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Inhibition of fatty acid synthase expression by 1α,25-dihydroxyvitamin D_3 in prostate cancer cells

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Abstract

1α,25-Dihydroxyvitamin D_3 (1α,25$(OH)_2D_3$) and its derivatives are a potential treatment of human prostate cancer. The antiproliferative action of 1α,25$(OH)_2D_3$ is mainly exerted through nuclear vitamin D receptor (VDR)-mediated control of target gene transcription. To explore the target genes which are regulated by 1α,25$(OH)_2D_3$ in human prostate cancer LNCaP cells, cDNA microarray was performed by using a chip that contains 3000 gene probes. The results showed that 24 genes were regulated by 1α,25$(OH)_2D_3$. Five of them encode proteins which belong to metabolic enzymes and fatty acid biosynthesis. Fatty acid synthase (FAS) was found to be down-regulated by 1α,25$(OH)_2D_3$, and the regulation was confirmed by real-time quantitative RT-PCR analysis. Inhibition of FAS expression by 1α,25$(OH)_2D_3$ in LNCaP cells was more than 50% at 6 h. Inhibitory effect of 1α,25$(OH)_2D_3$ on FAS expression was completely blocked in the presence of protein synthesis inhibitor cycloheximide, indicating that the down-regulation of FAS gene expression by 1α,25$(OH)_2D_3$ was indirect in LNCaP cells. An inhibition of FAS activity by cerulenin resulted in a strong inhibition of LNCaP cell proliferation. The inhibition of FAS expression and cell proliferation by 1α,25$(OH)_2D_3$ seemed to be androgen-dependent, since antiandrogen, casodex and DCC-treatment of serum blocked the vitamin D action. The findings suggest that FAS is involved in the antiproliferative effect of 1α,25$(OH)_2D_3$ in presence of androgens on prostate cancer LNCaP cells.

Keywords: Fatty acid synthase; Gene regulation; 1α,25-Dihydroxyvitamin D_3; Antiandrogen; Cell proliferation

1. Introduction

The protective function of vitamin D against prostate cancer is evident [1–3]. This function is thought to be exerted through vitamin D receptor (VDR)-mediated pathway controlling the target gene expression, resulting in G1/S-phase arrest in cell cycle, apoptosis and cell differentiation [2]. The knowledge of the vitamin D-regulated genes is necessary for understanding of prostate cancer development. LNCaP, a well established human prostate cancer cell line, is commonly employed to study the effects of vitamin D and its analogs on prostate cancer, since this cell line is sensitive to vitamin D.

Fatty acid synthase (FAS/FASN), a key metabolic enzyme involved in the de novo biosynthesis of fatty acids, has been found to be a potential anticancer target [4–6]. FAS expression in human normal tissues is under a strict hormonal control [7–9]. In contrast, FAS expression appears to be independent of hormonal regulation in many cancers, because its expression is commonly up-regulated in these cancers despite high levels of ambient fatty acids [10]. Epithelial cells in human endometrium, one of the fastest growing human tissues, express abundant FAS during their proliferation [9,11], and the regions of endometrial carcinomas with the highest FAS expression are those regions with highest cell proliferation [12] suggesting a correlation between FAS expression and cell proliferation. FAS was found to be overexpressed in breast, prostate, ovary, endometrium and colon cancers [13–18]. In several of these cancers, elevated FAS expression occurs early in cancer progression and is associated with poor prognosis [19,20]. In prostate cancer, fatty acid synthesis pathway was found to be selectively activated in a subset of prostate cancers, and FAS expression elevated markedly not only at mRNA level [21] but also at protein level [14,16]. Immunohistochemical studies showed that the overexpression of FAS tended to increase from low grade to high grade prostatic epithelial neoplasia (PIN), and to invasive carcinoma, whereas FAS expression was negative in normal or benign hyperplastic grandular structures. These

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findings indicate that overexpression of FAS is an early and common event in the development of prostate cancer, and suggest that antineoplastic therapy based on FAS inhibition may be a chemoprevention or a curative treatment for prostate cancer [22].

FAS has a specific tissue distribution, a low expression in normal tissues and a high expression in cancers, and it has a unique β-ketoacyl synthase activity. These characteristics make it an attractive target for cancer therapy by FAS inhibitors [10], since the inhibitor-induced cytotoxicity could be limited to cancer cells without significant effect on normal cells. Inhibition of FAS expression by its inhibitor cerulenin resulted in both breast and prostate cancer cell death [23,24]. In this study, we found for the first time that FAS expression was down-regulated by $1\alpha,25(OH)_2D_3$ in prostate cancer LNCaP cells.

2. Materials and methods

2.1. Reagents and buffers

Cy™3-dUTP (25 nM), Cy™5-dUTP (25 nM), dNTP (dATP, dCTP, dGTP, dTTP) and Oligo(dT)$_{(12–18)}$ primer (1 mg/ml) were purchased from Amersham Pharmacia Biotech (Piscataway, USA). COT-1 DNA (1 mg/ml), SuperScript II (200 U/μl) and Yeast tRNA (10 mg/ml) were purchased from Gibco BRL (Grand Island, USA). Ten times Dig blocking buffer was from Roche Diagnostics (Mannheim, Germany). Polyadenylic acid (PolyA) and cerulenin were from Sigma (Missouri, USA). rRNasin® RNase inhibitor (40 U/μl) was purchased from Promega (Madison, USA). TRIzoL Reagent was from Invitrogen (Carlshad, USA). High Capacity DNA Archive kit and SYBR Green PCR Master Mix kit were purchased from Applied Biosystems (Forster City, USA). $1\alpha,25(OH)_2D_3$ was obtained from Leo Pharmaceuticals (Ballerup, Denmark). FBS was from Gibco BRL (Life Technology, Paisley, Scotland). RPMI-1640 medium was purchased from Sigma–Aldrich (Saint Louis, USA). Hydroxyflutamide was from Schering-Plough Co. (Helsinki, Finland). Casodex was obtained from AstraZeneca (London, UK).

2.2. Cell culture, treatment and RNA isolation

Human prostate cancer LNCaP cells (ATCC, USA) were maintained in RPMI-1640 medium supplemented with 10% FBS, 3 mM L-glutamine, 100 μg/ml streptomycin and 100 U/ml penicillin at 37 °C in humidified atmosphere of 5% CO_2. LNCaP cells grown to 70% confluence were treated with $1\alpha,25(OH)_2D_3$ and other reagents at indicated concentrations. For vitamin D, cycloheximide, casodex and hydroxyflutamide treatments, 0.1% of ethanol was used as control. DMSO (0.1%) was applied as control for cerulenin treatment. RNA was isolated with TRIzoL Reagent following the instruction of manufacturer. RNA concentrations were measured at 260 nm with GeneQuant II (Pharmacia Biotech).

2.3. cDNA microarray and data analysis

cDNA microarray was performed according to manufacturer's instruction. In brief, 20 μg of RNA sample from $1\alpha,25(OH)_2D_3$-treated cells was labelled with Cy™5-dUTP (25 nM) by reverse transcription under Oligo(dT)$_{(12–18)}$ primer direction, in parallel, the equal amount of RNA sample from untreated cells was labelled with Cy™3-dUTP (25 nM) as control. The RNA labelling reactions were done at 42 °C for 80 min. After the labelling reaction, RNA was removed from synthesized cDNA by addition of small amount of NaOH solution (1 M) followed by neutralisation with Tris–HCl (1 M, pH 7.5). Cy3-cDNA and Cy5-cDNA were combined together in one Microcon Column (Millipore Corporation, Bedford, USA) and washed four times in TE buffer (pH 7.4) by centrifugation. In the final washing step, COT-1 DNA, PolyA and Yeast tRNA were added to washing buffer, and centrifuged to make the final volume of labelled cDNA mixture less than 10 μl. For hybridization, Human2-1 Glass chip containing 3000 cDNA probes (Turku Centre for Biotechnology. Turku, Finland) was pretreated with 0.1% SDS, sterile H_2O and 95% ethanol, respectively, and air-dried. The labelled cDNA mixture was hybridized with the chip in a humid chamber at 65 °C over night. After hybridization, the chip was washed four times with slight shaking, and spinned dry by centrifugation. The hybridized chip was scanned in ScanArray 4000 Series (Packard BioScience), the hybridization images were analyzed using QuantArray Microarray Analysis Software (Packard BioScience), and the data were finally normalized to median by using Excel Date Normalization Macro.

2.4. Real-time quantitative RT-PCR analysis

Real-time quantitative RT-PCR was performed in ABI PRISM 7000 Detection System (Applied Biosystems, USA). Reverse transcription (RT) and real-time PCR were done separately with High Capacity Archive kit (Applied Biosystems, USA) and SYBR Green PCR Master Mix kit (Applied Biosystems, USA) following the instruction of manufacturers. Briefly, for reverse transcription, 10 μg of RNA dissolved in 50 μl PCR-compatible buffer was combined with 50 μl of 2× RT Master Mix (10 μl of 10× reverse transcription buffer, 4 μl of 25× dNTPs, 10 μl of 10× random primers, 5 μl of multiscribe reverse transcriptase (50 U/μl) and 21 μl of nuclease-free H_2O), RT was performed at 25 °C for 10 min followed by 37 °C for 120 min. For real-time PCR, 20 ng of cDNA was combined with primers and 2× SYBR Green PCR Master Mix to the final volume of 50 μl per reaction. Real-time PCR was done at 95 °C for 10 min followed by 40 cycles at 95 °C, 15 s and 60 °C, 1 min. The PCR product was examined by dissociation

curve and agarose gel electrophoresis to ensure that band was visible only at the expected molecular weight. The data was analyzed by ABI Prism 7000 SDS Software and normalized to housekeeping gene porphobilinogen deaminase (PBGD) to verify uniform RNA loading in experiments. The final result was expressed as *N*-fold difference in gene expression between treated sample and control sample {*N*(fold) = [gene (treatment)/PBGD (treatment)]/[gene (control)/PBGD (control)]}. The values used for calculation in the formula were obtained from corresponding standard curve. The standard curves were made by series of dilution of untreated samples. All primers used were designed by using compatible software Primer Express for ABI PRISM 7000 Detection System (Applied Biosystems). Primers for human FAS cDNA were as follows: forward primer 5′-AACTCCAAGGACACAGTCACCAT-3′ (in exon 12), reverse primer 5′-CAGCTGCTCCACGAACTCAA-3′ (in exon 13), PCR product was 65 bp in length. Primers for human PBGD cDNA were as follows: forward primer 5′-CCACACACAGCCTACTTTCCAA-3′ (in exon 4), reverse primer 5′-TTTCTTCCGCCGTTGCA-3′ (in exon 5), PCR product was 69 bp.

2.5. Cell growth assay

LNCaP cells were grown in 96-well plates and treated with hormones and other reagents at indicated time. After the treatment, cell growth was assayed by using crystal violet staining. In brief, cells were fixed by 11% glutaraldehyde solution in plates for 15 min with shaking at 500 rpm, and air-dried after washing. Crystal violet solution (0.1%) was added to stain fixed cells for 20 min by shaking, excess dye was removed by extensive washing with deionized water, the plates were air-dried before addition of 10% acetic acid to withdraw cell-bound dye. The optical density of dye extracts was measured directly in plates at the wavelength of 590 nm by using the Microplate Reader (WALLAC, VICTOR 1420 MULTILABEL COUNTER, Turku, Finland).

2.6. Statistical method

Data collected in the study was analyzed by a paired two-tailed *t*-test. Each result was obtained from at least three independent experiments. $P < 0.05$ was considered as significant, and $P < 0.001$ as highly significant.

3. Results

3.1. 1α,25(OH)$_2$D$_3$-regulated genes revealed by cDNA microarray in LNCaP cells

To investigate the expression profile of the genes regulated by 1α,25(OH)$_2$D$_3$ in prostate cancer LNCaP cells, cDNA microarray was performed by using the chip containing 2500 known genes and 500 ESTs. Three independent cDNA microarrays were done with RNA samples from LNCaP cells treated and untreated by 10 nM 1α,25(OH)$_2$D$_3$ for 24 h. The microarray results showed that 24 genes expressed differentially between 1α,25(OH)$_2$D$_3$ treated and untreated cells. The genes which were up- or down-regulated more than 1.8-fold (including 1.8-fold) or less than 0.58-fold (including 0.58-fold) were considered. Of the 24 genes, 19 were down-regulated by 1α,25(OH)$_2$D$_3$ (Table 1), and 5 were up-regulated by 1α,25(OH)$_2$D$_3$ (Table 2).

Table 1
List of genes from cDNA microarray that are down-regulated by 10 nM 1α,25(OH)$_2$D$_3$ at 24 h in prostate cancer LNCaP cells

Accession no.	Description	Fold
N20036	ATPase, H^+ transporting lysosomal (vacuolar proton pump), α-polypeptide	0.49 ± 0.05
N69204	Chromosome segregation 1 (yeast homology)-like	0.45 ± 0.02
T50788	EST	0.40 ± 0.11
N73769	Eukaryotic translation initiation factor 4E binding protein 2	0.51 ± 0.20
H50323	Fatty acid synthase	0.41 ± 0.08
N53172	G protein-coupled receptor	0.56 ± 0.23
H56918	Human clone 23933 mRNA sequence	0.49 ± 0.08
	K-α-1	0.41 ± 0.04
AA491206	KIAA0217 protein	0.50 ± 0.16
R93829	Nucleosome assembly protein 1-like-1	0.54 ± 0.13
AA598659	Nuclear mitotic apparatus protein	0.53 ± 0.13
AA598487	Phosphoribosylglycinamide formyltransferase	0.56 ± 0.12
AA668595	Quinone oxidoreductase homology	0.48 ± 0.15
H78788	RAN binding protein 2	0.46 ± 0.13
AA281635	Suppression of tumorigenicity 16 (melanoma differentiation)	0.52 ± 0.08
R00707	Stearoyl-CoA desaturase (δ-9-desaturase)	0.40 ± 0.17
AA608548	SET translocation (myeloid leukemia-associated)	0.50 ± 0.07
AA599116	Small nuclear ribonucleoprotein polypeptides B and B1	0.46 ± 0.12
AA490213	Transducer of ERBB2	0.51 ± 0.09

Table 2
List of genes from cDNA microarray that are up-regulated by 10 nM $1\alpha,25(OH)_2D_3$ at 24 h in prostate cancer LNCaP cells

Accession no.	Description	Fold
H75862	KIAA0670 protein/acinus	2.0 ± 0.84
H65261	ESTs moderately similar to p76 (*H. sapiens*)	2.31 ± 1.05
W86776	Histidine ammonial-lyase	2.23 ± 0.15
AA478553	Dopachrome tautomerase	1.93 ± 0.35
AA479093	Inositol 1,4,5 triphosphate receptor	1.84 ± 0.65

3.2. Inhibition of FAS expression by $1\alpha,25(OH)_2D_3$ in LNCaP cells

On the basis of cDNA microarray result, the FAS expression was studied further by using real-time quantitative RT-PCR. RT-PCR results showed that FAS expression was significantly inhibited by 10 nM $1\alpha,25(OH)_2D_3$ at 24 h after the treatment (0.60 ± 0.02-fold in mRNA expression). To study the time course of the FAS expression, LNCaP cells were treated for 6, 24 and 48 h. The results showed

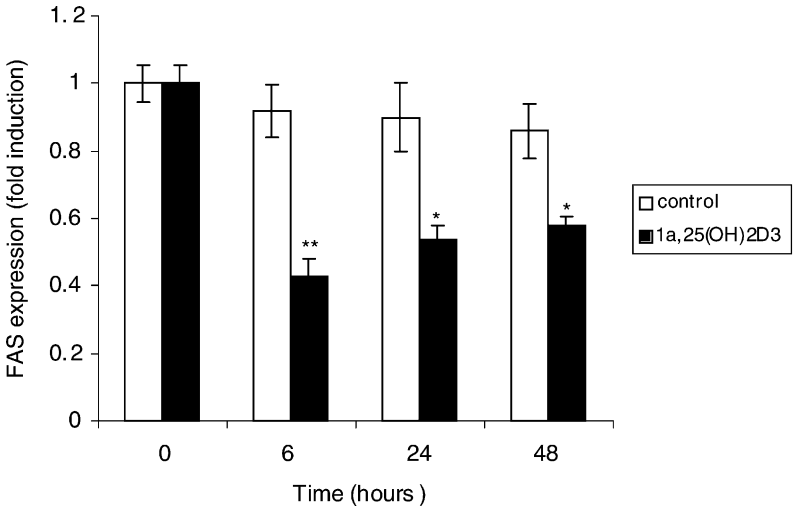

Fig. 1. Inhibition of FAS expression by $1\alpha,25(OH)_2D_3$ in prostate cancer LNCaP cells. LNCaP cells grown to about 70% confluence were treated with 10 nM $1\alpha,25(OH)_2D_3$ at indicated time, control cells received 0.1% ethanol only. FAS expression was analyzed by real-time quantitative RT-PCR. In three indicated time points, the strongest FAS inhibition by 10 nM $1\alpha,25(OH)_2D_3$ was at 6 h. At 24 and 48 h treatments, FAS inhibition were also statistically significant ($^{*}P < 0.05$, $^{**}P < 0.001$).

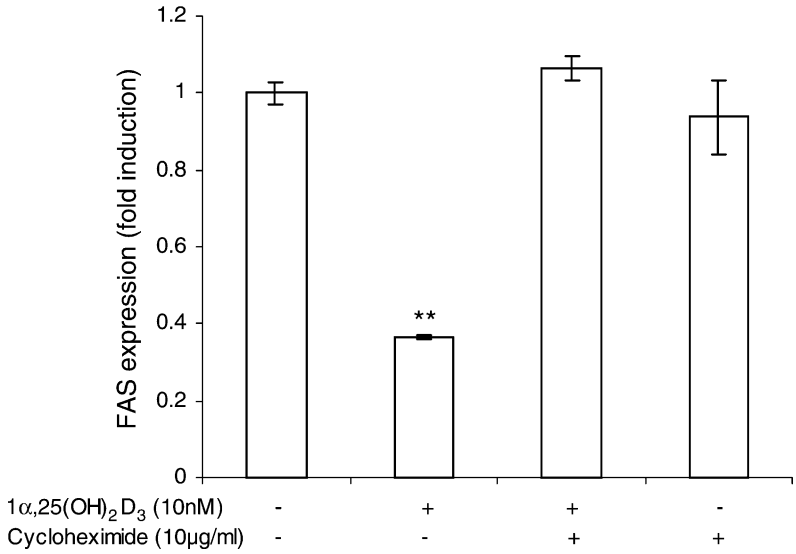

Fig. 2. Inhibition of FAS expression by $1\alpha,25(OH)_2D_3$ in the presence of cycloheximide in LNCaP cells. LNCaP cells were treated by 10 nM $1\alpha,25(OH)_2D_3$ in the presence or absence of 10 μg/ml cycloheximide for 6 h. FAS expression was analyzed by real-time quantitative RT-PCR. Inhibition of FAS expression by $1\alpha,25(OH)_2D_3$ alone was highly significant compared with both control and $1\alpha,25(OH)_2D_3$ plus cycloheximide treatment ($^{**}P < 0.001$).

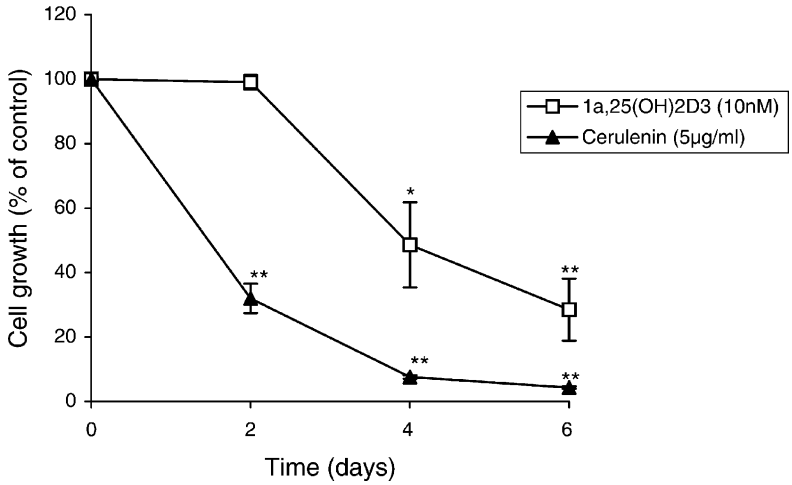

Fig. 3. Growth inhibition of LNCaP cells by FAS protein inhibitor cerulenin. LNCaP cells were seeded in 96-well plates at the density of 2500 cells per well in RPMI-1640 medium supplemented with 10% FBS. After 24 h growth for cell attachment, LNCaP cells were treated by cerulenin or $1\alpha,25(OH)_2D_3$ at indicated concentrations. For cerulenin treatment, cells received 0.1% DMSO only as control. Ethanol (0.1%) was used as control for vitamin D treatment. Cell growth was assayed by crystal violet staining at indicated time and expressed as % of control. Cerulenin strongly inhibited LNCaP cell growth. Inhibition of LNCaP cell growth by 10 nM $1\alpha,25(OH)_2D_3$ was significant at treatment days 4 and 6 ($^{*}P < 0.05$, $^{**}P < 0.001$).

that the strongest inhibition of FAS expression by 10 nM $1\alpha,25(OH)_2D_3$ was seen at 6 h ($P = 0.00076$). A statistically significant inhibition of FAS expression was also found at 24 h ($P = 0.0049$) and 48 h ($P = 0.0044$) (Fig. 1).

To study whether the down-regulation of FAS gene expression by $1\alpha,25(OH)_2D_3$ is direct, LNCaP cells were treated with $1\alpha,25(OH)_2D_3$ in the presence of protein synthesis inhibitor cycloheximide for 6 h. The result showed that inhibition of FAS expression by $1\alpha,25(OH)_2D_3$ was completely blocked by cycloheximide in LNCaP cells (Fig. 2), suggesting that inhibition of FAS expression might be indirect. To study the effect of FAS on cell proliferation, FAS protein inhibitor cerulenin was added in the culture medium. The growth of LNCaP cells was inhibited by cerulenin, and the inhibitory effect was time-dependent (Fig. 3). Casodex, an androgen antagonist, partially reversed growth inhibition of LNCaP cells by $1\alpha,25(OH)_2D_3$ (Fig. 4). In contrast, antiandrogen, hydroxyflutamide seemed to enhance the growth inhibition of LNCaP cells by $1\alpha,25(OH)_2D_3$ (Fig. 5). $1\alpha,25(OH)_2D_3$ failed to inhibit the FAS expression when the cells were grown in the serum treated with dextran coated charcoal (DCC) (data not shown).

4. Discussion

The result here is the first expression profile of vitamin D-regulated genes in prostate cancer cells revealed by cDNA

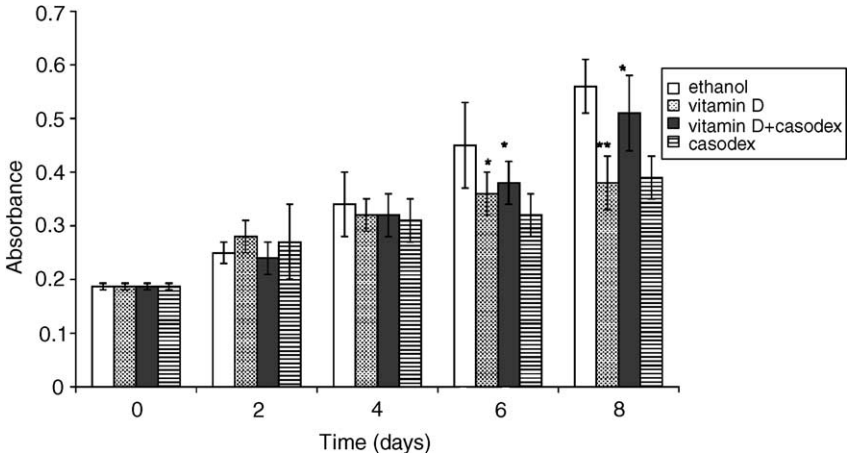

Fig. 4. The effect of androgen antagonist casodex on $1\alpha,25(OH)_2D_3$-repression of prostate cancer LNCaP cell proliferation. LNCaP cells were seeded in 96-well plates and treated with 10 nM $1\alpha,25(OH)_2D_3$ in the presence or absence of 1 μM casodex for indicated time. Cell proliferation was assayed by crystal violet staining. Inhibition of LNCaP cell growth by $1\alpha,25(OH)_2D_3$ was partially reversed by casodex (days 6 and 8). Increased blockage effect of casodex on vitamin D-inhibition of LNCaP cell growth was seen at the later time point ($^{*}P < 0.05$, $^{**}P < 0.001$).

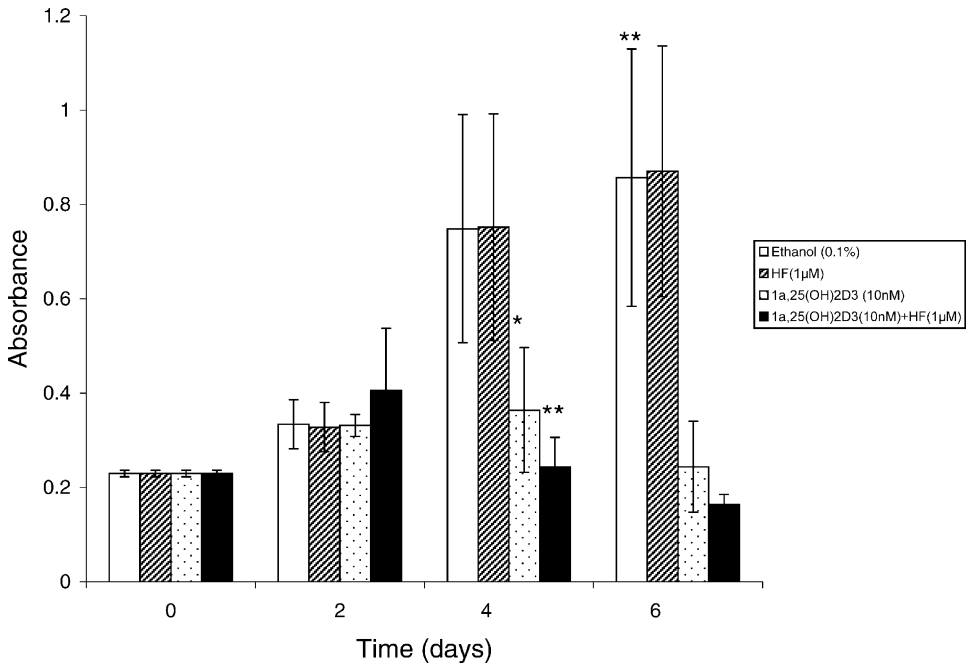

Fig. 5. The effect of antiandrogen hydroxyflutamide (HF) on $1\alpha,25(OH)_2D_3$-repression of prostate cancer LNCaP cell proliferation. LNCaP cells were seeded in 96-well plates and treated with 10 nM $1\alpha,25(OH)_2D_3$ in the presence or absence of 1 μM hydroxyflutamide at indicated time points. Cell proliferation was assayed by crystal violet staining. Hydroxyflutamide was not able to block the antiproliferative effect of $1\alpha,25(OH)_2D_3$ on LNCaP cells but enhanced vitamin D inhibitory effect (days 4 and 6) ($^*P < 0.05$, $^{**}P < 0.001$).

microarray. The microarray results showed that 24 genes were regulated by 10 nM $1\alpha,25(OH)_2D_3$ in prostate cancer LNCaP cells. Nineteen of these genes were down-regulated and five were up-regulated. Of the 24 vitamin D-regulated genes, 5 encode enzymes which belong to metabolic enzymes involved in fatty acid biosynthesis, purine nucleotide biosynthesis and histidine catabolism. FAS, phosphoribosylglycinamide formyltransferase (GART) and stearoyl-CoA desaturase (SCD) are down-regulated by $1\alpha,25(OH)_2D_3$. Histidine ammonial-lyase (HAL) and dopachrome tautomerase are up-regulated by vitamin D. This suggests that cellular metabolic pathways may play important roles in the action of $1\alpha,25(OH)_2D_3$. FAS and GART were found to be associated with cancer development [19,20,22,25]. HAL inhibits the growth of some cancers [26]. The regulation of these metabolic enzymes by vitamin D might be associated with its anticancer ability. Other genes such as G protein-coupled receptor and nuclear mitotic apparatus protein were also found to be regulated by $1\alpha,25(OH)_2D_3$ in LNCaP cells.

FAS is highly expressed in prostate cancer and correlates with cancer development [14,16,21,22]. In our study, FAS expression is found to be down-regulated by $1\alpha,25(OH)_2D_3$ in LNCaP cells. Inhibitory effect of vitamin D on FAS expression is the strongest at 6 h. Inhibition of FAS expression by $1\alpha,25(OH)_2D_3$ was completely blocked in the presence of protein synthesis inhibitor, cycloheximide, suggesting that down-regulation of FAS expression by vitamin D is not direct but might be mediated by a new protein synthesized during treatment. FAS protein inhibitor, cerulenin, was able to inhibit the proliferation of LNCaP cells suggesting that FAS is involved in the antiproliferative effect of $1\alpha,25(OH)_2D_3$ on LNCaP cells. An earlier study showed that inhibition of LNCaP cell growth by $1\alpha,25(OH)_2D_3$ was blocked by androgen antagonist casodex, and suggested that inhibition of LNCaP cell proliferation by vitamin D was dependent upon androgen action [27]. It has been demonstrated that another antiandrogen, hydroxyflutamide, acts as androgen in LNCaP cells due to a mutation in androgen receptor [28]. Similarly in our study, casodex antagonized vitamin D inhibition of LNCaP cell growth. This suggests that inhibition of FAS expression by $1\alpha,25(OH)_2D_3$ might be androgen-dependent. We also show that hydroxyflutamide is androgenic in LNCaP cells and it cannot antagonize the inhibition of LNCaP cell proliferation by $1\alpha,25(OH)_2D_3$.

Since androgen was found to up-regulate FAS expression in LNCaP cells [29], the androgens present in the serum added to the cell culture medium could affect vitamin D-inhibition of FAS expression. Therefore, we treated the serum with DCC to remove steroids, and the vitamin D effect on FAS expression was abolished. This suggests that the inhibition of FAS expression by $1\alpha,25(OH)_2D_3$ is androgen-dependent. This is in line with our epidemiological result suggesting that the protective action of vitamin D against prostate cancer is androgen-dependent [3]. Androgen receptor is up-regulated by $1\alpha,25(OH)_2D_3$ in LNCaP cells [30]. This might explain why the inhibition of FAS expression by vitamin D was slightly less pronounced at 24

and 48 h than at 6 h. Increased AR may enhance the effect of the small amount of androgens in the serum on stimulation of FAS expression, resulting in less inhibition of FAS expression by $1\alpha,25(OH)_2D_3$ at the later time point.

Inhibition of FAS expression might be a potential treatment for cancer. Human promyelocytic leukemia HL60 cells were arrested in G1-phase of cell cycle by inhibition of FAS expression [31]. C75, a synthetic inhibitor of FAS, abolished growth of human mesotheliomas xenografts in mice at the level without a significant systemic toxicity except for a reversible weight loss [5]. The inhibition of FAS expression by $1\alpha,25(OH)_2D_3$ provides a possibility of developing vitamin D-based FAS inhibitory drugs for prostate cancer therapy. These drugs may selectively inhibit the growth of prostate cancer cells with high level of FAS expression. The synthetic derivatives of vitamin D are most promising, since they do not possess the side effect of $1\alpha,25(OH)_2D_3$ such as hypercalcemia, which severely limits its clinical use as a drug for prostate cancer therapy.

In conclusion, FAS was for the first time found to be down-regulated by $1\alpha,25(OH)_2D_3$ in prostate cancer LNCaP cells. The regulation was shown to be indirect and androgen-dependent. Inhibition of FAS activity by its inhibitor resulted in a clear-cut suppression of LNCaP cell proliferation. These findings suggest that FAS is involved in the antiproliferative effect of $1\alpha,25(OH)_2D_3$ on prostate cancer LNCaP cells.

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