Signaling and Regulation

Activation of Androgen Receptor, Lipogenesis, and Oxidative Stress Converged by SREBP-1 Is Responsible for Regulating Growth and Progression of Prostate Cancer Cells

Wen-Chin Huang1, Xiangyan Li1, Jian Liu2, Jentai Lin3, and Leland W.K. Chung1

Abstract

We previously reported that sterol regulatory element-binding protein-1 (SREBP-1) is involved in the transcriptional regulation of androgen receptor (AR) and formation of fatty acid through altered expression of fatty acid synthase (FASN). In this article, we provide a new finding that SREBP-1 induced oxidative stress in prostate cancer cells through increased production of reactive oxygen species (ROS) and expression of NADPH oxidase 5 (Nox5). We have shown that (i) expression of SREBP-1 protein is positively associated with the clinical Gleason grades in human prostate cancer; (ii) genetic overexpression or knockdown of SREBP-1 in prostate cancer cells resulted in corresponding increased or decreased AR, FASN and Nox5 expression, fatty acid and lipid droplet accumulation, and ROS generation; and (iii) SREBP-1 induces and promotes the growth, migration, invasion, and castration-resistant progression of prostate cancer cells in vitro and in vivo. Our data show a novel molecular mechanism by which SREBP-1 promotes prostate cancer growth and progression through alterations in the concerted intracellular metabolic and signaling networks involving AR, lipogenesis, and ROS in prostate cancer cells. Mol Cancer Res; 10(1): 133–42. ©2011 AACR.

Introduction

Cancer progression is the underlying cause of mortality and morbidity in prostate cancer patients. Lethal progression of prostate cancer from androgen-dependent status to androgen-refractory (or castration resistant) status involves multiple mechanisms in which androgens and androgen receptor (AR)-mediated signaling play key roles (1–3). Blockade of androgen action and the AR signaling axis is currently the main treatment for prostate cancer and its progression. Several reports have shown that androgen biosynthesis and AR signaling in prostate cancer cells are intimately affected by lipogenesis (4–6). Lipid raft membrane-related intracellular signaling pathways have been shown to induce AR activity (7–9). High-fat diets were shown to promote prostate cancer cell growth and aggressiveness (10, 11), and drugs that interfere with fatty acid and cholesterol metabolism and absorption were shown to reduce prostate cancer growth, angiogenesis, and progression (12–14). Identifying the underlying molecular mechanisms linking lipogenesis and AR signaling could facilitate further development of promising therapeutic approaches for human prostate cancer.

Sterol regulatory element-binding protein-1 (SREBP-1) is a critical transcription factor for lipogenesis (15–17). Three major isoforms of SREBP have been identified, such as SREBP-1a, SREBP-1c, and SREBP-2. SREBP proteins (125 kDa) are anchored to the endoplasmic reticulum (ER) membrane. Through proteolytic cleavage (18), the activated amino-terminus (68 kDa) of SREBP translocates into the nucleus to bind SRE (sterol regulatory element) cis-acting elements and trigger gene expression. SRE is found in the promoter regions of genes encoding enzymes for fatty acid, lipid, and cholesterol biosynthesis, including fatty acid synthase (FASN), HMG CoA synthase (19, 20) and farnesyl diphosphate synthase (21). SREBP-1 and one of its target genes, FASN, reported to be a metabolic oncogene (22), have been shown to be involved in prostate cancer malignant progression (6, 23). Our recent results showed that SREBP-1 regulated AR promoter activity and expression and cell viability in prostate cancer cells (5). The data concurred with the observation of upregulation of SREBP-1 expression in human prostate cancer tissues during castration-resistant progression (23). These experimental and clinical data suggest that SREBP-1 may play an important role in the regulation of prostate cancer cell growth and progression to lethal castration-resistant disease.

In this study, we revealed a new molecular mechanism by which SREBP-1 promotes prostate cancer growth, survival,
and lethal progression. We genetically manipulated SREBP-1 using either an expression vector or a sequence-specific short hairpin RNA (shRNA) approach to show, respectively, increased or decreased AR expression, cell proliferation, migration, and invasion in prostate cancer cells. Through the induction of FASN expression, SREBP-1 induced fatty acid and lipid droplet formation and accumulation in prostate cancer cells. Furthermore, SREBP-1 increased reactive oxygen species (ROS) levels via increased NADPH oxidase 5 (Nox5) expression in prostate cancer cells. ROS has been shown to induce signal transduction, survival, and progression of cancer cells (24, 25). In mouse xenograft models, SREBP-1 promoted human prostate tumor growth and supported the development of a castration-resistant progression phenotype through the induction of AR, FASN, and Nox5 expression. SREBP-1 and its downstream AR/lipogenesis/ROS signaling axis, therefore, provide novel therapeutic targets for prostate cancer treatment.

Materials and Methods

Prostate cancer cell lines, cell culture, reagents, and materials

Human prostate cancer cell lines LNCaP and C4-2B (26) were cultured in T-medium (Invitrogen) supplemented with 5% FBS. SREBP-1 expression vector and shRNA were obtained from OriGene Technologies, Inc. and Santa Cruz Biotechnology, respectively. Oil Red O and diphenylerythioderivedone (DPI) were purchased from Sigma-Aldrich. Human prostate carcinoma tissue microarray (TMA) was obtained from IMGENEX.

Western blot analysis

Cell lysates were prepared from prostate cancer cells as previously described (27). Nuclear extracts were prepared by a NucBuster Protein Extraction kit (Novagen). Western blot analysis was done by NOVEX system (Invitrogen). Primary antibodies against human SREBP-1, FASN, AR, Nox5 (Santa Cruz Biotechnology), catalase (Abcam), Akt, and phospho-Akt (p-Akt, Ser 473; Cell Signaling Technology) were utilized.

Fatty acid content quantification and Oil Red O staining

The long chain fatty acid contents were determined by a Fatty Acid Quantiﬁcation Kit (MBL International Corporation). Lipid droplet formation was assayed by an Oil Red O staining method (28). Oil Red O staining was examined and recorded by a phase contrast microscope. For quantitation, Oil Red O retained in cells were extracted by 100% isopropanol and measured optical absorbance at 500 nm normalized by total cell numbers.

Cell proliferation, in vitro migration, and invasion assays

For cell proliferation assay, prostate cancer cells (1 × 10^5 cells per well) were seeded on 6-well plates for 3-day incubation. Cells were harvested and counted by hemocytometer. The Boyden chamber method was utilized to examine in vitro cell migration and invasion of prostate cancer cells. Briefly, the undersides of the upper Boyden chambers were precoated with collagen I (2.5 μg/cm^2) for migration assay or growth factor–depleted Matrigel matrix (1:4 dilution, for invasion assay). Cells (5 × 10^4 cells) were seeded inside the precoated upper chambers. After incubation at 37°C for 12 to 24 hours (migration), or 24 to 48 hours (invasion), the numbers of migrated or invading cells were measured by the crystal violet staining method (29).

Intracellular ROS determination

Superoxide or hydrogen peroxide was assayed by dihydroethidium (DHE) or 5-(and-6)-chloromethyl-2',7'-dichlorodihydrofluorescein diacetate, acetyl ester (CM-H2DCFDA; Invitrogen). DHE was oxidized to red fluorescent ethidium by superoxide, and CM-H2DCFDA was oxidized to green fluorescent dichlorofluorescein (DCF) by hydrogen peroxide. Cells were treated with 10 μmol/L DHE or 5 μmol/L CM-H2DCFDA for 30 minutes, respectively, at 37°C. Subsequently, treated prostate cancer cells were washed with PBS and cultured in T-medium for 30 minutes. The mean fluorescence intensity was determined by flow cytometry FACS Calibur (BD Bioscience) as relative ROS (superoxide or hydrogen peroxide) compared with controls.

Mouse xenograft experiments

All the mouse experiments were approved and carried out in accordance with institutional guidelines. Four-week-old athymic nu/nu male mice (Charles River) were inoculated subcutaneously with control or Neo or overexpressing SREBP-1 LNCaP (H2) with 1 × 10^6 cells per mouse. The tumor burdens were monitored by tumor volume [V = 4/3πX(D/2)^2 × D/2, in which D is the minor tumor axis and D is the major tumor axis] weekly. To determine the effects of surgical castration on the growth of LNCaP tumors, nu/nu male mice were subcutaneously inoculated with 3 × 10^6 Neo or H2 cells per mouse. After 6 weeks of tumor growth, mice were either surgically castrated or sham operated. Blood specimens were harvested and serum PSA was determined by AIA-360 Immunoassay Analyzer (Tosoh Bioscience) weekly. At the end of the animal experiments, mice were euthanized and prostate tumor tissues were harvested, fixed in 10% formalin, dehydrated in ethanol, embedded in paraffin, and sectioned for histomorphologic and immuno-histochemical (IHC) analyses (5).

Statistical analysis

Statistical analyses were done as described previously (30). Student’s t test and 2-tailed distribution were applied in the analysis of statistical significance. Statistical analysis of human prostate carcinoma TMA results was done using Fisher’s exact test.

Results

Overexpression of SREBP-1 is associated with aggressive pathologic features in human prostate cancer

To study the clinical significance of a lipogenic transcription factor, SREBP-1, in prostate cancer progression, we...
determined the expression of SREBP-1 protein in human prostate carcinoma TMA. We assayed SREBP-1 expression using a clinical prostate cancer progression set representative of tumors at different stages of the disease from normal/benign to localized cancer with different Gleason grades (Fig. 1 and Table 1). SREBP-1 showed only 20% (3/15) positive expression in normal/benign prostate tissues, whereas expression of SREBP-1 protein increased with higher Gleason grade of disease [from 50% (grade 3) to 71% (grade 5); Table 1]. Interestingly, nuclear SREBP-1 was detected prevalently in grade 4 and 5 prostate cancers (Fig. 1C and D). Statistical analysis revealed that overall SREBP-1 expression levels were strongly correlated with Gleason grades ($P = 0.003$). These results suggested that expression of SREBP-1 protein is closely linked with the development of aggressive pathologic features in human prostate cancer. SREBP-1 may be a potential prognostic biomarker for human prostate cancer.

**SREBP-1 induces expression of AR and FASN and increases formation of fatty acid and lipid droplets in prostate cancer cells**

We previously showed that SREBP-1 regulated AR transcriptional expression by binding the 5′-flanking AR promoter region in prostate cancer cells (5). To further investigate the biological functions of SREBP-1 in prostate cancer, we established LNCaP cells stably overexpressing SREBP-1 under the control of a universal CMV promoter (5) because LNCaP cells showed lower intrinsic SREBP-1 [both precursor SREBP-1 (125 kDa) and mature nuclear SREBP-1 (68 kDa)] than aggressive C4-2B cells (Fig. 2A; ref. 26). After antibiotic screening, we selected the 2 highest stably overexpressing both precursor and nuclear SREBP-1 LNCaP clones, H1 and H2 (Fig. 2B). Consistent with previous observations, SREBP-1 induced expression of AR (5) and FASN (31) in H1 and H2 cells (Fig. 2B). FASN is one of the SREBP-1 target proteins and has been shown as a metabolic oncogene in prostate cancer (32). Because FASN is a key enzyme for *de novo* synthesis of fatty acid and lipids, we subsequently examined the levels of long chain fatty acid and lipid droplets in cells. As shown in Fig. 2C, fatty acid contents were significantly increased in H1 and H2 compared with untransfected LNCaP and control Neo (empty expression vector transfected) cells. Also, lipid droplets were highly accumulated in both H1 and H2 compared with

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**Table 1. Expression of SREBP-1 in human prostate carcinoma TMA**

<table>
<thead>
<tr>
<th>Clinicopathologic characteristics</th>
<th>SREBP-1 expression, n (%)</th>
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<tr>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>Gleason grade</td>
<td></td>
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<tr>
<td>Normal/benign (n = 15)</td>
<td>3 (20)</td>
</tr>
<tr>
<td>Grade 3 (n = 14)</td>
<td>7 (50)</td>
</tr>
<tr>
<td>Grade 4 (n = 27)</td>
<td>19 (70)</td>
</tr>
<tr>
<td>Grade 5 (n = 14)</td>
<td>10 (71)</td>
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NOTE: n indicates the numbers of samples.
control cells (Fig. 2D). The results suggested that SREBP-1 induced expression of AR and FASN and increased accumulation of fatty acid and lipid droplets in prostate cancer cells.

**SREBP-1 regulates cell proliferation, migration, and invasion in prostate cancer cells**

Next, we determined the biological role of SREBP-1 in regulating cell proliferation, migration, and invasion in prostate cancer cells. Overexpressing SREBP-1 H1 and H2 cells showed increased cell proliferation compared with untransfected LNCaP and control Neo cells during a 3-day incubation (Fig. 3A). One of the hallmarks of progressive and metastatic cells is their ability to invade surrounding tissues and migrate efficiently. The assays of *in vitro* migration and invasion were conducted by the Boyden chamber method. H1 and H2 showed significantly increased migratory and invasive capabilities compared with the control groups (Fig. 3B). The results supported the conclusion that SREBP-1 promoted cell proliferation, migration, and invasion, the hallmarks of progressive cancer cells. Conversely, knockdown of SREBP-1 using a sequence specific shRNA with lentiviral delivery to C4-2B cells as known with high intrinsic SREBP-1 expression (Fig. 2A), showed decreased SREBP-1, AR, and FASN expression (Fig. 3C). SREBP-1 shRNA also significantly inhibited cell proliferation (Fig. 3D), *in vitro* migration (23.8% ± 10.0% inhibition, *P* = 0.04) and invasion (31.8% ± 10.3% inhibition, *P* = 0.01) in aggressive C4-2B cells. These data, in aggregate, revealed that SREBP-1 plays an important role in regulation of cell proliferation, migration, and invasion in prostate cancer cells.

**SREBP-1 induces cell proliferation and progression through increased Nox5 expression and intracellular ROS levels in prostate cancer cells**

ROS and Nox (a ROS generator) have been reported to regulate cell proliferation, progression, metastasis, and radiation resistance of prostate cancer cells (33–35). Our cDNA microarray data revealed that Nox5 was upregulated in overexpressing SREBP-1 prostate cancer cells compared with control cells (unpublished data). To further determine whether SREBP-1 induces prostate cancer cell proliferation and progression through activation of Nox5 and ROS, we first examined expression of Nox5 in control and SREBP-1stably overexpressing H1 and H2 cells. Consistent with
SREBP-1 Promotes Prostate Cancer Growth and Progression

Figure 3. SREBP-1 promotes cell proliferation, migration and invasion in prostate cancer cells. A, H1 and H2 showed increased cell proliferation compared with LNCaP and control Neo during 3-day incubation. The relative cell proliferation (%) was assigned as 100% in LNCaP cells. **, \( P < 0.005 \), significant differences from Neo. Data represent the mean ± SD of 2 independent quadruplicate experiments. B, SREBP-1 significantly induced in vitro cell migration and invasion in H1 and H2 compared with control groups. *, \( P < 0.05 \); **, \( P < 0.005 \) significant differences from Neo. C, knocking down SREBP-1 by shRNA with lentiviral delivery in high intrinsic SREBP-1 C4-2B cells showed decreased SREBP-1, AR, and FASN expression determined by Western blot. D, downregulation of SREBP-1 by shRNA showed decreased cell proliferation of C4-2B. The relative cell proliferation (%) was assigned as 100% in untransfected C4-2B cells (–). **, \( P < 0.005 \) significant differences from nonspecific control shRNA-transfected C4-2B cells (Con).

cDNA microarray results, Nox5 protein increased in H1 and H2 compared with Neo and untransfected LNCaP cells (Fig. 4A). We also found that SREBP-1 increased p-Akt expression (Fig. 4A), which is involved in prostate cancer cell proliferation, survival, and progression (36). Next, we assessed ROS (superoxide and hydrogen peroxide) levels in cells. The levels of hydrogen peroxide were increased in H1 and H2 cells (Fig. 4B, the right panel); the levels of superoxide, however, were not significantly changed by SREBP-1 (Fig. 4B, the left panel). We also observed that 2 superoxide-degraded enzymes, extracellular superoxide dismutase (SOD3) and mitochondrial SOD (SOD2), were upregulated in H1 and H2 cells (data not shown). In addition, catalase, an enzyme responsible for hydrogen peroxide degradation, was decreased in H1 and H2 cells (Fig. 4A). To further investigate whether SREBP-1 induces prostate cancer cell proliferation through activation of Nox5 and ROS, a Nox inhibitor and ROS scavenger, DPI, was used to treat these prostate cancer cells. As shown in Fig. 4C, cell proliferation in both Neo and H2 cells was affected by DPI in a concentration-dependent inhibition. H2 cells with high Nox5 and ROS levels exhibited a phenotype with increased resistance to DPI-mediated suppression of cell growth (Fig. 4C). Interestingly, DPI inhibited AR expression in prostate cancer cells (Fig. 4D). Hydrogen peroxide has been shown to affect AR expression in LNCaP cells (37). DPI inhibited AR expression could be through ROS by decreasing hydrogen peroxide in prostate cancer cells. These data collectively indicated that SREBP-1 induced prostate cancer cell proliferation and progression through increased Nox5 expression and intracellular ROS levels.

SREBP-1 promotes prostate tumor growth and castration resistance in subcutaneous xenograft mouse models

Because SREBP-1 expression is increased in advanced forms of human prostate cancer (Fig. 1; ref. 23), we sought to determine whether SREBP-1 confers growth advantages in hormone-naive mice and resistance to tumor shrinkage in surgically castrated mice. We found that SREBP-1 overexpressing H2 cells inoculated subcutaneously developed a 100% incidence of tumor formation (8/8) in mice. Control Neo cells only exhibited 50% incidence of tumor formation (4/8) during an 8-week observation period. LNCaP classically showed less aggressive and less tumorigenic characteristics in mouse models (26). Furthermore, H2 tumors exhibited a 14-fold increased growth rate over that of the Neo tumors, as assessed by tumor volumes (Neo: 8.8 ± 5.0 mm\(^3\) and H2: 124.0 ± 40.0 mm\(^3\)) after 8 weeks of \textit{in vivo} growth (Fig. 5A). Consistent with previous Western blot results, IHC data showed that H2 highly expressed SREBP-1 (mostly in nuclei), FASN (cytoplasm), Nox5 (cell membranes), and AR (mostly in nuclei) in comparison with Neo tumors harvested from mouse subcutaneous spaces (Fig. 5B). Next, we sought to determine whether SREBP-1 would mediate castration resistance in prostate tumor xenografts...
Figure 4. SREBP-1 induces cell proliferation and progression through alterations of Nox5 and ROS in prostate cancer cells. A, SREBP-1 induced Nox5 and p-Akt, and inhibited catalase expression in H1 and H2 cells determined by Western blot. B, SREBP-1 increased the levels of hydrogen peroxide in H1 and H2. The levels of superoxide were not significantly changed by SREBP-1. The relative DCF (%) and DHE (%) were assigned as 100% in LNCaP cells. **, P < 0.005, significant differences from Neo. Data represent the mean ± SD of 2 independent triplicate experiments. C, DPI, a Nox inhibitor and ROS scavenger, inhibited cell proliferation of Neo and H2 in a dose-dependent pattern (0–5 μmol/L) during a 2-day treatment. H2 cells showed increased resistance to DPI-mediated suppression of cell proliferation. The relative cell proliferation (%) was assigned as 100% for each cell without DPI treatment. **, P < 0.005, significant differences from cells without DPI treatment. Data represent the mean ± SD of 2 independent quadruplicate experiments. D, DPI also inhibited AR expression in H2 cells.

Discussion

Aberration of cellular metabolisms has been reported to be strongly linked with cancer. Cancer cells reprogram energy production through aerobic glycolysis followed by lactic acid fermentation in the presence of oxygen (the Warburg effect; ref. 38). Upregulation of de novo lipogenesis (fatty acid, lipid, and cholesterol biosynthesis) in cancer cells is associated with increased needs for membranes and energy storage, and activation of intracellular signaling pathways during uncontrolled cell proliferation and division as well as cancer development and progression (6, 7, 9, 39, 46). Lipogenic activation has also been shown to increase the biosynthesis of androgens in prostate cancer cells (4–6). Androgens and AR signaling are involved in the regulation of prostate cancer development and lethal castration-resistant progression. However, the mechanism of dysregulation of lipogenesis in prostate cancer cells and its contribution to prostate cancer development and progression remain unclear. We previously showed that a key lipogenic transcription factor (15–17), SREBP-1, regulated AR promoter activity and transcriptional expression as well as cell viability in prostate cancer cells (5). In this study, we further revealed a novel molecular mechanism by which SREBP-1 promotes prostate cancer growth and progression through collaborative induction of AR expression, lipogenesis and oxidative stress. The results were confirmed by genetic approaches in which SREBP-1 overexpression or knockdown affected AR, FASN, and Nox5 expression, fatty acid and lipid droplet accumulation, and ROS levels in prostate cancer cells. These biochemical alterations were found to be closely associated with cell growth and behavioral changes, which can be monitored by prostate cancer cell proliferation, migration, and invasion. The data in aggregate suggest that SREBP-1 not only is a crucial mediator for lipogenesis as previously described but also induces AR a known survival factor and increases oxidative stress through induction of Nox5, an important ROS generator, in prostate cancer cells.

ROS and Nox have been closely linked to the initiation and progression of cancer (34, 35, 41, 42). ROS are produced in cells when oxygen is metabolized, including superoxide, hydrogen peroxide, and hydroxyl radicals. Excessive ROS accumulation in cells can cause cell injury by damaging vital molecules such as DNA, RNA, and proteins. Increased intracellular ROS, however, often leads to the enhanced growth, survival, and progression of cancer cells (43–45).
Hydrogen peroxide has been shown to induce cell transformation of nontumorigenic urothelial cells (44) and increase prostate tumorigenicity (42). Significant spheroid growth stimulation occurred when cancer cells were exposed to hydrogen peroxide (46). Our results confirmed that overexpressing SREBP-1 in prostate cancer cells increased ROS (hydrogen peroxide) levels, induced cell proliferation, migration, and invasion in vitro, and promoted subcutaneous tumor growth in mice. Furthermore, SREBP-1 also increased p-Akt protein expression (Fig. 4A). The data are consistent with published results in which activation of an Akt survival signaling pathway can occur through ROS regulation in several cancer models (25, 47, 48). Together, our results reveal a previously unrecognized role of SREBP-1 promoting prostate cancer growth and survival and increasing prostate cancer cell migration and invasion through augmented Nox5 expression, intracellular ROS, and activation of AR and Akt signaling, which ultimately could be responsible for the increased aggressiveness and malignancy of prostate cancer cells commonly associated with castration resistance.

We propose that one of the key mechanisms by which SREBP-1 promotes prostate cancer progression is through the induction of Nox5 expression, which enhances ROS levels in cells. We identified a SREBP-1 binding site in the 5′-flanking Nox5 promoter region (data not shown), which suggests that SREBP-1 controls Nox5 expression via promoter transcriptional regulation. Studies using DPI, a Nox activity inhibitor and ROS scavenger, provide additional evidence that SREBP-1 increases ROS levels and promotes prostate cancer cell proliferation through Nox5. In addition, inhibition of Nox5 expression and decrease of ROS production by Nox5-specific antisense oligonucleotides caused...

Figure 5. SREBP-1 promotes human prostate tumor growth and castration resistance in mouse subcutaneous xenograft models. A, tumor growth was assayed by tumor volume after subcutaneous inoculation of H2 and control Neo cells in mice. SREBP-1 significantly induced the growth of H2 compared with Neo tumors. **, P < 0.005, significant differences from Neo tumors. B, IHC of subcutaneous Neo and H2 tumor specimens. H2 tumors highly expressed SREBP-1 (mostly in nuclei), FASN (cytoplasm), Nox5 (cell membranes), and AR (mostly in nuclei) proteins compared with Neo tumors. Scale bar = 100 μm. C, mouse castration study. Tumor volumes of subcutaneous H2 tumors continuously increased after mouse castration (week 6) compared with Neo tumors (top). Serum PSA levels of both Neo and H2 tumor-bearing mice dropped the first week postcastration (week 7, bottom). However, PSA levels of H2 mice significantly rebounded 4 weeks after castration (week 10) compared with the Neo group. *, P < 0.05, significant differences from Neo.
a reduction in the growth of prostate cancer cells (35). This article further supports our findings. Furthermore, DPI has been shown to inhibit cell migration and invasion and decrease matrix metalloproteinase (MMP)-2 and MMP-9 expression and activity in PC3 prostate cancer cells (34). Interestingly, by decreasing Nox activity and ROS levels, DPI greatly inhibited AR expression in LNCaP cells (Fig. 4D). It could be due to DPI decreasing intracellular hydrogen peroxide levels (42) because hydrogen peroxide has been shown to affect AR expression in LNCaP cells (37). These data are consistent with the suggestion that SREBP-1 increases ROS production through transcriptional regulation of Nox5 expression in prostate cancer cells. Targeting Nox, ROS, and AR by DPI may be a promising therapeutic approach for the treatment of lethal progression of human prostate cancer.

Very limited information is currently available on the SREBP-1 expression profile of clinical prostate cancer. One early study showed that SREBP-1 was elevated in human primary prostate tumors compared with benign prostatic hypertrophy (23). In addition, deregulated SREBP-1 expression may be relevant to prostate cancer castration-resistant progression (23). Our results in a human prostate carcinoma TMA further showed that overexpression of SREBP-1 is significantly associated with aggressive pathologic features in human prostate cancer (Fig. 1 and Table 1). Importantly, SREBP-1 was highly expressed in the nuclei of prostate tumor cells with higher Gleason grades (Fig. 1). The precursor of SREBP-1 protein is an ER membrane-bound form. Through a proteolytic process (18), the mature amino-terminal polypeptide is translocated to the nuclei to activate the expression of lipogenesis related and other genes containing SREBP-1 binding sites in their promoters, such as FASN (31), AR (5), and Nox5 (the present study). Furthermore, IHC results of mouse bearing subcutaneous human prostate tumor xenografts showed that SREBP-1 was highly expressed in the nuclei of H2 (i.e., LNCaP–SREBP-1) tumor specimens collected from both intact (Fig. 5B) and castrated (data not shown) mice, which exhibited higher tumor incidences, burdens, and serum PSA levels, when compared with control Neo tumor specimens. The clinical and animal data collectively indicate that SREBP-1 expression and nuclear translocation play a critical role in the regulation of prostate cancer development and progression to castration resistance. Further investigation of the regulatory mechanism of SREBP-1 nuclear translocation in prostate cancer cells might be of importance.

The present studies show for the first time that (i) analysis of a human prostate carcinoma TMA with varying grades of diseases revealed that SREBP-1 expression positively correlates with prostate cancer progression. Nuclear translocation of SREBP-1 may also be closely associated with the degree of prostate cancer malignancy. (ii) Genetic alterations of SREBP-1 expression led to coordinated regulation of FASN, AR, and Nox5 expression in prostate cancer cells. (iii) Through the dual induction of FASN and Nox5 expression, SREBP-1 increased fat fatty acid and lipid droplets) and ROS (hydrogen peroxide) accumulation in prostate cancer cells. (iv) SREBP-1 induced prostate cancer cell proliferation, migration, and invasion in vitro and promoted prostate tumor growth and castration-resistant progression in vivo. Collectively, the molecular mechanism by which SREBP-1 promotes prostate tumor growth and resistance to castration and androgen responsiveness is through a concerted activation of AR, lipogenesis, and ROS signaling networks (Fig. 6).

Data presented in this article are collected from the study of established AR-positive human prostate cancer cell lines, and further investigation of this concept in AR-negative prostate cancer cells might be of importance. Also, additional studies may be warranted to define whether the concerted signaling would function in tumor microenvironment, for example, tumor–stroma interaction. Taken together, we identified that SREBP-1, a transcription factor known to regulate fat biosynthesis and homeostasis, promotes and maintains prostate cancer growth and progression by activating and reprogramming the AR/lipogenesis/ROS signaling axis. SREBP-1 and its ancillary regulatory signaling pathways may, therefore, be novel promising therapeutic targets for the prevention and treatment of lethal progression in human prostate cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References


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