MINIREVIEW

Vitamin D and Prostate Cancer (44389)

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Abstract. **Classically, the actions of vitamin D have been associated with bone and mineral metabolism. More recent studies have shown that vitamin D metabolites induce differentiation and/or inhibit cell proliferation of a number of malignant and nonmalignant cell types including prostate cancer cells. Epidemiological studies show correlations between the risk factors for prostate cancer and conditions that can result in decreased vitamin D levels. The active metabolite of vitamin D, 1,25-dihydroxyvitamin D_3 (calcitriol), inhibits growth of both primary cultures of human prostate cancer cells and cancer cell lines, but the mechanism by which the cells are growth-inhibited has not been clearly defined. Initial studies suggest that calcitriol alters cell cycle progression and may also initiate apoptosis. One of the disadvantages of using vitamin D *in vivo* is side-effects such as hypercalcemia at doses above physiological levels. Analogs of calcitriol have been developed that have comparable or more potent antiproliferative effects but are less calcemic. Further research into the mechanisms of vitamin D action in prostate and identification of suitable analogs for use *in vivo* may lead to its use in the treatment or prevention of prostate cancer.**

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Vitamin D and Prostate Cancer

The idea that diet and nutrition are important risk factors in cancer incidence and progression is not new. Numerous epidemiological and experimental studies have been based on the hypothesis that cancer can be linked to risk factors that involve dietary components. Cell culture, animal model, and human epidemiological studies in cancers such as esophagus, lung, pancreas, stomach, colon, breast, cervix, prostate, kidney, and bladder have indicated that modifications in nutrition reduce cancer incidence (1). Vitamins A, B, C, D, E, and K have all been investigated for cancer preventive or cancer treatment potential in many cancers (1), and the lack of adequate vitamin D has been suggested as a risk factor for prostate cancer (2). Current research in this area has focused on using *in vivo* and *in vitro* models to study the effects of vitamin D on prostate cancer, investigating the mechanisms through which vitamin D inhibits prostate cancer cell growth, correlating vitamin D receptor alleles and prostate cancer incidence, and developing vitamin D analogs as potential therapeutic or preventative agents for the treatment of prostate cancer.

Vitamin D. Vitamin D is a key regulator of bone and mineral metabolism and it plays an important role in the regulation of calcium and phosphorus transport in the small intestine (3, 4). Vitamin D deficiencies in humans cause a variety of health problems including rickets, hypocalcemia, and bone loss in adults (5). More recently, investigators have found that vitamin D modulates growth and differentiation in a wide range of tissues including, but not limited to the breast, prostate, pancreas, and skin (6–9). The lack of vitamin D has been linked to a predisposition for the development of cancers in these tissues (1) suggesting that low levels of vitamin D may also play a role in prostate cancer etiology (2).

Prostate Cancer Etiology and Epidemiology. Prostate cancer is the second leading cause of death from cancer

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in American males, and over 11 million men in the United States have prostatic lesions that can be histologically classified as prostate cancer (10). Although prostate cancer grows more slowly than many cancers, the mortality rate due to the disease is on the rise. Consequently, prostate cancer has become a major heath concern in recent years.

There are three major risk factors for prostate cancer: age, race, and geography (11). Prostate cancer incidence increases with age, with over 80% of prostate cancers found in men over the age of 65 (2). Other epidemiological studies have clearly shown that the incidence of prostate cancer in African Americans is higher than in American Caucasians (2, 12) whereas the rates for Japanese men are 1/20 those of Americans (13). Another epidemiological study showed that mortality rates for the disease are high in North America and in northwest Europe but are low in Africa and in Central and South America (11). Thus far, there are no strong correlations linking prostate cancer incidence to diet, venereal disease, sexual habits, smoking, or occupational exposure (14–16). However, some reports have suggested that serum hormone levels of testosterone and prolactin may affect prostate cancer risk, but the studies on these effects are limited (16). The basis of these predispositions for prostate cancer development have not been determined. Although there are likely to be multiple factors involved, each of these risk factors can be correlated with changes in vitamin D as described below.

Vitamin D and the Vitamin D Receptor. Vitamin D is a secosteroid that can be obtained through dietary means or synthesized *in vivo* (5). For most Americans, the major source of vitamin D is endogenous synthesis in the skin where 7-dehydrocholesterol is converted through a photosynthetic reaction followed by a heat-catalyzed step to Vitamin D_3. Both endogenously synthesized and dietary Vitamin D_3 are transported to the liver by vitamin D-Binding Protein (DBP) where they are hydroxylated at the C-25 position (5). From the liver, the 25-hydroxylated form is transported to the kidney where it is hydroxylated a second time at the C-1 position to form the biologically active metabolite,1,25-dihydroxyvitamin D_3 (calcitriol) (5).

Calcitriol acts by binding to the vitamin D receptor (VDR), a ligand-activated transcription factor (3). VDR is a member of the steroid hormone receptor family of transcription factors that also includes the thyroid hormone receptors and the retinoic acid receptors (3). These receptors activate or repress the transcription of target genes upon binding their respective ligands (3). The VDR, which acts as a heterodimer with retinoid X receptor, regulates the transcription of numerous proteins including osteocalcin, calcium binding proteins, PTH, and c-*myc* (17, 18). The three human prostate cancer cell lines most widely used for *in vitro* studies of prostate cancer, LNCaP, PC3, and DU145, all contain a functional VDR (19, 20).

Vitamin D and Other Cancers. A number of epidemiological reports have suggested a link between low vitamin D levels and the incidence of cancer including breast cancer (21, 22). Like the epidemiological correlations found between vitamin D and prostate cancer (see below) (2, 23), calcitriol levels in women that were subsequently diagnosed with breast cancer were lower when compared to women who did not develop breast cancer (24). Over 80% of human breast tumors have been found to contain the VDR (8, 25) and thus would be candidates for treatment with calcitriol. *In vitro*, many of the established human breast cancer cell lines are growth inhibited by calcitriol (26, 27).

In addition to breast cancer, the VDR has been found in many tumors including lung carcinomas, malignant melanomas, osteosarcomas, and colon carcinomas (28, 29). Evidence that calcitriol can induce cellular differentiation *in vitro* was reported initially in a study of human normal and leukemic myeloid stem cells (30). As might be predicted, many of the cell lines developed from the various tumors are growth inhibited and/or differentiated *in vitro* by calcitriol (31). Moreover, *in vivo* studies using animal models of skin, colon, and breast cancer, have shown that calcitriol may have preventive and/or therapeutic effects in the treatment of these cancers (31).

Vitamin D and the Incidence of Prostate Cancer. Schwartz *et al.* (2) in 1990 hypothesized that vitamin D deficiency increases the risk of prostate cancer. Although dietary sources provide a small amount of the daily requirement for vitamin D, the major source of vitamin D for Americans is ultraviolet light–induced conversion of a precursor as a result of exposure to sunlight (31). Deficiency in vitamin D due to reduced synthesis of a previtamin D_3 is a potential common element among the three major risk factors for prostate cancer. First, older men generally are exposed to less sunlight compared to their younger counterparts (32), and their capacity to synthesize previtamin D_3 decreases with age (33). Second, the high melanin content of the skin of African Americans reduces the efficiency of synthesis of previtamin D_3 (34). Third, Hanchette *et al.* (11) showed that mortality rates for prostate cancer were inversely associated with the availability of sunlight. Interestingly, one study has shown that men with low serum levels of calcitriol were more likely to develop prostate cancer subsequently than men with higher levels of serum calcitriol (23). However, other studies failed to find a correlation (35, 36). The lower incidence of clinical disease in Japanese men may be related to the high amount of vitamin D obtained from fish oils in the traditional Japanese diet (13). Consistent with this is the finding that Japanese men who migrate to the United States and adopt a Western diet show a significant increase in the incidence of prostate cancer (13).

A Role for VDR Polymorphisms in Prostate Cancer? Several polymorphisms have been identified in the VDR gene locus (37, 38). These include a poly A microsatellite in the 3′ flanking region (38) ($L \geq 18$ poly A's or $S \leq 17$ poly A's), an alteration in intron 8 that generates a BsmI site (B = no site; b = BsmI site), and a polymorphism in exon 9 that generates a TaqI site (T = no site; t =

Taq site) without altering the amino acid sequence. Although there are a few studies suggesting association of L,T with increased risk of prostate cancer (37), a study comparing men who died from prostate cancer with healthy controls showed no correlation (39). Importantly, none of the polymorphisms found to date are associated with changes in receptor sequence and function. This suggests that it is more likely that an alteration linked to these polymorphisms, either within or near the gene encoding VDR, alters prostate cancer risk.

Vitamin D and Normal Prostate. Receptor expression studies in whole sections of the prostate show localization primarily in the secretory epithelial cells (40), the main site of cancer development (41). Cultures of human prostate cells from normal tissue express VDR with higher levels found in prostate epithelial cells compared to prostate fibroblasts (42). The hormone-binding affinity of the receptors is indistinguishable from VDR found in classical target tissues (42). Interestingly, under the cell culture conditions used, the cell lines derived from the normal epithelial and fibroblastic prostate tissue were irreversibly growth inhibited by treatment with calcitriol (42). Little is known about the effects of calcitriol on normal prostate *in vivo*. However, Konety *et al.* (43) found that calcitriol not only increased stromal cell proliferation and reduced epithelial cell proliferation in the prostate of castrated rats but also increased prostate cell differentiation when administered in combination with androgens.

Vitamin D and Prostate Cancer. Good animal models have been a limitation in studying prostate cancer. Although new models have been developed in recent years (44, 45), most of the evaluations of calcitriol and synthetic analogs have been done in prostate cancer cells using assays for cell growth, clonal expansion, or invasion. An additional limitation is the paucity of human or other prostate cancer cell lines that retain the characteristics of human prostate cancers, including expression of functional androgen receptor and androgen dependence for growth. A number of established cell lines lack androgen receptors (46) although the receptors are usually expressed even in androgen ablation–resistant disease (47, 48). Other cell lines contain mutations in known tumor suppressors such as p53 or retinoblastoma protein (Rb), both of which are not mutated at high frequencies in human prostate tumors (49–52). The majority of the studies have used human or rat prostate cancer cell lines to examine the growth properties and characteristics of prostate cancer cells *in vitro*. Table I illustrates characteristics of some of the more widely used cell lines. Some of these characteristics may contribute to the differences in response to calcitriol.

Most of the studies investigating the effects of calcitriol on prostate cancer cell growth *in vitro* have used growth assays to measure differences in cell number as a result of treatment with calcitriol or synthetic analogs. Of these studies, many have focused on LNCaP cells, which most closely mimic a human prostate cancer cell by being androgen responsive and secreting prostate-specific antigen (PSA), a prostate cell differentiation marker, in response to androgen. However, this line does contain a point mutation in its androgen receptor which broadens the range of hormones to which it responds (53, 54). Miller *et al.* (19) first showed an effect of calcitriol on LNCaP prostate cancer cells. Although the LNCaP cells grown in charcoal-stripped serum were somewhat growth stimulated by calcitriol, PSA expression was induced, suggesting induction of differentiation (19). Subsequent studies performed in medium containing fetal bovine serum showed that calcitriol substantially inhibits the growth of LNCaP cells (20). Figure 1 shows a typical growth curve for LNCaP cells grown in the absence or presence of calcitriol. Remarkably, this treatment essentially halts growth within a few days of initiating treatment. Moreover at limiting concentrations of calcitriol, Blutt *et al.* (55) found that retinoid X receptor ligands in combination with calcitriol synergistically inhibited growth of LNCaP cells.

The effect of calcitriol on other prostate cancer cell lines has also been studied. Skowronski *et al.* (20) found that PC3 cells are much less responsive to calcitriol, and DU145 cells are essentially unresponsive to growth inhibition by calcitriol although both lines contain functional

Table I. Characteristics of Available Prostate Cancer Cell Lines

Cell line	Source	VDR	Calcitriol growth inhibition	AR	Respond to androgen	p53	Rb
LNCaP	Human[53]	Y[19]	Y[20]	Y[46]	Y[53]	Y[131]	Y[105]
PC3	Human[132]	Y[20]	Y[20]	N[46]	N[132]	N[133]	Y[105]
DU145	Human[134]	Y[20]	N[20]	N[46]	N[134]	N[131]	N[105]
JCA-1	Human[135]	N[136]	N[136]	N/A	N[136]	Y[137]	Y[137]
ALVA-31	Human[138]	Y[136]	Y[136]	N[46]	N[138]	N/A	N/A
CPA	Canine[139]	N/A	N/A	Y[140]	Y[140]	N/A	N/A
NRP-152	Rat[141]	N/A	Y[141]	Y[141]	Y[141]	N/A	N/A
NRP-154	Rat[141]	N/A	N[141]	N[141]	N[141]	N/A	N/A
AT-2	Rat[142]	N/A	Y[62]	N[143]	N[143]	N/A	N/A
MLL	Rat[142]	N/A	Y[62]	N/A	N[142]	N/A	N/A

Note. Numbers refer to references listed at the end of the review. Y = present; N = absent; N/A = information not available; AR = androgen receptor; VDR = vitamin D receptor; Rb = retinoblastoma gene product.

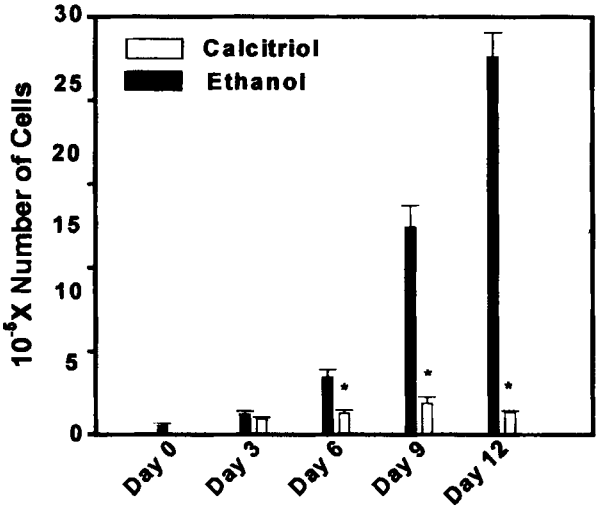

Figure 1. Effect of calcitriol on the growth of LNCaP human prostate cancer cells. Cells were plated in six well dishes at a density of 13,000 cells/well and treated with either ethanol (vehicle) or 100 n*M* calcitriol for the indicated number of days. Fresh medium and hormone were added every 3 days. Cells were trypsinized and counted using a Coulter Counter. Bars represent the mean ± SD ($n = 3$). *, $P \leq 0.05$ compared to vehicle-treated cells on the same day.

VDR. The reasons for these differences are, as yet, unknown.

Clonal expansion assays in soft agar and invasion assays have also been used to measure the effects of calcitriol. Calcitriol significantly inhibits the clonal proliferation of both the LNCaP and the PC3 cell lines (70% and 90% of control colonies after 14 days of treatment with 10 n*M* calcitriol, respectively) while minimally affecting the DU145 cell line (56). Although primary cultures of prostate cancer cells have been difficult to establish, Peehl *et al.* (42) have established cultures of human prostatic epithelial cells from malignant tissues. They detected VDR in all of the primary cultures established (10 strains tested) and also showed using clonal growth assays that these primary cultures are irreversibly growth inhibited by as little as 2 hr of calcitriol treatment (42). Other studies using invasion assays (Amgel assay) have revealed that although the LNCaP and PC3 cell line are not invasive in Amgel, the DU145 cells are highly invasive and are markedly inhibited (66%) by treatment with 100 n*M* calcitriol for 72 hr (57). The DU145 cell response to calcitriol in this assay suggests that the invasion assay is measuring an activity of calcitriol that differs from that which causes growth inhibition.

Analyses of the effects of VDR agonists in animals or humans to date are minimal, and unfortunately, prostate cancer models are quite limited. Dogs provide the best model for human prostate cancer, but they are expensive, and extensive studies are difficult because of inherent genetic variation (58). Various mouse and rat models for prostate cancer have been developed (44, 45, 59–61) that can be used to look at prostate cancer therapies *in vivo* while keeping genetic background constant and costs acceptable. Several of these models rely on implanting cells grown in tissue culture into genetically compatible hosts or immune-compromised mice to produce tumors. Getzenberg *et al.* (62) demonstrated that calcitriol inhibits the growth of the MLL and the AT-2 cell lines, both of which are highly metastatic Dunning rat prostate cancer cell lines. Very high concentrations (20 μ*M*) were needed in cell culture to obtain 50% growth inhibition, but calcitriol treatment of the rats with MLL tumors implanted subcutaneously in the flanks of Copenhagen rats resulted in a decreased tumor volume and a reduction in the size and number of lung metastases (62). However, the animals, which were given 1 μg of calcitriol three times a week for 3 weeks, had significantly elevated serum calcium levels, which would be unacceptable in treatment of humans (62).

Two reports have addressed the effects of calcitriol on prostate cancer in humans. In a small trial in 13 men with hormone refractory prostate cancer, only two men exhibited a decrease in PSA levels during the course of treatment with calcitriol (63). However, Gross *et al.* (64) showed that in six of seven asymptomatic patients with increasing PSA levels, calcitriol treatment significantly decreased the rate of PSA rise. A dose-dependent hypercalciuria as a result of treatment limited the amount of hormone administered (64).

Use of Analogs of Calcitriol. One of the limitations in using calcitriol as a treatment is that calcitriol can cause hypercalciuria and hypercalcemia, which limits the amounts administered to patients (5). The levels of calcitriol used in the cell studies are much higher than circulating levels of calcitriol (16–65 pg/ml) and cannot be safely achieved *in vivo* (65). Consequently, analogs of calcitriol that efficiently activate VDR, but are less calcemic are being developed and tested for both growth inhibitory and differentiation properties. A number of more potent analogs have been developed, some of which are less calcemic. Several studies have shown that many of these analogs can inhibit the growth of prostate cancer cells (66–69) as well as other cancer cells (6, 8, 9, 70) at lower concentrations than calcitriol. Schwartz *et al.* (67) showed that 16-diene analogs were more effective in inhibiting growth than calcitriol and that, interestingly, the DU145 cell line, which is unresponsive to calcitriol, was significantly growth inhibited by the analogs. Moreover, one diene analog, 1,24-dihydroxy-16-ene-23-yne-vitamin D_3, tested on the proliferation of human prostate cancer cells (PC3) in a nude mouse model, was found to significantly reduce the relative increase in tumor volume over a 22-day period without raising serum calcium levels (71). 24-Oxo metabolites of vitamin D have also been reported not only to inhibit the growth of LNCaP more effectively but also to inhibit growth of the DU145 cells (72). Fluorine derivatives of calcitriol also exhibit similar properties (66). One of the promising analogs is 1(S),3(R)-dihydroxy-20(R)-(5′-ethyl-5′-hydroxy-hepta-1′(E),3′(E)-dien-1′-yl)-9,10-secopregna-5(Z),7(E),10(19)-triene (EB1089), a deltanoid compound developed by Leo Pharmaceuticals (Ballerup, Denmark) (73). This analog, reported to be 2-fold more potent in displacing labeled calcitriol from the VDR, exhibited 3-fold greater inhibitory

activity when administered to LNCaP cells (74) and also inhibited the growth of PC3 cells (75).

Other deltanoid analogs, KH1060, HM, V, and MC 1288 can also inhibit the growth of LNCaP and PC3 cells at 10–100-fold lower concentrations (74). Importantly, some of the analogs can inhibit growth at concentrations that do not induce hypercalcemia. The basis for the increased separation between concentrations that activate VDR and those that induce hypercalcemia has not been clearly delineated, but may be related to the weaker affinity of DBP for these compounds, which increases the available circulating levels. Also, some of these analogs are not metabolized as quickly as calcitriol (76). A phase-I trial has been performed using EB1089 to treat patients with advanced breast and colorectal cancer with results suggesting that there is some stabilization of the disease with treatment (77).

Mechanism of Vitamin D Action in Prostate Cancer

Role of the Vitamin D Receptor. A number of studies have addressed the mechanism by which calcitriol reduces growth in cancer cells although more extensive studies have been done in nonprostate cells. In some cases the findings agree with studies performed in prostate cell systems, whereas in others they differ substantially. Calcitriol acts through poorly defined nongenomic pathways not involving the nuclear receptor as well as through pathways requiring the receptor (78, 79). Several studies have led to the conclusion that the VDR is required for calcitriol-mediated inhibition of prostate cancer cell growth. The JCA-1 human prostate cancer cell line, which does not express the VDR, is unresponsive to treatment with calcitriol, but JCA-1 cells stably transfected with a VDR-expression plasmid are growth inhibited by calcitriol (80). ALVA-31 human prostate cancer cells stably transfected with an antisense plasmid for VDR, which reduced VDR expression, showed a reduced growth inhibition in response to calcitriol (81). These studies showed that the antiproliferative effects of calcitriol require the VDR in prostate cancer cells. Whether nongenomic actions also contribute to the responsiveness remains to be determined. However, although the receptor appears to be required for response, the amount of receptor is not indicative of a cell's ability to respond, nor does the presence of a functional receptor necessarily confer response. The ALVA-31 cells contain more VDR than the LNCaP cells yet the LNCaP cells are much more sensitive to calcitriol (82). The DU145 cell line contains functional VDR yet is minimally responsive to calcitriol (20). These studies suggest that although the VDR is required for calcitriol action, the response probably involves multiple components rather than simple induction or repression of a single target gene.

Differentiation. One of the mechanisms by which calcitriol might inhibit tumor growth is through induction of differentiation with a concomitant reduction in proliferation. Calcitriol has been shown to induce differentiation of a number of normal cell types (83–87). Calcitriol treatment also induces expression of characteristic, differentiation-specific, cell surface markers in leukemic cells that are detected using flow cytometry (88, 89). One specific marker, Prostate Specific Antigen (PSA), is secreted by prostate cells that are differentiated (90, 91). Rising serum PSA levels are an indication of prostate cancer and result from an increase in the total number of cells (92, 93). Calcitriol treatment of prostate cancer cell lines results in the increase of the cellular synthesis of PSA, suggesting that calcitriol may be capable of causing differentiation of prostate cells (19, 20, 94). Although this appears to be inconsistent with the fact that rising serum PSA levels are an indication of prostate cancer, the ability of calcitriol to reduce cell number will result in an overall decrease in total PSA.

Cell Cycle. The means by which calcitriol inhibits proliferation is an active area of study. Both changes in cell cycle progression and changes in cell death have been investigated. Leukemia cells and some breast cancer cell lines treated with calcitriol accumulate in the G_1 phase of the cell cycle (95, 96). LNCaP prostate cancer cells also accumulate in the G_1 phase of the cell cycle as a result of treatment (55, 97). However, neither the ALVA-31 (97) cells nor the PC3 cells accumulate in G_1 (97) even though they are both growth inhibited by calcitriol. This suggests that calcitriol also acts through alternative pathways to inhibit growth observed in these cells. The means by which the G_1 accumulation is induced has not been determined, but the data support a role for retinoblastoma gene product (Rb), a critical regulator of the G_1 to S phase transition (98). The active form of Rb is underphosphorylated and binds to and sequesters the transcription factor E2F, which is important for transcription of proteins involved in cell proliferation (99, 100). Once Rb is inactivated by phosphorylation, E2F is released, and progression through S phase ensues (99, 100). Rb mutations have been identified in a variety of human cancers although their frequency is low in prostate cancer (101, 102). Regulation of Rb phosphorylation depends on the activity of cyclin-dependent kinases (cdk), which are regulated by cyclins, which activate the kinases, and by cyclin-dependent kinase inhibitors (cdki), which inhibit kinase activity (103). Zhuang *et al.* have demonstrated that Rb is in a hypophosphorylated state; E2F has decreased transcriptional activity; the levels of p21, a cdki, are elevated; and there is decreased cdk2 activity in LNCaP cells after treatment with calcitriol (97). Studies by Liu *et al.* (104) using leukemic U937 cells indicated that p21 is directly regulated by VDR through a putative vitamin D response element; however, p21 induction was not observed in PC3 cells, consistent with the lack of G_1 accumulation as a result of treatment (97). These data suggest that the regulation of the cell cycle by calcitriol is more complex in prostate cells than a simple direct induction. Interestingly, the DU145 cell line, which does not contain functional Rb (105) is not growth inhibited by calcitriol (20). Similarly, prostate cancer cells transformed with an SV40 large T antigen, which blocks Rb function, are also not growth inhibited by cal-

citriol (106). Collectively these data suggest that response to calcitriol in prostate cancer cells may depend on functional Rb.

Apoptosis. Apoptosis, or programmed cell death, is an alternate means for reducing cell accumulation. Calcitriol-induced apoptosis in cancer cells appears to be dependent on the cancer cell line studied. In some breast cancer cell lines, calcitriol induces apoptosis whereas in others, it does not (107, 108). Calcitriol clearly does not induce apoptosis in HL-60 leukemic cells (109), but calcitriol-induced induction of apoptosis in prostate cancer cells is more controversial. Zhuang *et al.* (97) did not detect apoptosis in LNCaP cells whereas Hsieh reported a small amount in a subpopulation of floating cells (110). In contrast, Fife *et al.* (6) reported extensive apoptosis under conditions that differed from those used by other investigators. Although VanWeelden demonstrated apoptosis in MCF-7 breast cancer cell tumors in nude mice (111), there are no reports of the effects of calcitriol or analogs on the induction of apoptosis in prostate cancer cells *in vivo*.

Growth Factors. Stromal–epithelial interactions are one of the most important regulators of growth in the prostate (112, 113). Androgens play a major role in the development and growth of the prostate (114). It is believed that the development of independence from these interactions occurs through expression of autocrine growth factors by the epithelium and contributes to cancer growth and progression. Some of the other critical factors for positive regulation of epithelial growth in the prostate include epidermal growth factor (EGF), keratinocyte growth factor (KGF), basic fibroblastic growth factor (bFGF), and insulin-like growth factor (IGF) (115, 116). Imbalances in the activity of these growth factors may contribute to the progression of prostatic disease.

Some evidence suggests that calcitriol can alter the activities of growth factors in the prostate. Calcitriol has been implicated in the positive regulation of IGF binding proteins, which regulate availability of IGF in ALVA-31 prostate cancer cells (117, 118). There is also evidence that TGFβ, an inhibitory growth factor, can be induced by calcitriol (119). TGFβ may contribute to the calcitriol-induced growth inhibition in PC3 cells (120, 121). However, LNCaP cells are not responsive to TGFβ (120), suggesting that calcitriol-induced growth inhibition in LNCaP cells occurs through a TGFβ-independent mechanism.

Several studies have suggested a potential relationship between response to calcitriol and androgens. Testosterone and its more potent metabolite, dihydrotestosterone (DHT), act by binding to the androgen receptor, which is a member of the same family of ligand-activated transcription factors as the VDR (122–125). Calcitriol induces androgen receptor expression as measured both by western blot and binding assays (126, 127). Moreover when calcitriol and DHT are administered in combination to LNCaP cells, the cells show a synergistic enhancement of PSA expression (127). This effect, interestingly, was much more pronounced when the cells were cultured in serum stripped with charcoal (126), which removes endogenous steroids as well as other small hydrophobic molecules including peptides. LNCaP cells are not growth inhibited by calcitriol when the cells are cultured in charcoal-stripped serum (19), but the antiproliferative ability of calcitriol was restored in charcoal-stripped serum when DHT was added back to the medium (94, 126). Remarkably, the androgen receptor antagonist, bicalutamide, partially reversed the growth inhibitory effects of calcitriol. These studies suggest that calcitriol action is closely linked to androgen action and that androgen regulation may contribute to the effectiveness of calcitriol in inhibiting growth.

Angiogenesis. Angiogenesis, the process through which new blood vessels are created, is a critical step in the progression and ultimate metastasis of tumors (128). Calcitriol has been shown to inhibit angiogenesis in an experimental tumor cell-induced angiogenesis assay in mice (129, 130), suggesting that cacitriol treatment may aid in reducing angiogenesis.

Summary

The epidemiological data are consistent with the hypothesis that low levels of vitamin D are a risk factor for prostate cancer. There have been numerous studies examining the effects of calcitriol and analogs on prostate cancer cell growth. In some cases (LNCaP cells (20) and primary prostate cancer cells (42)) growth inhibition is extensive, whereas in other cell lines, growth inhibition is modest. *In vivo* studies using the more responsive cells have not yet been described, so it is not known whether this remarkable growth inhibition will be reproduced *in vivo*. Whether the response of human prostate tumors will more closely match the response of primary cultures or of some of the more aggressive cell lines remains to be determined. The chief limitations in using calcitriol therapeutically are the adverse side effects of hypercalciuria and hypercalcemia. Nonetheless, Gross *et al.* (64) in a small study, were able to reduce the rate of PSA rise (which is used as a marker for prostate cancer) in a series of patients treated with calcitriol. The newly developed analogs that are less calcemic may permit treatment at levels sufficient to reduce tumor growth and provide an effective therapeutic or preventive agent as an alternative or addition to current prostate cancer therapies.

1. Doll R, Peto R. The causes of cancer. J Natl Cancer Inst **66:**1191–1308, 1991.
2. Schwartz GG, Hulka BS. Is vitamin D deficiency a risk factor for prostate cancer? (hypothesis). Anticancer Res **10:**1307–1312, 1990.
3. Haussler MR, Mangelsdorf DJ, Komm BS. Molecular biology of the vitamin D hormone. In: Clark JH, Ed. Recent Progress in Hormone Research: Proceedings of the 1988 Laurentian Hormone Conference. New York: Academic Press, Inc., pp263–305, 1988.
4. Anderson JJB, Toverud SU. Diet and vitamin D: A review with an emphasis on human function. J Nutr Biochem **5:**58–65, 1994.
5. Holick MF. The photobiology of vitamin D_3 in man. In: Kumar R, Ed. Vitamin D: Basic and Clinical Aspects. Boston: Martinus Nijhoff, pp197–216, 1984.

6. Fife Rs, Sledge GW, Proctor C. Effects of vitamin D_3 on proliferation of cancer cells *in vitro*. Cancer Lett **120:**65–69, 1997.
7. Colston KW, James SY, Ofori-Kuragu EA, Binderup L, Grant AG. Vitamin D receptors and antiproliferative effects of vitamin D derivatives in human pancreatic carcinoma cells *in vivo* and *in vitro*. Br J Cancer **76:**1017–1020, 1997.
8. Colston KW, Berger U, Coombes RC. Possible role for vitamin D in controlling breast cancer cell proliferation. Lancet **1:**188–191, 1989.
9. Colston KW, Colston JM, Feldman D. 1,25-Dihydroxyvitamin D_3 and malignant melanoma: The presence of receptors and inhibition of cell growth in culture. Endocrinology **108:**1083–1086, 1981.
10. Feldman D, Showronski RJ, Peehl DM. Vitamin D and prostate cancer. In: American Institute of Cancer Research, Ed. Diet and Cancer. New York: Plenum Press, pp53–63, 1995.
11. Hanchette CL, Schwartz GG. Geographic patterns of prostate cancer mortality. Evidence for a protective effect of ultraviolet radiation. Cancer **70:**2861–2869, 1992.
12. Wu LY, Semenya KA, Hardy RE, Hargreaves MK, Robinson SB, Pederson L, Sung JF, Haynes MA. Cancer rate differentials between blacks and whites in three metropolitan areas: A 10-year comparison. J Natl Med Assoc **90:**410–416, 1998.
13. Haenszel W, Kurihara M. Studies of Japanese migrants. I. Mortality from cancer and other diseases among Japanese in the United States. J Natl Cancer Inst **40:**43–68, 1968.
14. Giovannucci E. How is individual risk for prostate cancer assessed? Hematol Oncol Clin North Am **10:**537–548, 1996.
15. Zaridze DG. Cancer of the prostate: Epidemiology and aetiology. Br J Urol **59:**493–502, 1987.
16. Carter BS, Carter HB, Isaacs JT. Epidemiologic evidence regarding predisposing factors to prostate cancer. Prostate **16:**187–197, 1990.
17. Lian JB, Stein GS. Transcriptional control of vitamin D-regulated proteins. J Cell Biochem **49:**37–45, 1992.
18. Haussler MR, Jurutka PW, Hsieh J-C, Thompson PD, Selznick SH, Haussler CA, Whitfield GK. New understanding of the molecular mechanism of receptor-mediated genomic actions of the vitamin D hormone. Bone **17:**33S–38S, 1995.
19. Miller GJ, Stapleton GE, Ferrara JA, Lucia MS, Pfister S, Hedlund TE, Upadhya P. The human prostatic carcinoma cell line LNCaP expresses biologically active, specific receptors for 1 α,25-dihydroxyvitamin D_3. Cancer Res **52:**515–520, 1992.
20. Skowronski RJ, Peehl DM, Feldman D. Vitamin D and prostate cancer: 1,25-dihydroxyvitamin D_3 receptors and actions in human prostate cancer cell lines. Endocrinology **132:**1952–1960, 1993.
21. Newmark HL. Vitamin D adequacy: A possible relationship to breast cancer. Adv Exp Med Biol **364:**109–114, 1994.
22. Garland FC, Garland CF, Gorham ED, Young JF. Geographic variation in breast cancer mortality in the United States: A hypothesis involving exposure to solar radiation. Prev Med **19:**614–622, 1990.
23. Corder EH, Guess HA, Hulka BS, Friedman GD, Sadler M, Vollmer RT, Lobaugh B, Drezner MK, Vogelman JH, Orentreich N. Vitamin D and prostate cancer: A prediagnostic study with stored sera. Cancer Epidemiol Biomarkers Prev **2:**467–472, 1993.
24. Hiatt RA, Krieger N, Lobaugh B, Drezner MK, Vogelman JH, Orentreich N. Prediagnostic serum vitamin D and breast cancer. J Natl Cancer Inst **90:**461–463, 1998.
25. Eisman JA, Suva LJ, Sher E, Pearce PJ, Funder JW, Martin TJ. Frequency of 1,25-dihydroxyvitamin D_3 receptor in human breast cancer. Cancer Res **41:**5121–5124, 1981.
26. Chouvet C, Vicard E, Devonec M, Saez S. 1,25-dihydroxyvitamin D_3 inhibitory effect on the growth of two human breast cancer cell lines. J Steroid Biochem **24:**373–376, 1986.
27. Eisman JA, Koga M, Sutherland RL, Barkla DH, Tutton PJ. 1,25-dihydroxyvitamin D_3 and the regulation of human cancer cell replication. Proc Soc Exp Biol Med **191:**221–226, 1989.
28. Miller CW, Morosetti R, Campbell MJ, Mendoza S, Koeffler HP. Integrity of the 1,25-dihydroxyvitamin D_3 receptor in bone, lung, and other cancers. Mol Carcinog **19:**254–257, 1997.
29. Evans SR, Houghton AM, Schumaker L, Brenner RV, Buras RR, Davoodi F, Nauta RJ, Shabahang M. Vitamin D receptor and growth inhibition by 1,25-dihydroxyvitamin D_3 in human malignant melanoma cell lines. J Surg Res **61:**127–133, 1996.
30. Koeffler HP, Amatruda T, Ikekawa N, Kobayashi Y, DeLuca HF. Induction of macrophage differentiation of human normal and leukemic myeloid stem cells by 1,25-dihydroxyvitamin D_3 and its fluorinated analogues. Cancer Res **44:**5624–5628, 1984.
31. Studzinski GP, Moore DC. Sunlight: Can it prevent as well as cause cancer? Cancer Res **55:**4014–4022, 1995.
32. Lawson DE, Paul AA, Black AE, Cole TJ, Mandal AR, Davie M. Relative contributions of diet and sunlight to vitamin D state in the elderly. Br Med J **2:**303–305, 1979.
33. Baker MR, Peacock M, Nordin BE. The decline in vitamin D status with age. Age Ageing **9:**249–252, 1980.
34. Matusoka LY, Wortsman J, Haddad JG, Kolm P, Hollis BW. Racial pigmentation and the cutaneous synthesis of vitamin D. Arch Dermatol **127:**536–538, 1991.
35. Gann PH, Ma J, Hennekens CH, Hollis BW, Haddad JG, Stampfer MJ. Circulating vitamin D metabolites in relation to subsequent development of prostate cancer. Cancer Epidemiol Biomarkers Prev **5:** 121–126, 1996.
36. Braun MM, Helzlsouer KJ, Hollis BW, Comstock GW. Prostate cancer and prediagnostic levels of serum vitamin D metabolites. Cancer Causes Control **6:**235–239, 1995.
37. Taylor JA, Hirvonen A, Watson M, Pittman G, Mohler JL, Bell DA. Association of prostate cancer with vitamin D receptor gene polymorphism. Cancer Res **56:**4108–4110, 1996.
38. Ingles SA, Ross RK, Yu MC, Irvine RA, Lapera G, Haile RW, Coetzee GA. Association of prostate cancer risk with genetic polymorphisms in vitamin D receptor and androgen receptor. J Natl Cancer Inst **89:**166–170, 1997.
39. Kibel AS, Isaacs SD, Isaacs WB, Bova GS. Vitamin D receptor polymorphisms and lethal prostate cancer. J Urol **160:**1405–1409, 1998.
40. Kivineva M, Blauer M, Syvala H, Tammela T, Tuohimaa P. Localization of 1,25-dihydroxyvitamin D_3 receptor (VDR) expression in human prostate. J Steroid Biochem Mol Biol **66:**121–127, 1998.
41. Stevens A, Lowe JS. Male reproductive system. In: Stevens A, Lowe JS, Eds. Histology. London: Gower Medical Publishing, pp 304–321, 1992.
42. Peehl DM, Skowronski RJ, Leung GK, Wong ST, Stamey TA, Feldman D. Antiproliferative effects of 1,25-dihydroxyvitamin D_3 on primary cultures of human prostatic cells. Cancer Res **54:**805–810, 1994.
43. Konety BR, Schwartz GG, Acierno JS, Becich MJ, Getzenberg RH. The role of vitamin D in normal prostate growth and differentiation. Cell Growth Differ **7:**1563–1570, 1996.
44. Greenberg NM, DeMayo F, Finegold MJ, Medina D, Tilley WD, Aspinall JO, Cunha GR, Donjacour AA, Matusik RJ, Rosen JM. Prostate cancer in a transgenic mouse. Proc Natl Acad Sci U S A **92:** 3439–3443, 1995.
45. Greene DR, Taylor SR, Aihara M, Yoshida K, Egawa S, Park SH, Timme TL, Yang G, Scardino PT, Thompson TC. DNA ploidy and clonal selection in *ras* + *myc*-induced mouse prostate cancer. Int J Cancer **60:**395–399, 1995.
46. Tilley WD, Wilson CM, Marcelli M, McPhaul MJ, Androgen receptor gene expression in human prostate carcinoma cell lines. Cancer Res **50:**5382–5386, 1990.
47. Trapman J, Brinkmann AO. The androgen receptor in prostate cancer. Pathol Res Pract **192:**752–760, 1996.
48. Klocker H, Culig Z, Hobisch A, Cato ACB, Bartsch G. Androgen receptor alterations in prostatic carcinoma. Prostate **25:**266–273, 1994.
49. Byrne RL, Horne CH, Robinson MC, Autzen P, Apakama I, Bishop RI, Neal DE, Hamdy FC. The expression of *waf*-1, p53 and *bcl*-2 in prostate adenocarcinoma. Br J Urol **79:**190–195, 1997.
50. Kubota Y, Fujinami K, Uemura H, Dobashi Y, Miyamoto H, Iwasaki Y, Kitamura H, Shuin T. Retinoblastoma gene mutations in primary human prostate cancer. Prostate **27:**314–320, 1995.
51. Brooks JD, Bova GS, Ewing CM, Piantadosi S, Carter BS, Robinson JC, Epstein JI, Isaacs WB. An uncertain role for p53 gene alterations in human prostate cancers. Cancer Res **56:**3814–3822, 1996.
52. Grignon DJ, Caplan R, Sarkar FH, Lawton CA, Hammond EH, Pilepich MN, Forman JD, Mesic J, Fu KK, Abrams RA, Pajak TF, Shipley WU, Cox JD. p53 status and prognosis of locally advanced prostatic adenocarcinoma: A study based on RTOG 8610. J Natl Cancer Inst **89:**158–165, 1997.
53. Horoszewicz JS, Leong SS, Kawinski E, Karr JP, Rosenthal H, Chu

TM, Mirand EA, Murphy GP. LNCaP model of human prostatic carcinoma. Cancer Res **43:**1809–1818, 1983.
54. Veldscholte J, Ris-Stalpers C, Kuiper GGJM, Jenster G, Berrevoets C, Claassen E, van Rooij HCJ, Trapman J, Brinkmann AO, Mulder E. A mutation in the ligand binding domain of the androgen receptor of human LNCaP cells affects steroid binding characteristics and response to antiandrogens. Biochem Biophys Res Commun **173:**534–540, 1990.
55. Blutt SE, Allegretto EA, Pike JW, Weigel NL. 1,25-dihydroxyvitamin D_3 and 9-*cis*-retinoic acid act synergistically to inhibit the growth of LNCaP prostate cells and cause accumulation of cells in G_1. Endocrinology **138:**1491–1497, 1997.
56. de Vos S, Holden S, Heber D, Elstner E, Binderup L, Uskokovic M, Rude B, Chen D, Le J, Cho SK, Koeffler HP. Effects of potent vitamin D_3 analogs on clonal proliferation of human prostate cancer cell lines. Prostate **31:**77–83, 1997.
57. Schwartz GG, Wang MH, Zang M, Singh RK, Siegal GP. 1α,25-Dihydroxyvitamin D (calcitrol) inhibits the invasiveness of human prostate cancer cells. Cancer Epidemiol Biomarkers Prev **6:**727–732, 1997.
58. Water DJ, Bostwick DG. The canine prostate is a spontaneous model of intraepithelial neoplasia and prostate cancer progression. Anticancer Res **17:**1467–1470, 1997.
59. Pretlow TG, Delmoro CM, Dilley GG, Spadafora CG, Pretlow TP. Transplantation of human prostatic carcinoma into nude mice in matrigel. Cancer Res **51:**3814–3817, 1991.
60. Wang Y, Corr JC, Thaler HT, Tao Y, Fair WR, Heston WD. Decreased growth of established human prostate LNCaP tumors in nude mice fed a low-fat diet. J Natl Cancer Inst **84:**1456–1462, 1995.
61. Lubaroff DM, Canfield L, Rasmussen GT, Reynolds CW. An animal model for the study of prostate carcinoma. Natl Cancer Inst Monogr **49:**275–281, 1978.
62. Getzenberg RH, Light BW, Lapco PE, Konety BR, Nangia AK, Acierno JS, Dhir R, Shurin Z, Day RS, Trump DL, Johnson CS. Vitamin D inhibition of prostate adenocarcinoma growth and metastasis in the Dunning rat prostate model system. Urology **50:**999–1006, 1997.
63. Osborne JL, Schwartz GG, Smith DC, Bahnson R, Day R, Trump DL. Phase II trial of oral 1,25-dihydroxyvitamin D (calcitriol) in hormone refractory prostate cancer. Urol Oncol **1:**195, 1995.
64. Gross C, Stamey T, Hancock S, Feldman D. Treatment of early recurrent prostate cancer with 1,25-dihydroxyvitamin D_3 (calcitriol). J Urol **159:**2035–2040, 1998.
65. Holick MF, Krane SM, Potts JT Jr. Calcium, phosphorus, and bone metabolism: Calcium-regulating hormones. In: Fauci AS, Braunwald E, Isselbacher KJ, Martin JB, Eds. Principles of Internal Medicine (14th ed). New York: McGraw Hill, p. 2220, 1998.
66. Campbell NJ, Elstner E, Holden S, Uskokovic M, Koeffler HP. Inhibition of proliferation of prostate cancer cells by a 19-nor-hexafluoride vitamin D_3 analogue involves the induction of p21waf1, p27kip1 and E-cadherin. J Mol Endocrinol **19:**15–27, 1997.
67. Schwartz GG, Oeler TA, Uskokovic MR, Bahnson RR. Human prostate cancer cells: Inhibition of proliferation by vitamin D analogs. Anticancer Res **14:**1077–1081, 1994.
68. Kubota T, Koshizuka K, Koike M, Uskokovic M, Miyoshi I, Koeffler HP. 19-nor-26,27-bishomo-vitamin D_3 analogs: A unique class of potent inhibitors of proliferation of prostate, breast, and hematopoietic cancer cells. Cancer Res **58:**3370–3374, 1998.
69. Hedlund TE, Moffatt KA, Uskokovic MR, Miller GJ. Three synthetic vitamin D analogues induce prostate-specific acid phosphatase and prostate-specific antigen while inhibiting the growth of human prostate cancer cells in a vitamin D receptor–dependent fashion. Clin Cancer Res **3:**1331–1338, 1997.
70. Vandewalle B, Wattez N, Lefebvre J. Effects of vitamin D_3 derivatives on growth, differentiation, and apoptosis in tumoral colonic HT29 cells: Possible implication of intracellular calcium. Cancer Lett **97:**99–106, 1995.
71. Schwartz GG, Hill CC, Oeler TA, Becich MJ, Bahnson RR. 1,24-dihydroxy-16-ene-23-yne-vitamin D_3 and prostate cancer cell proliferation *in vivo*. Urology **46:**365–369, 1995.
72. Campbell MJ, Reddy GS, Koeffler HP. Vitamin D_3 analogs and their 24-oxo metabolites equally inhibit clonal proliferation of a variety of cancer cells but have differing molecular effects. J Cell Biochem **66:**413–425, 1997.
73. Hansen CM, Maenpaa PH. EB 1089, a novel vitamin D analog with strong antiproliferative and differentiation-inducing effects on target cells. Biochem Pharmacol **54:**1173–1179, 1997.
74. Skowronski RJ, Peehl DM, Feldman D. Actions of vitamin D_3 analogs on human prostate cancer cell lines: Comparison with 1,25-dihydroxyvitamin D_3. Endocrinology **136:**20–26, 1995.
75. Wang X, Chen X, Akhter J, Morris DL. The *in vitro* effect of vitamin D_3 analogue EB-1089 on a human prostate cancer cell line (PC-3). Br J Urol **80:**260–262, 1997.
76. Kissmeyer AM, Binderup E, Binderup L, Hansen M, Andersen NR, Makin HL, Schroeder NJ, Shankar VN, Jones G. Metabolism of the vitamin D analog EB 1089: Identification of *in vivo* and *in vitro* liver metabolites and their biological activities. Biochem Pharmacol **53:**1087–1097, 1997.
77. Gulliford T, English J, Colston KW, Menday P, Moller S, Coombes RC. A phase I study of the vitamin D analogue EB 1089 in patients with advanced breast and colorectoral cancer. Br J Cancer **78:**6–13, 1998.
78. Nemere I, Zhou LX, Norman AW. Nontranscriptional effects of steroid hormones. Receptor **3:**277–291, 1993.
79. Nemere I, Dormanen MC, Hammond MW, Okamura WH, Norman AW. Identification of a specific binding protein for 1α,25-dihydroxyvitamin D_3 in basal-lateral membranes of chick intestinal epithelium and relationship to transcaltachia. J Biol Chem **269:**23750–23756, 1994.
80. Hedlund TE, Moffat KA, Miller GJ. Stable expression of the nuclear vitamin D receptor in the human prostatic carcinoma cell line JCA-1: Evidence that the antiproliferative effects of 1α,25-dihydroxyvitamin D_3 are mediated exclusively through the genomic signaling pathway. Endocrinology **137:**1554–1561, 1996.
81. Hedlund TE, Moffatt KA, Miller GJ. Vitamin D receptor expression is required for growth modulation by 1α,25-dihydroxyvitamin D_3 in the human prostatic carcinoma cell line ALVA-31. J Steroid Biochem Mol Biol **58:**277–288, 1996.
82. Zhuang SH, Schwartz GG, Cameron D, Burnstein KL. Vitamin D receptor content and transcriptional activity do not fully predict antiproliferative effects of vitamin D in human prostate cancer cell lines. Mol Cell Endocrinol **126:**83–90, 1997.
83. Zhao X, Feldman D. Regulation of vitamin D receptor abundance and responsiveness during differentiation of HT-29 human colon cancer cells. Endocrinology **132:**1808–1814, 1993.
84. Owen TA, Aronow MS, Barone LM, Bettencourt B, Stein GS, Lian JB. Pleiotropic effects of vitamin D on osteoblast gene expression are related to the proliferative and differentiated state of the bone cell phenotype: Dependency upon basal levels of gene expression, duration of exposure, and bone matrix competency in normal rat osteoblast cultures. Endocrinology **128:**1496–1504, 1991.
85. Costa EM, Blau HM, Feldman D. 1,25-dihydroxyvitamin D_3 receptors and hormonal responses in cloned human skeletal muscle cells. Endocrinology **119:**2214–2220, 1986.
86. Suda T, Shinki T, Takahashi N. The role of vitamin D in bone and intestinal cell differentiation. Ann Rev Nutr **10:**195–211, 1990.
87. Suda T, Takahashi N, Martin TJ. Modulation of osteoclast differentiation. Endocrine Rev **13:**66–80, 1992.
88. Nakajima H, Kizaki M, Veno H, Muto A, Takayama N, Matasushita H, Sonoda A, Keda Y. All-*trans* and 9-*cis* retinoic acid enhance 1,25-dihydroxyvitamin D_3–induced monocytic differentiation of U937 cells. Leukemia Res **20:**665–676, 1996.
89. Abe E, Miyayra C, Sakagami H, Takeda K, Konno T, Yamazaki S, Suda T. Differentiation of mouse myeloid leukemia cells induced by 1α,25-dihydroxyvitamin D_3. Proc Natl Acad Sci U S A **78:**4990–4994, 1981.
90. Papsidero LD, Kuriyama M, Wang MC, Horoszewicz J, Leong SS, Valenzuela L, Murphy GP, Chu TM. Prostate antigen: A marker for human prostate epithelial cells. J Natl Cancer Inst **66:**37–41, 1991.
91. Sinha AA, Wilson MJ, Gleason DF. Immunoelectron microscopic localization of prostatic-specific antigen in human prostate by the protein A-gold complex. Cancer **60:**1288–1293, 1987.
92. Gleave ME, Hsieh JT, Wu H-C, Von Eschenbach AC, Chung LWK. Serum prostate-specific antigen levels in mice bearing human prostate LNCaP tumors are determined by tumor volume and endocrine and growth factors. Cancer Res **52:**1598–1605, 1992.
93. Stamey TA, Kabalin JN. Prostate-specific antigen in the diagnosis

and treatment of adenocarcinoma of the prostate. I. Untreated patients. J Urol **141:**1070–1075, 1989.
94. Esquenet M, Swinnen JV, Heyns W, Verhoeven G. Control of LNCaP proliferation and differentiation: Actions and interactions of androgens, 1α,25-dihydroxycholecalciferol, all-*trans* retinoic acid, 9-*cis* retinoic acid, and phenylacetate. Prostate **28:**182–194, 1996.
95. Zhang F, Godyn JJ, Uskokovic M, Binderup L, Studzinski GP. Monocytic differentiation of HL60 cells induced by potent analogs of vitamin D_3 precedes the G_1/G_0 phase cell cycle block. Cell Prolif **27:** 643–654, 1995.
96. Simboli-Campbell M, Narvaez CJ, VanWeelden K, Tenniswood M, Welsh J. Comparative effects of 1,25-$(OH)_2D_3$ and EB 1089 on cell cycle kinetics and apoptosis in MCF-7 breast cancer cells. Breast Cancer Res Treat **42:**31–41, 1997.
97. Zhuang SH, Burnstein KL. Antiproliferative effect of 1α,25-dihydroxyvitamin D_3 in human prostate cancer cell line LNCaP involves reduction of cyclin-dependent kinase 2 activity and persistent G_1 accumulation. Endocrinology **139:**1197–1207, 1998.
98. Bartek J, Lukas J, Strauss M. A common path to tumor growth: Deregulation of Rb-dependent restriction point contol (review). Oncology Reports **3:**237–240, 1996.
99. Sidle A, Palaty C, Dirks P, Wiggan O, Kiess M, Gill RM, Wong AK, Hamel PA. Activity of the retinoblastoma family proteins, pRB, p107, and p130, during cellular proliferation and differentiation. Crit Rev Biochem Mol Biol **31:**237–271, 1996.
100. Dyson N. The regulation of E2F by pRB-family proteins. Genes Dev **12:**2245–2262, 1998.
101. Sarkar FH, Sakr W, Li YW, Macoska J, Ball DE, Crissman JD. Analysis of retinoblastoma (RB) gene deletion in human prostatic carcinomas. Prostate **21:**145–152, 1992.
102. Carter BS, Ewing CM, Ward WS, Treiger BF, Aalders TW, Schalken JA, Epstein JI, Isaacs WB. Allelic loss of chromosomes 16q and 10q in human prostate cancer. Proc Natl Acad Sci U S A **87:**8751–8755, 1990.
103. Sherr CJ. G_1 phase progression: Cycling on cue. Cell **79:**551–555, 1994.
104. Liu M, Lee M-H, Cohen M, Bommakanti M, Freedman LP. Transcriptional activation of the Cdk inhibitor p21 by vitamin D_3 leads to the induced differentiation of the myelomonocytic cell line U937. Genes Dev **10:**142–153, 1996.
105. Bookstein R, Shew J-Y, Chen P-L, Scully P, Lee W-H. Suppression of tumorigenicity of human prostate carcinoma cells by replacing a mutated Rb gene. Science **247:**712–715, 1990.
106. Gross C, Skowronski RJ, Plymate SR, Rhim JS, Peehl DM, Feldman D. Simian virus 40-, but not human papillomavirus-, transformation of prostatic epithelial cells results in loss of growth-inhibition by 1,25-dihydroxyvitamin D_3. Int J Oncol **8:**41–47, 1996.
107. Simboli-Campbell M, Narvaez CJ, Tenniswood J, Welsh J. 1,25-dihydroxyvitamin D_3 induces morphological and biochemical markers of apoptosis in MCF-7 breast cancer cells. J Steroid Biochem Mol Biol **58:**367–376, 1996.
108. Narvaez CJ, Welsh J. Differential effects of 1,25-dihydroxyvitamin D_3 and tetradecanoylphorbol acetate on cell cycle and apoptosis of MCF-7 cell and a vitamin D_3-resistant variant. Endocrinology **138:**4690–4698, 1997.
109. Xu H-M, Tepper CG, Jones JB, Fernandez CE, Studzinski GP. 1,25-Dihydroxyvitamin D_3 protects HL60 cells against apoptosis but downregulates the expression of the *bcl*-2 gene. Exp Cell Res **209:** 367–374, 1993.
110. Hsieh T-C, Wu KJ. Induction of apoptosis and altered nuclear/cytoplasmic distribution of the androgen receptor and prostate specific antigen by 1α,25-dihydroxyvitamin D_3 in androgen-responsive LNCaP cells. Biochem Biophys Res Commun **235:**539–544, 1997.
111. VanWeelden K, Flanagan L, Binderup L, Tenniswood M, Welsh J. Apoptotic regression of MCF-7 xenografts in nude mice treated with the vitamin D_3 analog, EB1089. Endocrinology **139:**2102–2110, 1998.
112. Chung LW. Implications of stromal-epithelial interaction in human prostate cancer growth, progression, and differentiation. Semin Cancer Biol **4:**183–192, 1993.
113. Kooistra A, Konig JJ, Keizer DM, Romijn JC, Schroder FH. Inhibition of prostatic epithelial cell proliferation by a factor secreted specifically by prostatic stromal cells. Prostate **26:**123–132, 1995.
114. Sandberg AA. Endocrine control and physiology of the prostate. Prostate **1:**169–184, 1980.
115. Ware JL. Growth factors and their receptors as determinants in the proliferation and metastasis of human prostate cancer. Cancer Metastasis Rev **12:**287–301, 1993.
116. Peehl DM. Cellular biology of prostatic growth factors. Prostate (Suppl)**6:**74–78, 1996.
117. Huynh H, Pollak M, Zhang JC. Regulation of insulin-like growth factor (IGF)-II and IGF binding protein 3 autocrine loop in human PC-3 prostate cancer cells by vitamin D metabolite 1,25-(OH) $2D_3$ and its analog EB1089. Int J Oncol **13:**137–143, 1998.
118. Drivdahl RH, Loop SM, Andress DL, Ostenson RC. IGF-binding proteins in human prostate tumor cells: Expression and regulation by 1,25-dihydroxyvitamin D_3. Prostate **26:**72–79, 1995.
119. Koli K, Keski-Oja J. Vitamin D_3 regulation of transforming growth factor-β system in epithelial and fibroblastic cells: Relationships to plasminogen activation. J Invest Dermatol (Symp Proc) **1:**33–38, 1996.
120. Wilding G, Zugmeier G, Knabbe C, Flanders K, Gelmann E. Differential effects of transforming growth factor β on human prostate cancer cells *in vitro* Mol Cell Endocrinol **62:**79–87, 1989.
121. Desruisseau S, Ghazarossian-Ragni E, Chinot O, Martin PM. Divergent effect of TGF1 on growth and proteolytic modulation of human prostatic-cancer cell lines. Int J Cancer **66:**796–801, 1996.
122. Jenster G, van der Korput HA, van Vroonhoven C, van der Kwast TH, Trapman J, Brinkmann AO. Domains of the human androgen receptor involved in steroid binding, transcriptional activation, and subcellular localization. Mol Endocrinol **5:**1396–1404, 1991.
123. Trapman J, Klaassen P, Kuiper GG, van der Korput JA, Faber PW, van Rooij HC, Geurts van Kessel A, Voorhorst MM, Mulder E, Brinkmann AO. Cloning, structure, and expression of a cDNA encoding the human androgen receptor. Biochem Biophys Res Commun **153:**241–248, 1988.
124. Tilley WD, Marcelli M, Wilson JD, McPhaul MJ. Characterization and expression of a cDNA encoding the human androgen receptor. Proc Natl Acad Sci U S A **86:**327–331, 1989.
125. Brinkmann AO, Faber PW, van Rooij HC, Kuiper GG, Ris C, Klaassen P, van der Korput JA, Voorhorst MM, van Laar JH, Mulder E. The human androgen receptor: Domain structure, genomic organization, and regulation of expression. J Steroid Biochem Mol Biol **34:** 307–310, 1989.
126. Zhao X-Y, Ly LH, Peehl DM, Feldman D. 1α,25-Dihydroxyvitamin D_3 actions in LNCaP human prostate cancer cells are androgen-dependent. Endocrinology **138:**3290–3298, 1997.
127. Hsieh T-C, Ng C-Y, Mallouh C, Tazaki H, Wu JM. Regulation of growth, PSA/PAP and androgen receptor expression by 1α,25-dihydroxyvitamin D_3 in the androgen-dependent LNCaP cells. Biochem Biophys Res Commun **223:**141–146, 1996.
128. Folkman J. The role of angiogenesis in tumor growth. Semin Cancer Biol **3:**65–71, 1992.
129. Shokravi MT, Marcus DM, Alroy J, Egan K, Saornil MA, Albert DM. Vitamin D inhibits angiogenesis in transgenic murine retinoblastoma. Invest Opthalmol Vis Sci **36:**83, 1995.
130. Majewski S, Skopinska M, Marczak M, Szmurlo A, Bollag W, Jablonska S. Vitamin D_3 is a potent inhibitor of tumor cell-induced angiogenesis. J Invest Dermatol (Symp Proc) **1:**97–101, 1996.
131. Carroll AG, Voeller HJ, Sugars L, Gelmann EP. p53 Oncogene mutations in three human prostate cancer cell lines. Prostate **23:**123–134, 1993.
132. Kaighn ME, Narayan KS, Ohnuki Y, Lechner JF, Jones LW. Establishment and characterization of a human prostatic carcinoma cell line (PC-3). Investig Urol **17:**16–23, 1979.
133. Rubin SJ, Hallahan DE, Ashman CR, Brachman DG, Beckett MA, Virudachalam S, Yandell DW, Weichselbaum RR. Two prostate carcinoma cell lines demonstrate abnormalities in tumor suppressor genes. J Surg Oncol **46:**31–36, 1991.
134. Stone KR, Mickey DD, Wunderli H, Mickey GH, Paulson DF. Isolation of a human prostate carcinoma cell line (DU 145). Int J Cancer **21:**274–281, 1978.
135. Muraki J, Addonizio JC, Choudhury MS, Fischer J, Eshghi M, Davidian MM, Shapiro LR, Wilmot PL, Nagamatsu GR, Chiao JW. Establishment of a new human protatic cancer cell line. Invest Urol **36:**79–84, 1990.
136. Miller GJ, Stapleton GE, Hedlund TE, Moffat KA. Vitamin D recep-

tor expression, 24-hydroxylase activity, and inhibition of growth by 1α, 25-dihydroxyvitamin D_3 in seven human prostatic carcinoma cell lines. Clin Cancer Res **1:**997–1003, 1995.

137. Faiella L, Ng C, Hsieh TC, Wu JM, Mallouh C. Effects of IFN-β and 1,25-dihydroxyvitamin D_3 on cellular proliferation, induction of 2′,5′-oligoadenylate (2-5A) synthetase and changes in immunoreactive pRB/p53 in human prostatic JCA-1 cells. Biochem Mol Biol Int **39:**1085–1092, 1996.
138. Loop SM, Rozanski TA, Ostenson RC. Human primary prostate tumor cell line, ALVA-31: A new model for studying the hormonal regulation of prostate tumor cell growth. Prostate **22:**93–108, 1993.
139. Eaton CL, Pierrepoint CG. Growth of a spontaneous canine prostatic adenocarcinoma *in vivo* and *in vitro:* Isolation and characterization of a neoplastic prostatic epithelial cell line, CPA 1. Prostate **12:**129–143, 1988.
140. Castagnetta L, Carruba G, Granata OM, Lo Casto M, Arcuri F, Mesiti M, Pavone-Macaluso M. Prostate long-term epithelial cell lines: Biological and biochemical features. Ann N Y Acad Sci **595:**64, 1990.
141. Danielpour D, Kadomatsu K, Anzano MA, Smith JM, Sporn MB. Development and characterization of nontumorigenic and tumorigenic epithelial cell lines from rat dorsal-lateral prostate. Cancer Res **54:** 3413–3421, 1994.
142. Isaacs JT, Isaacs WB, Feitz WF, Scheres J. Establishment and characterization of seven Dunning rat prostatic cancer cell lines and their use in developing methods for predicting metastatic abilities of prostatic cancers. Prostate **9:**261–281, 1986.
143. Diamond DA, Barrack ER. The relationship of androgen receptor levels to androgen responsiveness in the Dunning R3327 rat prostate tumor sublines. J Urol **132:**821–827, 1984.