



Vitamin D and prostate cancer[☆]

P. Tuohimaa^{a,d,*}, A. Lyakhovich^a, N. Aksenov^a, P. Pennanen^a, H. Syväälä^a,
Y.R. Lou^a, M. Ahonen^a, T. Hasan^b, P. Pasanen^c, M. Bläuer^a, T. Manninen^a,
S. Miettinen^a, P. Vilja^a, T. Ylikomi^{a,d}

^a Medical School, University of Tampere, 33014 Tampere, Finland

^b Department of Dermatology, Tampere University Hospital, 33520 Tampere, Finland

^c Department of Chemistry, University of Turku, Turku, Finland

^d Department of Clinical Chemistry, Tampere University Hospital, 33520 Tampere, Finland

Abstract

Our recent epidemiological study (Ahonen et al., *Cancer Causes Control* 11(2000) (847–852)) suggests that vitamin D deficiency may increase the risk of initiation and progression of prostate cancer. The nested case–control study was based on a 13-year follow-up of about 19000 middle-aged men free of clinically verified prostate cancer. More than one-half of the serum samples had 25OH-vitamin D (25-VD) levels below 50 nmol/l, suggesting VD deficiency. Prostate cancer risk was highest among the group of younger men (40–51 years) with low serum 25-VD, whereas low serum 25-VD appeared not to increase the risk of prostate cancer in older men (> 51 years). This suggests that VD has a protective role against prostate cancer only before the andropause, when serum androgen concentrations are higher. The lowest 25-VD concentrations in the younger men were associated with more aggressive prostate cancer. Furthermore, the high 25-VD levels delayed the appearance of clinically verified prostate cancer by 1.8 years. Since these results suggest that vitamin D has a protective role against prostate cancer, we tried to determine whether full spectrum lighting (FSL) during working hours could increase serum 25-VD concentrations. After 1-month exposure, there was no significant increase in the serum 25-VD level, although there was a bias towards slightly increasing values in the test group as opposed to decreasing values in controls. There was no significant change in the skin urocanic acid production. The possibility to use FSL in cancer prevention is discussed. In order to clarify the mechanism of VD action on cell proliferation and differentiation, we performed studies with the rat and human prostates as well prostate cancer cell lines. It is possible that 25-VD may have a direct role in the host anticancer defence activity, but the metabolism of vitamin D in the prostate may also play an important role in its action. We raised antibodies against human 1 α -hydroxylase and 24-hydroxylase. Our preliminary results suggest that vitamin D is actively metabolised in the prostate. Vitamin D appears to upregulate androgen receptor expression, whereas androgens seem to upregulate vitamin D receptor (VDR). This may at least partially explain the androgen dependence of VD action. VD alone or administered with androgen causes a suppression of epithelial cell proliferation. VD can activate mitogen-activated kinases, erk-1 and erk-2, within minutes and p38 within hours. Also, auto/paracrine regulation might be involved, since keratinocyte growth factor (mRNA and protein) was clearly induced by VD. Based on these studies, a putative model for VD action on cell proliferation and differentiation is presented. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Prostate cancer; Vitamin D; Prevention; Growth; Differentiation; Full spectrum light

1. Introduction

Adenocarcinoma of the prostate gland is the most commonly diagnosed non-skin malignancy [1]. Mortality from the disease is continuously rising. It represents 14% of all cancer deaths projected for 1996 and makes prostate cancer the second leading cause of cancer deaths. Also in Finland, the annual number of prostate cancer cases is increasing with an accelerating rate

[☆] Proceedings of the 14th International Symposium of the Journal of Steroid Biochemistry and Molecular Biology “Recent Advances in Steroid Biochemistry and Molecular Biology” (Quebec, Canada 24–27 June 2000).

* Corresponding author.

E-mail address: pentti.tuohimaa@uta.fi (P. Tuohimaa).

[2](Fig. 1). When compared with Nigerian men, African-American men have a sixfold increased risk of developing clinically detectable prostate cancer [3]. African-American men have a higher incidence of prostate cancer than Caucasian-American men [4] and African-American have lower serum vitamin D levels as a result of their darker skin pigmentation [5]. Therefore, it has been hypothesised that vitamin D deficiency increases the risk of initiation and progression of prostate cancer [6]. Indeed, results from geographical studies suggest an inverse relationship between the level of solar radiation and prostate cancer mortality [7,8]. The major source of vitamin D is through sunlight (UV-B)-induced photobiosynthesis in the skin. However, analytical epidemiological studies on the role of vitamin D in the etiology of prostate cancer have been controversial [9–12]. Finland is an optimal country for an epidemiological study of serum vitamin D because, during the several months of the winter season, we receive practically no effective ultraviolet-B (UV-B) irradiation.

We recently reported an association between vitamin D status and risk of prostate cancer in a case-control study nested within a cohort of 18966 men aged 40–57 at the entry [13] who participated in the first health examination for the Helsinki Heart Study in 1981–1982 [14], and who had not previously had clinically detected prostate cancer. More than one-half of the serum samples had 25OH-vitamin D (25-VD) levels below 50 nmol/l, suggesting VD deficiency. There was a high seasonal variation in the serum 25-VD concentration; the highest values were found in September. Men with serum 25-VD levels below the median had an adjusted relative risk (OR) of 1.7 compared with men with

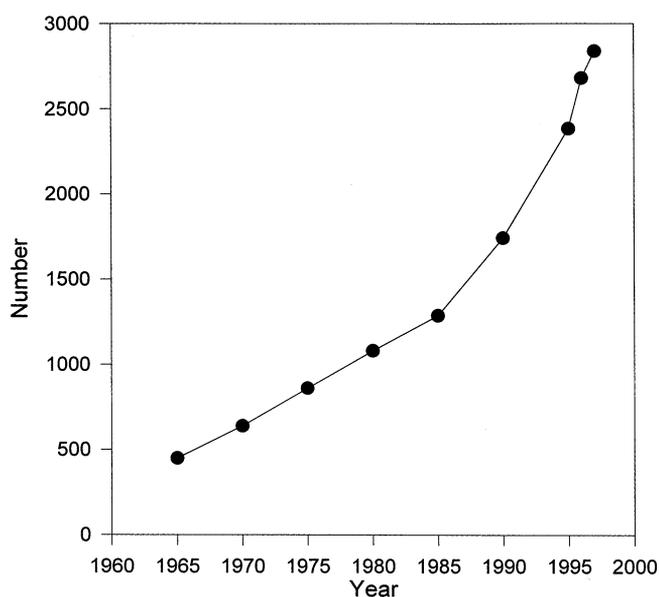


Fig. 1. The mean annual number of new prostate cancer cases in Finland diagnosed in 1965–1997, by primary site and period [1].

PROSTATE CANCER



Fig. 2. A summary of putative effects of vitamin D on prostate carcinogenesis based on our epidemiological data [13].

25-VD levels above the median. The highest prostate cancer risk was among the youngest men (40–51 years) with low serum 25-VD (OR = 3.1), whereas low serum 25-VD was no risk to older men (> 51 years) (OR = 1.2). This suggests that VD protects against prostate carcinogenesis only during the high androgen action before andropause. The lowest 25-VD concentrations in the younger men were associated with more aggressive prostate cancer (OR = 6.3). Furthermore, the high 25-VD levels delayed the clinically verified prostate cancer by 1.8 years. The results suggest that vitamin D has beneficial effects at all stages of cancer development (Fig. 2). It is obvious that, during the winter season in Finland, more natural or artificial UV-B light is needed, because dietary supply of vitamin D is not sufficient. Therefore, in this paper, we describe a preliminary study on whether full-spectrum light (FSL) including low-intensity UV-B irradiation could be effective to increase the serum vitamin D level. We also determined the *trans* to *cis* isomerisation of urocanic acid in the exposed skin area, since *cis*-UCA formation is greatly induced by UV-B radiation [15].

Experimental and *in vitro* studies suggest that vitamin D inhibits cell proliferation and induces cell differentiation in several target organs including prostate, and the effect is mediated by the vitamin D receptor (VDR) [16]. Both normal and cancerous prostate cells have the VDR [17–19], indicating that they are target cells that can respond to vitamin D. The active form of vitamin D, 1,25-dihydroxyvitamin D (1,25-VD), inhibits proliferation and induces differentiation in human prostate cancer cell lines [16,20]. Furthermore, the invasiveness of human prostate cancer cells is inhibited by 1,25-D [21]. In an experimental rat model, a vitamin D analog reduced prostate cancer incidence [22]. Because our epidemiological study already mentioned suggests that the serum concentration of a precursor of vitamin D, 25-VD, is associated with an increased prostate cancer risk, the local metabolism of VD in the prostate may play an important role. Therefore, we studied whether prostate has an active VD metabolism. Besides the involvement of VDR in the mitotic control system, little is known about the mechanism regulating growth control by vitamin D in the prostate. Therefore,

we have studied the regulation by vitamin D of the expression of growth factors and activity of mitogen-activated kinases.

2. Materials and methods

2.1. Full-spectrum lighting

Twenty-seven volunteers of the staff at the University of Tampere Medical School participated in the full-spectrum light study during 21 January–17 February 2000. They were interviewed for their vitamin D supply (travelling, vitamin D intake, etc). Four to 12 full-spectrum lamps (True-Light II DURO-TEST 36 W/5500/96, known also as True-Lite or Vita-Lite) according to the size of the room were installed giving an average light intensity of 10 W/m². The spectric irradiance of the lamps was measured at the distances of 80 cm (standing position) and 140 cm (sitting position) from the lamps horizontally and vertically using a spectroradiometer (Optronic 742). The vertical UV-B irradiances were 16.5 and 8.7 mW/m², respectively. The horizontal UV-B irradiance at 140 cm was 0.7 mW/m² (facial irradiance in the sitting position). A control group of 17 volunteers had normal fluorescent lamps (Osram Dulux S 11 W/31-830, Philips TDL 58 W/83, Sylvania Professional Décor 183, Sylvania Lux-Line Plus F85W/830 183) in their offices. No UV-B irradiation was detected from Osram and Philips lamps, but significant UV-B irradiation was emitted by both Sylvania lamps; 28 and 45 mW/m² at the distance of 80 cm, respectively. However, the total UV-B irradiation in the control group was variable, but significantly smaller or negligible as compared with the test group, because only a few Sylvania lamps were found in the rooms. The serum samples were assayed for concentrations of 25-hydroxyvitamin D (25-VD) by radioimmunoassay [13].

2.2. *Cis-Urocanic acid assay*

The *cis*-urocanic acid (*cis*-UCA) content of the back of the hand of the volunteers already mentioned was examined to find out whether the exposure for 4 weeks to full-spectrum light during working hours would enhance the synthesis of *cis*-UCA as compared with exposure to ordinary fluorescent light. None of them had exposed themselves to additional natural or artificial UVR for several months before the study period. During the study period, they were also asked to protect their hands with gloves outdoors. *Cis*-UCA was sampled using the non-invasive chamber sampling technique [23]. Briefly, nine small aluminium chambers (Finn Chambers[®], 8 mm in diameter; Epikon, Helsinki, Finland), with filters moistened with 0.1 M KOH, were attached to the skin and, after a 30-min occlusion time,

the filters were distributed, three filters per tube, into three tubes containing 0.1 M KOH. After a 24-h soaking time, the filters were removed and the samples were stored at –20°C and subjected to high-performance liquid chromatography analysis for *cis*-UCA, as outlined earlier [23].

2.3. Cell culture, growth assay and RNA isolation

Human LNCaP cells were routinely grown to near confluency for 1 week in phenol-red free RPMI 1640, supplemented with L-glutamine (2 mM), antibiotics, 5% dextran-coated charcoal-treated fetal calf serum (DCC-FCS) at 37°C and 5% CO₂. Twenty-four hours before experiments, the medium was replaced by medium containing 0.1% DCC-FCS to minimise effects of exogenous growth factors and hormones. Cells were treated with 1,25-dihydroxyvitamin D₃ (Leo Pharmaceutical Products, Denmark) or 17β-estradiol (Sigma, St Louis, MO, USA) (both 10 nM) for the indicated time periods. Medium was collected for protein isolation and cells were detached from the flasks using trypsin/ethylenediamine tetraacetic acid solution, then washed three times with ice-cold phosphate-buffered saline (PBS) and total RNA was extracted according to the standard manual of the TRIzol RNA isolation Kit (Gibco BRL). RNA electrophoresis was carried out to check the absence of RNA degradation.

Cells were seeded onto 96-well micro titer plates at a concentration of 3000 cells/well in 100 μl medium, containing appropriate amounts of VD, and were grown in 5% FCS or DCC-FCS medium. Cell growth was measured after 0, 2, 4, 6 days by crystal violet staining. Alternatively, after the indicated time interval, 10 μl WST-1 reagent was added and the cells were incubated for 4 h, then shaken thoroughly for 1 min on a shaker. Absorbances were measured with an enzyme-linked immunosorbent assay (ELISA) reader.

2.4. Antibodies

Several antibodies were raised in New Zealand rabbits against synthetic peptides coupled to polylysine. The following synthetic peptides were used: human 24-hydroxylase, CYP24_{287–298} (-N-R-L-E-K-Y-S-Q-Q-P-S-A-) for Ab129, hCYP24_{360–373} (-P-E-N-Q-R-P-R-E-E-D-L-R-N-M-) for Ab130; human 1α-hydroxylase, CYP27B_{100–113} and _{107–120} (-Q-E-G-P-R-P-E-R-C-S-F-S-P-W- and -R-C-S-F-S-P-W-T-E-H-R-R-C-R-) for Ab131 and hCYP27B_{167–178} (-R-R-L-R-R-Q-R-G-R-G-T-G-) for Ab132. The rabbits were injected first with 100 μg antigen with Freund's complete adjuvant and, thereafter, at least three times with 1-month intervals using Freund's incomplete adjuvant. The specificity of each antibody was tested by using peptide-ELISA and Western blot. Ab132 showed a very low IgG titer and

therefore it was not further analysed. Monoclonal antibody against rat CYP24 was from Dr K. Okuda [24]. Recombinant human FGF-7 (KGF) rabbit polyclonal antibody with epitope corresponding to amino acids 32–104 was purchased from Santa Cruz Biotechnology (Santa Cruz, USA); secondary anti-mouse and anti-rabbit IgG HRP-conjugates and enhanced chemiluminescence (ECL) detection reagents were from Amersham (Bucks, UK).

2.5. Immunohistochemistry

Tissue samples were immediately fixed with 4% paraformaldehyde (pH 7.4) in PBS at 4°C overnight, and subsequently embedded in paraffin. Paraffin sections (5 µm) were deparaffinised with xylene and rehydrated in a graded ethanol series. The antigens were retrieved by microwave oven heating. The sections were immersed in boiling 0.01 M sodium citrate buffer (pH 6.0) for 5 min, followed by cooling down for 20 min at room temperature and rinsing in TBS buffer (pH 7.6). The endogenous peroxidase activity was blocked for 30 min in 100% methanol with 0.5% hydrogen peroxide. After washing in PBS containing 0.1% Tween 20, the sections were incubated in 10% normal horse serum for 30 min at room temperature to block non-specific background staining. The primary antibody (1:500) was incubated with the sections overnight at 4°C. Biotinylated second antibody was incubated for 30 min and, finally, avidin biotin–peroxidase complex for 30 min. The sections were then stained with DAB substrate–chromogen solution for 5 min. The specimens were washed in PBS with 0.1% Tween 20 after every incubation step. For the negative control, another monoclonal antibody (PR22, anti-chicken progesterone receptor antibody) was used in place of the primary antibody.

2.6. Reverse transcriptase polymerase chain reaction amplification

Total RNA from LNCaP cells treated with either 1,25-dihydroxyvitamin D₃ or 17β-estradiol was extracted by means of TRIzol RNA isolation Kit. Reverse transcriptase polymerase chain reaction (RT-PCR) was carried out using the Smart cDNA syntheses KIT (Clontech Inc., Palo Alto, CA, USA) according to the manufacturer's manual. Samples of 3 µl each of the RT-PCR reaction were amplified in 50 µl volume in 1 × PCR Buffer for *taq* DNA polymerase (Perkin Elmer), 1.5 mM MgCl₂, 200 µM each dNTP and 0.5 µM primer mixture, and 2.5 U AmpliTaq DNA polymerase (Perkin Elmer) were added. PCR primers (Amersham Pharmacia Biotech) were as follows: FGF-7-1, 5'-AGAAACTAGAAAGAAGACTGCC-3'; FGF-7-2, 5'-GAAGCCTGACCTCAAACC-3'. The PCR profile was as follows: at 94°C for 3 min (one cycle), at

94°C for 30 s, at 55°C for 30 s, at 74°C for 1 min (35 cycles), and at 74°C for 5 min. A PE 9700 (Perkin Elmer) PCR amplifier was used for amplification.

2.7. Electrophoresis and immunoblotting

Cells were lysed in Laemmli application buffer (2 ×). The lysates were subjected to 7.5% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and were thereafter electrophoretically transferred onto the nitrocellulose membrane for 3 h at room temperature in transfer buffer containing 50 mM Tris, 380 mM glycine, 0.1% SDS and 20% methanol (pH 8.4). After transfer, membranes were soaked in 10 mM Tris–HCl, 150 mM NaCl, 0.05% Tween-20 (pH 8.0) (TBST buffer) containing 3% non-fat dry milk powder to block non-specific binding sites (blocking solution) and incubated for 1 h at room temperature. They were incubated for 1 h with E10 primary p44/42 antiphosphotyrosine monoclonal antibody E10 (1:2000), rabbit polyclonal anti-KGF antibody (1:2000) or polyclonal anti-human VDR antibody (1:200) [19]. Membranes were then washed three times for 5 min with TBST, and incubated for 1 h with secondary anti-mouse IgG antibodies (1:2000) or anti-rabbit IgG (1:1000) in blocking buffer with gentle agitation, washed three times for 5 min with TBS and subjected to ECL detection according to the instruction of the manufacturer. Intensities of scanned bands from the Western blots were detected by computer program for one-dimensional gel analysis (Pharmacia).

2.8. MAP kinase assay

MAP kinase (MAPK) assay was performed as follows. Myelin-binding protein (MBP) was used as the substrate for measuring MAPK activity of cell lysates at 37°C for 20 min in final volume of 50 µl containing 50 mM HEPES (pH 8.0), 10 mM MnCl₂, 1 mM dithiothreitol, 1 mM benzamidine, 0.3 mg/ml MBP, 50 µM [γ -³²P]ATP (10 µCi), 1 mM and cell lysate (10 µl). Reaction was stopped by adding excess of 10% TCA. The samples were spotted onto GFC phospho-cellulose filters. Each filter was rigorously washed three times with 5 ml of 10% TCA, dried in air and counted by liquid counter scintillation. Specific incorporation of ³²P into MBP was measured as the differences between samples incubated with and without MBP.

3. Results

3.1. Effect of FSL on serum 25-hydroxyvitamin D

There was considerable variation between individuals in serum 25-V_D concentrations (Fig. 3). However, all

the values except for those of two individuals are below 50 nmol/l, suggesting a common vitamin D deficiency during the midwinter season in Finland. We assayed 25-V_D from a few volunteers who visited Southern tourist resorts during the winter. All showed a clear-cut increase in 25-V_D after a 1–2 week visit, the values being above 50 nmol/l. The effect of a 4-week exposure to FSL caused an increase of 1 nmol/l in the average serum 25-V_D level. During the same period, the control group showed a slight decrease (3 nmol/l) in serum

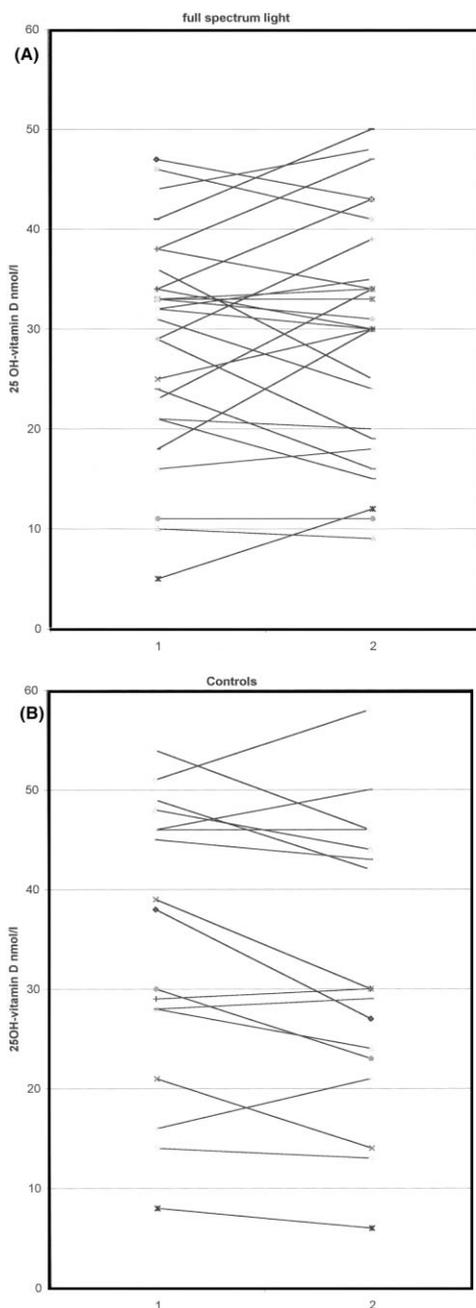


Fig. 3. The effect of 4-week full-spectrum lighting (FSL) on serum 25-hydroxyvitamin D concentration (nmol/l). (A) Test group ($N = 27$) and (B) control group ($N = 17$), normal fluorescent light. 1, 21 January 2000; 2, 17 February 2000.

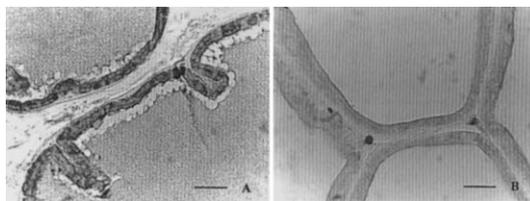


Fig. 4. Immunohistochemical localisation of 25-vitamin D₃ 24-hydroxylase in the rat lateral (A) and ventral (B) prostate. Monoclonal antibody against rat 24-hydroxylase is used. Immunoreactivity is seen in the type 1 and 2 epithelial cells (A). Ventral prostate shows very low immunoreactivity; only some macrophages show non-specific staining in the stroma. Bar, 50 μ m.

25-V_D. The changes were statistically non-significant due to high individual variation. There was, however, a bias towards increasing values in the FSL group and to decreasing 25-V_D levels among controls. In the FSL group, serum 25-V_D increased in 44%, remained equal in 27% and decreased in 29% of cases, corresponding values being 18, 29 and 53%, respectively, in controls. Thus results support the earlier finding of Gloth et al. [25] showing a 37% increase in serum 25-V_D after 1 week of FSL treatment.

3.2. *Cis-Urocanic acid*

The *cis*-UCA content of the back of the hand of the study group was 0.041 ± 0.019 nmol/cm² (mean \pm S.D.) and 0.042 ± 0.035 nmol/cm² before and after the FSL exposure, respectively, whereas the respective figures of the control group were 0.039 ± 0.020 and 0.043 ± 0.018 nmol/cm². These figures did not differ significantly between any groups.

3.3. Expression of CYP27B and CYP24 in the rat prostate

Strong immunostaining of CYP24 was seen in the type 1 and type 2 epithelial cells of the rat lateral prostate (Fig. 4A), and a weak immunoreaction was detected in the epithelial cells of the coagulating gland and the seminal vesicle as well as in the epithelial cells of the ventral lobe of the prostate (Fig. 4B). Our preliminary studies on human prostate and prostate cancer cell line, LNCaP, suggest significant expression of both CYP27B and CYP24 detected by Western blot and RT-PCR.

3.4. Effect of 1,25-V_D on cell proliferation, VDR and MAPK activity of LNCaP cells

We analysed the influence of 17 β -estradiol (E2) and 1,25-dihydroxyvitamin D₃ (1,25-D₃) alone or in combination with E2 on the proliferation of LNCaP cell line both in non-stripped 5% FCS and serum-stripped

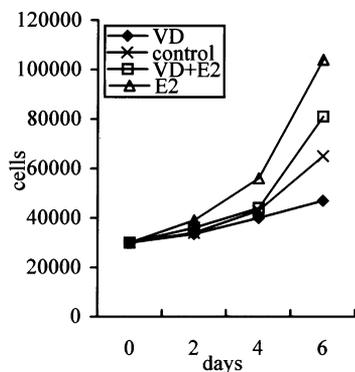


Fig. 5. Growth inhibition of LNCaP human prostate carcinoma cells by $1\alpha,25$ -dihydroxyvitamin D₃ (10 nmol) (VD) with or without 17β -estradiol (E2) (10 nmol). Steroids were present throughout the incubation time.

DCC-FCS medium using crystal violet staining. In case of serum-stripped medium, $1,25$ -VD inhibited and E2 increased cell proliferation (Fig. 5). Combination of these two compounds led to an inhibition of E2 effect on proliferation by VD. We found 35% of growth inhibition on day 6 of $1,25$ -VD treatment, whereas 60% growth increase after E2 treatment was found, compared with control cells grown in DCC medium. In non-stripped 5% FCS medium, the inhibitory effect of $1,25$ -VD was smaller. The $1,25$ -VD (10 nM) upregulated clearly VDR protein in LNCaP cells within 8 h (Fig. 6).

The ability of $1,25$ D₃ or E2 to activate MAPK in LNCaP cells was tested. Cells were grown for 1 week in the phenol red free DCC-FCS. The cells were treated with either $1,25$ -VD or E2 for 0, 1, 5, 20, and 180 min. After cell lysing, aliquots of samples were subjected to MAP kinase assay by incubation with MBP in the presence of [γ -³²P]-adenosine triphosphate

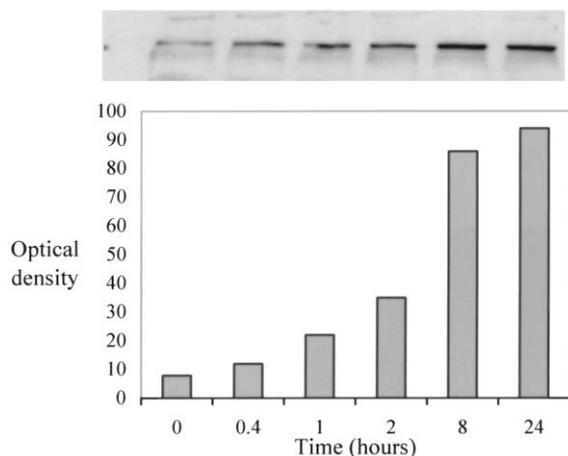


Fig. 6. Western blotting of vitamin D receptor in LNCaP cells with polyclonal anti hVDR antibody after $1\alpha,25$ -dihydroxyvitamin D₃ (10 nmol). For details, see Section 2.

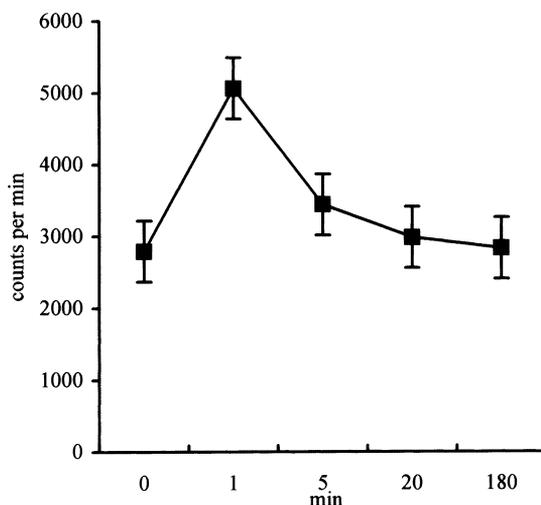


Fig. 7. Total MAPK activity in LNCaP cells after $1\alpha,25$ -dihydroxyvitamin D₃ (10 nmol). For details, see Section 2.

(ATP) as described in Section 2. In samples treated with either $1,25$ -VD or E2 for 1 min, a two- and threefold increase of MBP phosphorylation was observed, respectively (Fig. 7). The kinase activity decreased after 5 min of $1,25$ -VD or E2 treatment and returned to the basal level after 180 min.

To test which form of MAPKs, the erk-1 or erk-2, is activated after E2 stimulation, we treated LNCaP cells with E2 for 0, 1, 5, 20, and 120 min. After cell lysing, aliquots of samples were subjected to SDS-PAGE followed by Western blotting. Membranes were analysed using phospho-p44/42 MAP kinase (Thr202/Tyr204) E10 monoclonal antibody. Both erk-1 and erk-2 were rapidly activated after 1 min of E2 treatment (result not shown). The stimulatory effect of E2 was abolished by treating cells with anti-estrogen, ICI 182,780. Moreover, erk-2 form was predominantly phosphorylated. The MAP kinase activity was decreased after 5 min of E2 treatment and returned to the basal level after 120 min.

Another member of MAPK family, p38 is often activated along with increasing erk-1/erk-2 activity. To test this, we studied p38 MAPK phosphorylation after stimulation of LNCaP cells with $1,25$ -VD or E2. Both E2- and $1,25$ -VD-treated cells showed a twofold increase of p38 phosphorylation after 6 h of stimulation (data not shown).

To test how pretreatment of cells with $1,25$ -VD may influence E2-induced MAPK activation, both cell lines were pretreated with $1,25$ -VD and were then stimulated with E2. E2-stimulated erk-1/erk-2 activity was partially (15–20%) inhibited in $1,25$ -VD pretreated samples (Fig. 8). No significant difference in E2-induced activation of p38 of $1,25$ D₃-primed cells compared with $1,25$ D₃-non-treated cells was found (data not shown).

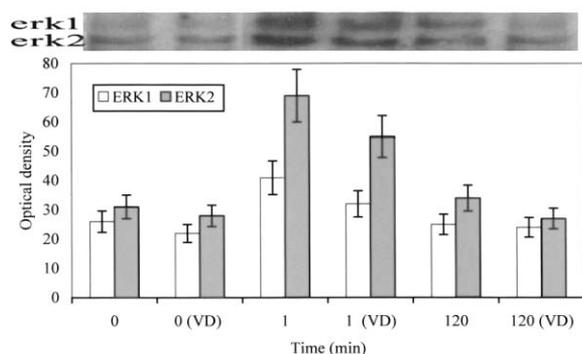


Fig. 8. Inhibition of erk-1/erk-2 stimulation after 17β -estradiol (10 nmol) by $1\alpha,25$ -dihydroxyvitamin D₃ (10 nmol) pretreatment (VD). Open bars, erk-1 activity; shaded bars, erk-2 activity.

3.5. Effect of $1,25$ -VD on keratinocyte growth factor (KGF/FGF-7) expression in LNCaP cells

To study the role of $1,25$ D₃ in regulation of expression of growth factors, MCF-7 cells growing in the presence of 17β -estradiol were treated with $1,25$ -VD for 0, 1, 6, and 48 h (100 nM) and then subjected to cDNA micro array assay [26]. Among a number of regulated genes, described earlier by other workers, we detected an upregulation of FGF-7 gene expression (threefold) as early as 6 h after $1,25$ -VD₃ treatment.

To study vitamin D-induced FGF-7 expression in LNCaP cells, we used RT-PCR amplification. To analyse regulation of FGF-7 mRNA expression in $1,25$ -VD or 17β -estradiol-treated cells, two primers, FGF-7-1 and FGF-7-2, corresponding to the 117 bp fragment of human cDNA FGF-7, 3' end, were used. There was no visible signal in RT-PCR amplification of FGF-7 mRNA in the control (Fig. 9). However, the evident signal of FGF-7 mRNA expression was significantly higher in $1,25$ -VD-treated samples, while in the 17β -

estradiol-treated sample only a slight band of the 117 bp fragment was seen.

4. Discussion

Our epidemiological study suggests that low levels of 25-hydroxyvitamin D are associated with an increased risk for subsequent earlier appearance and more aggressive development of prostate cancer, especially before the andropause [13]. Therefore, prevention of prostate cancer by VD administration would seem possible. However, a risk of an overdose in parenteral or oral administration is apparent and the adverse effects are serious. The endogenous metabolism is tightly regulated and, therefore, there is no risk of overdose. On the other hand, we receive two-thirds of our daily VD via the sunlight-induced synthesis in the skin and only one-third comes from the food [27]. The increase of serum 25-VD caused by FSL in the present study was small, but promising. It is not possible to increase the intensity of the exposure, since the intensity used was close to the maximum intensity allowed by law. The skin area exposed was minimal (face and hands). An increase of the exposed skin area is possible when short sleeve shirts are used. In our study, a few persons were using short sleeves, which did slightly enhance VD synthesis. In a recent study, a 36% increase in serum 25-VD values was obtained [25], which suggests that studies on FSL should be continued. In contrast, a study in submarines did not show significant effect of FSL on VD status [28]. In our study, the exposure to FSL was limited to the offices of the test persons. When they spent much time outside their offices during the work hours, general FSL in the institute would increase the dose of UV-B significantly. It seems, however, that FSL cannot fully solve the problem of vitamin D insufficiency in Nordic countries with regard to cancer prevention, but it could be used as a supportive treatment to increase vitamin D supply. FSL might be useful for long-term hospital patients, when an exposure of larger skin areas is possible.

Upon absorption of ultraviolet radiation (UVR), urocanic acid (UCA), a normal constituent of human skin, undergoes a *trans* to *cis* isomerisation. Therefore, the content of *cis*-UCA is significantly higher in both naturally and artificially ultraviolet radiation-exposed than in non-exposed skin [15]. *Cis*-UCA has been widely studied due to its immunosuppressive effects. It has been shown to be able to suppress both delayed and contact hypersensitivity, to enhance UVR-induced carcinogenesis and to prolong allograft acceptance in animal models in vivo. According to our calculations, the intensity of the UV-B radiation of FSL should be effective. However, according to the present pilot study, FSL did not induce the *cis*-UCA content of human

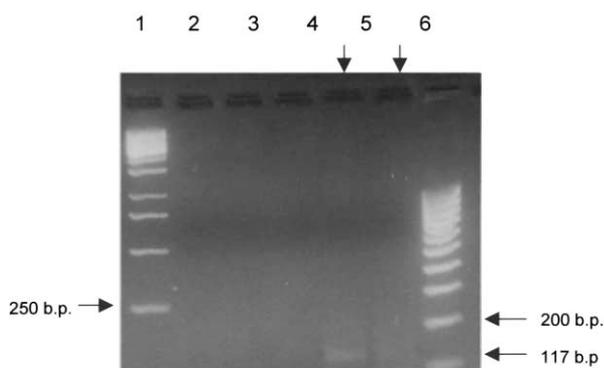


Fig. 9. FGF-7/KGF expression regulated by 17β -estradiol (10 nmol) or $1\alpha,25$ -dihydroxyvitamin D₃ (10 nmol) in LNCaP human prostate cancer cells. RT-PCR of 117 bp KGF fragment. Lanes 1 and 7, DNA ladders; lane 2, control, non-stimulated cells; lanes 3 and 5, 6 and 36 h after $1,25$ -VD stimulation; lanes 4 and 6, 6 and 36 h after estradiol stimulation.

skin during the 4-week exposure time, which suggests that the dose of UV-B was insufficient to induce the isomerisation of trans-UCA. Since *cis*-UCA formation is considered to be one of the very first photobiological events after UV-radiation exposure, we can conclude that radiation intensity should be higher than in this experiment in order to obtain more beneficial effects on serum 25-VD concentration. On the other hand, increase of skin *cis*-UCA might be harmful due to its immunosuppressive properties, and therefore its increase should be minimised. Therefore, the goal of cancer prevention with FSL would be normalisation of serum 25-VD without significant increase of *cis*-UCA. Whether this is possible remains to be shown.

It is of considerable interest that, on the basis of our epidemiological study, the risk of prostate cancer turned out to be associated with vitamin D precursor, namely 25-VD, which has a biological activity 500- to 1000-fold less than 1,25-VD [27]. However, concentrations of 25-VD typically exceed that of 1,25-VD by about 1000-fold. Therefore, it can be concluded that 25-VD might be biologically as important as 1,25-VD, both acting through VDR at their physiological concentrations. Both 25-VD and 1,25-VD have a complex substrate–product relationship. If vitamin D stores are adequate, an increasing 25-VD concentration does not elevate 1,25-VD level appreciably. Persons accidentally intoxicated with 30-fold 25-VD levels show no measurable influence on circulating 1,25-VD [27]. This might be the reason why many studies on association between 1,25-VD and cancer risk have been inconclusive [9–12].

25-VD is metabolised to the biologically more active 1,25-VD by 1α -hydroxylase (CYP27B). 1,25-VD is inactivated by 24-hydroxylase (CYP24). CYP24 has been identified as a candidate oncogene [29]. It is possible that local VD metabolism may explain the correlation between prostate cancer risk and serum VD precursor, 25-VD. Indeed, there are studies on prostate cell lines suggesting an active VD metabolism in the prostate [30]. Our preliminary studies support the idea that both rat and human prostates are able to activate and inactivate VD metabolites. It seems that the expression of the enzymes is relatively high in the prostate and may vary according to part of the prostate as well as to the physiological status. In cell lines, activity of CYP24 might be crucially important for the inhibition of proliferation by VD, since the cells with high expression show no growth inhibition by VD [31]. Estrogen and androgen are shown to stimulate expression of CYP24 [32], whereas their effect on CYP27B is minimal if any. However, modulation of VD metabolism is a promising target for cancer prevention and, therefore, further studies on human prostate are needed.

Our epidemiological study showed that a normal serum VD level prevents prostate carcinogenesis only before the andropause, suggesting that the phenomenon

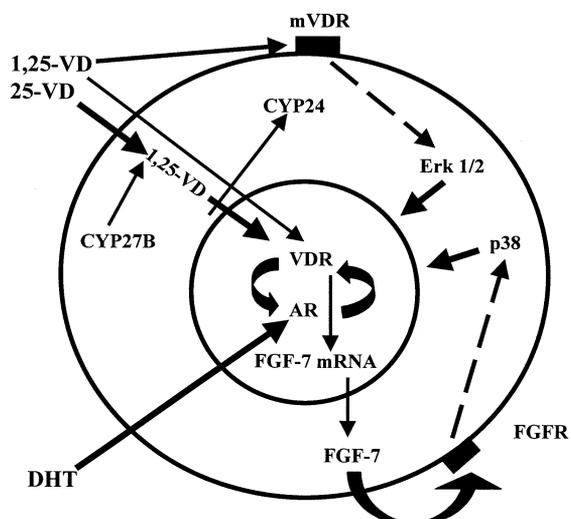


Fig. 10. Model of vitamin-D3 action in the prostate. 1,25-dihydroxyvitamin D3 (1,25-VD) may act directly on membrane or nuclear vitamin D receptor (VDR). Alternatively, 25-hydroxyvitamin D3 (25-VD) is activated by 1α -hydroxylase (CYP27B). Both metabolites are inactivated by 24-hydroxylase (CYP24). Androgens, especially dihydrotestosterone (DHT), upregulates through its cognate nuclear receptor VDR expression. Nuclear VDR regulates the fibroblast growth factor-7 (FGF-7)/keratinocyte growth factor (KGF) gene, which may act via p38 MAPK (late response). Vitamin D metabolites may act within minutes via the cell membrane on the erk-1/erk-2-MAPK system (rapid response).

is dependent on a relatively high circulating serum androgen level. When the antiproliferative action of VD is very dependent on VDR expression, it is possible that VDR expression in the prostate may vary in different physiological situation, the two key hormones being vitamin D and testosterone. Here, we demonstrate using Western blotting that VD induces clearly VDR expression at protein level, which is required for the mitotic inhibition by VD [33]. The relatively high concentration of VDR in the human and rat prostate [19] might be due to the upregulation of VDR by the high concentration of VD caused by local metabolism in the prostate as already mentioned. Recent studies showed that androgens upregulate VDR in ovarian cancer [34] as well as in prostate cancer [35]. The mechanism of how vitamin D action is dependent on VD metabolism and androgen action is depicted in Fig. 10.

1,25-VD is usually a potent inhibitor of cell proliferation, but it may either stimulate or inhibit cell proliferation, depending on the degree of cell differentiation, the concentration of vitamin D and the type of hormone metabolites [35]. In our study, 1,25-VD was inhibitory on proliferation of LNCaP cells. Therefore, it is surprising that 1,25-VD induced erk-1/erk-2 MAPK rapidly and p38 slowly, the induction being similar to that by E2. However, 2-day pretreatment with 1,25-VD decreased the E2-induced erk-1/erk-2 ac-

tivation, but not p38 activation. Possible explanation for that dual action may involve rapid (non-genomic) and slow (genomic) responses by 1,25-VD. Rapid response, mediated by Raf-Ras-MAPK cascade, was shown to include 1,25D3 triggering of specific membrane-localised molecules (possibly the membrane receptor for VD) [36]. Genomic response involves interaction of 1,25D3 with its nuclear VDR.

There are some studies on the role of VD in the regulation of growth factors [37–39]. Transforming growth factor β is one of the candidate growth factors involved in VD-induced growth and differentiation [38,40]. Our study on FGF-7/KGF was the first report on 1,25-VD induction of FGF-7 in MCF-7 cells. In the present study, we show that a similar induction occurs also in LNCaP cells. FGF-7 mRNA transcripts seem to be absent in fetal and adult prostatic tissues, and some prostatic cell lines, where FGF-7 receptor was present in cultured cells [40]. Surprisingly, 1,25-VD, an inhibitor of cell proliferation in the present study, induces expression of FGF-7, which is known to increase the proliferation of cancer cells [41,42]. Our preliminary study suggests that FGF-7/KGF could be involved in VD-dependent mitotic inhibition, since antibodies against FGF-7 added in the culture medium seem to block partially mitotic inhibition by VD (unpublished result). Because time course of the induction of p38 correlates well with FGF-7 induction by VD, it might be possible that p38 could mediate the effect of FGF-7 (slow genomic response), whereas erk-1/erk-2 could be involved in the non-genomic response (Fig. 10). However, details of our model need to be further studied in detail.

In summary, vitamin D might provide a potential treatment of prostate cancer [43] as well as an effective prevention of carcinogenesis.

Acknowledgements

This study was supported by grants from the Finnish Cancer Foundation, Medical Research Fund of Tampere University Hospital, and Tampere University. True-Lite full spectrum lamps were kindly donated by Dr. Ilkka Pekanheimo, AD-Lux, Turku, Finland. We thank Dr. K. Okuda, Miyazaki Medical College, Kiyotake, Miyazaki, Japan for the supply of the monoclonal antibody against CYP24.

References

- [1] S.L. Parker, T. Tong, S. Bolden, P.A. Wingo, Cancer statistics, *CA Cancer J. Clin.* 46 (1996) 5–27.
- [2] L. Teppo, Cancer incidence in Finland 1996 and 1997, *Cancer Soc. Finland Publ.* 61 (2000) 8.
- [3] J.M. Kozlowski, J.T. Grayhack, Carcinoma of the prostate, in: J.Y. Gillenwater, J.T. Grayhack, S.S. Howards, J.W. Duckett (Eds.), *Adult and Pediatric Urology*, 2nd ed., Mosby Year Book, St Louis, MI, 1995, pp. 1575–1713.
- [4] G.P. Studzinski, D.C. Moore, Sunlight — can it prevent as well as cause cancer?, *Cancer Res.* 55 (1995) 4014–4022.
- [5] N.H. Bell, A. Greene, S. Epstein, M.J. Oexmann, S. Shaw, J. Shary, Evidence of alteration of the vitamin D–endocrine system in blacks, *J. Clin. Invest.* 76 (1985) 470–473.
- [6] C. Gross, D. Peehl, D. Feldman, Vitamin D and prostate cancer, in: D. Feldman, F.H. Glorieux, J.W. Pike (Eds.), *Vitamin D*, Academic Press, San Diego, CA, 1997, pp. 1125–1139.
- [7] G.G. Schwartz, B.S. Hulka, Is vitamin D deficiency a risk factor for prostate cancer? (Hypothesis), *Anticancer Res.* 10 (1990) 1307–1311.
- [8] C.L. Hanchette, G.G. Schwartz, Geographic patterns of prostate cancer mortality. Evidence for a protective effect of ultraviolet radiation, *Cancer* 70 (1992) 2861–2869.
- [9] E.H. Corder, H.A. Guess, B.S. Hulka, G.D. Friedman, M. Sadler, R.T. Vollmer, B. Lobaugh, M.K. Drezner, J.H. Vogelmann, N. Orentreich, Vitamin D and prostate cancer: a prediagnostic study with stored sera, *Cancer Epidemiol. Biomarkers Prev.* 2 (1993) 467–472.
- [10] M.M. Braun, K.J. Helzlsouer, B.W. Hollis, G.W. Comstock, Prostate cancer and prediagnostic levels of serum vitamin D metabolites (Maryland, United States), *Cancer Causes Control* 6 (1995) 235–239.
- [11] P.H. Gann, J. Ma, C.H. Hennekens, B.W. Hollis, J.G. Haddad, M.J. Stampfer, Circulating vitamin D metabolites in relation to subsequent development of prostate cancer, *Cancer Epidemiol. Biomarkers Prev.* 5 (1996) 121–126.
- [12] A.M.Y. Nomura, G.N. Stemmermann, J. Lee, L.N. Kolonel, T.C. Chen, A. Turner, M.F. Holick, Serum vitamin D metabolite levels and the subsequent development of prostate cancer (Hawaii, United States), *Cancer Causes Control* 9 (1998) 425–432.
- [13] M.H. Ahonen, L. Tenkänen, L. Teppo, M. Hakama, P. Tuohimaa, Prostate cancer risk and prediagnostic serum 25-hydroxyvitamin D levels (Finland), *Cancer Causes Control* 11 (2000) 847–852.
- [14] M.H. Frick, O. Elo, K. Haapa, O.P. Heinonen, P. Heinsalmi, P. Helo, J.K. Huttunen, P. Kaitaniemi, P. Koskinen, V. Manninen, H. Mäenpää, M. Mälkönen, M. Mänttari, S. Norola, A. Pasternack, J. Pikkariainen, M. Romo, T. Sjöblom, E.A. Nikkilä, Helsinki Heart Study: primary-prevention trial with gemfibrozil in middle-aged men with dyslipidemia. Safety of treatment, changes in risk factors, and incidence of coronary heart disease, *N. Engl. J. Med.* 317 (1987) 1237–1245.
- [15] T. Mohammad, H. Morrison, H. HogenEsch, Urocanic acid photochemistry and photobiology, *Photochem. Photobiol.* 69 (1999) 115–135.
- [16] D.D. Bikle, Clinical counterpoint: vitamin D: new actions, new analogs, new therapeutic potential, *Endocr. Rev.* 13 (1992) 765–784.
- [17] R.J. Skowronski, D.M. Peehl, D. Feldman, Vitamin D and prostate cancer: 1,25-dihydroxyvitamin D3 receptors and actions in human prostate cancer cell lines, *Endocrinology* 132 (1993) 1952–1960.
- [18] D.M. Peehl, R.J. Skowronski, G.K. Leung, S.T. Wont, T.A. Stamey, D. Feldman, Antiproliferative effects of 1,25-dihydroxyvitamin D3 on primary cultures of human prostatic cancer cells, *Cancer Res.* 54 (1994) 805–810.
- [19] M. Kivineva, M. Bläuer, H. Syväälä, T. Tammela, P. Tuohimaa, Localization of 1,25-dihydroxyvitamin D3 Receptor (VDR) expression in human prostate, *J. Steroid Biochem. Mol. Biol.* 66 (1998) 121–127.

- [20] G.G. Schwarz, T.A. Oeler, M.R. Uskovic, R.R. Bahnson, Human prostate cancer cells: inhibition of proliferation by vitamin D analogs, *Anticancer Res.* 14 (1994) 1077–1082.
- [21] G.G. Schwartz, M.-H. Wang, R.K. Zhang, R.K. Singh, G.P. Siegal, $1\alpha,25$ -dihydroxyvitamin D (calcitriol) inhibits the invasiveness of human prostate cancer cells, *Cancer Epidemiol. Biomarkers Prev.* 6 (1997) 727–732.
- [22] M.S. Lucia, M.A. Anzano, M.V. Slayter, M.R. Anver, D.M. Green, M.V. Shrader, D.L. Logsdon, C.L. Driver, C.C. Brown, C.W. Peer, A.B. Roberts, M.B. Sporn, Chemopreventive activity of tamoxifen, *N*-(4-hydroxyphenyl)retinamide, and the vitamin D analogue Ro24-5531 for androgen-promoted carcinomas of the rat seminal vesicle and prostate, *Cancer Res.* 55 (1995) 5621–5627.
- [23] C.T. Jansen, K. Lammintausta, P. Pasanen, K. Neuvonen, E. Varjonen, K. Kalimo, P. Äyräs, A non-invasive chamber sampling technique for HPLC analysis of human epidermal urocanic acid isomers, *Acta Derm. Venereol.* 71 (1991) 143–145.
- [24] K. Iwata, A. Yamamoto, S. Satoh, Y. Ohyama, Y. Tashiro, T. Setoguchi, Quantitative immunoelectron microscopic analysis of the localization and induction of 25-hydroxy D3 24-hydroxylase in rat kidney, *J. Histochem. Cytochem.* 43 (1995) 255–262.
- [25] F.M. Gloth, W. Alam, B. Hollis, Vitamin D vs broad spectrum phototherapy in the treatment of seasonal affective disorder, *J. Nutr. Health Aging* 3 (1999) 5–7.
- [26] A. Lyakhovitch, N. Aksenov, P. Pennanen, S. Miettinen, M.H. Ahonen, H. Syväälä, T. Ylikomi, P. Tuohimaa, Vitamin D induced upregulation of keratinocyte growth factor (KGF/FGF-7) in MCF-7 human breast cancer cells, *Biochem. Biophys. Res. Commun.* 273 (2000) 675–680.
- [27] H. Reichel, H.P. Koeffler, A.W. Norman, The role of vitamin D endocrine system in health and disease, *N. Engl. J. Med.* 320 (1989) 980–991.
- [28] S.M. Luria, Effects of full spectrum lightning in submarines, *Government Reports Announcements and Index* 17 (1987).
- [29] D.G. Albertson, B. Ylstra, R. Segraves, C. Collins, S.H. Dairkee, D. Kowbel, W.L. Kuo, J.W. Gray, D. Pinkel, Quantitative mapping of amplicon structure by array CGH identifies CYP24 as a candidate oncogene, *Nat. Genet.* 25 (2000) 144–146.
- [30] G.G. Schwartz, L.W. Whitlatch, T.C. Chen, B.L. Lokeshwar, M.F. Holick, Human prostate cells synthesize $1,25$ -dihydroxyvitamin D₃ from 25-hydroxyvitamin D₃, *Cancer Epidemiol. Biomarkers Prev.* 7 (1998) 391–395.
- [31] G.J. Miller, G.E. Stapelton, T.E. Hedlund, K.A. Moffatt, Vitamin D receptor expression, 24-hydroxylase expression, and inhibition of growth by $1\alpha,25$ -dihydroxyvitamin D₃ in seven human prostatic carcinoma cell lines, *Clin. Cancer Res.* 1 (1995) 997–1003.
- [32] J. Omdahl, B. May, The 25-hydroxyvitamin D 24-hydroxylase, in: D. Feldman, F.H. Glorieux, J.W. Pike (Eds.), *Vitamin D*, Academic Press, San Diego, CA, 1997, pp. 69–85.
- [33] T.E. Hedlund, K.A. Moffatt, G.J. Miller, Vitamin D receptor expression is required for growth modulation by $1\alpha,25$ -dihydroxyvitamin D₃ in the human prostatic carcinoma cell line ALVA-31, *J. Steroid Biochem. Mol. Biol.* 58 (1996) 277–288.
- [34] M.H. Ahonen, Y.H. Zhuang, R. Aine, T. Ylikomi, P. Tuohimaa, Androgen receptor and vitamin D receptor in human ovarian cancer: growth stimulation and inhibition by the ligands, *Int. J. Cancer* 86 (2000) 40–46.
- [35] X.-Y. Zhao, L.H. Ly, D.M. Peehl, D. Feldman, $1\alpha,25$ -Dihydroxyvitamin D₃ actions in LNCaP human prostate cancer cells are androgen-dependent, *Endocrinology* 138 (1997) 3290–3298.
- [36] A. Norman, Rapid biological responses mediated by $1\alpha,25$ -dihydroxyvitamin D₃: a case study of transcalcachia (rapid hormonal stimulation of intestinal calcium transport), in: D.M. Feldman, F.H. Glorieux, J.W. Pike (Eds.), *Vitamin D*, Academic Press, San Diego, CA, 1997, pp. 233–256.
- [37] E. Nolan, M. Donepudi, K. VanWeelden, L. Flanagan, J. Welsh, Dissociation of vitamin D₃ and anti-estrogen mediated growth regulation in MCF-7 breast cancer cells, *Mol. Cell. Biochem.* 188 (1998) 13–20.
- [38] J.D. Haugen, M.R. Pittelkow, A.R. Zinsmeister, R. Kumar, $1\alpha,25$ -dihydroxyvitamin D₃ inhibits normal human keratinocyte growth by increasing transforming growth factor (2) release, *Biochem. Biophys. Res. Commun.* 229 (1996) 618–623.
- [39] I.M. Musiol, D. Feldman, $1,25$ -dihydroxyvitamin D₃ induction of nerve growth factor in L929 mouse fibroblasts: effect of vitamin D receptor regulation and potency of vitamin D₃ analogs, *Endocrinology* 138 (1997) 12–18.
- [40] R. Dahiya, C. Lee, P.C. Haughney, R. Chui, R. Ho, G. Deng, Differential gene expression of transforming growth factors alpha and beta, epidermal growth factor, keratinocyte growth factor, and their receptors in fetal and adult human prostatic tissues and cancer cell lines, *Urology* 48 (1996) 963–970.
- [41] K. Kiguchi, L. Beltran, T. Rupp, J. DiGiovanni, Altered expression of epidermal growth factor receptor ligands in tumor promoter-treated mouse epidermis and in primary mouse skin tumors induced by an initiation-promotion protocol, *Mol. Carcinogen.* 22 (1998) 73–83.
- [42] D.I. Kitsberg, P. Leder, Keratinocyte growth factor induces mammary and prostatic hyperplasia and mammary adenocarcinoma in transgenic mice, *Oncogene* 13 (1996) 2507–2515.
- [43] S.E. Chen, J. Wietrzyk, A. Chrobak, et al., The in vitro evaluation of 25-hydroxyvitamin D₃ and 19-nor- $1\alpha,25$ -dihydroxyvitamin D₂ as therapeutic agents for prostate cancer, *Clin. Cancer Res.* 6 (2000) 901–908.