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Sex Hormone Metabolism in Prostate Cancer Cells during Transition to an Androgen-Independent State

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The progression of prostate cancer during androgen deprivation therapy is a serious clinical problem. Little is known, however, about the mechanisms behind the transition of the disease to an androgen-independent stage. In the present report, we provide evidence of substantial changes in both estrogen and androgen metabolism during the transition of cultured prostate cancer LNCaP (lymph node carcinoma of the prostate) cells. The results of enzyme activity measurements performed using HPLC suggest that, related to the transition, there exists a remarkable decrease in the oxidative 17β-hydroxysteroid dehydrogenase (17HSD) activity, whereas the reductive 17HSD activity seems to increase. Relative quantitative RT-PCR revealed that the decrease in oxidative activity largely coincided with the remarkable decrease in the expression of the *HSD17B2* gene. Furthermore, the present data suggest that the observed increasing activity of 17HSD type 7 could lead to the increased intracellular production of 17β-estradiol during disease progression. This was supported by the cDNA microarray screening results, which showed a considerable overexpression of several estrogen up-regulated genes in the LNCaP cell line variant that represents progressive prostate cancer. Because 17HSDs critically contribute to the control of bioavailability of active sex steroid hormones locally in the prostate, the observed variation in intraprostatic 17HSD activity might be predicted to be crucially involved in the regulation of growth and function of the organ. (*J Clin Endocrinol Metab* 88: 705–712, 2003)

PROSTATE CANCER IS one of the most common forms of cancer in men in industrialized countries, and its incidence has been continuously increasing (1). Despite the substantial clinical importance of the disease, the mechanisms underlying the development and progression of prostate cancer are poorly understood. Androgens have long been considered to play a major role in the growth and function of the prostate by primarily mediating the cellular differentiation and proliferation of prostatic epithelium (2). With regard to prostatic carcinogenesis, the majority of prostate tumors arise from the secretory, androgen-dependent epithelial cells (3). The mainstay treatment of prostate cancer, endocrine therapy, aims to eliminate androgenic activity from the circulation as well as from the prostatic tissue (4). Most prostate carcinomas are initially responsive to such androgen withdrawal but eventually begin to grow androgen-independently, thus also becoming refractory to androgen deprivation therapy (5). Androgen withdrawal has been used in the treatment of prostate cancer patients since 1941 (6), but the failure of this therapy still represents a serious clinical problem. Unless improved therapies can be found, the frequency of mortality associated with prostate cancer is expected to increase (7).

The possibility exists that, apart from androgens, other steroid hormones or locally produced factors that interact with nuclear receptors are involved in cell proliferation, differentiation, and apoptosis in normal prostate. These factors have also been suggested to be involved in the growth stimulation of androgen-independent (AI) prostate tumors (8). The results of epidemiological and experimental studies have suggested that estrogens would be involved in the induction of prostate cancer (9). Estrogen receptor (ER)β is suggested to play a significant role in the differentiation and proliferation of prostate cells as well as to possibly modulate both the initial phases of prostate carcinogenesis and AI tumor growth (10). However, despite the fact that estrogens have been used in the treatment of prostate cancer because of their negative feedback on the hypothalamic-pituitary-gonadal axis, which leads to decreased serum testosterone (T) levels, the exact effects of estrogens on prostate epithelium are still primarily unknown.

In addition to classical steroidogenic tissues, a large series of human peripheral tissues possess all the enzymatic systems required for the formation of active androgens and estrogens from a relatively large supply of precursor steroids provided by the adrenals (11). In androgen and estrogen metabolism, the reactions between the active 17β-hydroxysteroids and the less active 17-ketosteroids are catalyzed by 17β-hydroxysteroid dehydrogenase (17HSD) enzymes (Fig. 1). 17HSD activity is present not only in classical steroidogenic tissues, such as human placenta, ovary, and testis, but also in several peripheral tissues, including breast, prostate, gastrointestinal tract, liver, and kidney (12). Thus, 17HSDs are of crucial importance in the regulation of the intracellular levels of biologically active steroid hormones in a variety of tissues. Generally, the reduction step is essential for the formation of active estrogens as well as active androgens, whereas the oxidative reaction is required for the inactivation of potent sex steroids into compounds having only low bi-

Abbreviations: 3αA-diol, 5α-Androstane-3α, 17β-diol; 3βA-diol, 5α-androstane-3β, 17β-diol; A-dione, androstenedione; AI, androgen-independent; CFE, colony-forming efficiency; DHT, dihydrotestosterone; E1, estrone; E2, 17β-estradiol; ER, estrogen receptor; EST, expressed sequence tag; FCS, fetal calf serum; HSD, hydroxysteroid dehydrogenase; LNCaP, lymph node carcinoma of the prostate; PSA, prostate-specific antigen; T, testosterone; tPA, tissue-type plasminogen activator.

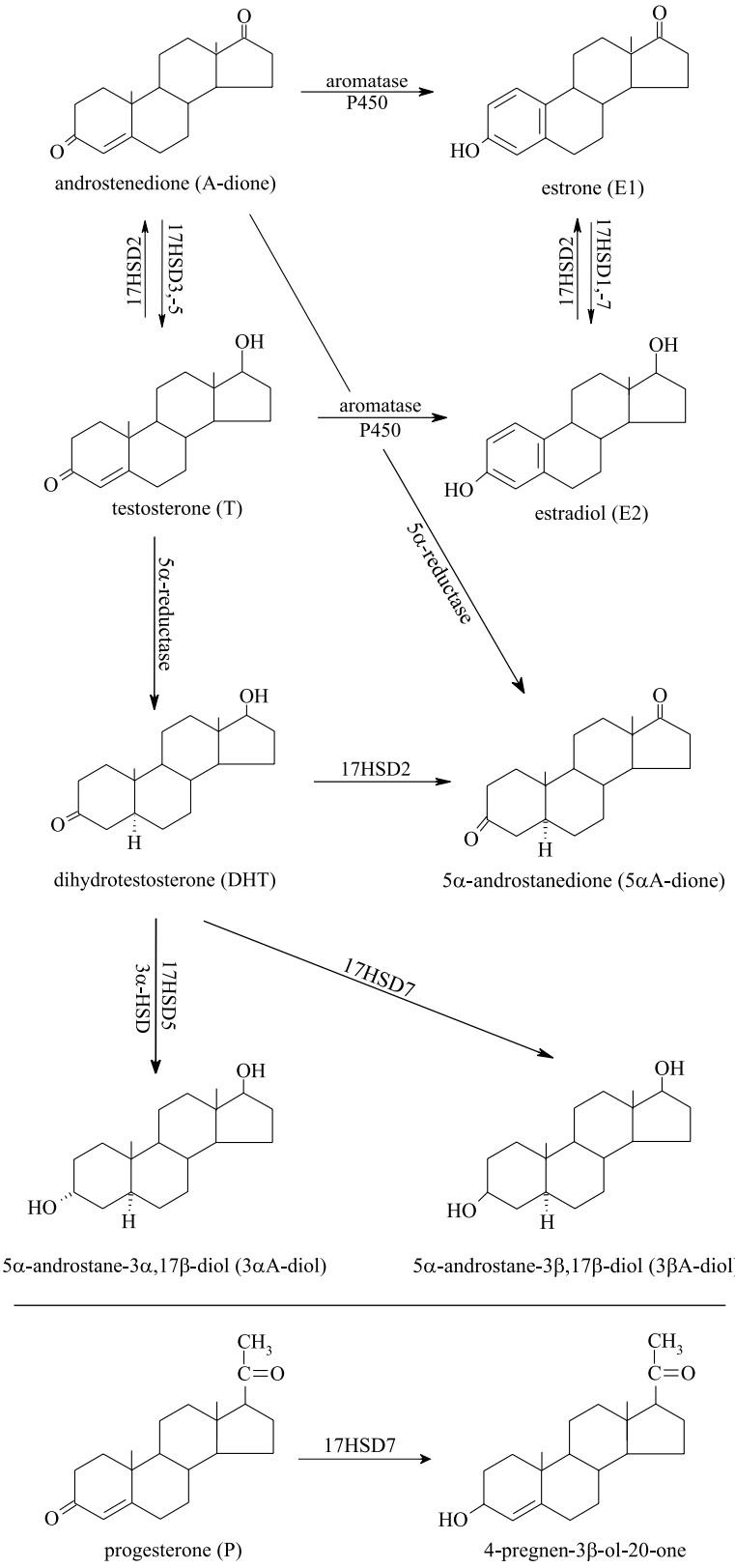


FIG. 1. Reactions catalyzed by different types of 17HSD enzymes.

ological activity or no activity at all (13). In intact cells, the activity catalyzed by each type of 17HSD enzymes is almost exclusively unidirectional: for example, the 17HSD types 1, 3, 5, and 7 catalyze the reductive reaction, whereas the types 2, 4, and 8 catalyze the reaction into the oxidative direction (13, 14).

The present study was performed to identify mechanisms related to the progression of prostate cancer into an AI state. To this end, the variations in cellular characteristics, as well as in estrogen and androgen metabolism during the transformation of prostate cancer LNCaP (lymph node carcinoma of the prostate) cells (15), were investigated. The enzyme activities that essentially take part in sex hormone metabolism were analyzed by following the conversion of substrates into specific products using HPLC, and the expression levels of genes for the different candidate types of 17HSD enzymes were quantified using the relative quantitative RT-PCR method. To identify the changes in the expression of estrogen up-regulated genes during prostate cancer progression, the cDNA microarray screening results of two LNCaP cell line variants (16) were analyzed in this respect.

Materials and Methods

Cell culture

The human prostate carcinoma cell line LNCaP (CRL-1740) was obtained from American Type Culture Collection (ATCC; Manassas, VA). First, cells were cultured in cell culture flasks in RPMI-1640 medium (ATCC) supplemented with 10% fetal calf serum (FCS), 100 U/ml penicillin, and 100 μg/ml streptomycin. When the transformation of cells was started and the cells were able to grow anchorage-independently, they were transferred to grow in large-scale suspension cultures. LNCaP cells, from 6–8 cell culture flasks with the culture area of 175 cm^2, were trypsinated with trypsin/EDTA solution (Clonetics, BioWhittaker, Inc., Walkersville, MD) and transferred into a spinner flask to a culture volume of 300 ml. In this culture, cell density was $6–8 \times 10^5$/ml. For spinner flasks, 0.1% Synperonic F68 (Serva Electrophoresis, Heidelberg, Germany) was added into the culture medium to reduce the shear force effects. Production of prostate-specific antigen (PSA; μg/liter) was determined using the DELFIA PSA kit (Wallac, Inc., Turku, Finland).

Cell growth

Both PSA-producing and non-PSA-producing LNCaP cells were seeded in RPMI-1640 medium (ATCC) containing 10% FCS, 100 U/ml penicillin, and 100 μg/ml streptomycin, in cell culture flasks with a culture area of 25 cm^2, at a density of 2×10^4 cells/cm^2. The cells were cultured for 8 d, during which the medium was replaced at d 4 and 6. The cells were trypsinated daily. Trypan blue (Sigma, St. Louis, MO) was added to detect cell viability, and the cell number was determined using Bürker's cell-counting chamber. The doubling time of the cells was determined from the logarithmic phase of the growth curve. The assays were performed in triplicate.

Colony-forming assay

A single cell suspension (10^2, 5×10^2, 10^3, and 5×10^3 cells/ml) in growth medium was plated on 35-mm tissue-culture dishes. After incubation for 1 wk at 37 C in a 5% CO_2 atmosphere, the colonies were fixed with 80% ethanol for 30 min and stained with 1% crystal violet in a 10% ethanol solution for 5 min. Colony-forming efficiency (CFE) was measured as: (number of colonies formed/number of cells plated) $\times$ 100%.

Enzyme activity measurements

For enzyme activity measurements, the cells were transferred into five spinner flasks. At this stage, the culture medium of the cells was replaced by charcoal-dextran-treated 5% FCS. The cell density used was 2×10^6/ml, and the volume of the medium in each spinner was 50 ml. The metabolism of the steroids was followed by adding ^{3}H-labeled substrates ([2,4,6,7-^{3}H]oestrone at 94–95 Ci/mmol; [2,4,6,7-^{3}H]estradiol at 88 Ci/mmol; [1,2,6,7-^{3}H]T at 95 Ci/mmol; [1,2,6,7-^{3}H]androst-4-ene-3,17-dione at 99 Ci/mmol) or 5α-dihydro[1,2,4,5,6,7-^{3}H]testosterone at 125 Ci/mmol); 200,000 cpm/ml; Amersham Pharmacia Biotech, Little Chalfont, UK) and corresponding unlabeled steroids estrone (E1), 17β-estradiol (E2), T, androstenedione (A-dione), or 5α-dihydrotestosterone (DHT) (Steraloids, Inc., Newport, RI) to a final concentration of 1 nM. The cell suspensions were incubated for the indicated times (1, 2, 4, 6, 10, and 20 h) at 37 C in a 5% CO_2 atmosphere, after which the reactions were stopped by immediately freezing the medium samples in an ethanol-dry ice bath. The steroids were extracted into an organic phase (diethyl ether:ethyl acetate, 9:1) from 1-ml aliquots. Enzyme activities were analyzed by determining the conversion of substrates to specific products using an HPLC system (Waters Corp., Milford, MA). The substrates and products were separated in a Symmetry C18 reverse-phase chromatography column (3.9 $\times$ 150 mm) using an acetonitrile/water (48:52, vol/vol) solution as a mobile phase, and radioactivity was measured by an on-line β-counter (150TR, FLO-ONE Radiomatic, Packard, Meriden, CT) connected to the HPLC system. Ecoscint A (National Diagnostics, Atlanta, GA) was used as a scintillation solution.

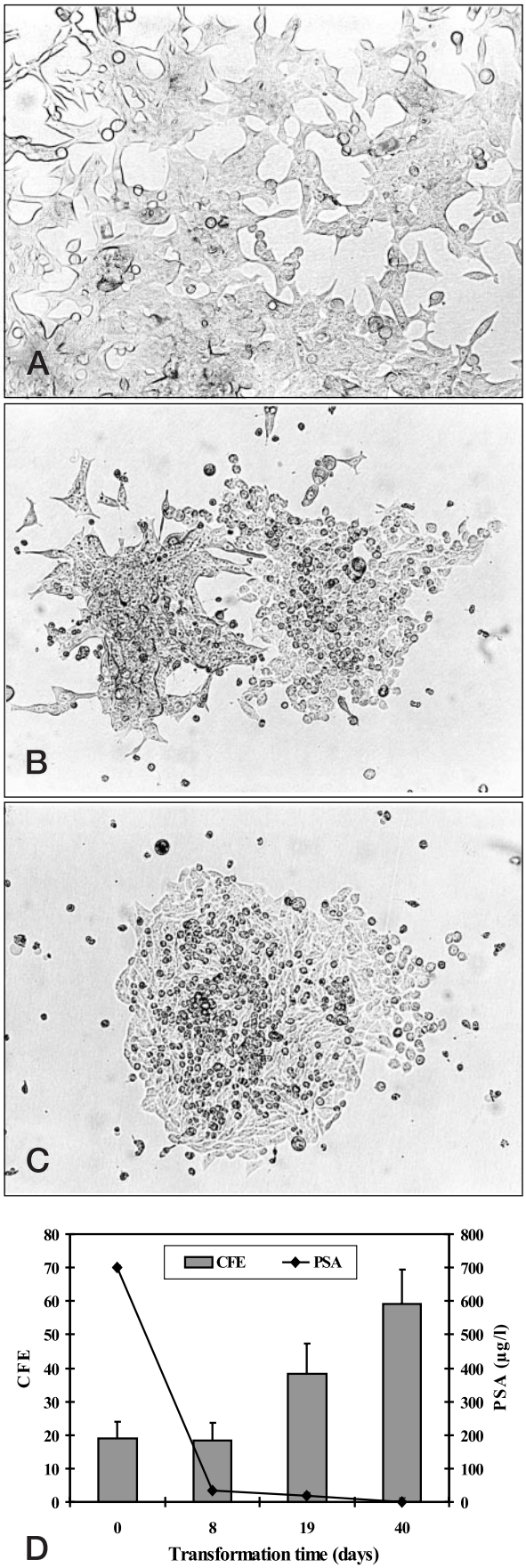
To identify the substrate specificity, human 17HSD type 7 was produced as a recombinant protein in *Spodoptera fugiperda* cells according to the method described in earlier studies (17). The activity of purified 17HSD type 7 was, with minor modifications, measured *in vitro* as previously described (18). Briefly, ^{3}H-labeled substrates (5α-dihydro[1,2-^{3}H]testosterone (125 Ci/mmol), [2,4,6,7-^{3}H]oestrone (94–95 Ci/mmol), [1,2,6,7-^{3}H]progesterone (86 Ci/mmol), or [1,2,6,7-^{3}H]androst-4-ene-3,17-dione (99 Ci/mmol)); 300,000 cpm/ml; Amersham Pharmacia Biotech, Little Chalfont, UK) were mixed with the corresponding unlabeled substrates (Steraloids, Inc.), the final concentration of which was 0.5 μM, and the cofactor NADPH (Boehringer Ingelheim GmbH, Mannheim, Germany) was added to a final concentration of 1 mM. The reactions were initiated by adding the samples of purified recombinant protein, which were diluted in 50 mM Tris-HCl (pH 7.4) containing 150 mM NaCl, 1 mM EDTA, and 0.01% BSA, and the mixtures were incubated at 37 C for 10 min. The stopping of the reactions and all the stages after that were performed following the procedure described above.

Isolation of RNA and RT-PCR analysis

Total RNA from LNCaP cells was extracted using the TRIzol reagent (Life Technologies, Inc., Gaithersburg, MD), and poly(A)-enriched RNA was extracted using oligo(dT)-cellulose (Amersham Pharmacia Biotech, Uppsala, Sweden) according to standard protocols. For relative quantitative RT-PCR, the GeneAmp RNA PCR Kit and GeneAmp 9600 thermal cycler (Perkin-Elmer Corp., Foster City, CA) were used, starting with 200 ng poly(A)-RNA and random hexamers. Reverse transcription was as follows: 22 C, 10 min; 42 C, 15 min; 99 C, 5 min; and 5 C, 5 min. The gene-specific primers (4 μM) used in PCR were 5′-AGTTGCTTCCATCCAACCTGGA-3′ and 5′-TTCCATTGCCTAGGTGGCCTTT-3′ for *HSD17B2*; 5′-GGTGTCAAACTTCAACCGCA-3′ and 5′-GGATAATTAGGGTGGCTAGCA-3′ for *HSD17B5*; and 5′-CCACCAAAAGCCTGAATCTCTCA-3′ and 5′-GAGTGTCATTGTCAGCTCTGGTT-3′ for *HSD17B7*. PCR consisted of denaturation (95 C, 15 sec), annealing (62 C, 64 C, and 62 C for *HSD17B2*, *-B5*, and -B7, respectively; 30 sec), and elongation (72 C, 30 sec). The linear range for each gene/sample was determined by removing aliquots at various PCR cycles (27, 23, and 25 cycles in the linear range for *HSD17B2*, *-B5*, and -B7, respectively), and the optimal ratio of the 18S primers:competimers (Classic II 18S Internal Standards; Ambion, Inc., Austin, TX) was adjusted for each gene-specific primer pair (1:7, 1.5:9, and 2:7 for *HSD17B2*, *-B5*, and -B7, respectively). Finally, the volume of 40 μl from each of the PCR reactions was run in 1.5% agarose gel containing 0.1% ethidium bromide (wt/vol). After the gel had been photographed, the optical densities for the PCR products were determined using a laser scanner (Molecular Dynamics, Inc., Sunnyvale, CA). The ratios of optical densities for gene-specific and 18S PCR products were counted for each specimen.

cDNA microarray and analysis of the expression of estrogen up-regulated genes

To analyze the expression of estrogen up-regulated genes in PSA-producing and non-PSA-producing LNCaP cells, the cDNA microarray screening data of the analysis performed earlier by our research group (16) were reanalyzed in this respect. For the cDNA microarray, total RNA from the LNCaP cells was extracted using TRIzol (Life Technologies, Inc.), and poly(A)-enriched RNA was extracted using a QuickPrep



Micro mRNA Purification Kit (Amersham Pharmacia Biotech, Uppsala, Sweden). mRNA from the cells was used for Human UniGEM V v1.0 Custom Screening (GenomeSystems, St. Louis, MO). mRNAs were labeled with different fluorescent labels, and average signals for the elements in the array were achieved for both probes. For additional information, see http://www.incyte.com.

For the analysis of potential changes in the expression of estrogen up-regulated genes during the progression of prostate cancer, the genes showing at least 8.5-fold overexpression in either the PSA-producing or the non-PSA-producing LNCaP cell line were gathered up from the cDNA microarray screening data. The results are presented as probe-average signal values and as expression folds between the LNCaP cell line variants.

Results

Characterization of cellular properties during transition to an AI state

During the growth of LNCaP cells under cell culture conditions, the cells entered the more aggressive, AI stage. In the course of the transition to the AI state, LNCaP cells converted themselves from originally net-forming cells, in close contact with each other, into small round-shaped cells that had lost their contact and were able to grow anchorage-independently in the suspension culture (Fig. 2, A–C). The higher growth potential of AI cells when compared with parental LNCaP cells was also observed as a decrease in their doubling time (from 30 to 16 h) during the transformation. Regarding the clonogenicity of the cells, their CFE was found to increase during the transformation process (Fig. 2D). Concomitantly with the development of the AI stage, furthermore, LNCaP cells lost their ability to produce detectable amounts of PSA (Fig. 2D). Additional culture of five LNCaP sublines produced strains with similar properties, even though the rate of transformation varied from one strain to another (data not shown). The cellular transformation was found to be irreversible, *i.e.* neither morphological properties nor PSA production of the cells was returned despite the treatment with androgen analog R1881 (17β-hydroxy-17α-methyl-estra-4,9,11-trien-3-one) (data not shown). The recent cDNA microarray screening data indicated that the expression of androgen receptor gene decreases to an extremely low level during the transformation of LNCaP cells (unpublished data). Whereas PSA-producing LNCaP cells serve as a model of well-differentiated prostate cancer, non-PSA-producing LNCaP cell variants represent progressive prostate cancer, with several changes in gene expression (16). This model allows us a continuous supply of cells during the transformation, thus enabling studies regarding the progression of prostate cancer in cell culture conditions.

Enzyme activity measurements

To gain insight into the changes in enzyme activities that have a substantial role in steroid hormone metabolism during the cellular transformation, the conversion of steroid

FIG. 2. Phase-contrast light microscopy of morphological changes in LNCaP cells before (A), during (B), and after (C) the transformation (magnification, ×100). CFE and the concentration of PSA during the transformation of LNCaP cells (D). CFE values are presented as a mean ± SD of the result from two to three cell densities (10^2, 5×10^2, 10^3, and 5×10^3 cells/ml), each of which was analyzed as a triplicate.

substrates E1, E2, T, A-dione, and DHT into their specific products was followed using the HPLC approach. The data indicate that androgen-dependent LNCaP cells possess predominant oxidative 17HSD activity, converting active steroids E2, T, and DHT into their less active 17-keto derivatives E1, A-dione, and 5αA-dione, respectively (Fig. 3, A–B). Oxidative activity decreases the potency of estrogens and androgens, thus possibly protecting tissues from excessive steroid hormone action (19). At a PSA-producing stage, after 1 wk of growth in a suspension culture, E2 was totally converted to E1 in 2 h; whereas, in transformed cells, after 5 wk of growth in a suspension culture, it took 10 h to convert 9% of E2 to E1. At the same time points during the cellular transformation, the oxidative activity converting T to A-dione decreased from 96% to 9%, regarding 10-h reaction time. With the DHT substrate, the formation of 5αA-dione in 10 h decreased from 72% to 6% during the transformation process. The trend of the decrease in oxidative 17HSD activity, concomitantly with the progression of LNCaP cells to an AI state, was observed in four of five cell lots studied (data not shown).

At the transformed stage, LNCaP cells possess remarkable reductive 17HSD activity, leading to the formation of active estrogen E2 and, to a lesser extent, active androgen T, from their less active 17-keto metabolites E1 and A-dione, respectively (Fig. 3, C–D). During the transformation process, the biosynthesis of estradiol was activated so that, within the 10 h, a maximum of 62% of E1 was converted to E2; while, at the PSA-producing stage of the same cell lot, there was not detectable production of E2 (0%). Regarding the androgenic substrate A-dione, the formation of T at the transformed stage reached a maximum of 26% in 10 h and 37% in 20 h *vs.* only 3% at the PSA-producing stage. With the DHT substrate, the formation of 5α-androstane-3α, 17β-diol (3αA-diol) in 10 h increased from 7% to 90% during the transformation process. The formation of 5α-androstane-3β, 17β-diol (3βA-diol) reached its peak value in a 2-h reaction; and during the period in question, the production of this metabolite, which has been suggested to act as an estrogen in the prostate (20), increased from 0 to 11%. The trend of increasing reductive activity during the cellular transformation was also noted in other lots of cultured LNCaP cells. In another cell lot, the conversion of E1 to E2 in 10 h increased from the starting point of only 3%, through 51%, up to 58%, while the production of PSA simultaneously decreased from 44.6 μg/liter, through 5.2 μg/liter, to an undetectable level (<0.5 μg/liter), respectively. The maximum level of the formation of E2 from E1 was detected in a cell lot showing 89% conversion during the incubation time of 20 h. By the time point of the measurement, the production of PSA had decreased to an undetectable level, less than 0.5 μg/liter.

Characterization of 17HSD gene expression

To gain a more detailed insight into the potential enzymes responsible for the detectable oxidative and reductive activities, the expression levels of genes for the different candidate types of 17HSDs (namely, the 17HSD types 2, 5, and 7) were determined at the same time points as the enzyme activity measurements, using the relative quantitative RT-PCR

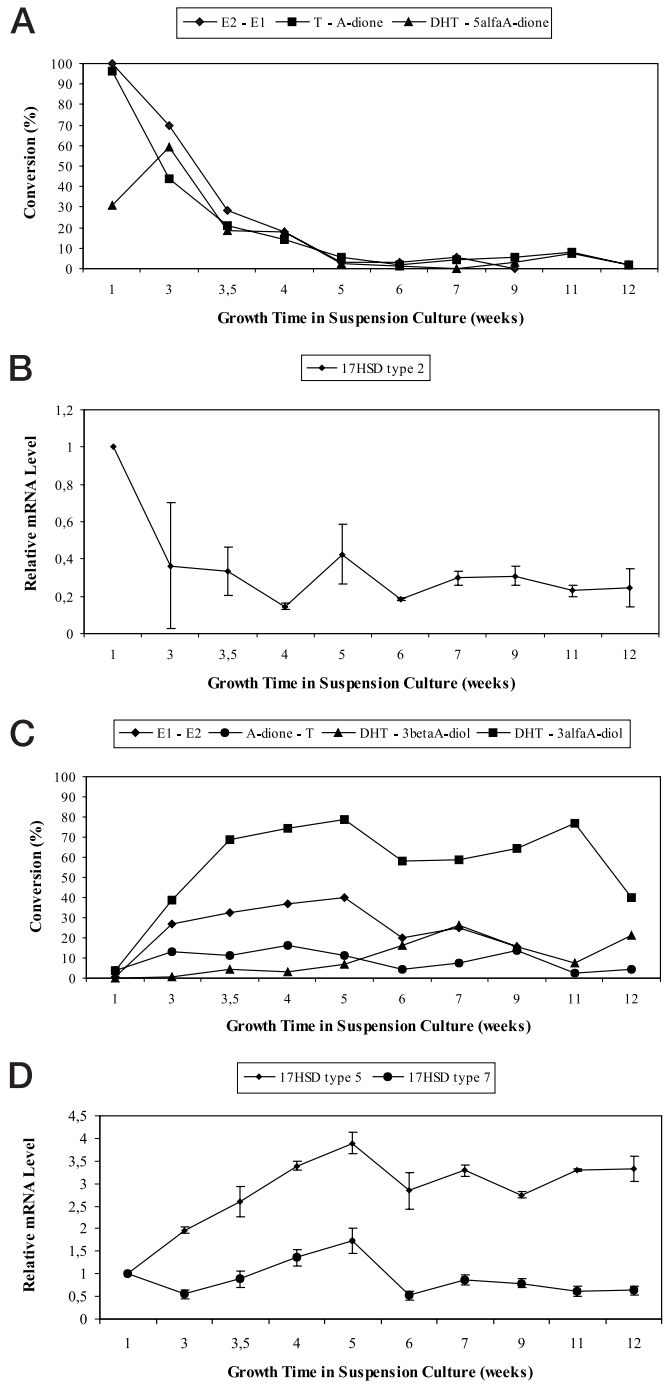


FIG. 3. Variation in 17HSD enzyme activity and gene expression levels during the transition of the human prostate cancer cell line LNCaP into an AI state. In enzyme activity measurements, E1, E2, T, A-dione, and DHT were used as substrates. At each of the time points during the transformation, oxidative and reductive 17HSD activities are expressed as a percentage of the specific product formed in 4-h reactions (A and C). During the follow-up period of cellular transformation, the production of PSA decreased from the starting point of 31.8 μg/liter (after 1 wk of growth in a suspension culture), through 24.4 μg/liter (3 wk of growth in a suspension culture), to an undetectable level, less than 0.5 μg/liter (after 3.5 wk of growth in a suspension culture). The expression of genes for the 17HSD types 2, 5, and 7 is shown as means of the relative mRNA level ± range (B and D). Duplicate or triplicate analyses were performed using the relative quantitative RT-PCR method.

method. The present data suggest a remarkable decrease in the oxidative 17HSD type 2 activity during the cellular transformation. At the time point of maximum decrease in the relative expression of *HSD17B2*, the inactivating reactions converting E2 to E1, T to A-dione, and DHT to 5αA-dione decreased from 100% to 14%, from 96% to 5%, and from 72% to 5%, respectively. During this period, the relative expression of *HSD17B2* decreased from 100% to 18% (Fig. 3B).

The results of relative quantitative RT-PCR revealed that, maximally, *HSD17B5* reached the level of 3.9-fold expression, compared with the level at the PSA-producing stage of the same cell lot (Fig. 3D). Regarding the simultaneous variation in the potential reactions for the 17HSD type 5 enzyme (Fig. 1), the conversion of DHT to 3αA-diol in 10-h reactions increased from 7% to 90%. In addition, the conversion of A-dione to T reached a value of 26%, compared with 4% at the PSA-producing state.

At the time point of maximal *HSD17B5* expression, the expression of *HSD17B7* also reached its peak value, showing a 1.7-fold level when compared with that at the starting point (Fig. 3D). At the same time, the conversion of E1 to E2, in 10 h, increased from 0 to 62%; and the conversion of DHT to 3βA-diol, in 2 h, from 0 to 11%. Purified human 17HSD type 7 was determined to predominantly catalyze the reductive reactions converting DHT to 3βA-diol and E1 to E2 (Fig. 4). A weaker reductive activity converting progesterone to 4-pregnen-3β-ol-20-one was also detected, but no conversion was observed when A-dione was used as a substrate.

cDNA microarray and analysis of the expression of estrogen up-regulated genes

To identify potential differences in the expression of estrogen up-regulated genes between PSA-producing LNCaP cell line and non-PSA-producing cell line variant, the cDNA microarray screening data (16) were analyzed in this respect. Estrogen up-regulation of the genes in question has usually been shown in typical estrogen target tissues or carcinoma cell lines. The screening data revealed that, in the non-PSA-producing LNCaP cell line variant, there were estrogen up-regulated genes among the most highly overexpressed genes (more than 8.5-fold); whereas in PSA-producing cells, none was found. The most prominent overexpression was seen with tissue-type plasminogen activator (tPA), which showed a 40.9-fold expression in non-PSA-producing LNCaP cells when compared with the expression in the PSA-producing cell line. The other genes that showed more than a 20-fold overexpression were expressed sequence tag (EST; similar to galectin-1), phospholipase D, and follistatin. The summary of the estrogen up-regulated genes observed to be overexpressed in the non-PSA-producing LNCaP cell line is expressed in Table 1.

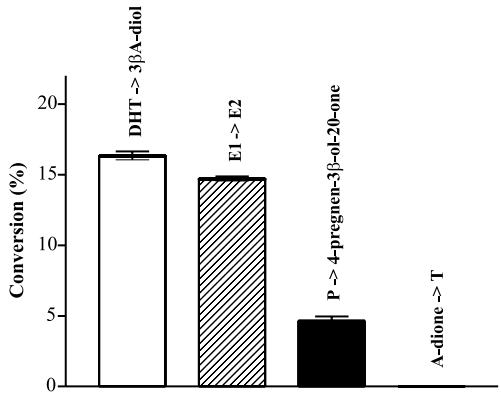


FIG. 4. Substrate specificity of purified human 17HSD type 7, produced as a recombinant protein in *Spodoptera fugiperda* cells (17). DHT, E1, progesterone (P), and A-dione were used as substrates. The results are given as the amounts of substrates converted by 12 μg recombinant h17HSD7 in 10 min at 37 C using NADPH as a cofactor (averages of triplicate measurements, repeated three times with similar results). The recombinant protein with 6× HIS-tag was purified using immobilized metal affinity resin (TALON Cell Thru; CLONTECH Laboratories, Inc., Palo Alto, CA).

Discussion

The progression of prostate cancer during endocrine therapy is a serious clinical problem, the molecular mechanisms of which largely remain to be clarified. The present study was carried out to explore the mechanisms related to the transition of prostate cancer into an AI state during the progression of the disease. To this end, we identified the variations in estrogen and androgen metabolism, as well as in cellular characteristics, during the transformation of prostate cancer LNCaP cells. Furthermore, we characterized the relative expression levels of genes for the different types of 17HSD enzymes, which are importantly involved in the regulation of the intracellular levels of biologically active steroids.

As a general trend of the present study, the variation in the metabolic pattern of LNCaP cells during their transformation seems to lead to increased production of active estrogens via an activation of E1 to E2 as well as via an inactivation of DHT into its estrogenic metabolite 3βA-diol. In addition, the concentration of active estrogens might be suggested to increase, on the basis of the observed remarkable decrease in the conversion of E2 to E1 during the cellular transformation. Regarding the potential enzymes responsible for these reactions, purified human 17HSD type 7 was determined to predominantly catalyze the reductive reactions converting DHT to 3βA-diol, and E1 to E2. The reaction converting DHT to 3βA-diol was also reported to be catalyzed by 17HSD type 7 in another recent study (14). The conversion of E1 to E2, instead, could alternatively be catalyzed by the 17HSD types 1 (21) and 3 (22). However, only low constitutive levels of the 2.3 kb mRNA for 17HSD type 1, which has not been reported to be translated into protein, have been detected in prostatic tissue specimens (23, 24). Expression of 17HSD type 3 has been detected only in the testis (22), and none was found in the prostate (23). Thus, according to the present data, 17HSD type 7 could be largely responsible for the increased estrogen production locally in prostate cancer cells.

Activity measurements *in vitro* have revealed that 17HSD type 2 preferentially catalyzes the oxidative reactions of both androgens and estrogens, converting T, DHT, and E2 into their cognate inactive metabolites, A-dione, 5αA-dione, and E1, respectively (25, 26). In the present study, the decreased inactivation of E2 to E1, observed concomitantly with the cellular transformation, is probably attributable to the decrease in the oxidative 17HSD2 activity, which was also

TABLE 1. Estrogen up-regulated genes in LNCaP (PSA −) cells

Expression fold		Signal value			GenBank accession no.	Gene product known as
PSA−	PSA+	PSA−	PSA+			
40.9	1.0	18986	464	Plasminogen activator, tissue	D01096	tPA
25.9	1.0	7567	292	ESTs, similar to Galectin-1	AA340061	Galectin 1
25.1	1.0	4262	170	Phospholipase D, phosphatidylcholine-specific	U38545	PLD1
21.0	1.0	3672	175	Follistatin	M19481	Follistatin
17.6	1.0	4513	257	Cytochrome P450, subfamily XXVIIB	AB006987	1-Hydroxylase
8.7	1.0	2820	325	IGF-binding protein 10	AF031385	IGFBP10, cyr61

shown as a reduced relative expression of *HSD17B2* gene. The observation of a remarkable decrease in the oxidative 17HSD type 2 activity during the cellular transformation is in line with our recent results suggesting an association between a chromosomal deletion at 16q24.1-q24.2, including the *HSD17B2* gene, and clinically aggressive features of prostate cancer (27, 28). In earlier studies, reduced expression of 17HSD type 2 mRNA has also been detected in prostate cancer specimens (23). Additionally, down-regulation of *HSD17B2* expression has been observed to be related to other forms of cancer (for example, to colon cancer development) (29). By inactivating the active androgens T and DHT and active estrogen E2 in peripheral tissues, 17HSD type 2 possibly protects the steroid target tissues from excessive steroid hormone action (19). During the disease progression, decreased inactivation of active androgens T and DHT in the prostatic epithelium could shift the balance toward increase in the proliferative pressure of cells and, furthermore, to unregulated prostatic growth. On the other hand, however, the expression level of androgen receptor gene has been detected to be lower in non-PSA-producing LNCaP cells when compared with the PSA-producing cell line (16). Additionally, the expression of *HSD17B5* reached the level of 3.9-fold expression at the non-PSA-producing state, compared with the level at the PSA-producing state of the same cell lot. Type 5 17HSD is able to function as bidirectional 3α-, 17β-, and 20α-HSD (30), but studies in a cellular context have suggested that this enzyme could function as a reductase (31). Examination of k_{cat}/K_m for the reactions has indicated that, as a reductase, it prefers DHT and 5αA-dione as substrates to A-dione. Therefore, the presence of type 5 17HSD in the prostate has been suggested to favor the formation of inactive androgens (30). Furthermore, most of the genes known to be androgen up-regulated have been shown to be overexpressed in PSA-producing LNCaP cells when compared with the non-PSA-producing cell line variant (16). In several independent studies, the level of PSA mRNA has been detected to be up-regulated by the action of androgens (32–35).

The present data provide support for the notion that prostate cancer is possibly independent not only of androgens but also of estrogens. It could be proposed that the increased activity of estrogen-producing enzymes would lead to increased availability of active estrogens concomitantly with the progression of the disease and, through this, would induce the expression of genes under estrogenic control locally in the prostate. This hypothesis is supported by the notion that there were several estrogen up-regulated genes significantly overexpressed in the non-PSA-producing LNCaP cells. The most prominent overexpression was seen with tPA, which has previously been investigated with regards to its association with several types of cancer, including prostate cancer (36–38). Among the most overexpressed estrogen up-regulated genes detected in non-PSA-producing cells were also phospholipase D, EST similar to galectin-1, follistatin, and cytochrome P450. Of these, the immediate product of phospholipase D activity, phosphatidate acid, has been suspected to act as a potential mitogenic signal in the cell (39). Galectins, instead, are a family of lectins that are potentially involved in cell adhesion as well as in the regulation of growth and in the trigger or inhibition of apoptosis. The expression pattern of some galectins has been observed to be altered in several types of cancer, including prostate cancer (40). The observed overexpression of follistatin in non-PSA-producing LNCaP cells is in line with the theory of follistatin overproduction favoring AI growth of prostate cancer (41). The product of cytochrome P450, 1-hydroxylase, catalyzes the conversion of 25-hydroxyvitamin D3 to 1,25-dihydroxyvitamin D3. Vitamin D and its receptor belong to the genes known to be associated with prostate cancer risk, like the genes encoding androgen receptor, 5α-reductase, and IGF (42).

Taken together, regulation of the intraprostatic concentrations of active androgens is suggested to maintain organ homeostasis by modulating the balance between proliferation and apoptotic death of prostatic epithelial cells (43). In addition to androgens, estrogens have been suggested to be involved in the abnormal growth of the prostate (44), even though the precise role of the hormones remains undefined. The observed remarkable changes in the 17HSD enzyme activities that critically impact on steroid hormone activation and inactivation may lead to considerably changing bioavailability of sex steroid hormones locally in prostate cells during the progression of prostate cancer. Consequently, the metabolic changes of sex steroid hormones may have a significant role in the biological behavior of prostate cancer and, furthermore, be of importance for the therapeutic management of the disease.

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