Dysregulation of Sterol Response Element-Binding Proteins and Downstream Effectors in Prostate Cancer during Progression to Androgen Independence


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ABSTRACT

Androgen ablation, the most common therapeutic treatment used for advanced prostate cancer, triggers the apoptotic regression of prostate tumors. However, remissions are temporary because surviving prostate cancer cells adapt to the androgen-deprived environment and form androgen-independent (AI) tumors. We hypothesize that adaptive responses of surviving tumor cells result from dysregulated gene expression of key cell survival pathways. Therefore, we examined temporal alterations to gene expression profiles in prostate cancer during progression to androgen independence at several time points using the LNCaP xenograft tumor model. Two key genes, sterol response element-binding protein (SREBP)-1 and -2 (SREBP-1a,-1c, and -2), were consistently dysregulated. These genes are known to coordinately control the expression of the groups of enzymes responsible for lipid and cholesterol synthesis. Northern blots revealed modest increased expression of SREBP-1a, -1c, and -2 after castration, and at androgen independence (day 21–28), the expression levels of both SREBP-1a and -1c were significantly greater than precastrate levels. Changes in SREBP-1 and -2 protein expression were observed by Western analysis. SREBP-1 68-kDa protein levels were maintained throughout progression, however, SREBP-2 68-kDa protein expression increased after castration and during progression (3-fold). SREBP-1c is a transcriptional regulator that coordinately activates the expression of the groups of genes associated with lipid and cholesterol synthesis as depicted in Fig. 1 (8). SREBP-1 and -2 transcriptional activity, decreased after castration and increased significantly at androgen independence. Levels of SREBP cleavage-activating protein, a regulator of SREBP transcriptional activity, decreased after castration and increased significantly at androgen independence. In clinical prostate cancer specimens from patients with varying grades of disease, the stained tissue sections showed high levels of SREBP-1 protein compared with noncancerous prostate tissue. As a result of the metabolic pathways of lipogenesis and cholesterol synthesis, tumor levels of SREBP-1 decreased significantly after 6 weeks. AI tumors expressed significantly higher levels of SREBP-1c. In summary, the LNCaP xenograft model of human prostate cancer as well as clinical specimens of prostate cancer demonstrated an up-regulation of SREBPs and their downstream effector genes during progression to androgen independence. As the AI phenotype emerges, enzymes critical for lipogenesis and cholesterol synthesis are activated and likely contribute significantly to cell survival of AI prostate cancer.

INTRODUCTION

Androgen ablation is the only form of systemic therapy demonstrated to prolong life in men with advanced prostate cancer. Removal of androgen induces apoptosis of prostate epithelial cells and regression of prostate tumors. Despite high initial response rates, remissions are temporary because surviving prostate cancer cells emerge with an androgen-independent (AI) phenotype. Thus, one of the main obstacles for curing advanced prostate cancer by androgen ablation is AI progression, a complex process involving dysregulated gene expression pathways that result in adaptive up-regulation of cell survival genes, ligand-independent androgen receptor transactivation, and activation of alternative growth factors or metabolic pathways (1).

Because prostate cancer varies in its biological aggressiveness, androgen sensitivity, and histological appearance, a single model that precisely mimics the diverse human condition does not exist. However, the LNCaP xenograft model does reflect many aspects of AI progression because it is androgen sensitive and prostate-specific antigen (PSA)-secreting (2, 3). As in human prostate cancer, serum PSA levels in this model are regulated by androgens and proportional to tumor volume in the intact animal (4). After castration, serum and tumor cell PSA levels decrease up to 80% and remain suppressed for 2–3 weeks. Beginning 3–4 weeks after castration, however, PSA production gradually increases above precastrate levels in the absence of testicular androgens, indicating the onset of AI PSA gene expression (5–7). AI progression in the LNCaP model is defined by the up-regulation of PSA in the absence of androgens. How PSA is re-expressed in androgen independence remains an intensive area of research focus. In general, it is hypothesized that escape from androgen-regulated PSA production may occur by either ligand-independent activation of the androgen receptor or up-regulation of alternative nonandrogenic pathways of signal transduction (5).

Characterization of the drift in gene expression caused by androgen ablation in the LNCaP tumor model mediating tumor progression to androgen independence provides insights into biological mechanisms and may facilitate the identification of prognostic indicators and therapeutic targets. We compared gene expression profiles at specified time points before and after castration in androgen-dependent, early postrcastration, and AI recurrent LNCaP tumors to identify genes and gene clusters that become dysregulated during AI progression in the LNCaP tumor model. Several genes that are known to be androgen-regulated (including PSA) decreased after androgen withdrawal but were re-expressed during AI progression, indicating that perhaps all androgen receptor-regulated genes are up-regulated in androgen independence. Such androgen receptor-regulated genes include the ste- role response element-binding protein (SREBP) family of transcriptional regulators that coordinate the expression of the cascades of genes associated with lipid and cholesterol synthesis as depicted in Fig. 1 (8). SREBP-1 and -2 protein isoforms (which share 45% homology) are derived from separate genes [SREBP-1a and -1c are splice variants (9, 10)]. SREBPs are transcription factors of the basic helix-loop-helix-Zip family and consist of approximately 1150 amino acids organized into three functional domains, an NH2-terminal WD (tryptophan aspartic acid) repeat domain of SREBP cleavage-activating protein (SCAP) that is functionally regulated in part by...
sterol levels (14). The SREBP/SCAP complex is thought to move to the Golgi, where an enzyme, site-1-protease, cleaves SREBP between the lysine and serine residues of the RSVLS (arginine, serine, valine, leucine, and serine) domain within the lumen of the Golgi when sterols are limiting (15). This cleavage enables a second enzyme to cleave a site within the membrane, resulting in the release and translocation of a 68-kDa NH2-terminal domain (basic helix-loop-helix) peptide to the nucleus, where it transactivates genes containing sterol-regulatory elements (16). Alternatively SREBP may be cleaved by caspase 3 to activate its nuclear translocation and subsequent transcriptional activation (17, 18). In the nucleus, SREBPs bind the sterol-regulatory element in the enhancer region of several genes encoding enzymes of cholesterol biosynthesis, unsaturated fatty acid biosynthesis, triglyceride biosynthesis, and lipid uptake [low-density lipoprotein (LDL) receptor; Ref. 19]. As part of a feedback mechanism, high cholesterol levels inhibit the SREBP/SCAP complex from recruiting or activating site-1-protease and therefore prevent release of the active basic helix-loop-helix fragment (20). SREBPs are androgen-regulated members of a pathway regulating cholesterol and acyl-CoA transport and fatty acid synthesis (8, 21) that are critical to cell proliferation and survival. We hypothesize that dysregulated expression of SREBPs after castration may help mediate AI progression through the downstream pathways regulated by SREBPs that ensure maintenance of cholesterol and fatty acid synthesis.

This report provides the first demonstration in an in vivo prostate cancer tumor model that expression of SREBPs as well as some of their downstream effectors [acyl-CoA-binding protein/diazepam-binding inhibitor (ACBP/DBI), fatty acid synthase (FAS), and farnesyl diphosphate synthase (FDPS)] becomes dysregulated during progression to androgen independence. Additionally, the expression of a posttranslational regulator of SREBPs, SCAP, was increased during progression. Western analysis and immunohistochemistry demonstrated that SREBP-1 (68 kDa) protein level was maintained in LNCaP tumors after castration and during progression to androgen independence. SREBP-2 (68 kDa) protein expression increased significantly after castration and during progression. Interestingly, staining of human prostate tumors showed elevated levels of SREBP-1 protein compared with normal prostate tissue. Androgen ablation by neoadjuvant hormone therapy before radical prostatectomy resulted in decreased protein levels of SREBP-1; however, after several months, when tumors progressed to androgen independence, SREBP-1 protein levels once again increased. These results provide the first in vivo demonstration in both xenograft tumors and clinical specimens of human prostate cancer of the dysregulation of SREBPs and their downstream effector gene targets during progression to androgen independence.

MATERIALS AND METHODS

In Vitro Model: Cell Culture. Cultured LNCaP cells (derived from the lymph node metastasis of a prostate cancer patient), passage 35–45, were maintained in RPMI 1640 with 5% FCS and antibiotic/antimycotic at 37°C and 5% CO2 (22).

In Vivo Model: LNCaP Tumor Progression to Androgen Independence. LNCaP cells (2 × 106) were grown in vitro as described above, trypsinized, pelleted, and coinoculated with 120 μl of Matrigel (50:50 volume ratio of cells to Matrigel; Becton Dickinson Labware). Cells were equally distributed at six sites by s.c. injection (right and left shoulder, right and left flank, and right and left hip) in 6–8-week-old athymic nude mice (BALB/c strain; Charles River Laboratory) or severe combined immunodeficient mice under methoxyflurane anesthesia. Mice were housed in a barrier unit and monitored every 3–5 days for PSA levels and tumor growth.

Serum PSA Levels. Blood samples were collected by tail vein incision, and the serum was stored at −20°C until assayed for PSA. Serum PSA was determined using an enzymatic immunoassay kit (Abbot IMX) according to the manufacturer’s protocol. Time to AI PSA expression was defined as the duration of time required after castration for serum PSA levels to return to or increase above precastrate levels.

Tumor Harvest. When tumor size reached approximately 1.5 cm in diameter (6–8 weeks after injection), the sera were collected and stored at −70°C. Tumors were resected, minced, and subjected to a 10% acetic acid wash to remove excess extracellular matrix. Tumoral androgen receptor (AR) was determined using an immunoblotting assay kit (Amersham; Ref. 23).

**Fig. 1.** The roles and regulation of sterol response element-binding proteins (SREBPs) in prostate cancer cells. SREBP cleavage-activating protein and SREBP, both normally regulated by androgens, become dysregulated during progression to androgen independence. After two cleavage events initiated by SREBP cleavage-activating protein/site-1-protease, the 68-kDa NH2 domain of SREBP is transported into the nucleus, where it binds the sterol response element and transports fatty acid synthase (FAS), acyl-CoA-binding protein (ACBP), and farnesyl diphosphate synthase (FDPS). Other coregulatory factors are also required for transcription of these genes. Fatty acid synthase enzyme generates long-chain fatty acids including acyl-CoA (a source of energy and membrane phospholipid). Acyl-CoA-binding protein/diazepam-binding inhibitor transports acyl-CoA to the mitochondria, where it undergoes β-oxidation to provide energy for the cell. It is also a component of mitochondrial membrane phospholipids. Farnesyl diphosphate synthase plays a role in cholesterol synthesis and membrane production. SREBP-1a and -1c transcriptionally regulate enzymes in both pathways, whereas SREBP-2 is thought to primarily regulate cholesterol synthesis (8).
animals were sacrificed by carbon dioxide asphyxiation, and the tumors were resected. The tumor was further dissected, and segments were either placed in TRIzol (Invitrogen, Carlsbad, CA) and frozen immediately at −80°C or placed in formalin or liquid nitrogen. The remaining animals were surgically castrated under methoxyflurane anesthesia and monitored for PSA levels for up to 5 weeks. Tumors were harvested at several points along the PSA curve. The epithelial-stromal ratio is consistent within this tumor model and varies <10% between tumors, as assessed by immunohistochemistry of cytokeratin 14 (epithelium) and vimentin (stroma) [2].

Northern Analysis. Total RNA (15 µg) isolated in TRIzol (prepared according to the manufacturer’s protocol) was denatured in deionized formaldehyde/formamide/3-(N-morpholino)propanesulfonic acid (Fisher Biotech) sample buffer and subjected to electrophoresis through a denaturing 1% agarose, 3-(N-morpholino)propanesulfonic acid, and deionized formaldehyde gel at 75 V for 2 h in 1× 3-(N-morpholino)propanesulfonic acid buffer. The RNA was transferred to a nylon membrane (Biodyne B; Pall Gelman Laboratory, East Hills, NY) overnight in 20× SSC (pH 7.0). The RNA was UV cross-linked to the membrane (UV Stratalinker 1800; Stratagene) according to the manufacturer’s instructions. The membrane was hydrated with diethyl pyrocarbonate-treated water and then prehybridized in Expresshyb (Clontech) according to the manufacturer’s instructions. The membrane was preincubated with Tris buffer [20 mM Tris, 146 mM NaCl (pH 7.4)] and 5% skim milk at room temperature for 3 min before loading on 10% polyacrylamide gels. Proteins were boiled at 90°C for 5 min, and equal amounts of protein (10 µg per lane) were separated by polyacrylamide gel electrophoresis (PAGEL; National Diagnostics). Proteins were transferred electrophoretically onto polyvinylidene difluoride (Millipore, Temecula, CA) and stained with Coomassie blue R-250. The gels were scanned using a Molecular Dynamics (Sunnyvale, CA) Imaging System and analyzed using Scion Image software. Protein bands were quantitated using a Bio-Rad Gel Doc 2000 system. Protein expression levels were normalized to tubulin before statistical analyses were performed (n = 3; Student’s paired t test).

Tissue Microarray Slide Preparation. For human prostate cancer Gleason grade arrays, a total of 400 tumors were arrayed: 34 benign tumors; 70 Gleason grade 2 tumors; 235 Gleason grade 3 tumors; 34 Gleason grade 4 tumors; and 27 Gleason grade 5 tumors. The human prostate cancer tissue array comprised 150 specimens (2 samples/tumor specimen) from either patients who had not received hormone therapy or patients who had received neoadjuvant hormone therapy for 3, 6, or 8 months before radical prostatectomy. Tumors that had acquired AI growth were also included. AI status of the AI clinical specimens was based on biochemical progression in which patients had rising PSA values despite castrate levels of testosterone while undergoing androgen ablation therapy and clinical progression including local bladder outlet obstruction and/or bone scan progression. Sections (5 µm) were cut with a microtome by use of an adhesive-coated tape sectioning system (Instruments, Hackensack, NJ) to support the adhesion of the array elements.

Immunohistochemistry. Tissues were dehydrated, and endogenous peroxidase activity was blocked with methanol:30% H2O2 (9:1). Antigen retrieval was enhanced using commercial antigen unmasking solution (Dako Target Retrieval Solution; Dako Corp., Carpinteria, CA) with the autoclave method. BSA was applied for 1 h at 25°C to block the nonspecific binding sites on the slides, which were then incubated in a humidified chamber overnight at 4°C with a 1:100 dilution of SREBP-1 antibody (commercial antibodies tested did not detect SREBP-2 by immunohistochemistry). After primary incubation, tissue sections were washed three times with PBS and incubated with biotinylated horseradish peroxidase-conjugated IgG secondary antibody (Upstate Biotechnology, Lake Placid, NY) at a concentration of 30 min at 25°C. The antigen was visualized by a subsequent 5-min incubation with diaminobenidine tetrahydrochloride before counterstaining with hematoxylin. Tissues were covered with mounting media (Pervanad, Fisher Scientific, Fair Lawn, NJ) and a coverslip.

Negative control slides were processed in an identical fashion to those described above, with the substitution of normal goat nonimmune serum for the primary antisera. No color reactions were observed in negative control slides. Photomicrographs were taken through a Leica DMLS microscope coupled to a digital camera (Photometrics CoolSNAP; Roper Scientific, Inc., Glenwood, IL) and the corresponding computer software.

Scoring of SREBP-1 Staining. The stained area of SREBP-1 was evaluated and scored as follows: specimens were graded from 0 to +4 intensity, representing the range from no staining to intense staining. All comparisons of staining intensity and percentages were made at ×400 magnification. Simultaneously expression of SREBP-1 protein was recorded as nuclear, cytoplasmic, and both. The percentage of stained area (combination of intensity and surface area) was measured by Image Plus software (Media Cybernetics) and assessed by the pathologist (M. A.). ANOVA was performed.

RESULTS

To examine changes in gene expression during prostate cancer progression to androgen independence, LNCaP tumors were harvested at various times before and after castration throughout the progression time series. RNA was initially analyzed using cDNA microarrays to derive gene expression profiles. Alteration to the expression levels of key transcription factors was studied in greater depth to determine potential cascades of gene activation that may functionally impact prostate cancer progression to androgen independence. Of particular interest was the observation that SREBPs appeared to be up-regulated during prostate cancer progression in the LNCaP tumor model. SREBPs are transcription factors that are known to coordinately regulate genes involved in two major pathways including enzymes of fatty acid synthesis as well enzymes of cholesterol synthesis and transport as depicted in Fig. 1 (8).
Serum PSA Levels Change during Tumor Progression to Androgen Independence. Serum PSA levels were decreased 3–7 days after castration (Fig. 2). After 21–28 days, serum PSA levels increased significantly (P < 0.05), indicating an AI phenotype. The PSA level of intact hosts increased at a continuous rate for 21 days and then reached a plateau by 28 days.

SREBP-1 and SREBP-2 mRNA and Protein Expression Were Altered during Progression to Androgen Independence. To verify the array-based observations, we performed Northern blot analysis on total RNA isolated from several LNCaP tumor series with probes specific for SREBP-1a, -1c, and -2. These results demonstrated that SREBP-1a, -1c, and -2 were up-regulated during progression. RNA expression of SREBP-1a and -1c, at androgen independence (day 28 postcastration), was significantly increased compared with expression in tumors from intact mice (Fig. 3, A–D). The elevated expression of these transcription factors was modest but significant in six separate tumor progression series (P < 0.05).

The expression of SREBP-2 was also moderately increased after 21–28 days, compared with the levels determined in tumors from intact mice (Fig. 3, E and F); however, these differences were not significant. Generally, expression of SREBPs displayed trends to increased levels at androgen independence that paralleled significant trends found in downstream effectors as documented below.

SREBP-1 and -2 Protein Expression Was Altered during Progression to Androgen Independence. Western analysis of SREBP-1 revealed that the uncleaved 125-kDa product changed with progression. Levels were decreased at 3–7 days and reached a nadir at 14 days. By 21–28 days, protein expression increased compared with day 14, but not significantly, and did not reach precastrate levels (Fig. 4, A and B). Expression levels of the cleaved form of SREBP-1 (68 kDa) changed slightly during progression (Fig. 4, A and B). The increased levels of cleaved protein at day 14 correlated with decreased levels of full-length protein at this time. The antibody does not distinguish between SREBP-1a and -1c isoforms; therefore, we cannot determine their specific expression profiles or determine correlation between mRNA and protein. Unlike SREBP-1, SREBP-2 protein expression of the cleaved form (68 kDa) increased significantly during progression to levels 350% above precastrate levels (P < 0.05; Fig. 4, C and D).
Despite modest changes in SREBP-2 mRNA expression after castration, there were significant increases in 68-kDa protein levels during progression. These changes in 68-kDa SREBP-2 were greater than those documented for 68-kDa SREBP-1. We have been unable to obtain a specific antibody for mature (125-kDa) SREBP-2 that detects protein on Western blots, so we are unsure of the kinetics of cleavage events during progression. We have shown that throughout progression, levels of both 68-kDa cleavage products of SREBP-1 and -2 are present to interact with sterol response elements. Because SREBPs are regulated in part by the androgen receptor in prostate cancer cells, perhaps the reactivation of SREBPs at androgen independence occurs through a similar process, as does PSA expression in androgen independence.

To determine whether the up-regulation of SREBPs during progression functionally impacted the expression of genes known to be regulated by SREBPs, we examined the expression of key genes within the cascades of lipogenesis and cholesterol synthesis and acyl-CoA transport (ACBP, FAS, and FDPS; Fig. 1).

ACBP/DBI Expression Was Altered during Progression to Androgen Independence. It is known that ACBP/DBI is regulated in part indirectly by androgens in prostate cancer cells through the direct up-regulation of SREBP-1a mRNA (24). It is unclear whether other SREBP isoforms are equally effective in regulating ACBP/DBI expression. ACBP/DBI functions as a transporter of acyl-CoA and cholesterol into the mitochondria and thus plays a role in cholesterol-based biosynthesis. The profile of ACBP/DBI mRNA expression during progression correlated somewhat with the expression pattern seen for PSA, another androgen-regulated gene. After castration, ACBP/DBI mRNA expression was significantly decreased after 3–14 days compared with precastrate controls (P < 0.05). Levels returned to normal after 21–28 days (P < 0.05; Fig. 5, A and B), when androgen independence was reached. This may indicate a response to increased SREBPs at this time. Specific levels of SREBP-1a and -1c protein could not be measured; therefore, it was unclear which isoform was involved in the regulation of ACBP/DBI during progression to androgen independence.

FAS Expression Is Increased at Androgen Independence. As shown in Fig. 1, SREBPs coordinately regulate transcription of several enzymes involved in the biosynthesis of cholesterol and fatty acids. FAS is a key enzyme in the fatty acid synthesis cascade and has been shown to be regulated by SREBP-1a (21). Expression of FAS decreased after a 14-day period following castration and by 21 days had increased to levels greater than that found in tumors from intact mice (P < 0.05; Fig. 5, C and D). The levels continued to increase significantly during the 28-day follow-up. The kinetics of increased expression were similar to those of SREBP-1a and -1c (days 21–28), inferring a coordinated regulation during tumor progression to androgen independence of this pathway of genes involved in fatty acid synthesis.

FDPS Expression Increased during Progression to Androgen Independence. A downstream target of SREBPs involved in the cholesterol synthesis cascade, shown in Fig. 1, is FDPS, which is critical for metabolism and membrane production (8, 21). Expression levels of FDPS did not decrease significantly after castration but did increase gradually and, by 21–28 days, had increased to levels that were greater than those in tumors of intact mice (Fig. 5, E and F). The levels at 28 days were significantly elevated (P < 0.05). The profile of FDPS mRNA expression was similar to that of SREBP-2 mRNA and protein in that both were increased at androgen independence.

SCAP Expression Was Dysregulated during Tumor Progression. The observed increases in levels of downstream targets were greater than changes in levels of SREBPs. It was shown that in addition to up-regulation of SREBPs at the transcriptional level, the activation of SREBP protein was also being stimulated. Therefore, we examined the expression of a protein activator of SREBP function, the SREBP cleavage-activating protein (SCAP), to determine whether it was also aberrantly expressed during progression to androgen independence.
DISCUSSION

Through array-based analysis, we have identified genes in the LNCaP tumor model that become dysregulated after castration and during progression to androgen independence. Data presented here characterize the cascades of genes coordinately regulated by the SREBPs, SREBP-1a, -1c, and SREBP-2, including ACBP/DBI, FDPS, FAS, and a posttranslational regulator of SREBP activity, SCAP, all of which are components of a pathway critical to survival. Tumor cells are highly metabolically active and proliferative; thus they require increased energy metabolism (citrate cycle) and membrane production (fatty acid and cholesterol synthesis). The proteins regulating fatty acid biosynthesis and cholesterol levels in prostate cells are regulated by androgens, including SREBPs (8, 21). SREBPs in turn regulate ACBP/DBI (24–26), FDPS, and FAS (8, 11, 21, 23, 27, 28). Recently, SCAP was shown to be regulated by androgens in prostate cells (23).

Androgens stimulated the expression of SREBP transcripts and precursor proteins in LNCaP cells in vitro and increased the nuclear content of the active 68-kDa fragment of SREBP (8, 11). Expression of SREBP-1a and -1c mRNA in our in vivo tumor progression series increased significantly when tumors reached androgen independence. There was a trend of increased expression of SREBP-2 mRNA during AI progression that did not quite reach significance. Protein expression of the uncleaved SREBP-1 125-kDa product was decreased at 14 days postcastration compared with precastrate levels but increased after 21–28 days. SCAP may play an important role in augmenting SREBP activity in tumor progression to androgen independence, but it may not be the only mechanism regulating SREBP function.

SREBP-1 Protein Expression Is Altered in Human Prostate Cancer Specimens. To determine whether SREBP expression is altered in clinical prostate cancer, a human prostate cancer tissue array comprised specimens from four types of prostatic tissue: (a) benign prostatic hypertrophy; (b) tumors from patients who had not received hormonal therapy; (c) tumors from patients who had received neoadjuvant hormonal therapy for 3, 6, or 8 months; and (d) tumors from patients who had not received neoadjuvant hormone therapy (c). Tissue specimens were stained for SREBP-1. As can be seen in Fig. 7, A, sections of benign prostatic hypertrophy showed a low level of staining for SREBP-1, compared with sections of untreated prostate cancer, which showed intense staining (Fig. 7B). After 6 weeks to 3 months of neoadjuvant hormonal therapy (Fig. 7, C and D), the prostate sections showed very little SREBP-1 staining. However, by 8 months of neoadjuvant hormonal therapy (Fig. 7E), levels of SREBP-1 had increased, and when tumors had reached androgen independence (Fig. 7F), the staining was very intense. Morphological changes related to androgen withdrawal therapy precluded assessing an exact Gleason grade, but in Fig. 7, the sections were all equivalent to Gleason grade 3. As seen in Fig. 7G, neoadjuvant hormone therapy administered for 6 weeks to 8 months significantly decreased the amount of SREBP-1 present in patient tumor samples (P < 0.001), but at androgen independence, SREBP-1 increased to levels seen without androgen ablation treatment.

By Northern analysis, SCAP expression was significantly decreased 14 days after castration (P < 0.05) and significantly up-regulated by 21 days and 28 days (P < 0.05; Fig. 6, A and B). The increased expression at 21 and 28 days correlated with significant increases in mRNA SREBPs at androgen independence. Expression of both SREBP and SCAP has been shown to be androgen regulated (23). Interestingly, the mRNA expression profile of SCAP closely mimics the expression profile of ACBP/DBI and FAS, downstream effectors of SREBPs. The lowest level of expression of ACBP/DBI, FAS, and SCAP occurred at day 14, followed by significant increases at 21–28 days. SCAP may play an important role in augmenting SREBP activity in tumor progression to androgen independence, but it may not be the only mechanism regulating SREBP function.

ACBP/DBI, fatty acid synthase (FAS), and farnesyl diphosphate synthase (FDPS) expression increases during prostate cancer progression to androgen independence. ACBP/DBI (A), FAS (C), and FDPS (E) mRNA expression as a percentage change (mean ± SE) from precastrate (P) expression in LNCaP tumors was assessed after postcastration intervals of 3–7, 14, 21, and 28 days by Northern analysis and quantified by phosphorimaging (n = 4 for each time point). Representative blots of ACBP/DBI (B), FAS (D), and FDPS (F) and ribosomal 18S RNA expression in LNCaP tumors harvested during progression (precastrate to 28 days postcastration) are shown. *, statistical significance (P < 0.05).

DYSREGULATION OF SREBP IN PROSTATE CANCER PROGRESSION

Fig. 5. Acyl-CoA-binding protein/diazepam-binding inhibitor (ACBP/DBI), fatty acid synthase (FAS), and farnesyl diphosphate synthase (FDPS) expression increases during prostate cancer progression to androgen independence. ACBP/DBI (A), FAS (C), and FDPS (E) mRNA expression as a percentage change (mean ± SE) from precastrate (P) expression in LNCaP tumors was assessed after postcastration intervals of 3–7, 14, 21, and 28 days by Northern analysis and quantified by phosphorimaging (n = 4 for each time point). Representative blots of ACBP/DBI (B), FAS (D), and FDPS (F) and ribosomal 18S RNA expression in LNCaP tumors harvested during progression (precastrate to 28 days postcastration) are shown. *, statistical significance (P < 0.05).

Fig. 6. A, sections of benign prostatic hypertrophy showed a low level of staining for SREBP-1, compared with sections of untreated prostate cancer, which showed intense staining (Fig. 7B). After 6 weeks to 3 months of neoadjuvant hormonal therapy (Fig. 7, C and D), the prostate sections showed very little SREBP-1 staining. However, by 8 months of neoadjuvant hormonal therapy (Fig. 7E), levels of SREBP-1 had increased, and when tumors had reached androgen independence (Fig. 7F), the staining was very intense. Morphological changes related to androgen withdrawal therapy precluded assessing an exact Gleason grade, but in Fig. 7, the sections were all equivalent to Gleason grade 3. As seen in Fig. 7G, neoadjuvant hormone therapy administered for 6 weeks to 8 months significantly decreased the amount of SREBP-1 present in patient tumor samples (P < 0.001), but at androgen independence, SREBP-1 increased to levels seen without androgen ablation treatment.

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Androgens stimulated the expression of SREBP transcripts and precursor proteins in LNCaP cells in vitro and increased the nuclear content of the active 68-kDa fragment of SREBP (8, 11). Expression of SREBP-1a and -1c mRNA in our in vivo tumor progression series increased significantly when tumors reached androgen independence. There was a trend of increased expression of SREBP-2 mRNA during AI progression that did not quite reach significance. Protein expression of the uncleaved SREBP-1 125-kDa product was decreased at 14 days compared with precastrate levels but increased after 21–28 days. Interestingly, the cleaved 68-kDa product remained relatively con-
DYSREGULATION OF SREBP IN PROSTATE CANCER PROGRESSION

Androgen enhances the expression of several lipogenic enzymes including those in the fatty acid synthesis pathway (FAS) and the nuclear factor Y, binds SREBP-1α, and disrupts binding to sterol response elements and thus may play a regulatory role in the transcription of some SREBP-responsive genes (31). Recent studies have shown that inhibition of the transcriptional activity of SREBP prevents its degradation, resulting in accumulation of SREBP proteins (32). This lack of degradation may account for the presence of mature SREBP proteins detected in our studies during the early postcastration period, when mRNA levels of SCAP, ACBP/DBI, and FAS were low.

In summary, in our in vivo model of prostate cancer, SCAP, SREBPs, and downstream effectors ACBP/DBI, FAS, and FDPS were all significantly elevated compared with intact controls when androgen independence was reached. The magnitude of expression and the temporal changes in expression of SREBPs differed from that seen in downstream effector genes during the early postcastration interval, indicating that several pathways were likely involved during this time to regulate the expression of these androgen-regulated genes.

Specific regulation of SREBP-1 and -2 appears to occur by different mechanisms during progression. Defining which isoform regulated specific genes was beyond the scope of this work; however, there appear to be several mechanisms to regulate transcription and processing of SREBPs including androgen levels, sterols, fatty acids, coregulators, and insig-1 and -2 (33). Differential regulation of SREBP-1 and -2 was reported by others previously. Together, these data suggest that, in general, SREBP-2 appears to be the main isoform that transcriptionally regulates the cholesterol biosynthesis genes and the LDL receptor, whereas SREBP-1a and -1c are transcriptional activators of fatty acid synthesis [in addition to cholesterol synthesis (28, 34)].

ACBP/DBI expression is regulated indirectly by androgen through the up-regulation and activation of SREBPs (24). ACBP/DBI is a multifunctional, highly conserved 10-kDa protein expressed in various tissues including rat brain and pancreas, bovine liver, Leydig cells, and glial cells as well as in yeast and bacteria (25). In our studies, LNCaP tumor ACBP/DBI levels decreased within a week of castration but then increased significantly over the next several weeks. Whereas the function of ACBP/DBI in tumors during progression to androgen independence is unknown, several potential roles may include supporting energy metabolism, steriodogenesis, membrane production, and transcription. ACBP/DBI binds with high affinity to cellular acyl-CoA (35, 36) and transports acyl-CoA to the mitochondria (37). Once inside the mitochondria, acyl-CoA undergoes β-oxidation to acetyl-CoA, which then generates ATP during the citric acid cycle and respiratory chain (37). ACBP/DBI may also play a role in steroid production because it transports cholesterol to the inner mitochondrial membrane, where it is loaded onto cholesterol side chain cleavage cytochrome-P450, initiating steroidogenesis (38–40).

Also within the mitochondria, ACBP/DBI has been shown to transport cholesterol to the peripheral benzodiazepine receptor, which is part of a heteromeric complex involved in the formation of a mitochondrial permeability transition pore (20). The peripheral benzodiazepine receptor/ACBP complex may play a role in preventing apoptosis by stabilizing the mitochondrial membrane by ensuring an adequate supply of cholesterol (41–43). Interestingly, a correlation of breast cancer cell aggressive phenotype with peripheral benzodiazepine receptor expression in the nuclear membrane has been documented (44). Peripheral benzodiazepine receptor in aggressive breast tumor cells regulated cell proliferation and cholesterol transport into the nucleus; however, the role of nuclear cholesterol is unknown. Nuclear ACBP/DBI is known to interact with the nuclear binding protein HNF-4α and thus may play a role in regulating transcription of genes involved in glucose and lipid metabolism (45).
cholesterol synthesis pathway [FDPS (8)]. FAS is a key enzyme involved in de novo biosynthesis of fatty acids. Most normal adult tissues express very low levels of FAS and, instead, use dietary circulating fatty acids for biosynthesis (46). We report here that FAS mRNA expression in human prostate xenograft tumors decreased for 14 days after castration and then increased as the tumor progressed to androgen independence. In another prostate xenograft tumor (CWR22), castration caused decreases in FAS protein expression, which increased again when androgen was administered 21–28 days after castration (47). Additionally, others have reported that in LNCaP cells, androgens stimulated the expression of FAS as well as other lipogenic genes (8, 48). In human ovarian, endometrial, breast, and prostate cancers, overexpression of FAS was correlated with advanced pathological stage (49). Furthermore, the role of FAS in cancer cells was critical to their survival because it was demonstrated that administration of an inhibitor of FAS, cerulenin, induced apoptosis of an AI human prostate carcinoma cell line (50). In vivo, a 4-fold reduction of tumor growth in an AI human prostate cancer xenograft after treatment with the FAS inhibitor c75 (51) was reported. Interestingly, cell lines that overexpressed FAS were more sensitive to cerulenin than cells that did not overexpress FAS, suggesting that cancer cells require endogenous fatty acid biosynthesis because of increased demand (52–55). This increased requirement by cancer cells of fatty acid synthesis may prove to be a target for therapeutics in prostate cancer.

FDPS plays a critical role in cholesterol homeostasis. Interestingly, the FDPS expression profile in the LNCaP tumor progression series paralleled that of SREBP-2, gradually increasing during progression. Increased SREBP-2 and FDPS may help maintain high levels of cholesterol in prostate tumors despite the loss of androgen. Some types of cancer cells lack the feedback regulatory systems for cholesterol and fatty acid uptake (56). In normal human prostate epithelial cells, SREBP-2 transcriptionally regulated expression of LDL receptor (carrier of cholesterol and fatty acids) mRNA, which was feedback-regulated by LDL and cholesterol. However, in human prostate cancer cell lines that are not androgen responsive (PC-3 and DU145), expression of SREBP-2 and LDL receptor was not down-regulated by cholesterol (56). This loss of feedback regulation of SREBP2 and its downstream effector LDL receptor, in advanced cancer cells, may result in increased fatty acid uptake, providing energy and structural lipids for membranes (52).

SCAP contains a sterol-sensing domain and is regulated by sterol levels. SCAP loses its proteolytic activity when sterols reach high levels within the cell (19). Recently, it has been demonstrated in vitro in LNCaP cells that androgens induced an increased expression of SCAP, which enhanced the production of cleaved SREBP and stimulated lipogenic gene expression (23). As we have shown in this present work, removal of androgen (castration) caused a decreased RNA expression of SCAP. Interestingly, this decreased expression was eventually reversed as the tumor progressed to an AI phenotype similar to other androgen-regulated genes. The mature forms of SREBPs did not decrease after castration as mentioned above. Cleaved product of SREBP-2 protein was significantly increased during progression, and the cleaved product of SREBP-1a and...
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Dysregulation of Sterol Response Element-Binding Proteins and Downstream Effectors in Prostate Cancer during Progression to Androgen Independence


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