluorescence intensities of the immobilized targets were measured using a laser confocal microscope (MoTo-International). Intensity data were integrated at a pixel resolution of 10 μm. Hybridizations were performed on LNCaP cDNA array slides (Clontech) with the 6A4 cDNA probe representing the 3′ end of the PSDR1 cDNA. Two separate rounds of library screening identified 14 partial-length cDNA clones. Searches of dbEST identified seven IMAGE cDNA clones (IMAGE CloneID: 360400, 109237, 1130518, 1401718, 1337270, 1723130, 1703429) that contained sequences homologous to PSDR1. All of the clones were sequenced and assembled using the Sequencer software (Gene Codes, Corp.). To clone the 5′ end of the cDNA, 5′-RACE was performed on human prostate Marathon-Ready cDNA (Clontech) using primers 6A3.RC3 (5′-GGACAGCATTCTCTCCTAGTTGGGGC-3′) and 6A4.RC4 (5′-CAGAGGGAGGACACAGCGG-GAAC-3′). The RACE products were subcloned into PCR2.1-TOPO (Invitrogen) and sequenced.

Phage plaques (1,200,000) from a human prostate 5′-STRETCH cDNA library (Clontech) were screened with PSDR1 12P-cDNA probes according to the manufacturer’s instructions. Eleven additional cDNA clones were isolated, subcloned and sequenced. RACE reactions were performed using the human prostate Marathon-ready cDNA cloning kit (Clontech) following the manufacturer’s instructions. Templates for RACE reactions were prostate Marathon-ready cDNA (Clontech) and androgen-stimulated LNCaP cDNA prepared using Marathon cDNA amplification kit (Clontech). Nested 5′-RACE reactions were performed according to the manufacturer’s instructions; first with primers 6A4RC4, 5′-CAGAGGGAGGACACAGCGG-GAAC-3′ and AP1 (Clontech) and then a nested RACE reaction with primers 6A4RC3, 5′-GGACAGCATTCTCTCCTAGTTGGGGC-3′ and AP2 (Clontech). The RACE products were subcloned into PCR2.1-TOPO vectors with the TOPO TA cloning kit (Invitrogen) and sequenced.

Chromosomal Localization of PSDR1 by Radiation Hybrid Panel Mapping. The G3 Gene bridge radiation hybrid panel (Research Genetics, Huntsville, AL) was used to map the chromosomal localization of PSDR1 with primers 6A4F (5′-GGGCGATTCCTCCTACATGGTCTT-3′) and 6A4R (5′-CCTCCTAAACAGTGTGGACACAG-3′). After 35 cycles of amplification, the reaction products were separated on a 1.2% agarose gel, and the resulting product pattern was analyzed through the Stanford genome center's webserver1 to determine the probable chromosomal location.

In Situ Hybridization. For mRNA in situ hybridization, recombinant plasmid pCRII-TOPO (Invitrogen), containing a 400-bp PSDR1 fragment was linearized to generate sense and antisense digoxigenin-labeled RNA probes. In situ hybridization was performed according to the manufacturer’s protocol on the Ventana GenII automated instrument (Ventana Medical Systems, Tucson, AZ). Tissue sections (5 μm) were mounted onto Chroma plus slides (VWR Scientific), deparaffinized in a 65°C oven for 2 h followed by three 5-min soaks in xylene and rehydrated through graded alcohol with a final rinse in 2× SSC. Before hybridization, sections were digested with proteinase 1 cocktail for 12 min at 37°C, then 10 ng of either sense or antisense probe in the hybridization buffer was applied. Programmed recipe files consisting of buffer rinses, protease digestion, hybridization, detection, and counterstains were optimized for the PSDR1 probe. Digoxigenin-labeled RNA probe was added manually. Antidigoxigenin was used as the primary antibody. The probe was denatured at 65°C, and hybridization was carried out at 42°C for 360 min. Washes were performed at 37°C with 2×, 1×, and 0.1× SSC. The system uses a cocktail of antirabbit and antiswine secondary IgG-biotinylated antibody with an indirect biotin-avidin diaminobenzidine detection system. The sections were counterstained with hematoxylin.

RESULTS

Identification of a Novel Androgen-regulated cDNA, PSDR1, by Microarray Expression Analysis. Microarrays comprised of cDNA clones derived from prostate tissues were hybridized with total cDNA probes synthesized from androgen-stimulated and androgen-starved LNCaP prostate cancer cells. Four independent data points for each arrayed cDNA were generated. The hybridization ratios for 20 distinct cDNAs were consistently increased by >2-fold in androgen-stimulated relative to androgen-starved cells. We did not observe any cDNAs with consistent hybridization ratios <0.5, a ratio that would indicate down-regulated expression. The genes induced by androgens included hK2 (23), hK3, also known as PSA (24), NKX3.1 (25), prostate/PRTSS17 (26), TMPRSS2 (13), PART-1 (27), several genes involved in lipid metabolism, and several anonymous ESTs. The expression level of

1 Internet address: http://shgc.stanford.edu.
the cDNA clone corresponding to one of these ESTs, 6A4, increased 3-fold in androgen-stimulated LNCaP cells relative to androgen-deprived cells as assayed by microarray hybridization (Fig. 1A). Sequence comparisons against the GenBank and dbEST databases revealed homology only to uncharacterized partial-length ESTs (e.g., AA657851, IMAGE ID:1207405). Full-length cloning of the corresponding cDNA and subsequent nucleotide and amino acid sequence comparisons revealed significant homology to conserved motifs of the SDR family of proteins. We have named this gene **PSDR1** for Prostate Short-chain Dehydrogenase/Reductase 1.

**Cloning of the Full-Length **PSDR1** cDNA.** We screened a human prostate cDNA library with the 6A4 cDNA probe representing the 3' end of the **PSDR1** cDNA and identified 16 partial-length cDNA clones. Searches of the dbESTs initially identified seven IMAGE cDNA clones (IMAGE Clone ID: 360400, 109237, 1130518, 1401718, 1337270, 1723130, 1703429) that contained sequence homologous to **PSDR1**. To clone the 5' end of the cDNA, 5'-RACE was performed on cDNA from normal human prostate (Clontech) and the LNCaP cell line. All of the clones were sequenced and subsequently assembled using the Sequencher software (Gene Codes, Corp.). A total of 2539 bp were obtained, which corresponds to the 2.5-kb band of Fig. 1A, a representative microarray hybridization section showing the androgen-stimulated expression of **PSDR1**. cDNAs from androgen-stimulated (A+) and androgen starved (A-) LNCaP cells were labeled and hybridized to cDNA microarrays. **Arrows**, the location of **PSDR1** cDNA on the microarray. **B**, Northern analysis of the same RNAs used in the microarray experiment hybridized with **PSDR1**, **PSA**, and **G3PDH** probes. **LNCaP(1)**, LNCaP cells at steady state grown in 10% serum without additional androgens and harvested at 70% confluence. **C**, Northern analysis demonstrating **PSDR1** expression in the prostate cancer cell lines LNCaP, DU145, and PC3 grown in 10% serum (SS) or with additional androgen (+A), three normal prostate tissue samples (NP), three primary prostate adenocarcinoma samples (CAP) and androgen-dependent (XE-AD), and androgen-independent xenografts (XE-AI). **D**, Northern analysis demonstrating the **PSDR1** expression profile in normal human tissues. **E**, a multiple tissue dot blot (Clontech) containing 50 human tissue RNAs was hybridized with **PSDR1** probe. Signal intensities were captured with phosphor screen and scanned with a phosphorimager. **Bar graph**, signal intensities calculated with ImageQuant program. The 50 human tissues are: A1, whole brain; A2, amygdala; A3, caudate nucleus; A4, cerebellum; A5, cerebral cortex; A6, frontal lobe; A7, hippocampus; A8, medulla oblongata; B1, occipital lobe; B2, putamen; B3, substantia nigra; B4, temporal lobe; B5, thalamus; B6, acumen; B7, spinal cord; C1, heart; C2, aorta; C3, skeletal muscle; C4, colon; C5, bladder; C6, uterus; C7, prostate; C8, stomach; D1, testis; D2, ovary; D3, pancreas; D4, pituitary gland; D5, adrenal gland; D6, thyroid gland; D7, salivary gland; D8, mammary gland; E1, kidney; E2, liver; E3, small intestine; E4, spleen; E5, thymus; E6, peripheral leukocyte; E7, lymph node; E8, bone marrow; F1, appendix; F2, lung; F3, trachea; F4, placenta; G1, fetal brain; G2, fetal heart; G3, fetal kidney; G4, fetal liver; G5, fetal spleen; G6, fetal thymus; G7, fetal lung; H1, yeast total RNA; H2, yeast (RNA); H3, E. Coli rRNA; H4, E. coli DNA; H5, poly (A); H6, human C'p1 DNA; H7, human DNA; H8, human DNA. B8, F5–F8 and G8 contain no RNAs. The units on the Y axis are relative intensity units.
which is in agreement with the size of its transcript as determined by Northern hybridization (Fig. 1D). The cDNA sequence was submitted to GenBank under the accession no. AF167438.

The PSDR1 cDNA encodes a putative protein of 318 amino acids. The start codon, GAGATGG matches in a strong context to the Kozak translation initiation consensus sequences (RRNATGG, where R is a purine; Ref. 28). Two potential polyadenylation signals were identified at nucleotide positions 2439 and 2481. IMAGE clone 1703429 has a poly(A) stretch that uses the AAATTT polyadenylation signal at 2419, and our original cDNA clone 6A4 uses the AAATTA signals at 2481. However, we were not able to find a polyadenylation site that would produce the 900-bp band seen in testis tissue. PCR primers flanking the start and stop codons were designed, and an expected size band encompassing the entire coding region was amplified from human prostate Marathon-Ready cDNA (Clontech; data not shown).

Comparisons of the assembled cDNA sequences indicated several polymorphic sites. Five distinct single nucleotide polymorphisms were recognized between the three independent prostate tissue sources used for PSDR1 cloning. Three occur in the coding region of the PSDR1 sequence; nucleotide 379, ggc to ggg; nucleotide 916, gtc to gtc; and nucleotide 921, gtc to gcc. The first two are conserved changes, whereas the latter results in a valine to alanine amino acid substitution. Alignments of sequences in dbEST with homology to PSDR1 identified more than 20 distinct nucleotide differences in tissue sources presumably derived from different individuals.

Prostate-localized and Androgen-regulated Expression of PSDR1. The androgen-regulated expression of PSDR1 was confirmed by Northern analysis using the same LNCaP RNA that was used for microarray analysis. PhosphorImage quantitation of the Northern analysis demonstrated a 3-fold induction of PSDR1 expression after 72 h of androgen exposure relative to 72 h of androgen starvation (Fig. 1B). PSA expression increased 25-fold, and the expression of the G3PDH loading control did not change significantly. Interestingly, Northern analysis with androgen-independent prostate cancer cell lines DU145 and PC3 demonstrated PSDR1 expression in both cell types (Fig. 1C) indicating a mechanism of PSDR1 transcription in these cells that is independent of androgen requirements.

The distribution of PSDR1 transcripts in normal human tissues and prostate carcinoma was determined by Northern analysis and mRNA dot blot. Of 16 adult tissues examined by Northern, a PSDR1 message of 2.5 kb was predominantly expressed in prostate (Fig. 1D). In testis, the PSDR1 probe hybridized to an additional band at about 900 bp (Fig. 1D), which could indicate cross-hybridization, alternate splicing, or alternate usage of polyadenylation signals. The PSDR1 expression profile was confirmed using an RNA Master dot blot (Clontech) comprised of RNA from 50 different tissues. PSDR1 expression was detected predominantly in prostate with a very low relative level of expression in spleen, thymus, testis, ovary, small intestine, colon, peripheral blood leukocyte, and kidney, adrenal gland, and fetal liver (Fig. 1E). PSDR1 expression was at least 4-fold higher in prostate relative to any other human tissue examined. PSDR1 expression was detected in all of the normal and neoplastic prostate tissue samples examined. These included three normal whole prostate tissues; three primary prostate adenocarcinomas, androgen-dependent and androgen-independent prostate cancer xenografts; and three prostate cancer cell lines (Fig. 1C).

PSDR1 Shares Homology with Members of the SDR Family. We used the nucleotide and translated the 318-amino acid PSDR1 sequence to search the National Center for Biotechnology Information sequence databases by using BLAST and BEAUTY algorithms (29). Partial homology was seen with several oxidoreductases from bacteria and plant sources. To see whether the homology was significant, we searched the protein sequence of PSDR1 against the BLOCKS database (30). The PSDR1 protein has three blocks that all match to the SDR family protein signature BLOCK (BL00061; Ref. 31) with a significant combined E-value of 2.6e-06. The SDR family are NAD- or NADP-dependent oxidoreductases (31), which include enzymes involved in steroid metabolism such as estradiol 17-β-dehydrogenase (also called 17-β-hydroxysteroid dehydrogenase; EC 1.1.1.62), 15-hydroxyprostaglandin dehydrogenase (NAD+; EC 1.1.1.141) from human and 11-β-HSD (EC 1.1.1.146; 11-DH; Ref. 31). A multiple sequence alignment of the PSDR1 protein with different members of the human HSD family and a prokaryotic 20-β-HSD (Streptomyces 3α/20β-HSD) is shown in Fig. 2.

Only two motifs are highly conserved in the SDR family. The first is a common GlyXXXGlyXXXGly pattern, in which the coenzyme NAD(H) or NADP(H) binds at the NH2 terminus of the SDR enzyme (31). The second motif is a segment, TyrXXXLys, believed to be involved in the catalytic activity of the enzyme (32). The PSDR1 protein contains these two signatures (as shown by asterisk in Fig. 2). Sequence alignments reveal that proteins in the SDR family exhibit residue identities of only about 15–30%, probably because of their early divergence and remote origin (31). PSDR1 shows ~25% amino acid identity with other members of the SDR family.

Searches against prosite patterns database revealed that PSDR1 contains two Asn-glycosylation sites at amino acid (aa) position 174 and 198. These two sites are also conserved among SDR family proteins (Fig. 2). In addition, two protein kinase C (PKC) phosphorylation sites (aa 57 and 106), a casein kinase II phosphorylation site (aa 57), and a 7 N-myristoylation site are identified in the protein.

PSDR1 Genomic Organization and Promoter Sequence Analysis. BLAST searches with the full-length PSDR1 cDNA identified homology with nucleotide sequence derived from a recently deposited unannotated 197-kb chromosome 14 BAC clone, R-1012A1, sequenced by the National Sequencing Center-Genoscope in France (GenBank accession no. AL049779). Alignment with the PSDR1 sequence demonstrated that this BAC contains the entire PSDR1 cDNA and allowed for the determination of the PSDR1 genomic structure. The PSDR1 gene comprises 7 exons and 6 introns. The sizes of exons and introns and the exon/intron junctional sequences are listed in Fig. 3B. All of the intron/exon junctions conform to the 5′-gt...3′-ag consensus (33) except intron 2. Intron 2 has a 5′-gc...3′-ag splicing signal, a structure that has been identified in other genes (34).

We examined the 5′ genomic sequences for potential transcriptional start sites using a neural network promoter prediction program (35) and for potential transcriptional factor binding site using the TESS (Transcription Element Search Software) program (36). We identified a strong promoter sequence with a score of 0.87 (a score of 0.85 has a 0.1–0.4% false positive prediction rate). The predicted transcription start site is 167 bp 5′ of the ATG start codon. A TATA box (TATAAT) is found 30 bp 3′ of the putative transcriptional initiation site (Fig. 3A). A sequence that has 86.7% homology (13 of 15 nucleotides) to the consensus ARE, 5′-GGA/TACAnntTGTTCT-3′, (37) was identified (Fig. 3A). Two sequences that have 86.7% (13 of 15 nucleotides) homology to the consensus sequence of PREs (38) were also identified (Fig. 3A). An interleukin-6 response element binding protein site, TTCCCAGAA, (39) was identified 281 bp 5′ of the transcriptional initiation site.

Chromosomal Localization of PSDR1. The medium-resolution Stanford G3 radiation hybrid panel was used to determine the chro-
mosomal localization of PSDR1 using gene-specific PCR primers 6A4F and 6A4R. Analysis of the typing results on the Stanford Human Genome Center Radiation Hybrid Panel server indicated that PSDR1 is located closest to Stanford Human Genome Center Radiation Hybrid Panel-255 between two cytogenetically mapped markers D4S63 (mapped to 14q23) and D4S258 (mapped to 14q24.3). Therefore, PSDR1 is mapped to 14q23-24.3, consistent with the BAC clone mapping data localizing BAC R-1012A1 to chromosome 14q.

**PSDR1 Expression in Normal and Neoplastic Prostate Epithelium.** Normal prostate contains two major epithelial cell populations, the luminal secretory cells and the basal cells. In situ hybridizations were performed on sections of normal prostate by using an antisense RNA probe specific for PSDR1 to localize its expression. PSDR1 was expressed in both normal basal and luminal cell populations. (Fig. 4, A and C). Little to no staining was seen in fibromuscular stromal cells, endothelial cells, or infiltrating lymphocytes. Hybridization with sense PSDR1 RNA probes showed no background staining (Fig. 4B).

**DISCUSSION**

In a search for genes regulated by androgens in the human prostate, we have identified a new member of the SDR superfamily. SDRs encompass a large group of functionally diverse proteins in pro- and eukaryotes (31). Enzymes in this family typically exhibit residue identities of only 15–30%, indicating early gene duplication events and subsequent extensive divergence (31). Regions of high conservation are restricted to specific segments, which indicate a possible common fold, active site, reaction mechanism, and coenzyme and substrate binding regions (40). Of relevance for the study of androgen-mediated effects in prostate carcinoma is the classification of several key enzymes involved in steroid biosynthesis, HSDs, within the SDR family. This group of HSDs includes 17β-HSD types 1–4 and 6, 15-hydroxyprostaglandin dehydrogenase, and 11β-HSD (31). 17β-HSD 3 converts androstenedione to testosterone (42); 17β-HSD 6 converts 5-androstane-3α,17β-diol (3-adiol) to androsterone (43). In prostate cancer cells, 17β-HSD type 2 exclusively converts 5α-DHT and testosterone into the less potent 17-keto compounds 5α-androstanedione and 4-androstenedione, respectively (44). This suggests
that 17HSD type 2 plays a part in the androgen metabolic pathway, resulting in the inactivation of testosterone and 5α-DHT locally in the prostate. Enzyme expression in the prostate could, therefore, protect cells from excessive androgen action. Because PSDR1 shows significant homology to HSD members of the SDR family, we hypothesize that PSDR1 is also involved in prostate cellular steroid metabolism that may include biosynthesis and/or degradation. The pharmacological modulation of PSDR1 activity could thus influence prostate cellular growth and provide a new target for prostate cancer therapy.

Numerous studies support associations between molecular variations involving genes of the androgen metabolic pathway and the development and progression of prostate cancer (45). In addition to environmental influences, racial and international variation in prostate cancer incidence suggests that inheritable genetic factors such as those that influence androgen biosynthesis, activation, transport, and metabolism are operative (45). In addition to polymorphic variation in the AR itself (46), specific polymorphisms in the 5α-reductase type 2 (SRD5A2) gene, the enzyme converting testosterone to the more bioactive DHT, result in increased enzyme activity and confer up to a 7-fold increased risk for the development of prostate cancer in African-American men (47). Allelic variants in the 3-β HSD type II gene, encoding one of two enzymes that initiates the inactivation of DHT, have been identified and are currently under assessment for a role in racial/ethnic differences in prostate carcinogenesis (48). Polymorphisms in PSDR1 could influence enzyme activity and consequently result in variations in steroid metabolism between individuals. Our preliminary analysis of the PSDR1 sequence from three different prostate tissue sources identified five distinct single nucleotide polymorphisms. Three occur in the coding region of the PSDR1 sequence, one of which results in a valine to alanine amino acid substitution. An alignment of sequences in dbEST with homology to PSDR1 identified more than 20 distinct nucleotide differences in tissue sources presumably derived from different individuals. Although some of these differences may represent sequencing artifacts, these findings warrant a more directed study of PSDR1 variation in different ethnic populations and in samples of prostate carcinoma.

The expression of PSDR1 is induced by synthetic androgens in LNCaP cells. The mechanism of androgen-mediated regulation of PSDR1 expression is unknown and could involve either direct AR binding to PSDR1 promoter regions or indirect activation through the modulation of intermediary transcription factors or via posttranscriptional mechanisms. Androgens have been shown to regulate expression of other oxidoreductases. The mouse alcohol dehydrogenase ADH1, which belongs to the long-chain dehydrogenase family, is induced 10–12 fold by androgens in mouse kidney cells (49). The induction of mouse ADH1 gene by androgens seems to be AR de-
pendent because the ADH1 gene in Tmj mice lacking functional AR was not responsive to androgens (49). 17-ß-HSD1, which belongs to the SDR family, is stimulated by androgen through AR-mediated mechanism (50). We have identified a putative ARE and two putative PRE sites that demonstrate a high degree of homology to the respective consensus hormone receptor binding sites. Whether these responsive elements are functional awaits further investigation. In addition, PSDR1 transcription is not entirely mediated by androgens as demonstrated by a low level of detectable PSDR1 message in androgen-starved LNCaP cells and in the androgen-independent PC3 and DU145 prostate cancer cell lines. Studies of the PSDR1 protein may identify additional mechanisms of functional regulation.

The localized expression of PSDR1 in prostate epithelium is interesting because, to our knowledge, PSDR1 is the first member of the human SDR family that is expressed predominantly in the prostate gland. Other members of SDR family exhibit tissue-restricted patterns of expression. For example, 17-ß-HSD1 is predominantly expressed in placenta and ovary (51, 52); 17-ß-HSD2 is expressed in placenta and liver (53); 17-ß-HSD3 is expressed in testis (42); 17-ß-HSD4 is primarily expressed in liver and kidney (54); 17-ß-HSD5 is expressed most abundantly in liver and testis (41, 55); and 17-ß-HSD6 is expressed equally in liver and prostate (43).

The identification of genes with selective expression in specific organs or cell types provides an entry point for understanding biological processes that occur uniquely within a particular tissue. Genes and their cognate proteins whose expression is specific for the prostate have greatly aided the diagnosis and treatment of prostate carcinoma. The significance of the prostate predominant expression pattern of PSDR1 remains to be determined. If the tissue expression profile of the PSDR1 protein corresponds to the transcript expression profile, then PSDR1 may represent an additional target for prostate cancer diagnostic and therapeutic interventions. Cellular or humoral immunotherapy could be designed to exploit the tissue expression differential. Our hypothesis is that PSDR1 is involved in steroid synthesis and/or degradation in normal and neoplastic prostate epithelium, and, as such, it may be a key enzyme involved in maintaining intracellular balance of steroid hormones in these cells. Additional studies including the expression of PSDR1 protein and the analysis of substrate specificity and kinetics are needed to address this question.

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