

The Role of Vitamin D in Prostate Cancer

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Abstract

Prostate cancer (PCa) cells harbor receptors for vitamin D (VDR) as well as androgens (AR). 1,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃] increases AR expression and enhances androgen actions linking the two receptor systems. 1,25(OH)₂D₃ exhibits antiproliferative activity in both AR-positive and AR-negative PCa cells. Less calcemic analogs of 1,25(OH)₂D₃, with more antiproliferative activity, are being developed and will be more useful clinically. The mechanisms underlying differential analog activity are being investigated. In target cells, 1,25(OH)₂D₃ induces 24-hydroxylase, the enzyme that catalyzes its self-inactivation. Co-treatment with 24-hydroxylase inhibitors enhances the antiproliferative activity of calcitriol. Primary cultures of normal or cancer-derived prostatic epithelial cells express α -hydroxylase, the enzyme that catalyzes the synthesis of 1,25(OH)₂D₃, the levels being much lower in the cancer-derived cells and in PCa cell lines. This finding raises the possibility of using 25-hydroxyvitamin D₃ [25(OH)D₃] as a chemopreventive agent in PCa. In LNCaP human PCa cells, 1,25(OH)₂D₃ and its analogs exert antiproliferative activity predominantly by cell cycle arrest, but also induce apoptosis, although to a much lesser degree. Growth arrest is mediated by induction of IGF binding protein-3 (IGFBP-3), which in turn increases the expression of the cell cycle inhibitor p21, leading to growth arrest. Other actions of 1,25(OH)₂D₃ in PCa cells include promotion of pro-differentiation effects and inhibition of tumor cell invasion, metastasis and angiogenesis. Combination therapy with retinoids, other anticancer agents or 24-hydroxylase inhibitors augments the inhibitory activity of 1,25(OH)₂D₃ in PCa and provides another effective approach in PCa treatment. Small clinical trials have shown that 1,25(OH)₂D₃ can slow the rate of prostate specific antigen (PSA) rise in PCa patients, demonstrating proof of concept that 1,25(OH)₂D₃ or its analogs will be clinically effective in PCa therapy. Current research involves further investigation of the role of 1,25(OH)₂D₃ and its analogs for the therapy or chemoprevention of PCa.

Introduction

1 α ,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃), the active form of vitamin D, is a secosteroid hormone that plays an important role in calcium and phosphate homeostasis through its actions in the intestine, kidney, bone and parathyroid glands (Feldman et al. 2001). In addition to its classic actions on calcium regulation, 1,25(OH)₂D₃ also exerts antiproliferative and pro-differentiating effects in many tumors and malignant cells, including prostate cancer cells (van Leeuwen and Pols 1997). PCa is the second most common malignancy in American men after skin cancer and causes approximately 40,000 deaths per year. Circulating androgens promote PCa growth and androgen deprivation therapy remains the mainstay of PCa treatment (Hellerstedt and Pienta 2002). However, many patients eventually fail this therapy and develop androgen-independent PCa (AIPC) and metastatic disease that is not amenable to current therapies (Denmeade and Isaacs 2002). One of the goals of current research on PCa is the elucidation of pathways by which AIPC develops and the identification of new agents that would prevent and/or slow down the progression of AIPC (Feldman and Feldman 2001). In recent years, 1,25(OH)₂D₃ has emerged as a promising therapeutic agent in the treatment of prostate cancer (PCa) (Gross et al. 1997; Blutt and Weigel 1999; Konety et al. 1999; Miller 1999; Feldman et al. 2000). This concept is based on a large body of evidence from epidemiological, cell culture, animal and clinical studies that shows anti-tumor activity of vitamin D in PCa.

Vitamin D Metabolism and Action

The first step in the synthesis of the active form of vitamin D begins in the skin (Feldman et al. 2001). Ultraviolet (UV) irradiation converts the precursor 7-dehydrocholesterol to cholecalciferol (vitamin D₃). Dietary forms of vitamin D include calciferol in supplemented milk, ergocalciferol (vitamin D₂) in plants, and vitamin D₃ in animal products. Endogenously synthesized and dietary vitamin D are transported to the liver, where they are hydroxylated at the C-25 position to form the pro-hormone, 25-hydroxyvitamin D₃ (25(OH)D₃) (Feldman et al. 2001). The active hormone 1,25(OH)₂D₃ is then synthesized in the kidney by the hydroxylation of 25(OH)D₃ at the C-1 position by the enzyme 25-hydroxyvitamin D₃ 1 α -hydroxylase (1 α -hydroxylase). 1,25(OH)₂D₃ initiates its actions in target cells by binding to the vitamin D receptor (VDR), which is a member of the steroid hormone receptor gene superfamily (Mangelsdorf et al. 1995). Ligand binding activates the VDR, which forms a heterodimer with the retinoid X receptor (RXR). The VDR-RXR dimer binds to vitamin D response elements (VDRE) in the promoters of vitamin D target genes and regulates their expression (Malloy et al. 1999). Transcriptional activation by nuclear hormone receptors such as VDR requires their interactions with coactivators and corepressors (Hausler et al. 1998). In

target cells 1,25(OH)₂D₃ induces the expression of the enzyme 25-hydroxyvitamin D₃ 24-hydroxylase (24-hydroxylase), which catalyzes the initial step in the conversion of the active molecule 1,25(OH)₂D₃ into less active metabolites.

Vitamin D and Prostate Cancer

Epidemiological Studies

Several epidemiological studies have found correlations between exposure to UV irradiation and PCa risk. Schwartz and Hulka have demonstrated that mortality rates from prostate cancer in the US are inversely correlated with UV irradiation (Schwartz and Hulka 1990). Race is also a risk factor for prostate cancer, with the clinical incidence in American Blacks twice that of Caucasians. This may also be related to vitamin D insufficiency because high melanin levels in darkly pigmented skin block UV irradiation and inhibit the formation of vitamin D₃ (Mittra and Bell 1997). Although some studies suggest that lower serum 1,25(OH)₂D₃ levels are a risk factor for PCa (Corder et al. 1993), others do not support this hypothesis (Gross et al. 1997; Blutt and Weigel 1999; Konety et al. 1999; Miller 1999; Feldman et al. 2000).

VDR Polymorphisms and Prostate Cancer Risk

Several polymorphisms have been identified in the VDR gene (Uitterlinden et al. 2001). Some of these polymorphisms may contribute to PCa risk, although not all studies could confirm this finding (reviewed in Feldman 1997; Ingles et al. 1998). The role of VDR polymorphisms in diseases such as osteoporosis and PCa is being actively investigated and it is proposed that differences in the functional activity of the different VDR alleles might contribute to the risk of these diseases (Jurutka et al. 2000).

Vitamin D Actions in Prostate Cells

There is strong evidence supporting a role for 1,25(OH)₂D₃ as a critical regulator of growth and differentiation in the prostate (Gross et al. 1997; Blutt and Weigel 1999; Konety et al. 1999; Miller 1999; Feldman et al. 2000). 1,25(OH)₂D₃ inhibits the growth of primary prostatic epithelial cells derived from normal tissue benign prostatic hyperplasia (BPH) and cancer (Peehl et al. 1994). The well-known human PCa cell lines such as LNCaP, PC-3 and DU 145 (Skowronski et al. 1993) as well as other human PCa cell lines such as ALVA 31, PPC-1 (Miller et al. 1995) and MDA PCa 2a and 2b (Zhao et al. 2000) also respond to 1,25(OH)₂D₃ with growth inhibition. Interestingly, the vitamin D-mediated growth inhibition in the primary prostatic epithelial cells appears

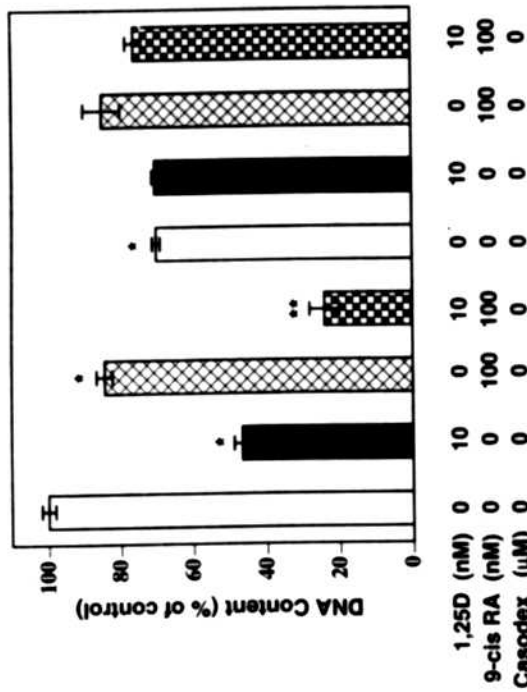


Fig. 1 Effect of casodex on 1,25(OH)₂D₃ and 9-cis RA -induced growth inhibition of LNCaP cells. LNCaP cells were treated with ethanol vehicle, 10 nM 1,25(OH)₂D₃, 100 nM 9-cis RA individually or together in the presence or absence of Casodex at 1 µM, for 6 days. Cellular DNA contents were determined by Burton's method. The data are expressed as percent of control, a mean of three triplicate samples ± SEM; **p* < 0.01 compared with vehicle treated control group. [Reproduced with permission from Zhao et al. (1999) *Endocrinology* 140:1205-1212]

to be irreversible (Peehl et al. 1994). Among the well-studied PCa cell lines, 1,25(OH)₂D₃ exerts a substantial inhibitory effect on LNCaP cell growth, has an intermediate effect to inhibit PC-3 cells and only minimally inhibits DU 145 cells (Skowronski et al. 1993).

Vitamin D and Androgen Interactions

Androgens acting through the androgen receptor (AR) regulate prostate growth and play an important role in the development and progression of PCa (Hellerstedt and Pienta 2002). Studies from our lab (Zhao et al. 1997b) have shown that there is cross-talk between 1,25(OH)₂D₃ and androgen signaling in the androgen-responsive LNCaP human PCa cells. 1,25(OH)₂D₃ up-regulates AR gene expression at both mRNA and protein levels in LNCaP cells, and consequently the secretion of PSA by these cells is synergistically enhanced when the cells are exposed to a combination of androgens and 1,25(OH)₂D₃ (Zhao et al. 1999). The antiproliferative action of 1,25(OH)₂D₃ in LNCaP cells appears to be androgen dependent. As shown in Fig. 1, the 1,25(OH)₂D₃-mediated growth inhibition could be blocked by the AR antagonist Casodex (Zhao et al. 1997b). The retinoid X receptor (RXR) ligand 9-cis retinoic acid (9-cis RA) also inhibits LNCaP cell growth and a combination of 1,25(OH)₂D₃ and 9-cis RA shows a synergistic enhancement of the growth inhibition (Zhao et al.

1997b). This androgen-dependent mechanism of 1,25(OH)₂D₃ action may be specific to LNCaP cells because 1,25(OH)₂D₃ also inhibits the growth of other PCa cells, which do not express the AR (Skowronski et al. 1993; Peehl et al. 1994). Zhao et al. (2000) have shown that 1,25(OH)₂D₃ inhibits the growth and up-regulates AR expression in MDA PCa 2a and 2b cells that were recently established from the bone metastasis of a patient who exhibited advanced AIPC. In contrast to LNCaP, the growth inhibitory action of 1,25(OH)₂D₃ in the MDA PCa cells appears to be androgen independent (Zhao et al. 2000). Importantly, these findings support the potential therapeutic role of vitamin D in the treatment of AIPC.

Vitamin D Analogs

The concentrations of 1,25(OH)₂D₃ required to produce a significant antiproliferative effect in vivo (see "Clinical Studies" below) cause hypercalcemia and/or hypercalciuria and renal stone formation, which limits its therapeutic use (Gross et al. 1998). Therefore, a number of structural analogs with less calcemic activity and equal or greater antiproliferative activity than 1,25(OH)₂D₃ have been developed (Feldman et al. 1997; Hisatake et al. 1999). We compared the biological actions of some of these analogs on LNCaP cells to those of 1,25(OH)₂D₃ (Skowronski et al. 1995). Several analogs exhibited up to fourfold greater inhibitory activity than 1,25(OH)₂D₃. Interestingly, we noted that the potency of antiproliferative activity did not directly correlate with affinity of the analogs for VDR, indicating that other factors are involved (Zhao et al. 1997a). Several mechanisms appear to mediate the differential activity of the analogs (Feldman et al. 1997) and include differences in pharmacokinetics, metabolism, structural interactions with the VDR and recruitment of comodulators to the ligand-VDR complex. It is hoped that vitamin D analogs with enhanced antiproliferative activity and with reduced calcemic side effects will emerge as clinically useful anticancer agents (Gross et al. 1997; Blutt and Weigel 1999; Konety et al. 1999; Miller 1999; Feldman et al. 2000).

Vitamin D Metabolism Modulates Cellular Responsiveness to the Hormone

The key enzymes involved in vitamin D metabolism are 24-hydroxylase, which catalyzes the initial step in the conversion of 1,25(OH)₂D₃ to less active metabolites, and 1 α -hydroxylase, which catalyzes the synthesis of 1,25(OH)₂D₃ from 25(OH)D₃. As discussed in the following sections, the level of expression of these enzymes in target cells such as PCa cells influences the magnitude of the growth inhibitory responses to vitamin D metabolites.

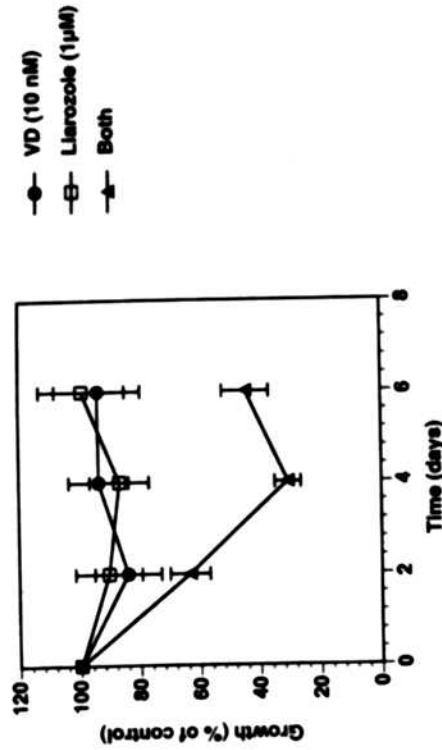


Fig. 2 Effect of 1,25(OH)₂D₃ (10nM), liarazole (1 μM) or a combination of both on DU 145 cell growth over a time course of 6 days with the fresh medium containing the agents replenished every 2 days. Cell proliferation was estimated using the MTS assay. Data are expressed as mean±SD of three experiments; **p*<0.05 compared with vehicle treated control group. [Reproduced with permission from Ly et al. (1999) *Endocrinology* 140:2071–2076]

25-Hydroxyvitamin D₃ 24-Hydroxylase

1,25(OH)₂D₃ induces the expression of the enzyme 24-hydroxylase in target cells, which catalyzes the initial step in the conversion of the active molecule 1,25(OH)₂D₃ into less active metabolites. In prostate cells, the degree of growth inhibition by vitamin D appears to be inversely proportional to the 24-hydroxylase activity in the cells. Among the human PCa cell lines DU 145, PC-3 and LNCaP, DU 145 cells exhibit a high level of 24-hydroxylase induction and are least responsive to 1,25(OH)₂D₃ in terms of growth inhibition (Skowronski et al. 1993; Miller et al. 1995). On the other hand, the basal and induced expression of 24-hydroxylase is very low in LNCaP cells and growth inhibition by 1,25(OH)₂D₃ is substantial. Investigators from our laboratory (Ly et al. 1999) have examined the possibility that inhibition of 24-hydroxylase activity would render DU 145 cells more sensitive to the antiproliferative action of 1,25(OH)₂D₃. Results of this investigation show that in DU 145 cells, liarazole (an imidazole derivative which inhibits P450 hydroxylases) causes significant inhibition of 24-hydroxylase activity leading to increased 1,25(OH)₂D₃ half-life in these cells and enhanced up-regulation of VDR. The study further demonstrates that in the presence of liarazole, 1,25(OH)₂D₃ could elicit a significant growth inhibitory response in DU145 cells. As shown in Fig. 2, the growth inhibition due to 1,25(OH)₂D₃ treatment alone is minimal in DU 145 cells. However, a combination of 1,25(OH)₂D₃ and liarazole causes appreciable growth inhibition. Miller et al. have also demonstrated that the differences in 1,25(OH)₂D₃-mediated growth inhibition between various PCa cell lines correlate inversely to 24-hydroxylase expression in these cells (Miller

et al. 1995). A recent study by Pechl et al. (2002) has shown that in primary human PCa cells, the use of the P450 inhibitor ketoconazole potentiates the growth inhibitory effects of 1,25(OH)₂D₃ or its structural analog EB 1089 by inhibiting the 24-hydroxylase activity in these cells. Thus combinations of 1,25(OH)₂D₃ with inhibitors of 24-hydroxylase such as ketoconazole or liarazole may enhance its antitumor effects in PCa therapy. The combination approach may also allow the use of 1,25(OH)₂D₃ at lower concentrations, thereby reducing the hypercalcemic side effects.

25-Hydroxyvitamin D₃ 1α-Hydroxylase

Endogenously synthesized and dietary vitamin D are transported to the liver where they are hydroxylated at the C-25 position to form the pro-hormone 25(OH)D₃ (Feldman et al. 2001). The active hormone 1,25(OH)₂D₃ is then formed in the kidney by the hydroxylation of 25(OH)D₃ at the C-1 position by the enzyme 1α-hydroxylase. The kidneys are the major source of circulating 1,25(OH)₂D₃ in the body. In recent years, however, the presence of extrarenal 1α-hydroxylase has been demonstrated and contributes to the local production of 1,25(OH)₂D₃ within the tissue. Schwartz et al. (1998) have shown that human prostatic epithelial cells express 1α-hydroxylase and raised the possibility that treatment with 25(OH)D₃ could potentially inhibit the growth of PCa, due to production of 1,25(OH)₂D₃ within the prostate, without the systemic side effect of hypercalcemia. The ability of 25(OH)D₃ to cause hypercalcemia is much reduced because of its lower affinity for the VDR. A recent study from our laboratory (Hsu et al. 2001) quantitated the levels of 1α-hydroxylase in primary prostatic epithelial cells derived from normal tissue, BPH or cancer as well as in established PCa cell lines. This study shows that epithelial cells from normal prostate have more 1α-hydroxylase activity than those derived from BPH or cancer. The activity in primary cancer cells is lower than BPH and the PCa cell lines express the lowest 1α-hydroxylase activity. Whitlatch et al. (2002) similarly found reduced 1α-hydroxylase activity in prostate cancer cells compared to normal prostatic cells. In a recent study Sergersten et al. (2002) have examined 1α-hydroxylase expression by RT-PCR and immunohistochemical analyses and report that the expression of 1α-hydroxylase is lower in parathyroid carcinomas than in normal parathyroid tissue. However, studies on tissues derived from normal colon and colon carcinoma show elevated levels of 1α-hydroxylase in colon carcinoma (Bareis et al. 2001). Our study also shows that the antiproliferative effect of 25(OH)D₃ correlates with the endogenous 1α-hydroxylase activity in prostate cells. As illustrated in Fig. 3, the growth of primary epithelial cells from normal tissue or BPH is inhibited by 25(OH)D₃ to an extent similar to 1,25(OH)₂D₃, as it could be converted to 1,25(OH)₂D₃ by endogenous 1α-hydroxylase activity. In contrast, in primary epithelial cells from cancer or in the human PCa cell line LNCaP, with very low endogenous 1α-hydroxylase activity, the antiproliferative action of 25(OH)D₃ is much less pronounced than that of 1,25(OH)₂D₃.

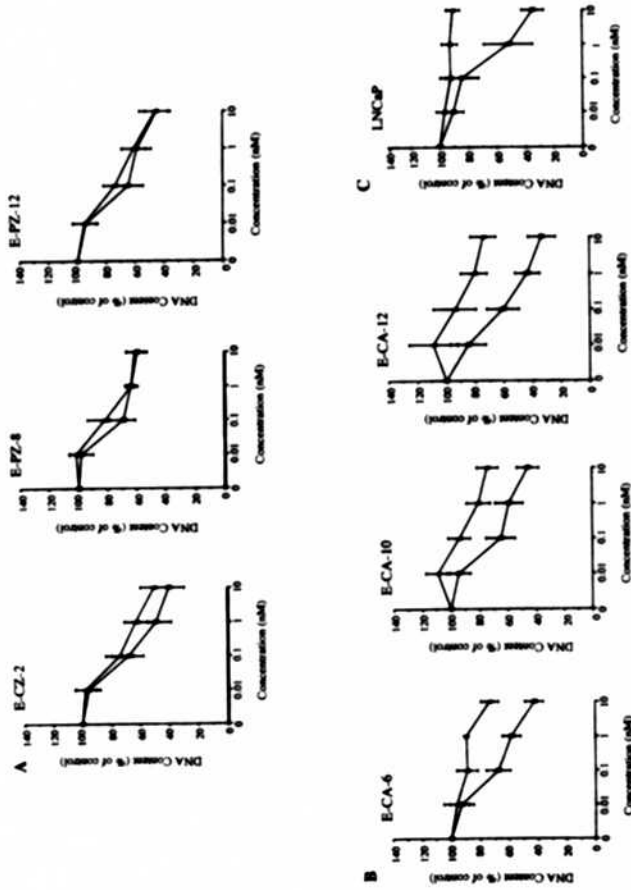


Fig. 3A-C Growth inhibition by 25(OH) $_2$ D $_3$ (triangles) in comparison with 1,25(OH) $_2$ D $_3$ (circles) in prostate epithelial cells. **A** In normal prostatic epithelial cells (E-CZ-2, E-PZ-8 and E-PZ-12), 25(OH) $_2$ D $_3$ and 1,25(OH) $_2$ D $_3$ were equally growth inhibitory at concentrations between 0.01 and 10 nM. **B** In primary cultures of cancer cells (E-CA-6, E-CA-10, E-CA-12), 1,25(OH) $_2$ D $_3$ induced ~20%–40% more growth inhibition than 25(OH) $_2$ D $_3$. **C** The prostate cancer cell line LNCaP, although fully responsive to 1,25(OH) $_2$ D $_3$, was resistant to treatment with 25(OH) $_2$ D $_3$. (Reproduced with permission from Hsu et al. (2001) Cancer Res 61:2852–2856)

Our findings of reduced 1 α -hydroxylase in cancer-derived prostatic epithelial cells raises the possibility that this difference may endow the malignant cells with an intrinsic growth advantage because of the resultant decrease in the local production of the growth inhibitory agent 1,25(OH) $_2$ D $_3$. In addition, local deficiency of 1,25(OH) $_2$ D $_3$ may allow cellular de-differentiation and invasion, hallmarks of malignancy (see "Mechanisms of Vitamin D Action" below). We conclude that a decrease in 1 α -hydroxylase activity may represent an important mechanism in PCa development and/or progression and suggest that the administration of 25(OH) $_2$ D $_3$ might be an effective chemopreventive approach, while 1 α -hydroxylase is initially still high within the prostate.

Mechanisms of Vitamin D Action

Several studies have investigated the molecular mechanisms by which 1,25(OH) $_2$ D $_3$ mediates growth inhibitory and pro-differentiating effects on prostate cells (Gross et al. 1997; Blutt and Weigel 1999; Konety et al. 1999;

Morrison 1997; Feldman et al. 2000). As summarized below, 1,25(OH) $_2$ D $_3$ seems to have multiple and diverse actions, often cell-specific, including effects on cell cycle arrest, apoptosis, inhibition of metastasis and angiogenesis.

Cell Cycle Arrest

In LNCaP PCa cells 1,25(OH) $_2$ D $_3$ treatment has been shown to result in the accumulation of cells in the G $_1$ phase of the cell cycle (Blutt et al. 1997). 1,25(OH) $_2$ D $_3$ treatment of LNCaP cells causes an increase in the expression of the cyclin-dependent kinase (CDK) inhibitor p21, a decrease in CDK2 activity leading to a decrease in the phosphorylation of the retinoblastoma protein (Rb) and repression of E2F transcriptional activity, resulting in G $_1$ arrest of the cells (Zhuang and Burnstein 1998). Liu et al. (1996) have shown that 1,25(OH) $_2$ D $_3$ directly up-regulates p21 gene expression in U937 leukemia cells, by binding to the VDR and acting through a putative vitamin D response element (VDRE) in the promoter of the p21 gene. However, in LNCaP cells, the regulation of p21 gene expression appears to be indirect. Studies from our lab (Boyle et al. 2001) have shown that 1,25(OH) $_2$ D $_3$ induces IGFBP-3 gene expression, which, in turn, results in increased p21 protein levels. Our study shows that the up-regulation of IGFBP-3 expression is essential for 1,25(OH) $_2$ D $_3$ -mediated inhibition of LNCaP cell growth as addition of IGFBP-3 antisense oligonucleotides abrogates 1,25(OH) $_2$ D $_3$ -mediated growth inhibition. 1,25(OH) $_2$ D $_3$ does not increase p21 expression in PC-3 cells, which is consistent with the lack of G $_1$ accumulation of these cells following 1,25(OH) $_2$ D $_3$ treatment. The mechanism of action in PC-3 cells appears to be different. In these cells, 1,25(OH) $_2$ D $_3$ increases the expression of transforming growth factor β (TGF- β), which mediates the antiproliferative effects of 1,25(OH) $_2$ D $_3$ (Wilding et al. 1989). Thus, the regulation of cell cycle distribution by 1,25(OH) $_2$ D $_3$ appears to be cell specific and may involve multiple pathways of action.

Apoptosis

Induction of apoptosis or programmed cell death by 1,25(OH) $_2$ D $_3$ is not uniformly seen in all cancer cells (Gross et al. 1997; Blutt and Weigel 1999; Konety et al. 1999; Miller 1999; Feldman et al. 2000). Blutt et al. (2000) showed evidence of apoptosis in LNCaP cells treated with 1,25(OH) $_2$ D $_3$ for 6 days. They also demonstrated the down-regulation of the pro-apoptotic proteins Bcl-2 and Bcl-X $_L$ following 1,25(OH) $_2$ D $_3$ treatment. In LNCaP cells, therefore, 1,25(OH) $_2$ D $_3$ stimulates both growth arrest and to a lesser extent, apoptosis.

Differentiation

1,25(OH)₂D₃ has been found to induce the differentiation of a number of normal and malignant cells (Feldman et al. 1997). Konety et al. (1996) showed that when histological examination of the rat prostate tissue was carried out, a greater degree of epithelial cellular differentiation was seen in rats treated with testosterone (T) and 1,25(OH)₂D₃ compared to rats treated with T alone. In the PCa cells LNCaP and MDA PCa 2a and 2b, 1,25(OH)₂D₃ increased the expression of PSA (Zhao et al. 1999, 2000) which may be considered as a differentiation marker for prostatic epithelial cells.

Reduction of Metastatic Potential

In addition to the inhibition of proliferation, 1,25(OH)₂D₃ decreases the tumor size and lung metastasis of the highly metastatic Mat-Ly-Lu and R 3327-AT-2 Dunning rat PCa cells in vivo (Schwartz et al. 1995). A recent study from our laboratory (Sung and Feldman 2000) has shown that in DU 145 and PC-3 human PCa cells, 1,25(OH)₂D₃ inhibits invasiveness, cell adhesion and migration (Fig. 4) to the basement membrane matrix protein laminin, due in part to decreasing the expression of $\alpha 6$ and $\beta 4$ integrins. In LNCaP and PC-3 cells, 1,25(OH)₂D₃ and its analogs have also been shown to increase the expression of E-cadherin, a tumor suppressor gene whose expression is inversely correlated to the metastatic potential of the cells (Campbell et al. 1997).

Angiogenesis

Angiogenesis, or the process of new blood vessel formation, is critical for tumor progression and metastasis. 1,25(OH)₂D₃ has been shown to inhibit tumor cell-induced angiogenesis in mice (Majewski et al. 1996; Mantell et al. 2000), and therefore may have a therapeutic application in advanced metastatic cancer.

Vitamin D-Regulated Gene Expression

Research from many laboratories has attempted to identify novel 1,25(OH)₂D₃ target genes mediating its various actions, especially the regulation of cell growth (Freedman 1999). We have analyzed the patterns of gene expression in 1,25(OH)₂D₃-treated primary prostatic epithelial cells as well as PCa cell lines, using cDNA microarrays carrying 20,000 genes. Our analyses have revealed several novel putative vitamin D target genes in primary epithelial cells. In general, there is an appreciable overlap in the profiles of 1,25(OH)₂D₃-regulated genes in normal and cancer-derived primary cells. In both of these cultured cells, the expression of dual specificity phosphatase 10 shows maximal up-reg-

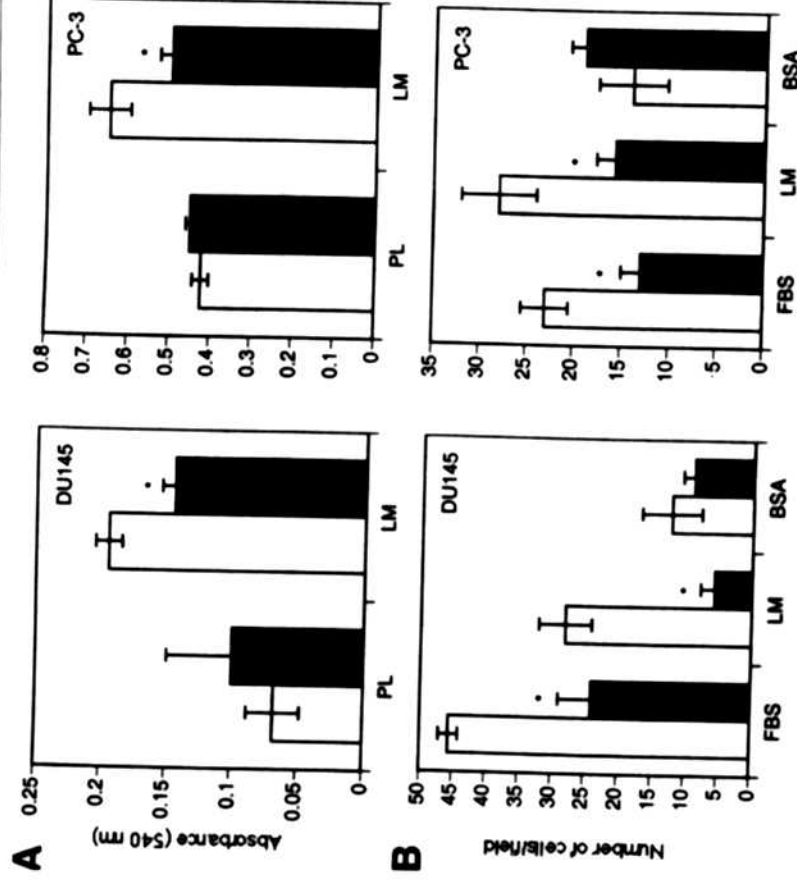


Fig. 4A, B 1,25(OH)₂D₃ decreases the migration of DU 145 and PC-3 cells to fetal bovine serum (FBS) and laminin. Cells were treated with ethanol vehicle (white bars) or 50 nM 1,25(OH)₂D₃ (black bars) for 72 h prior to assay. Migration of 1,25(OH)₂D₃ treated cells toward 10% FBS, 0.1% bovine serum albumin (BSA) and 100 μ M laminin (LM) is expressed as percent of migrating cells compared to vehicle treated controls. Number of vehicle-treated migrating cells were 46 \pm 6, 28 \pm 6 and 19 \pm 6 for DU 145 cells (FBS, LM and BSA, respectively) and 25 \pm 8, 30 \pm 9 and 18 \pm 6 for PC-3 cells. (*n*=3; **p*<0.05 compared to vehicle treated control cells). [Reproduced with permission from Sung and Feldman (2001) Mol Cell Endocrinol 164:133-143]

ulation. Dual specificity phosphatase 10 inactivates mitogen-activated protein kinase (MAPK), suggesting that an important feature of the growth inhibitory activity of 1,25(OH)₂D₃ in these cells may be an inhibition of the growth-promoting effect of MAPK. Early up-regulation of the kinase anchoring protein gravin is seen. Our data support the role of vitamin D as an antioxidant in primary prostate cells. Thioredoxin reductase 1, involved in redox balance, is an early response gene in both normal and cancer cells. Up-regulation of superoxide dismutase 2 is also indicative of protection from oxidative damage. The regulation of the expression of metallothionein genes by 1,25(OH)₂D₃ is different between the normal and cancer-derived primary cells, the former showing an up-regulation and the latter a significant down-regulation. Metallothioneins constitute the majority of intracellular protein thiols and as such are

considered to act as cell survival factors. Up-regulation of metallothioneins in normal prostatic epithelial cells is consistent with the anti-apoptotic effect of $1,25(\text{OH})_2\text{D}_3$ in these cells. Certain metallothioneins have been reported to be overexpressed in PCa (Zhang et al. 1996) and hence a down-regulation of their expression in the cancer-derived cells may be therapeutically beneficial.

In summary, our analysis has revealed biologically important targets of $1,25(\text{OH})_2\text{D}_3$ in prostate cells and has provided a starting point for additional investigations into the molecular mechanisms underlying the anticancer effect of $1,25(\text{OH})_2\text{D}_3$ and its analogs.

In Vivo Studies

Rodent Models

Although several mouse and rat models of PCa have been developed (Lucia et al. 1998; Sharma and Schreiber-Agus 1999), there is still a lack of a perfect model for human PCa. Many in vivo studies have investigated the effects of $1,25(\text{OH})_2\text{D}_3$ or its analogs on the establishment and growth of human PCa cells as xenografts in immune-compromised mice and showed that $1,25(\text{OH})_2\text{D}_3$ or its analogs inhibit the growth of PCa xenografts, causing significant reductions in tumor size and volume (Gross et al. 1997; Blutt and Weigel 1999). Although imperfect, xenograft models provide an in vivo system to validate the antitumor effects of $1,25(\text{OH})_2\text{D}_3$ or its analogs and monitor their ability to elevate serum calcium levels.

Clinical Studies

A few investigators have undertaken clinical trials in PCa patients to evaluate the safety and efficacy of treatment with vitamin D or its analogs. Osborn et al. (1995) reported a small phase II trial of $1,25(\text{OH})_2\text{D}_3$ in 13 patients with hormone refractory metastatic PCa. No objective responses (>50% reduction in serum PSA levels or >30% reduction in measurable tumor mass) could be seen, and the median time to progression was 10.6 weeks. A pilot study from our laboratory (Gross et al. 1998) used increasing concentrations of $1,25(\text{OH})_2\text{D}_3$ to treat seven patients with early recurrent PCa following radiation or surgery. We compared the doubling time of serum PSA before and after $1,25(\text{OH})_2\text{D}_3$ treatment in the same patient. In all seven patients, the rate of PSA rise was substantially decreased by $1,25(\text{OH})_2\text{D}_3$ and in the case of patient 1, the serum PSA levels actually decreased, registering a negative doubling time (Table 1). Due to hypercalcemia, vitamin D therapy was discontinued in three of the seven patients. Withdrawal from therapy resulted in increases in PSA doubling time, with the values reaching those of pretreatment levels in these patients (Table 1). This study provides evidence that vitamin D could be effective in slowing the progression of PCa in patients. Both these

Table 1 PSA doubling time in months before and after $1,25(\text{OH})_2\text{D}_3$ treatment (from Gross et al. 1998, with permission)

Patient no.	Before therapy	During therapy	P value	Therapy discontinued
1	17.1	-3.3	0.005	10.8
2	5.5	12.5	<0.0001	2.1
3	12.0	37.6	0.009	13.7
4	10.8	15.8	0.002	-
5	23.2	43.0	0.029	-
6	12.5	16.7	0.453	-
7	4.1	9.4	0.0009	-

clinical trials found a high incidence of hypercalcemia or hypercalcemia and the development of renal stones in some patients. This finding underscores the fact that development of hypercalcemia would impact the use of very high doses of $1,25(\text{OH})_2\text{D}_3$ and therefore limits the therapeutic benefit that may only be realized at high doses.

Combination Therapy

As discussed above, several structural analogs of $1,25(\text{OH})_2\text{D}_3$, which are more potent as antiproliferative agents but have less calcemic effects, are being developed so that hypercalcemic side effects do not limit the therapeutic application of vitamin D metabolites (Gross et al. 1997; Blutt and Weigel 1999; Konecny et al. 1999; Miller 1999; Feldman et al. 2000). In addition to searching for less calcemic vitamin D analogs, investigators have been testing a variety of factors for additive or synergistic antiproliferative activity for use in combination with $1,25(\text{OH})_2\text{D}_3$. Clinically relevant drugs that enhance the activity of $1,25(\text{OH})_2\text{D}_3$ on prostatic epithelial cells include platinum drugs (Moffatt et al. 1999) and paclitaxel (Hershberger et al. 2001). Using primary cultures of prostate cancer cells, we found enhanced activity of $1,25(\text{OH})_2\text{D}_3$ when combined with suramin (Peehl et al. 1995). Suramin is a polysulfonated naphthylurea compound, which is used with some efficacy to treat patients with advanced prostate cancer. For the most part, the mechanism of additive or synergistic activity of $1,25(\text{OH})_2\text{D}_3$ with these drugs is not understood. As we discussed earlier, combination of $1,25(\text{OH})_2\text{D}_3$ with inhibitors of 24-hydroxylase such as liarozole or ketoconazole would increase the half-life of $1,25(\text{OH})_2\text{D}_3$ in target cells and lead to VDR up-regulation. This would allow the use of $1,25(\text{OH})_2\text{D}_3$ at lower concentrations, thereby possibly reducing the hypercalcemic side effects.

Interactions between vitamin D and retinoids have been recognized for some time and are believed to occur through RXR, with which both VDR and RAR dimerize (Mangelsdorf et al. 1995). When we tested a variety of factors for their ability to enhance the response of primary cultures of normal and malignant prostatic epithelial cells to vitamin D, we found synergistic activity

between *all-trans*-retinoic acid (RA) and 1,25(OH)₂D₃ (Peehl et al. 1995). Another study from our laboratory (Zhao et al. 1999) has shown synergistic inhibition of LNCaP cell growth when a combination of 1,25(OH)₂D₃ and 9-*cis* RA is used, as illustrated in Fig. 1. Other studies (Esquenet et al. 1996; Blutt et al. 1997) have found similar synergistic growth-inhibitory activity of 9-*cis* RA and 1,25(OH)₂D₃ on LNCaP cells. Various other retinoids sensitize prostate cancer cell lines to 1,25(OH)₂D₃ or analogs (Campbell et al. 1998; Elstner et al. 1999). Retinoids have been tested in clinical trials for prostate cancer but, as for 1,25(OH)₂D₃, therapeutic efficacy is limited by toxicity. A combination of vitamin D metabolites with retinoids would enable either class of compounds to be used at lower concentrations, thereby reducing toxic side effects while achieving synergistic anticancer activity.

Conclusions

1,25(OH)₂D₃ is a potent antiproliferative agent in normal and malignant prostate cells. The potency of growth inhibition by vitamin D metabolites is modulated by the presence and activity of key enzymes involved in vitamin D metabolism, namely 24-hydroxylase and 1 α -hydroxylase. The mechanisms underlying the anticancer effects of vitamin D in PCa cells are varied and cell specific and include growth arrest, apoptosis, pro-differentiation effects, modification of growth factor activity, inhibition of tumor cell invasiveness and interactions with androgen signaling. Investigators are making progress in identifying 1,25(OH)₂D₃-regulated genes and understanding their role in the mediation of the above-mentioned effects. Such studies would unveil novel 1,25(OH)₂D₃-regulated genes and provide new therapeutic targets. Combination of vitamin D metabolites with other growth inhibitory agents such as taxol derivatives, retinoids or imidazole compounds that inhibit 24-hydroxylase such as liarozole or ketoconazole, is a therapeutic approach that might limit the hypercalcemic side effects. Investigators are also developing structural analogs of 1,25(OH)₂D₃ with less calcemic activity and equal or greater antiproliferative activity than 1,25(OH)₂D₃. Another challenging area of research involves defining the mechanisms by which various vitamin D analogs maintain potent growth inhibitory effects and yet are less calcemic. We expect that progress in these areas of research will lead to the development of more potent and safer vitamin D compounds to be employed in the treatment of PCa, alone or in combination with other anticancer agents.

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