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Vitamin D and prostate cancer prevention and treatment

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Human prostate cells contain receptors for 1α,25-dihydroxyvitamin D, the active form of vitamin D. Prostate cancer cells respond to vitamin D_3 with increases in differentiation and apoptosis, and decreases in proliferation, invasiveness and metastasis. These findings strongly support the use of vitamin D-based therapies for prostate cancer and/or as a second-line therapy if androgen deprivation fails. The association between either decreased sun exposure or vitamin D deficiency and the increased risk of prostate cancer at an earlier age, and with a more aggressive progression, indicates that adequate vitamin D nutrition should be a priority for men of all ages. Here we summarize recent advances in epidemiological and biochemical studies of the endocrine and autocrine systems associated with vitamin D and their implications for prostate cancer and in the evaluation of vitamin D_3 and its analogs in preventing and/or treating prostate cancer.

Vitamin D_2 (ergocalciferol) is derived from fungi and plants, whereas vitamin D_3 (cholecalciferol) is produced in the skin. Both forms (referred to here as vitamin D) are hydroxylated to create the active hormone. The first hydroxylation step, which forms 25(OH)D (see Glossary), occurs in the liver. 25(OH)D is then further hydroxylated at the 1α-position by 25(OH)D-1α-hydroxylase (1α-OHase, also known as CYP27B1) in the kidney to form $1\alpha,25(OH)_2D$, the active form of vitamin D (Fig. 1) [1]. The cDNAs that encode 1α-OHase in mice, rats and humans have been cloned [2,3], and $1\alpha,25(OH)_2D$ is now known to play important roles in the regulation of >60 genes, including those associated with calcium homeostasis, immune responses, blood pressure control, cell proliferation, differentiation and apoptosis. [1,3,4].

Prostate cancer is the most commonly diagnosed and the second most fatal cancer in American men. The inverse correlation ($P < 0.0001$) between the mortality rate of prostate cancer and exposure to ultraviolet radiation (UVR) in the US population, as well as the greater risk of prostate cancer in Afro-Caribbean men indicate that one precipitating factor for prostate cancer might be vitamin D insufficiency [5]. The biochemical evidence to support a role for vitamin D in prostate cancer includes the demonstration of VDR and the antiproliferative, apoptotic and prodifferentiation activities of $1\alpha,25(OH)_2D$ and its analogs in prostate cells *in vitro* and *in vivo* [6–8].

Here we summarize recent findings of: (1) the association between vitamin D deficiency, UVR exposure and the risk of prostate cancer; (2) the mechanism of $1\alpha,25(OH)_2D$ action; (3) the identification of 1α-OHase in the prostate and its implications; (4) the evaluation of antiproliferative activity of $1\alpha,25(OH)_2D_3$ and its analogs in prostate cells in culture, in animal models and in clinical trials; and (5) the controversy that surrounds the association between VDR polymorphism and the risk of prostate cancer.

Vitamin D deficiency, UV exposure and the risk of prostate cancer

An association between vitamin D deficiency and prostate cancer was reported by Ahonen *et al.* in a 13-year follow-up of 19 000 middle-aged men in the Helsinki Heart Study [9]. In this study, 149 cases of prostate cancer were identified and matched to 566 sample controls. The study showed that low circulating levels of 25(OH)D (<40 nmol l^{-1} or 16 ng ml^{-1}) were associated with an increased risk of subsequent earlier onset and more aggressive progression of prostate cancer, especially before the age of 52.

UVR exposure has a significant protective effect in prostate cancer [10,11]. Luscombe *et al.* [10] showed that cancer patients with the lowest quartile of sun exposure developed cancer at a median age of 67.7 years compared with 72.1 years in patients in other quartiles. Although the mechanism of this association is unclear, it is likely that increased cutaneous synthesis of vitamin D_3 increases the circulating levels of $25(OH)D_3$ and the subsequent formation of $1\alpha,25(OH)_2D_3$ in the prostate by prostatic 1α-OHase [12]. $1\alpha,25(OH)_2D_3$ then interacts with VDR in

Glossary

$1\alpha,25(OH)_2D_3$: $1\alpha,25$-dihydroxyvitamin D_3
$25(OH)D_3$: 25-hydroxyvitamin D_3
EB1089: Seocalcitol, $1\alpha,25$-dihydroxy-22,24-diene-24,26,27-trihomovitamin D_3
CDK: cyclin-dependent kinase
CKI: cyclin-dependent kinase inhibitor
E2F: early gene 2 factor
IGFBP: insulin-like growth factor binding protein
$p21^{waf1}$: cyclin-dependent kinase inhibitor p21Cip1/Waf1
p27: cyclin-dependent kinase inhibitor p27Kip1
p53: p53 tumor suppressor
RFLP: restriction fragment length polymorphism
VDR: vitamin D receptor
VDRE: vitamin D response element

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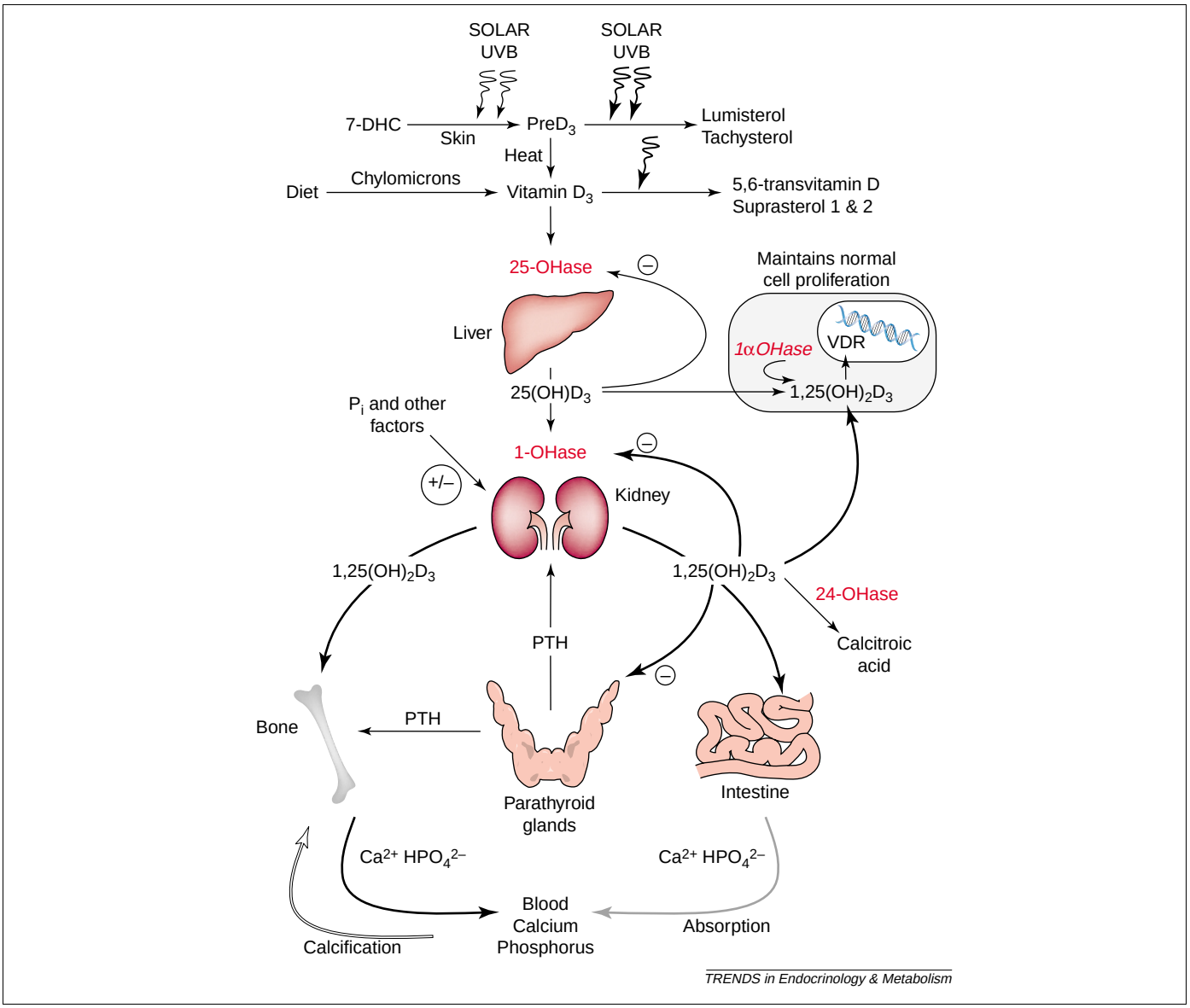

Fig. 1. Synthesis and metabolism of vitamin D_3. Vitamin D_3 is either ingested in the diet or produced in the skin after exposure to the ultraviolet B portion (UVB) of the solar spectrum (290–315 nm), which converts 7-dehydrocholesterol (7-DHC) to previtamin D_3 ($PreD_3$). The cutaneous synthesis of vitamin D_3 is inversely related to latitude, skin pigmentation and age. To be biologically active, vitamin D_3 must be hydroxylated, first in the liver by vitamin D-25-hydroxylase (25-OHase, also known as CYP27A1) to form 25-hydroxyvitamin D_3 [$25(OH)D_3$], the major circulating metabolite of vitamin D_3, and then in the kidney at the 1α-position, which is catalyzed by 25(OH)D-1α-hydroxylase (1α-OHase, also known as CYP27B1) to form 1α,25-dihydroxyvitamin D_3 [$1\alpha,25(OH)_2D_3$], the active form of vitamin D_3. Both 25-OHase and 1α-OHase are mitochondrial cytochrome P-450 enzymes. Parathyroid hormone (PTH) and low serum concentrations of phosphorus (P_i) both enhance the production of $1\alpha,25(OH)_2D_3$. Once formed, $1\alpha,25(OH)_2D_3$ regulates serum concentrations of Ca^{2+} and phosphorus by increasing the efficiency of Ca^{2+} and phosphorus absorption from the intestine and by mobilizing Ca^{2+} stores from bone. $1\alpha,25(OH)_2D_3$ also downregulates the expression of PTH and 1α-OHase in a feedback mechanism that regulates the synthesis of $1\alpha,25(OH)_2D_3$. Ultimately, vitamin D maintains Ca^{2+} and phosphorus levels within the normal serum range, to sustain a variety of metabolic functions, physiologic functions and bone health. Reproduced with permission from the *American Journal of Clinical Nutrition* © Am J Clin Nutr. American Society for Clinical Nutrition [67].

the prostate and induces cell-cycle arrest and apoptosis [6–8]. Alternatively, either vitamin D photoisomers that are produced in the skin [1] or humoral factors that are unrelated to vitamin D could be responsible for the UVR effect.

Mechanism of vitamin D action in prostate cancer cells

The antiproliferative effect of vitamin D in prostate cells is mediated through VDR, which is a member of the steroid/nuclear receptor superfamily. In target cells, VDR binds $1\alpha,25(OH)_2D$ with high affinity and specificity, and then interacts with the retinoid X receptor (RXR). This heterodimeric complex contains two characteristic zinc-finger motifs that bind to a specific DNA-sequence motif, called a vitamin D-response element (VDRE) in the promoter region of vitamin D-regulated genes and they ultimately regulate the rate of RNA polymerase II-mediated transcription of these genes (Fig. 2) [13].

Evidence that VDR is required for the antiproliferative effects of $1\alpha,25(OH)_2D_3$ in prostate cancer-cell lines has been obtained using stable transfection of cDNA that encodes the VDR into JCA-1 cells, a human prostatic carcinoma cell line [6]. This causes proportional increases in antiproliferative effects and activity of 25(OH)D-24-hydroxylase (24-OHase, also known as CYP24), a mitochondrial cytochrome P-450 enzyme, by $1\alpha,25(OH)_2D_3$. Conversely, stable transfection of antisense VDR cDNA into ALVA-31 cells derived from human prostate cancer

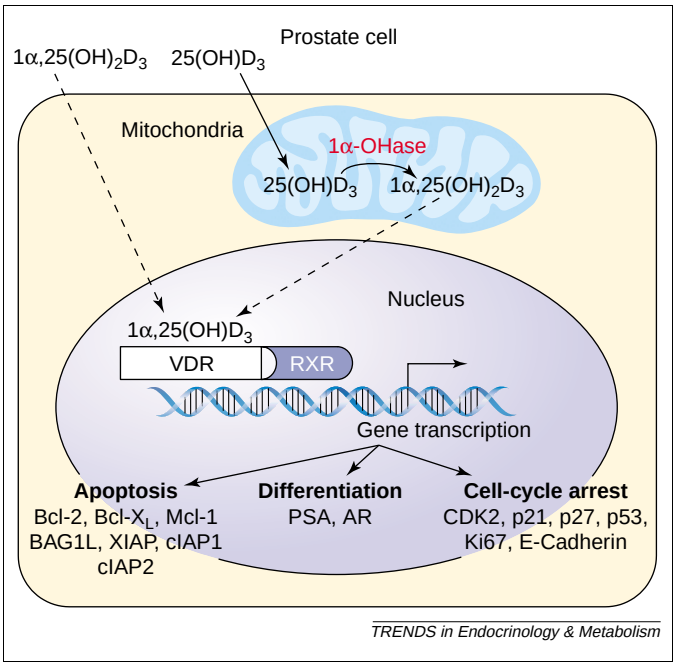

Fig. 2. Mechanism of vitamin D_3 activity. Both 25-hydroxyvitamin D_3 [25(OH)D_3] and 1α,25-dihydroxyvitamin D_3 [1α,25(OH)$_2D_3$] are transported into prostate cells. 25(OH)D_3 is then converted to 1α,25(OH)$_2D_3$ by 25(OH)D-1α-hydroxylase (1α-OHase) in mitochondria. Binding of 1α,25(OH)$_2D_3$ to the vitamin D receptor (VDR) causes the VDR to heterodimerize with the retinoid X receptor (RXR). The VDR–RXR heterodimer binds to specific vitamin D-response elements in the promoter region of vitamin D_3-responsive genes and induces gene transcription. The gene products include proteins involved in apoptosis (Bcl-2, Bcl-X_L, Mcl-1, BAG1L, XIAP, cIAP1 and cIAP2), differentiation [prostate specific antigen (PSA) and the androgen receptor (AR)], and cell-cycle regulation, including cyclin-dependent kinase (CDK), CDK inhibitors (p21 and p27), tumor suppression (p53 and E-Cadherin), and cell proliferation-associated nuclear antigen (Ki 67).

attenuates the ability of 1α,25(OH)$_2D_3$ to inhibit cell growth and induce 24-OHase [6].

Antiproliferative actions

The precise pathways through which 1α,25(OH)$_2$D transduces signals in prostate cells are less well understood. Recent studies indicate that 1α,25(OH)$_2$D might act through different pathways to inhibit cell proliferation in different cell types. For example, LNCaP cells, which are androgen-sensitive prostate-cancer cells, accumulate in the G0–G1 phase of the cell cycle after treatment with 1α,25(OH)$_2D_3$ [8,14] but no such accumulation is observed in ALVA-31 and PC-3 cells, even though the growth of both is inhibited by 1α,25(OH)$_2D_3$ [14]. 1α,25(OH)$_2D_3$-induced cell-cycle arrest of LNCaP cells in the G0–G1 phase involves decreased phosphorylation of the retinoblastoma gene-encoded protein (Rb). This is followed by a reduction in the activity of the E2F transcription factor, which leads to increased activity of $p21^{waf1}$, the CDK inhibitor, and decreased CDK2 activity. 1α,25(OH)$_2D_3$-induced arrest of LNCaP cells in G0 might also require functional p53 [15]. In p53-negative PC-3 cells and a line of LNCaP cells (called LN-56) in which p53 function is impaired by stable transfection with the genetic suppressor element 56, 1α,25(OH)$_2D_3$ does not cause G0 arrest. This allows these cells to quickly regain normal growth capabilities when 1α,25(OH)$_2D_3$ is withdrawn from the media [15]. Although abrogating Rb function with the SV40 large-T antigen compromises the ability of 1α,25(OH)$_2D_3$ to inhibit the growth of prostate-cancer cells [7], 1α,25(OH)$_2D_3$ does inhibit prostate-cell growth in a G^{γ}/T-15 transgenic mouse line that contains the human fetal globin promoter linked to the SV40 large-T antigen [16]. Moreover, the growth of DU145 cells, which lack functional Rb and have high levels of 24-OHase, is inhibited by 1α,25(OH)$_2D_3$ in the presence of Liarozole [an inhibitor of 24-OHase that prevents the 24-hydroxylation of 1α,25(OH)$_2D_3$ and so prolongs the half-life of 1α,25(OH)$_2D_3$ [7]. These findings indicate that 1α,25(OH)$_2D_3$-mediated growth inhibition in DU145 cells and in cell lines generated using the SV40 large-T antigen might be mediated by an alternative mechanism.

Apoptotic actions

Under some experimental conditions 1α,25(OH)$_2$D also induces apoptosis in LNCaP cells [8,15,17,18]. Using the terminal deoxynucleotide transferase-mediated dUTP nick end labeling (TUNEL) assay followed by flow cytometric analysis to quantify DNA fragmentation, Blutt *et al.* [18] have observed apoptosis after treating LNCaP cells with 1α,25(OH)$_2D_3$. This is accompanied by downregulation of two antiapoptotic proteins, Bcl2 and $BclX_L$, and is prevented by overexpression of the gene that encodes Bcl2. Other antiapoptotic proteins (Mcl-1, BAG1L, XIAP, cIAP1 and cIAP2) are also downregulated by 1α,25(OH)$_2D_3$ in LNCaP cells but proapoptotic Bax and Bak are unaltered [17]. This downregulation leads to the activation of caspase-3 and caspase-9, the apical proteases in the mitochondrial pathway for apoptosis [17]. Neither apoptosis nor changes in synthesis of pro-apoptotic protein have been observed in DU145 cells treated with 1α,25(OH)$_2D_3$. Thus, both growth arrest and apoptosis are involved in growth regulation of LNCaP cells in response to 1α,25(OH)$_2D_3$.

Interaction between vitamin D and other hormones

1α,25(OH)$_2$D does not act alone in regulating prostate cell proliferation. RARs and ARs are involved in regulating the growth of some cancer cell lines [7]. Weigel and associates [8] have demonstrated that 1α,25(OH)$_2D_3$ and 9-*cis* RA, a ligand of RXR, act synergistically to inhibit the growth of LNCaP cells and cause cells to accumulate in G0. This appears to be dependent on functional p53 [15]. Zhao *et al.* showed that 1α,25(OH)$_2D_3$ and 9-*cis* RA increase the expression of mRNA that encodes the androgen receptor (AR) and act synergistically to inhibit LNCaP cell growth [19]. Because both actions are prevented by the pure AR antagonist, Casodex, they concluded that growth inhibition of LNCaP cells by 1α,25(OH)$_2D_3$ and/or 9-*cis* RA is mediated by an AR-dependent mechanism and preceded by the induction of AR gene expression. To re-examine the role of androgens in the antiproliferative effects of 1α,25(OH)$_2D_3$ in prostate cancer cells, Yang *et al.* [20] have utilized two androgen-independent cell models of prostate cancer, ALVA-AR and LNCaP-104R1, that contain functional ARs and VDRs. They found that neither growth of ALVA-AR nor of control ALVA-NEO cells is inhibited substantially by 1α,25(OH)$_2D_3$ either in the presence or absence of androgen, which indicates that the resistance of ALVA-AR to 1α,25(OH)$_2D_3$-mediated growth inhibition is not caused by lack of AR. They also found that

$1\alpha,25(OH)_2D_3$ inhibits the growth of LNCaP-104R1 cells by increasing the concentration of P27 and its subsequent association with CDK2, which leads to an increase in the proportion of cells in the G0–G1 phase of the cell cycle in the absence of androgen. This effect is not blocked by Casodex, which indicates that AR is not required for the effects of $1\alpha,25(OH)_2D_3$ in LNCaP-104R1 cells. Thus, $1\alpha,25(OH)_2D_3$ can inhibit the growth of prostate-cancer cells by both androgen-dependent and androgen-independent mechanisms [7].

Prodifferentiation and other actions

In addition to inhibiting cell growth and causing apoptosis, $1\alpha,25(OH)_2D_3$ stimulates the secretion of prostate specific-antigen (PSA) in LNCaP cells [6,7] and the expression of E-cadherin [21], a tumor-suppressor gene. It also inhibits angiogenesis [22] and reduces the invasiveness of DU-145 prostate cancer cells in an *in vitro* cell-invasion model [23].

Autocrine function of 1α-OHase

The inverse relationship between lower serum levels of $1\alpha,25(OH)_2D$ and higher prostate cancer risk documented in initial reports [24] have not been observed by other investigators [7,8]. This discrepancy highlights the possible importance of intraprostate concentrations of $1\alpha,25(OH)_2D$ rather than serum levels as the risk factor for prostate cancer. Under physiological conditions, $1\alpha,25(OH)_2D$ in the serum is produced mainly by the renal 1α-OHase, which is tightly regulated [1,3]. The levels of $1\alpha,25(OH)_2D$ do not fluctuate significantly with changing levels of serum 25(OH)D, except during vitamin D insufficiency [1]. Therefore, it is difficult to understand why vitamin D deficiency with low circulating levels of 25(OH)D and normal $1\alpha,25(OH)_2D$ levels is associated with the rate of prostate cancer mortality. One explanation is that prostate cells contain 1α-OHase that converts 25(OH)D to $1\alpha,25(OH)_2D$ locally. Thus, the concentration of $1\alpha,25(OH)_2D$ in the prostate might be influenced by the serum level of 25(OH)D.

Extrarenal synthesis of $1\alpha,25(OH)_2D$ from 25(OH)D in, for example, skin and activated macrophages is well known [1,3], and it is now recognized that two human prostate cancer cell lines, DU145 and PC-3, as well as cells derived from a normal prostate and a prostate with BPH also have 1α-OHase activity and synthesize $1\alpha,25(OH)_2D_3$ from $25(OH)D_3$. However, 1α-OHase activity has not been detected in LNCaP cells [12].

Comparing the activity of 1α-OHase in primary cultures of prostate epithelial cells derived from four patients with prostate cancer (CaP), two BPH patients and three normal donors demonstrates that the normal cultures had an average activity of 3.0 ± 0.36 pmol mg protein^{-1} h^{-1} (mean ± SD), whereas BPH and prostate cancer cultures had an average activity of 1.2 ± 0.28 and 0.46 ± 0.15 pmol mg protein^{-1} h^{-1}, respectively. Therefore, compared with primary cultures of normal prostate cells, enzyme activity is 60% and 85% lower in the primary cultured BPH and prostate cancer cells, respectively [25,26]. Similar results have been reported by Hsu *et al.* [27]. These findings have important implications and indicate that the loss of 1α-OHase activity might be associated with the initiation and progression of prostate cancer.

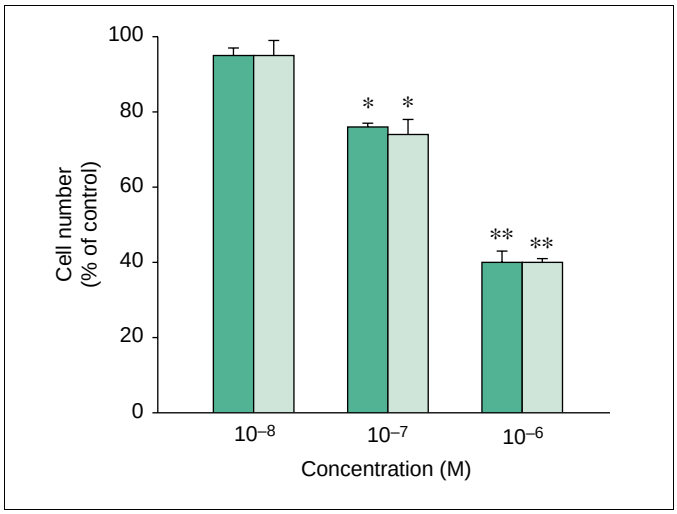

Fig. 3. Effect of $1\alpha,25(OH)_2D_3$ (dark green) and $25(OH)D_3$ (light green) on cell proliferation in primary cultures of prostate cells. Data are mean ± SD of nine determinations. *$P < 0.05$, ** $P < 0.001$ versus controls. There is no significant difference between $1\alpha,25(OH)_2D_3$ and $25(OH)D_3$ at the doses studied. Reproduced with permission from *Clinical Cancer Research* [28].

The importance of 1α-OHase in regulating the growth of prostate cells is substantiated by the findings that both $1\alpha,25(OH)_2D_3$ and $25(OH)D_3$ cause a dose-dependent growth inhibition of the primary cultured cells derived from human prostate tissue (Fig. 3) [27–29]. Because $25(OH)D_3$ has relatively low binding affinity for the VDR [1/500 that of $1\alpha,25(OH)_2D_3$], it has little or no antiproliferative activity in cells with little or no 1α-OHase activity, such as LNCaP cells [30]. The most likely explanation for the $25(OH)D_3$ response in the primary cell cultures is that $25(OH)D_3$ is converted to $1\alpha,25(OH)_2D_3$ by an 1α-OHase that is present in prostate cells.

To further investigate the association between the loss of 1α-OHase activity and prostate cancer, we transfected LNCaP cells with a human 1α-OHase–green fluorescent protein (GFP) fusion construct to confirm that the protein is expressed and appears in the mitochondria (Fig. 4). Alternatively, cells were transfected with cDNA encoding human 1α-OHase to study their responses to 25(OH)D (Fig. 5). Transient and stable transfection markedly increases the activity of 1α-OHase and so confers inhibition of cell growth by $25(OH)D_3$ (Fig. 5) [26].

Are vitamin D analogs useful for treating prostate cancer?

Numerous reports demonstrate that $1\alpha,25(OH)_2D_3$ stimulates differentiation and inhibits the proliferation, invasiveness and metastasis of prostate cancer cells [6–8,21–23]. In addition, $1\alpha,25(OH)_2D_3$ and its synthetic analogs prolong survival time in murine models of leukemia, and have been used successfully for treating psoriasis [1]. These findings strongly support the use of $1\alpha,25(OH)_2D_3$ and its analogs to treat prostate cancer and/or $1\alpha,25(OH)_2D_3$ as a second line of therapy when androgen deprivation fails. However, the results of several clinical trials indicate that the dose of $1\alpha,25(OH)_2D_3$

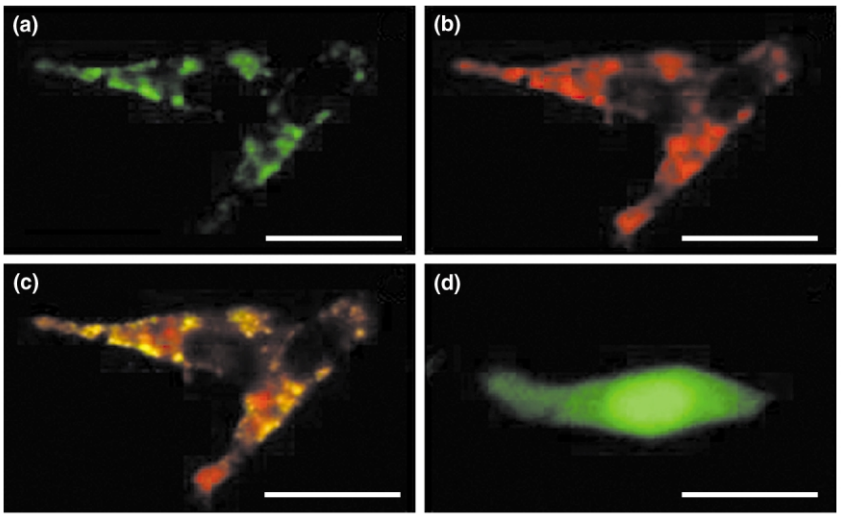

Fig. 4. Location of a fusion protein between 25-hydroxyvitamin D_3-1α-hydroxylase (1α-OHase) and green fluorescent protein (GFP) (1α-OHase–GFP) in LNCaP cells. (a-c) LNCaP cells were transfected with either the 1α-OHase–GFP plasmid or (d) GFP plasmid for 24 hours. Cells transfected with 1α-OHase–GFP were treated with MitoTracker Orange (400 nM) for 15 min and observed live with scanning laser confocal microscopy (600 ×). (a) The green fluorescence (530-nm filter) is perinuclear and punctuate, consistent with localization in the mitochondria. (b) The cell in (a), stained with the mitochondria-specific red fluorescent indicator MitoTracker (580-nm filter). (c) images (a) and (b) superimposed. Colocalization of the 1α-OHase–GFP green fluorescence with the mitochondrial red fluorescence, which appears yellow-green, confirms that 1α-OHase–GFP is synthesized in the mitochondria. (d) A live LNCaP cell transfected with the control GFP plasmid, viewed using a fluorescein filter. There is uniform green fluorescence throughout the cytoplasm, consistent with synthesis of GFP in the cytoplasm. Scale bar, 50 μm. Reproduced with permission from [26].

cannot be increased to >0.5 μg twice a day because of hypercalcemia and hypercalciuria [7,8,31,32]. However, the time taken for PSA to double is at least doubled after treatment with 1α,25$(OH)_2D_3$ [32]. Therefore, analogs of 1α,25$(OH)_2D_3$ that have less calcemic activity and more potent antiproliferative and prodifferentiatory activity are attractive therapeutic agents.

During the past two decades, >2000 analogs of 1α,25$(OH)_2D$ have been synthesized chemically [31,33] and their biological properties evaluated systematically in a variety of assay systems, with the goal of enhancing their antiproliferative and prodifferentiating activities, and reducing or eliminating their calcemic effects [7,21,28–35]. Several studies have been published that investigate the *in vivo* response of vitamin D analogs in prostate cancer [35–38]. In general, the analogs are either slightly more potent than or equipotent to 1α,25$(OH)_2D_3$, but slightly less calcemic than 1α,25$(OH)_2D_3$. A phase I trial of 1α-hydroxyvitamin D_2 in patients with advanced, hormone-refractory prostate cancer has been conducted, which shows that five out of 25 patients achieved disease stabilization for ≥6 months, with main toxicities being hypercalcemia and renal insufficiency [39]. So far, no analogs of 1α,25$(OH)_2D_3$ effectively prevent or inhibit prostate-cancer growth without significant calcemic side-effects.

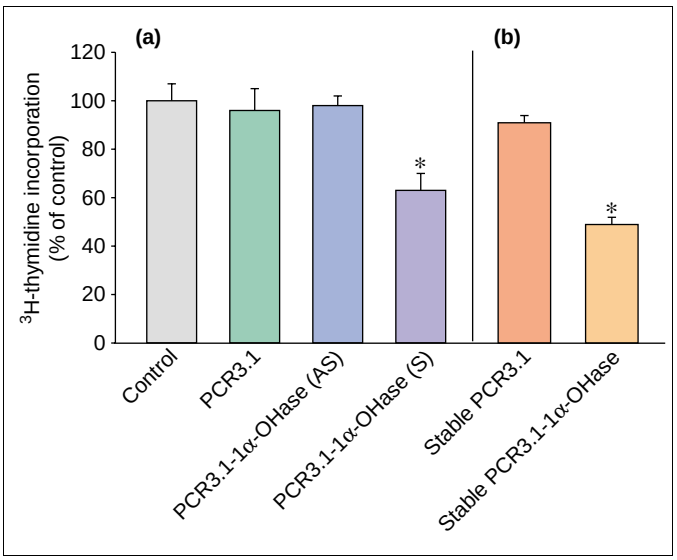

Fig. 5. Effect of 25-hydroxyvitamin D_3 [25$(OH)D_3$] (10^{-8} M) on the incorporation of ^{3}H-thymidine into DNA of LNCaP cells transfected with cDNA encoding 25(OH)D-1α-hydroxylase (1α-OHase). (a) LNCaP cells were transfected transiently with PCR 3.1 vector, antisense (AS) or sense (S) 1α-OHase cDNA. (b) LNCaP cells were stably transfected with either PCR 3.1 vector or with sense 1α-OHase cDNA. Data are presented as % of mock transfected control in the absence of 25$(OH)D_3$. Data are mean ± SD, n = 8, *$P < 0.05$. Reproduced with permission from [26].

Another approach to decreasing the side-effects of 1α,25$(OH)_2D_3$ and increasing its antiproliferative potency is to use 1α,25$(OH)_2D_3$ in combination with other agents, such as retinoids [8], platinum compounds [40], inhibitors of histone deacetylase (sodium butyrate and trichostatin A) [41] and docetaxel [42]. It has been shown that 1α,25$(OH)_2D_3$ and cisplatin, the most widely used platinum-based chemotherapeutic agent, act synergistically to inhibit the growth of PC3 and DU-145 cancer cells [40], and that cisplatin enhances 1α,25$(OH)_2D_3$-induced apoptotic signaling through mitogen-activated protein kinase kinase kinase (MEKK-1) [43]. Synergistic growth inhibition of LNCaP, PC-3 and DU-145 prostate cancer cells by 1α,25$(OH)_2D_3$ and its 19-nor-hexafluoride analogs in combination with either sodium butyrate or trichostatin A has also been observed [41]. The mechanism, which involves histone deacetylation, appears to induce apoptosis by restoring the normal 1α,25$(OH)_2D_3$-mediated pro-apoptotic signals that are lost during prostate cancer development. The combination of a weekly, oral high-dose (0.5 μg kg^{-1}) of calcitriol and weekly docetaxel

(36 mg m^{-2}) is well tolerated and effective in achieving a PSA response in 30 out of 37 metastatic, androgen-independent prostate-cancer patients [42].

VDR polymorphism and prostate cancer risk

Following the initial observation that indicated an association between polymorphisms in the VDR gene and the risk of osteoporosis [44], many studies have examined whether the same polymorphisms are related to the risk of prostate cancer [45]. Polymorphisms have been identified in exons 2, 8, and 9 of the VDR gene, and involve FokI, BsmI, and TaqI RFLPs, respectively. The FokI RFLP generates a VDR with three additional amino acids at the N terminus, whereas the BsmI, and TaqI RFLPs do not affect the coding sequence. A microsatellite polymorphism in the 3′-untranslated region that does not alter the VDR coding sequence has also been identified. Following the first report that indicated a positive association between TaqI RFLP and prostate-cancer risk in a study from North Carolina [46], there have been at least seven other studies that show a positive association between prostate cancer and TaqI [47–49], BsmI [50–51], FokI, [52] and poly-A microsatellite [51,53] polymorphisms (Table 1). However, the associations between prostate-cancer risk and polymorphisms are called into question in a similar number of studies [54–60]. There are several explanations for these conflicting findings. For example, they could be caused by: (1) differences in the selection of the patient and control groups; (2) limitations in the sample size; (3) inadequate control of confounding factors; and (4) variation in the prevalence of environmental risk factors and etiological factors across populations. In addition, the skin types of patients and controls that determine the pigmentation response and vitamin D_3 synthesis in response to solar UVB irradiation has not been identified, and this might be crucial for determining the outcome of polymorphism studies [61].

Regarding gene–gene interaction, the combined effects of the insulin-like growth factor (IGF) system and vitamin D on prostate cancer risk have been investigated in a population-based case-control study in Shanghai, China [59]. No significant association was observed between either BsmI or FokI polymorphisms in the VDR gene and prostate cancer risk. However, there was a decreased risk of prostate cancer in men with the highest tertile of plasma IGFBP-1 or -3 who have the ff FokI genotype but not FF and Ff genotypes. These results indicate that the IGF and vitamin D systems might interact to affect prostate cancer risk [62].

Conclusion and perspectives

It has been known for more than two decades that $1\alpha,25(OH)_2D_3$ is one of the most effective compounds for inhibiting proliferation and inducing terminal differentiation of normal and cancer cells that contain VDRs, including prostate cells. There has been progress in understanding how $1\alpha,25(OH)_2D_3$ inhibits cell growth and causes apoptosis in LNCaP cells but not in cells from other cancer cell lines, primary cultures and *in vivo*. More studies are required to examine the *in situ* interaction between vitamin D and other hormones and/or growth factors in the prostate.

If a positive association between polymorphisms in the VDR gene and prostate-cancer risk is established, the VDR genotype could potentially be used to identify men who are more likely to develop clinically significant prostate cancer and to intervene in these men to reduce the morbidity and mortality that result [63].

It is also recognized that an increase in the incidence and mortality of many common solid tumors, including prostate cancer is associated with both limited exposure to sunlight and vitamin D deficiency [1,9–11,64]. However, the exact association between latitude, sun exposure and increased concentrations of 25(OH)D was not well understood until the relatively recent observation that prostate cells contain the enzyme that converts 25(OH)D to $1\alpha,25(OH)_2D$ [12]. Synthesis of $1\alpha,25(OH)_2D$ in the prostate indicates that increasing circulating levels of 25(OH)D, either by adequate exposure to sunlight or oral supplementation, might provide a simple way to increase

Table 1. Polymorphisms in the vitamin D receptor gene and prostate cancer risk

Location	No. cases/controls	Polymorphism	Odds ratio	95% CI	Refs
Positive association					
North Carolina	108/170	TaqI	0.34	0.16–0.76 ($P < 0.01$)	[46]
France/Germany	105/132	TaqI	0.5	0.27–0.92 ($P < 0.026$)	[47]
Japan	115/133	TaqI	2.52	1.21–5.27 ($P < 0.009$)	[48]
Portugal	163/211	TaqI	2.11	1.15–3.88 ($P < 0.015$)	[49]
Los Angles	151/174	BsmI/Poly-A	0.7	0.3–1.6	[50]
Japan	222/326	BsmI	3.31	2.05–5.32 ($P < 0.0001$)	[51]
		Poly–A	0.44	0.198–0.966 ($P < 0.041$)	[51]
Los Angles	57/169	Poly–A	4.61	1.34–15.82	[53]
California	191/191	FokI	0.43	0.428–0.438 ($P = 0.015$)	[52]
Negative association					
US	372/591	BsmI, Taq I	0.86–0.92	0.57–1.29	[54]
Maryland	41/41	Poly-A	1.3	0.4–4.3	[55]
		Taq I	0.7	0.2–2.6	[55]
Japan	60/60	TaqI	1.3	0.6–2.8	[56]
Japan	100/202	TaqI	0.9	0.67–1.01	[57]
		Poly-A	0.9	0.67–1.01	[57]
North Carolina	77/183	Taq I	1.4	0.7–2.8	[58]
		Poly-A	1.2	0.6–2.5	[58]
China	191/304	BsmI, FokI	1.01–1.13	0.3–3.67	[59]
Austria	190/190	Taq I	1.76	0.9–3.45 ($P = 0.07$)	[60]

synthesis of 1α,25(OH)$_2$D in the prostate and, therefore, decrease the risk of prostate cancer. Chronic vitamin D insufficiency in young and middle-aged men [65] might increase their risk of prostate cancer. Similar to the recommendation that men >50 years of age should be screened for PSA, surveillance of serum 25(OH)D should be performed annually in men >30 years, especially those who are at higher risk of chronic vitamin D deficiency, such as African Americans and indoor workers. Thus, adequate vitamin D nutrition should be maintained, not only for bone health in men and women, but because it might decrease the risk of prostate cancer in men and mitigate metastatic activity should it develop.

The knowledge that the prostate synthesizes 1α,25(OH)$_2$D and that prostate-cancer cells respond to 1α,25(OH)$_2$D offers new strategies to help reduce the incidence of this devastating disease. The promising results of EB1089 in treating liver cancer offers hope that noncalcemic analogs of 1α,25(OH)$_2$D$_3$ can be developed that might be combined with other chemo-preventing agents to treat prostate cancer without serious side-effects [66].

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