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Inhibition of Fatty Acid Synthase Activity in Prostate Cancer Cells by Dutasteride

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PURPOSE. With malignant progression to androgen independence, prostate cancer cells develop resistance to apoptosis and exhibit a variety of gene expression changes, including increased fatty acid synthase (FASN) expression. Increased FASN expression has been shown to correlate with poor prognosis, and correspondingly, the FASN gene has been proposed as a therapeutic target. Because FASN is an androgen regulated gene in the prostate, we have examined the effects of dutasteride on FASN in prostate cancer cells in vitro. Dutasteride is a novel dual inhibitor of the 5 alpha-reductase enzymes and is currently in use both for treatment of benign prostate hyperplasia (BPH) and in the reduction by dutasteride of prostate cancer events (REDUCE) prostate cancer prevention trial.

METHODS. Microarray analysis was used to identify genes affected by treatment with dutasteride, followed by real time PCR confirmation. FASN expression at the protein level was examined using Western blotting and immunocytochemistry. Enzymatic activity of FASN was assayed by ^{14}C-labeled malonyl-CoA incorporation. Viability after dutasteride treatment was assayed by MTS (Promega) and apoptosis via caspase 3/7 by DEVD cleavage assay.

RESULTS. We have demonstrated that the 5 alpha-reductase inhibitor dutasteride, at clinically relevant levels, inhibits FASN mRNA, protein expression and enzymatic activity in prostate cancer cells.

CONCLUSIONS. This is the first study to examine the effects of clinically relevant levels of dutasteride on prostate cancer cells at the molecular level and specifically, demonstrating the inhibition of FASN in these cells. *Prostate 67: 1111–1120, 2007.* © 2007 Wiley-Liss, Inc.

KEY WORDS: **prostate cancer; fatty acid synthase; chemoprevention**

INTRODUCTION

While androgen ablation therapy remains the main treatment for advanced prostate cancer, the overall survival rate is only 2–3 years from initiation [1]. The success of androgen ablation therapy is often terminated by the emergence of androgen-depletion-independent (ADI) disease. With malignant progression to androgen independence, prostate cancer cells develop resistance to apoptosis and exhibit a variety of gene expression changes, including increased FASN expression [2–6].

Fatty acid synthase is an androgen-regulated enzyme involved in lipogenesis, specifically in the de novo synthesis of fatty acids. In prostate cancer, increased FASN expression has been shown to correlate with poor prognosis [2] and correspondingly, the FASN gene has been proposed as a therapeutic target.

In our previous publication using 10 μM dutasteride [9], Affymetrix U95Av arrays were used which contained the FASN gene listed as NULL, Hs.351958. FASN expression was also down-regulated in that study with a P-value of 0.018.

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The 5 alpha-reductase inhibitor dutasteride has been in use clinically to treat BPH since 2002 and the REDUCE trial [7] now underway is testing the efficacy of dutasteride in the prevention of prostate cancer. Dutasteride has been shown to cause cell death in prostate cancer cells in vitro at relatively high levels [8,9]. In this report, we have treated prostate cancer cells with clinically relevant levels of dutasteride and monitored genes and pathways affected by microarray analysis.

FASN was initially identified as one of the most significant genes affected by dutasteride treatment in these cells. This observation was confirmed by real time PCR, followed by examination of FASN protein expression and enzyme activity in LNCaP cells. We have demonstrated that the 5 alpha-reductase inhibitor dutasteride, at clinically relevant levels, inhibits FASN mRNA, protein expression and enzyme activity in prostate cancer cells in vitro.

MATERIALS AND METHODS

Cell Culture

Cell lines LNCaP, PC3 and VCaP were purchased from ATCC (Manassas, VA) and maintained in the following media: LNCaP and PC3 in RPMI 1640 (Invitrogen) +9% FBS (Biofluids), VCaP in DMEM high glucose (Invitrogen) +9% FBS. LNCaP-C4-2 was purchased from UroCor (Oklahoma City, OK) and maintained in RPMI 1640 +9% FBS. LAPC4 was obtained from Charles L. Sawyers and maintained in DMEM high glucose +9% FBS. The level of testosterone in FBS (Biofluids Lot 115090) was determined by ELISA to be <30 ng/dL. Dutasteride was provided by GlaxoSmithKline (Research Triangle Park, NC) and dissolved in DMSO at time of use.

Microarray

Affymetrix U133 2.0 chip arrays (Affymetrix, Santa Clara, CA) were probed with duplicate samples of total RNA according to manufacturer's instructions. Briefly, RNA from either vehicle control or dutasteride treated cells was isolated using Trizol (Invitrogen), further cleaned with RNEasy (Qiagen) and checked with Agilent (Affymetrix) before probe labeling. The microarray data was normalized using fastlo normalization [10,11]. Genes were identified as being differentially expressed between the cell lines with a linear mixed model using only PM values, similar to that proposed by Chu et al. [12]. The genes were ranked according to *P*-value (smallest to largest). Data was then further sorted using Ingenuity Pathways Analysis program (http://www.ingenuity.com). Real-time PCR was used to confirm array data for selected genes.

Real-Time PCR

Two-step real-time PCR was performed using cDNA prepared from RNA described above using Transcriptor First Strand cDNA Synthesis Kit (Roche) and SYBR Green PCR Master Mix (Applied Biosystems) on an ABI PRISM 7700 SDS following manufacturer's instructions. Primers for SYBR green amplification were designed using the Primer3 software (http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi) and both forward and reverse primers were used at a final concentration of 900 nM. PCR products (120–150 bp) were run on 1.2% agarose gels to check for nonspecific amplification.

Western Blotting

Protein extracts were prepared as whole cell lysates and run on 3–8% Tris acetate gels (Invitrogen). FASN rabbit polyclonal antibody (Novus) was used at 1:1,000 and SREBP1 mouse monoclonal (Abcam) at 1:100. Secondary HRP-linked antibodies were from Amersham/GE Healthcare. ERK2 (Santa Cruz Biotechnology) was used as a loading control for blots.

FASN Enzyme Activity

Assay for FASN activity was performed as described by Swinnen, et al. [13], using 40 μg of protein extract from vehicle or dutasteride-treated cells.

Cell Viability

Cell viability after dutasteride treatment was determined using CellTiter 96 Aqueous One Solution Cell Proliferation Assay (Promega) following manufacturer's instructions.

Apoptosis Assays

Activity of Caspase-3/7 was determined using Apo-ONE Homogeneous Caspase-3/7 Assay and CaspaseGLO assay kits (both Promega) following manufacturer's instructions.

Gene specific Arrays

Oligo GEArray Human Apoptosis Microarrays (OHS-012) from SuperArray Bioscience were probed with Biotin-16-UTP (ENZO) labeled cRNA. Chemiluminescent detection was by X-ray film and images were scanned and analyzed, all following manufacturer's instructions.

RESULTS

Gene Expression Changes in LNCaP Prostate Cancer Cells With Dutasteride Treatment

Dutasteride causes cell death in LNCaP cells either in the presence or absence of androgens, indicating that

dutasteride is affecting more than the androgen pathway via reduction in dihydrotestosterone (DHT) levels by inhibition of 5-alpha-reductase [8,9]. For this reason we sought to delineate other genes and pathways that might be involved by treating LNCaP cells with dutasteride and performing microarray analysis. We previously have described changes in viability and gene expression levels in LNCaP prostate cancer cells after treatment with 10 μM dutasteride [9]. Analysis revealed genes involved in multiple metabolic pathways, in addition to the androgen-signaling pathway. In the current study we have chosen to examine changes occurring in LNCaP cells after treatment with clinically relevant levels of dutasteride, as shown in Table IA, and extended our analysis of specific genes in these cells.

Preliminary time course and dose response experiments were done using real-time PCR to monitor genes we had identified as being affected by dutasteride at higher concentrations [9]. We found that as little as 670 pM dutasteride treatment for 48 hr was starting to up-regulate genes involved in apoptosis and 67 nM treatment for longer periods of time was consistently affecting viability (Fig. 1A, B and data not shown). Based on these results, we chose to examine effects of dutasteride treatment at 67 nM—a level, that is, clinically relevant (Table IA) and at the same time causing detectable changes in LNCaP cell viability and gene expression profile.

LNCaP cells were treated with vehicle alone (DMSO) or 67 nM dutasteride for a period of 10 days at which point total RNA was harvested in duplicate and used to probe DNA microarrays. Probing and data analysis of Affymetrix U133 2.0 chip arrays were performed as described previously [9]. The normalized array data was further sorted using Ingenuity Pathways Analysis software. It was found that while the rank of individual genes involved in response to dutasteride varies depending on the dose used (10 μM vs. 67 nM), the pathways or overall patterns of gene expression involved do not differ substantially, lending validity to our earlier findings [9]. As illustrated in Table IB, genes affected by dutasteride fall into categories regulating a variety of cellular processes ranging from cell cycle and cellular growth and proliferation to lipid metabolism.

Table II lists the top genes (P-values ≤ 0.01) with altered expression when LNCaP cells were treated with the lower dose of 67 nM dutasteride for 10 days. Among the genes most affected by this clinically relevant level of dutasteride at the RNA level is FASN, the gene chosen for the rest of this study.

Dutasteride Affects Genes in the SREBP Pathway

FASN is an androgen regulated gene in prostate cells and in normal cells is up-regulated in response to androgens through activation of the sterol regulatory element binding protein (SREBP) pathway [14–18]. The SREBP family (−1a, −1c, and −2) is a group of helix-loop-helix leucine zipper transcription factors that are synthesized in their inactive precursor (125 kDa) form and are attached to the endoplasmic reticulum (ER). SREBPs interact with SREBP-cleavage-activating protein (SCAP), forming a complex that is then stabilized on the ER by INSIG retention proteins. When activated by androgens, SCAP levels increase dramatically. The resulting shift in equilibrium between SCAP and INSIG proteins allows SREBP precursors to translocate to the Golgi apparatus where they are cleaved and activated. The active form of SREBP1 (60–70 kDa) directs synthesis of several lipogenic genes, one of which is FASN. It has been demonstrated that androgen stimulation of FASN is abolished when the SREBP binding sites in the promoter are deleted [15–17].

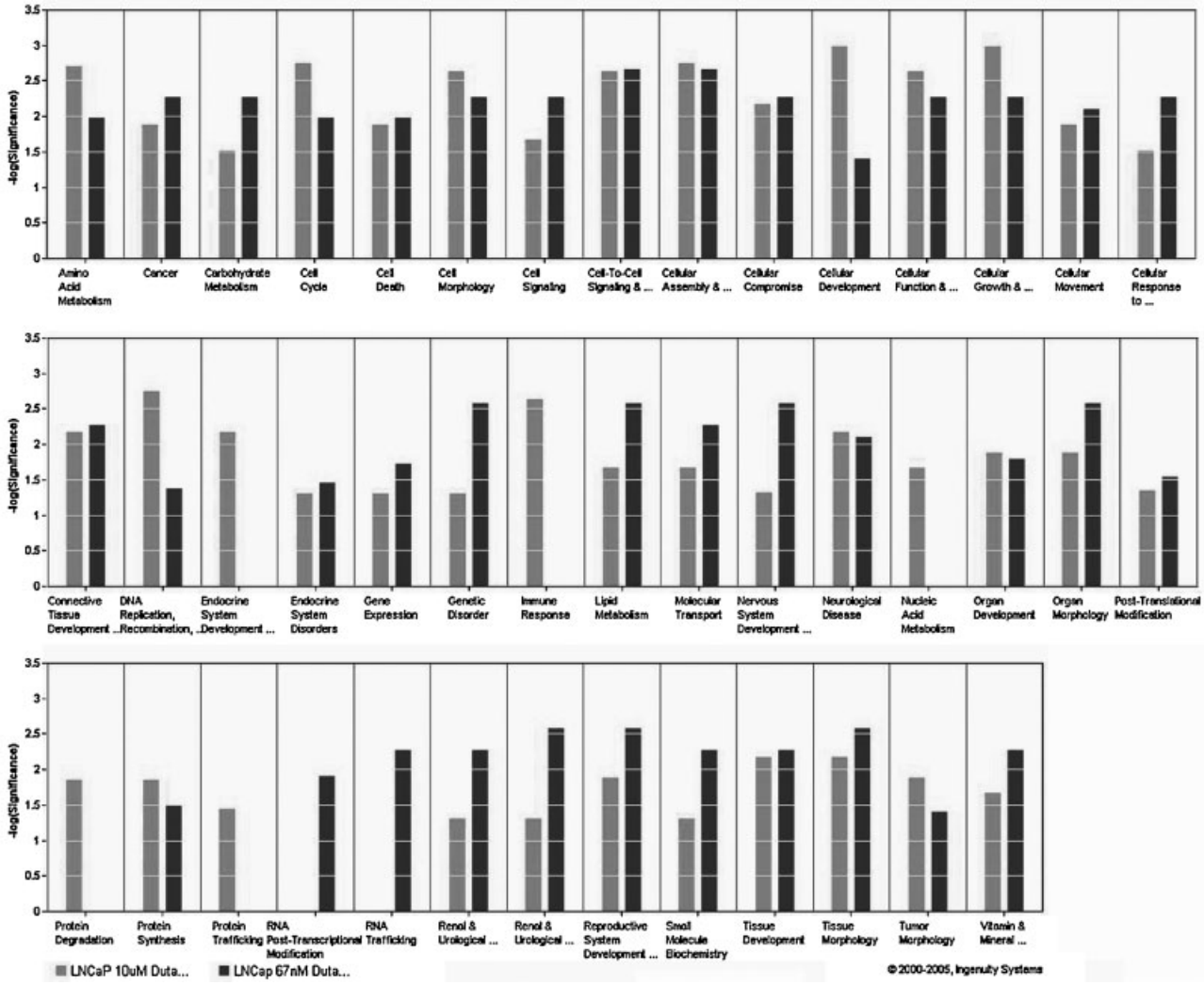
In addition to FASN, microarray analysis revealed a significant change in expression levels of six of the known components of the SREPB pathway (Fig. 2A). The relative expression levels of these were further examined using real-time PCR. LNCaP cells were treated for 96 hr with vehicle alone or increasing doses

TABLE IA. Dutasteride Concentrations for In Vivo and In Vitro Use

Treatment		Dutasteride level achieved		Use
0.05 mg	In vivo	1.1 ± 6 ng/ml	2.0 nM	Clinical trials [21]
0.5 mg	In vivo	38 ± 13 ng/ml	*70* nM	Clinical trials [21]
				REDUCE trial [7]
2.5 mg	In vivo	272 ± 94 ng/ml	*500* nM	Clinical trials [21]
5.0 mg	In vivo	535 ± 183 ng/ml	*1.0* μM	Clinical trials [21]
	In vitro	535 ng/ml		Schmidt et al. [9]
5.3 μg/ml	In vitro	5.3 μg/ml	10 μM	Schmidt et al. [9]

Concentrations of dutasteride used for in vivo and in vitro studies are listed; range of concentrations used in the present study is noted in italics.

TABLE IB. Ingenuity Pathways Analysis: Comparison of 10 μM and 67 nM Arrays



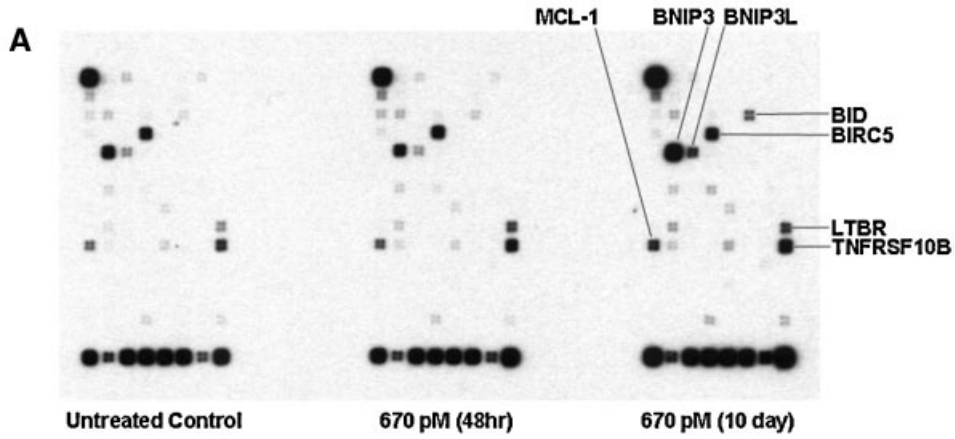
Affymetrix array data from two separate analyses using different levels of dutasteride to treat LNCaP cells were compared using Ingenuity Pathways analysis software. Data was first normalized and sorted as described in Materials and Methods and Table II, then a comparison was run with the data from cells treated with 10 μM dutasteride for 48 hr (see Schmidt et al. [9] for detailed array data) and 67 nM dutasteride for 10 days.

of dutasteride. As illustrated in Figure 2B, expression of SREBP-1a, SCAP, INSIG1, and ADD1 (SREBP-1c) decreased with treatment, while RNA levels of INSIG 2 increased, indicating that FASN transcription is down-regulated by dutasteride through this pathway in LNCaP cells.

Next, the expression of both FASN and its androgen regulator SREBP1 were examined at the protein level following dutasteride treatment. As shown in Figure 3A, FASN protein levels decreased steadily in a time and in a dose dependent manner starting as early as 48 hr. SREBP1 levels were also diminished, where bands indicative of both the precursor (125 kDa) and active (60–70 kDa) forms decreased by 96 hr, lending further evidence for involvement of this pathway in dutasteride treated cells (Fig. 3B).

Dutasteride Affects FASN Enzyme Activity in PCa Cells

To determine if the lower FASN protein levels were biologically relevant, we performed ^{14}C-malonyl-CoA incorporation assays as described by Swinnen et al. [13] to assess FASN enzyme activity. Cell extracts were made from LNCaP cells treated with increasing doses of dutasteride for 96 hr and FASN activity monitored by conversion of fatty acid precursors, acetyl-CoA, NADPH and ^{14}C-labeled Malonyl-CoA to fatty acid.



SYMBOL	GENE NAME	DESCRIPTION
BNIP3	Nip3	BCL2/adenovirus E1B 19kDa interacting protein 3
BNIP3L	BNIP3L	BCL2/adenovirus E1B 19kDa interacting protein 3-like
BIRC5	Survivin/API4	baculoviral IAP (inhibitor of apoptosis) repeat-containing 5
TNFRSF10B	Killer/DR5/TRAILR2	Tumor necrosis factor receptor superfamily, member 10b
MCL1	MCL-1	Myeloid cell leukemia sequence 1 (BCL2-related)
BID	Bid	BH3 interacting domain death agonist
LTBR	LTbR	TNFR superfamily, member 3

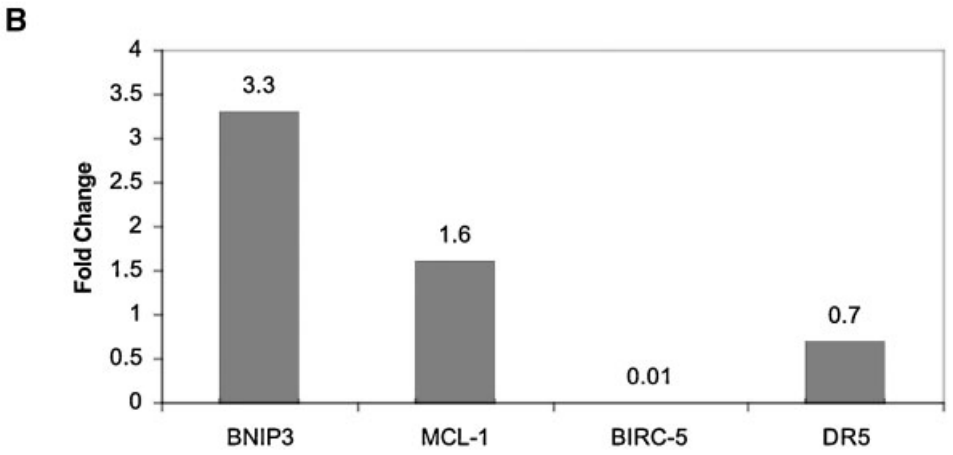


Fig. 1. Apoptosis-specific arrays probed with RNA from LNCaP cells treated with dutasteride. **A**: Previous work [9] indicated that genes in the FasL/TNFα pathway are being affected when LNCaP cells are undergoing apoptosis after dutasteride treatment. Here, we probed Human Apoptosis-specific arrays with RNA from LNCaP cells treated with doses of dutasteride ranging from 67 pM to 67 nM in order to delineate events taking place in LNCaP cells after treatment with these lower levels of dutasteride. Results presented are of arrays probed with 670 pM dutasteride and resemble our earlier findings, with seven genes in this pathway appearing to be up-regulated at the mRNA level. **B**: Real-time PCR was used to confirm array results. Relative quantitation was used to determine fold change in expression levels by the comparative C_T method using the formula $2^{-\triangle\triangle C}{}_T$ where C_T is the threshold cycle of amplification.

As illustrated in Figure 3C, enzyme activity dropped dramatically with as little as 67 nM dutasteride by 96 hr of treatment, indicating that the decreased levels of FASN at both the mRNA and protein levels are relevant to LNCaP cells' metabolism.

To determine if this finding was specific only to LNCaP cells, we extended our study to additional prostate cancer cell lines. We examined both FASN protein expression and activity in androgen responsive LAPC4 and androgen independent lines LNCaP–C4-2, VCaP and PC3. As demonstrated in Figure 4A, the basal level of FASN protein expression varies widely between cell lines, with the lowest expression seen in VCaP cells, but dutasteride treatment decreased the

TABLE II. Genes Significantly Affected by Dutasteride Treatment

Rank	AffyID	CytoBand	GeneSymbol	UnigeneID
1	41386_i_at	17p13.1	KIAA0346	Hs.103915
2	202102_s_at	19p13.1	BRD4	Hs.278675
3	212218_s_at	17q25	FASN	Hs.83190
4	215696_s_at	9q34.3	KIAA0310	Hs.396443
5	78047_s_at	20q11.2	MMP24	Hs.212581
6	37462_i_at	19p13.3-p13	SF3A2	Hs.115232
7	211947_s_at	1q23.3	XTP2	Hs.446197
8	214494_s_at	16q24.3///1	SPG7	Hs.311765
9	64899_at	19p13.2	LPPR2	Hs.6846
10	206929_s_at	19p13.3	NFIC	Hs.436639
11	210150_s_at	20q13.2-q13	LAMA5	Hs.11669
12	201516_at	1p36-p22	SRM	Hs.76244
13	208156_x_at	—	—	—
14	208979_at	20q11	NCOA6	Hs.435788
15	203704_s_at	6p25	RREB1	Hs.171942
16	210672_s_at	16p13.3	C16orf35	Hs.19699
17	201828_x_at	Xq26	CXX1	Hs.250708
18	202219_at	Xq28	SLC6A8	Hs.388375
19	212770_at	15q22	TLE3	Hs.287362
20	220584_at	19p13.3	FLJ22184	Hs.288540
21	210995_s_at	5q12.3	ARFD1	Hs.792
22	201356_at	—	—	—
23	212152_x_at	1p35.3	SMARCF1	Hs.170333
24	207435_s_at	16p13.3	SRRM2	Hs.433343
25	210994_x_at	5q12.3	ARFD1	Hs.792
26	217755_at	17q25.2	HN1	Hs.109706
27	213360_s_at	7q11.23	—	Hs.450237
28	209869_at	10q24-q26	ADRA2A	Hs.249159
29	212643_at	14q22.2	C14orf32	Hs.406401
30	36994_at	16p13.3	ATP6V0C	Hs.389107
31	218552_at	1p32.3	FLJ10948	Hs.170915
32	201225_s_at	1p36.11	SRRM1	Hs.18192
33	213244_at	19p13.3	SCAMP-4	Hs.144980
34	203062_s_at	6pter-p21.31	MDC1	Hs.433653
35	202804_at	16p13.1	ABCC1	Hs.391464
36	202805_s_at	16p13.1	ABCC1	Hs.391464
37	200675_at	11p15.5	CD81	Hs.54457
38	211382_s_at	10q26	TACC2	Hs.23196
39	212957_s_at	Xq11.2	LOC92249	Hs.31532
40	202801_at	19p13.1	PRKACA	Hs.194350
41	202260_s_at	9q34.1	STXBP1	Hs.325862
42	212124_at	10q23.1	RAI17	Hs.438767
43	214246_x_at	17p13-p12	CHRNE	Hs.313227
44	212193_s_at	5q33.2	LARP	Hs.6214
45	212787_at	14q24.3	C14orf170	Hs.303775
46	201872_s_at	4q31	ABCE1	Hs.12013
47	208033_s_at	16q22.3-q23	ATBF1	Hs.108806
48	209333_at	12q24.3	ULK1	Hs.47061
49	212599_at	7q11.23	AUTS2	Hs.296720
50	207760_s_at	12q24	NCOR2	Hs.287994

Affymetrix U133 2.0 chip arrays were probed with RNA isolated from LNCaP cells treated either with vehicle alone or 67 nM dutasteride for 10 days. The microarray data was normalized using Fastlo (Schmidt et al. [9]). Genes were identified as being differentially expressed between the cell lines with a linear mixed model (Chu et al. [12]). This table lists the top 50 genes as ranked by *P*-value.

A

Gene Symbol	Unigene ID	Ratio of Means	p-value
FASN	Hs.83190	0.6208	0.003
SREBF1	Hs.426528	0.6363	0.034
ADD1	Hs.183706	0.8089	0.033
SCAP	Hs.437096	0.7647	0.029
INSIG1	Hs.416385	1.1329*	0.383*
INSIG2	Hs.7089	1.3807	0.014
SREBF2	Hs.108689	0.8518	0.029

B

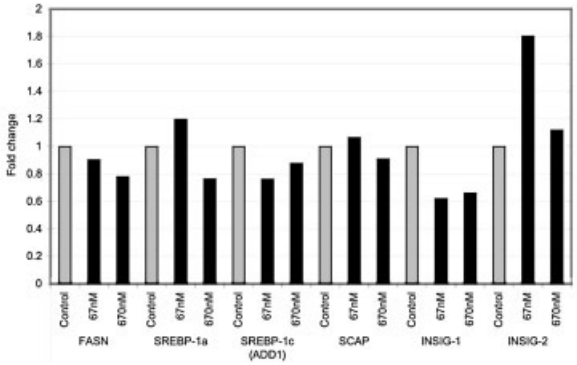


Fig. 2. Real-time PCR examining genes in the SREBP pathway. **A**: Affymetrix array data for genes in SREBP pathway in LNCaP cells following treatment with 67 nM dutasteride. *Array values for INSIGI were not significant. **B**: Real-time PCR was used to confirm array data and further examine relative expression of six of the genes. LNCaP cells were treated for 96 hr with vehicle alone (control) or increasing doses of dutasteride, with fold change being calculated using the comparative C_T method as detailed in legend for Figure I. This experiment was performed two times; data represents one set of amplifications for each gene.

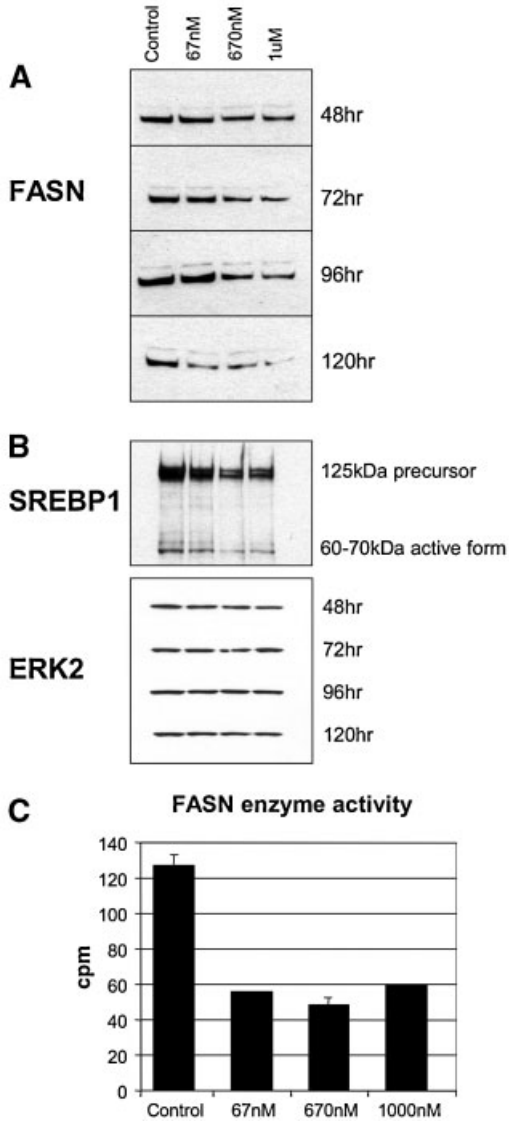


Fig. 3. FASN expression and enzyme activity in LNCaP cells treated with dutasteride. **A**: Time course and dose response of FASN protein levels in LNCaP cells with dutasteride treatment as determined by western blotting. FASN protein is decreased as early as 48 hr with 670 nM dutasteride; by 120 hr, even 67 nM is having a marked affect on protein levels. **B**: Levels of SREBPI were examined at 96 hr by Western blot and expression correlates with that of FASN at this time-point. ERK2 was used as a loading control for all blots. **C**: Assay for FASN enzyme activity was performed as described by Swinnen et al. [13], using 40 μg of protein extract from vehicle or treated LNCaP cells. Plotted values represent the average of triplicate samples assayed for each concentration of dutasteride. Standard deviations for 67 nM and 1,000 nM values are negligible, and therefore, not visible on graph.

level significantly in cells expressing androgen receptor (AR), regardless of androgen sensitivity. Enzyme activity mirrored the protein expression profiles, as shown in Figure 4B. This is consistent with our data demonstrating decreased viability and proliferation with 10 μM dutasteride treatment in LAPC4, while no effect was seen in AR-negative PC3 cells (unpublished data). The fact that dutasteride down-regulates FASN activity in androgen-independent AR positive cell lines is significant with regards to its potential use in prostate cancer treatment, where androgen antagonists are ineffective.

Dutasteride Induces Apoptosis Through the Caspase 3/7 Pathway

We and others have demonstrated previously that treatment of LNCaP cells at high concentrations of dutasteride (≥1 μM) causes apoptosis [8,9,19] by induction of genes in the FasL/TNFa pathway, ultimately leading to an increase in caspase 3/7 activity. In order to examine the pharmacological

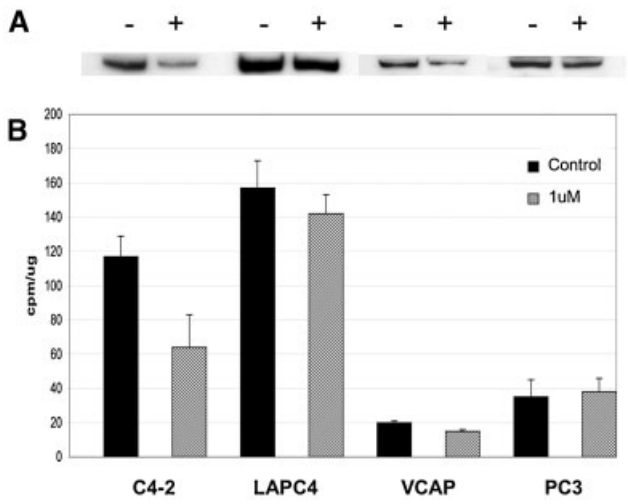


Fig. 4. FASN expression and enzyme activity in androgen dependent and independent PCa cell lines treated with dutasteride. **A**: Levels of FASN protein as examined by Western blot. Cells were treated for 120 hr with vehicle control (−) or 1 μM dutasteride (+). ERK2 was used as loading control (data not shown). **B**: Assay for FASN enzyme activity was performed as described by Swinnen et al. [13], using 40 μg of protein extract from cells treated for 120 hr with 1 μM dutasteride.

relevance of these observations, lower doses of dutasteride were tested on both cell viability and apoptosis. To assess the effects of dutasteride on cell viability, an MTS assay (Promega) was used. As seen in Figure 5A, cell growth was significantly affected by treatment with 670 nM by 96 hr, although not as dramatically as that which was observed with 5–10 μM dutasteride [8,9].

Apoptosis was assessed by DEVD substrate cleavage in two different assay systems. As shown in Figure 5B,C, a slight, yet significant, increase in Caspase3/7 activity was seen with the Apo-1 assay after 670 nM dutasteride and with 1 μM using the CaspaseGLO assay at 48 hr. As demonstrated earlier (Fig. 1), analysis of apoptotic pathways was performed by probing apoptosis-specific DNA microarrays with cRNA from LNCaP cells treated with 670 pM and 67 nM dutasteride for times ranging from 24 hr to 10 days. Figure 1A illustrates that genes in the TNF-receptor and Bcl-2 families were up-regulated in response to dutasteride treatment, even at the lowest levels. Expression levels of several of these genes were confirmed using real-time PCR (Fig. 1B).

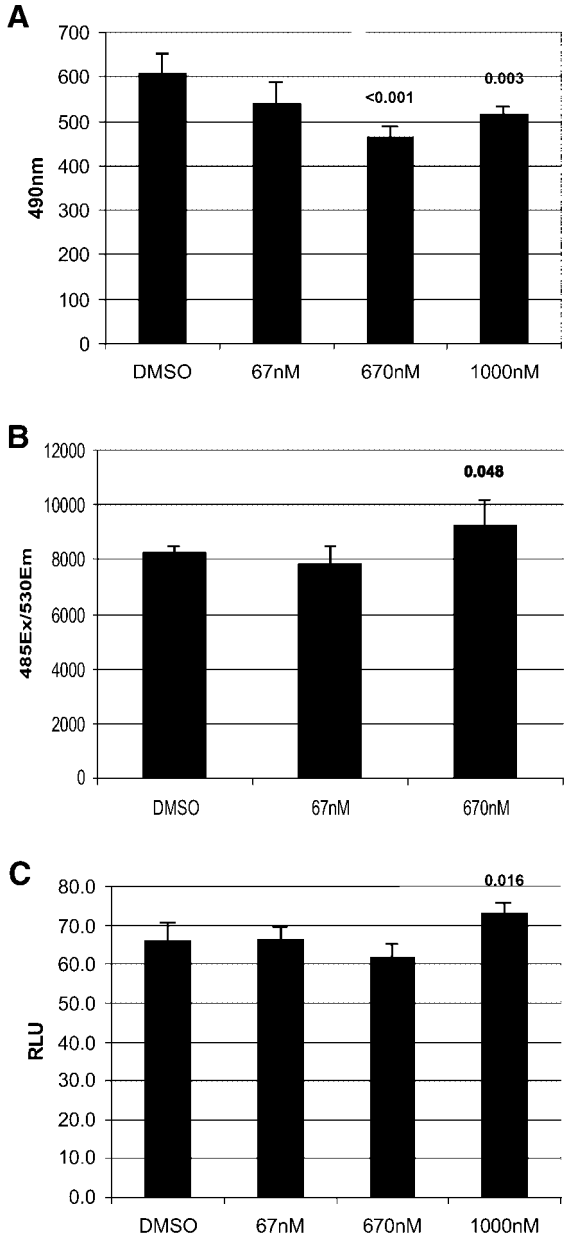


Fig. 5. Viability and apoptosis in LNCaP cells treated with dutasteride. **A**: LNCaP cells were treated with increasing doses of dutasteride and viability measured by MTS assay. Plotted values are the average and standard deviation of five samples per point. Statistical analysis was performed using a two-sample *t*-test with significant *P*-values plotted for each point. **B**,**C**: Apoptosis in LNCaP cells with dutasteride treatment was assayed by DEVD fluorescent substrate cleavage with both Apo-ONE and CaspaseGLO assays (Promega). Plotted values are the average and standard deviation of five samples per point. Statistical analysis was performed using a two-sample *t*-test with significant *P*-values plotted for each point.

DISCUSSION

Dutasteride is very effective at blocking the conversion of testosterone to the more active DHT in the prostate [20,21] and hence is currently in use for the treatment of BPH. Moreover, it is being tested in a Phase III clinical trial (REDUCE) for its ability to prevent PCa. There is mounting evidence that dutasteride acts on pathways in addition to the androgen receptor pathway. In an effort to better understand how this drug is working in prostate cells,

we have performed microarray analysis and extended our findings on its regulation of specific genes. We identified the FASN gene as one that is acutely sensitive to dutasteride. We demonstrate here for the first time that dutasteride, a drug already being proposed as a chemopreventive agent for prostate cancer, dramatically reduces FASN expression and most importantly FASN enzyme activity in prostate cancer cells in vitro.

Although initially effective, treatment with anti-androgens ultimately results in androgen-independent disease for which only palliative treatment is available. With malignant progression to androgen independence, prostate cancer cells develop resistance to apoptosis and exhibit a variety of gene expression changes, including increased FASN expression. FASN has not only been shown to be up-regulated with progression, but additionally, higher expression correlates with a poor outcome. FASN has been proposed to be a ''metabolic oncogene'' in cancer cells due to its ability to be epigenetically regulated by extra-cellular acidosis [22,23]. In light of this finding, various groups are examining the effects of various FASN inhibitors, from anti-FASN drugs such as C75 to siRNA directed to FASN [24–27].

Activation of the SREBP pathway by androgens, resulting in an increase in FASN activity has been demonstrated previously in LNCaP cells [17]. Our results demonstrate that removal of androgens, via dutasteride inhibition of the conversion of testosterone to DHT, also engages genes in this pathway. Ettinger et al. [28] demonstrated that in androgen independent (AI) LNCaP xenograft and patient tumors, the expression of SREBPs as well as their downstream effectors, SCAP and INSIG-1 and -2 become dysregulated during progression to androgen independence, with SREBP1 and SCAP being maintained at a high level even after castration or androgen ablation. The fact that dutasteride decreases the levels of these dysregulated, highly expressed genes in prostate cancer cells indicates it may prove to be therapeutically beneficial in the prevention or treatment of PCa.

In conjunction with down-regulating FASN activity, dutasteride induces apoptosis in LNCaP cells by activating genes in the TNFa pathway, such as BNIP3 and TNFRSF10B leading to caspase 3/7 activation. A connection between FASN inhibition and apoptosis has been demonstrated by a number of other groups [25,26,29,30]. De Schrijver et al. [26] performed studies showing that FASN inhibition by siRNA causes apoptosis in LNCaP cells. Flavanoids, such as EGCG, also cause inhibition of fatty acid synthesis, resulting in decreased growth and induction of apoptosis in prostate cancer cells [25]. Zhou et al. [30] induced apoptosis during S Phase in breast cancer cells with C75, an inhibitor of FASN, and a recent study by Bandyopadhyay et al. [29] also proposes a mechanism for the apoptotic pathway induced by inhibiting FASN. They have shown that in breast cancer tumor cells, inhibition of FASN by siRNA results in an accumulation of malonyl-CoA, which leads to an inhibition of carnitine palmitoyltransferase-1 (CPT-1), up-regulation of ceramide and ultimately, induction of the proapoptotic genes BNIP3, TRAIL, and DAPK2. It is tempting to speculate that a similar situation is occurring in prostate cancer cells and needs to be tested.

CONCLUSIONS

We initially identified FASN as one of the genes whose expression was most significantly affected by dutasteride treatment in LNCaP PCa cells. This observation was confirmed by real-time PCR, followed by examination of FASN protein expression and enzyme activity in LNCaP and additional PCa cell lines. We have demonstrated for the first time that the 5 alpha-reductase inhibitor dutasteride, at clinically relevant levels, inhibits FASN mRNA, protein expression and importantly, enzyme activity, in prostate cancer cells in vitro through the SREBP pathway and induces expression of proapoptotic genes BNIP3, TNFRSF10B, and DR5 leading to increased apoptosis. It will be important to discover whether this reduction of FASN activity with dutasteride treatment is also occurring in vivo. Experiments with xenografts and with prostate tissues from patients treated with dutasteride as part of pre-clinical trials should generate more insight into the effects of dutasteride on prostate cancer cells at the molecular level.

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