MINIREVIEW

Vitamin D and Prostate Cancer

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Vitamin D and its metabolites are best known for their actions in calcium and bone metabolism. However, epidemiological studies have suggested that an increased prostate cancer risk is associated with decreased production of vitamin D. *In vitro* and *in vivo* studies have shown that the biologically active form of vitamin D, 1α,25-dihydroxyvitamin D_3 (1,25D), inhibits proliferation of cancer cells derived from multiple tissues, including the prostate. Although the mechanisms underlying the growth inhibitory effects of 1,25D have not been fully elucidated, in prostate cancer cells 1,25D reduces cell growth via a number of cellular pathways, including cell cycle arrest, induction of apoptosis, and altered activation of growth factor signaling. The hypercalcemia induced by 1,25D *in vivo* limits its use clinically as a therapeutic agent. However, several 1,25D analogs have been developed that reduce prostate tumor growth in rodent xenograft models without causing hypercalcemia. Additional studies are required in order to determine whether these 1,25D analogs will be useful therapeutic agents for the treatment of prostate cancer. Exp Biol Med 229:277–284, 2004

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Prostate cancer is the most commonly diagnosed cancer in American men. Estimates indicate that 220,900 new cases of prostate cancer will be diagnosed in the United States in the year 2003 (1). Localized prostate cancer is routinely treated with surgery or radiation therapy, while the standard therapy for metastatic prostate cancer is androgen ablation. Although androgen ablation is initially successful at reducing prostate tumor growth, androgen-independent forms of the tumor develop approximately 18–24 months after the start of therapy (2). Presently, there are no effective treatments for these more aggressive, androgen-independent forms of prostate cancer. As a result, prostate cancer has become the second leading cause of cancer death among American men (1). Novel therapeutic strategies are therefore required in order to decrease incidence as well as the morbidity and mortality associated with prostate cancer. As described below, data from several studies suggest that derivatives of vitamin D_3 may serve as effective chemopreventive and/or therapeutic agents for prostate cancer.

Vitamin D and Vitamin D Receptor

Vitamin D is well known for its role in the regulation of calcium and phosphate homeostasis (3). However, a number of studies have shown that vitamin D metabolites also regulate growth and differentiation of other cell types. Cholecalciferol (vitamin D_3) can be obtained from foods such as fortified dairy products, fish oils, and egg yolks. However, the major source of vitamin D_3 is the skin, where the precursor 7-dehydrocholestrol is converted to vitamin D_3 upon exposure to ultraviolet radiation in sunlight. In the liver, the enzyme 25-hydroxylase converts vitamin D_3 to 25-hydroxyvitamin D_3 (25D), the major circulating form of vitamin D_3. 25D is further hydroxylated in the kidney by the enzyme 1α-hydroxylase to form the biologically active form of vitamin D, 1α,25-dihydroxyvitamin D_3 (1,25D). While the kidney is the primary site of 1,25D production, 1α-hydroxylase is also expressed in extrarenal sites, including the prostate (4), allowing for local synthesis of 1,25D.

The biological actions of 1,25D are primarily mediated by the vitamin D receptor (VDR), a member of the nuclear receptor superfamily of ligand-activated transcription factors (5). VDR normally resides in the nucleus. Upon binding 1,25D, the VDR, as part of a heterodimer complex with the retinoid X receptor (RXR), interacts with regions of DNA, termed vitamin D response elements (VDREs), and recruits coactivators that aid in regulating target gene transcription.

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Vitamin D and Normal Prostate

VDR is expressed in normal prostate epithelial and stromal cells (6). 1,25D inhibits the growth of prostate epithelial cell lines and primary cultures of epithelial and stromal cells derived from normal prostate tissue (6–11). The precursor 25D is as effective as 1,25D in inhibiting growth of normal prostate epithelial cells (9, 10, 12), because of the fact that 1α-hydroxylase is expressed in the normal prostate.

Vitamin D and the Incidence of Prostate Cancer

The major risk factors associated with prostate cancer are age, race, and geography (13). The incidence of prostate cancer increases with age, with greater than 70% of all prostate cancers diagnosed in men over 65 years old (1). It is most common in northern geographic regions such as North America and Northwestern Europe, with fewer cases found in South America and Asia (1). The incidence of prostate cancer also varies among ethnic groups, with the highest incidence of prostate cancer in the world found in African American males (1). The increased risk associated with these factors may be linked to low levels of vitamin D. Prostate cancer mortality increases with decreased exposure to sunlight (13, 14). Circulating levels of vitamin D metabolites also decrease with increasing age (15). Finally, high levels of melanin reduce vitamin D synthesis in the skin of African American males (16). Although some studies have detected correlations between serum levels of vitamin D metabolites and risk of prostate cancer, others have found no association (17–20). In a 13-year study involving approximately 19,000 Finnish men, an increased risk for prostate cancer was found in men with low serum 25D levels, with the highest prostate cancer risk in young men with low 25D levels (21). When compared with normal prostate, prostate cancer tissue expresses lower levels of 1α-hydroxylase (12, 22). The development of prostate cancer may therefore be enhanced not only by reduced circulating levels of 1,25D but also by decreased local production of 1,25D.

Vitamin D and Prostate Cancer

The effects of 1,25D on the growth of human prostate cancer cells vary widely. Of the cell lines tested, the one most responsive to 1,25D is the androgen-dependent LNCaP cells, which were established from a lymph node metastasis and express both VDR and the androgen receptor (AR) (23). 1,25D treatment for 6 days leads to a >60% decrease in LNCaP cell growth (24, 25) and the growth rate remains depressed after 1,25D has been removed from the culture media (26). The AR-positive MDA PCa 2a and 2b cells are also sensitive to growth inhibition by 1,25D (27). AR-negative, androgen-independent prostate cancer cell lines such as PC-3 cells are generally less responsive (25, 26).

Mechanisms of Vitamin D Action in Prostate Cancer

VDR reduces cell growth and facilitates differentiation in many tissue types through the regulation of target gene expression. In contrast with a drug that targets a single protein, the consequences of VDR activity in tumor tissue will depend not only on its own receptor activity, but also on the changes in VDR effector gene expression unique to the individual tumor. Thus, although VDR may be active in most prostate cancer cells, the response of the cells will vary depending on whether the cells retain intact signaling, cell cycle regulation, and apoptotic pathways.

Role of the Vitamin D Receptor

Although the best characterized activities of 1,25D are mediated by the nuclear VDR, 1,25D also induces rapid signaling events that may be independent of the nuclear receptor and could contribute to responsiveness to 1,25D (28, 29). Two complementary studies demonstrate that the VDR is required for 1,25D-induced growth inhibition in prostate cancer cells. First, ALVA 31 cells expressing antisense VDR mRNA expressed lower levels of VDR protein and were resistant to the growth inhibitory effect of 1,25D (30). In the second study, the level of VDR expression in the 1,25D-resistant JCA-1 cell line was increased via stable transfection of cells with wild-type VDR producing a line, the growth of which was inhibited by 1,25D (31). While VDR is required for 1,25D growth inhibition, the ability of 1,25D to decrease prostate cancer cell growth is not solely determined by the amount of VDR expressed in the cells. Although ALVA 31 cells express higher VDR protein levels than LNCaP cells, the LNCaP cell line is more sensitive to the growth inhibitory effects of 1,25D (32). Furthermore, DU145 cells, which express functional VDR, are minimally growth inhibited by 1,25D (25, 32).

A Role for the Androgen Receptor in Response to 1,25D

The androgen receptor (AR) plays a key role in the regulation of prostate cancer cell growth. In LNCaP cells, growth inhibition by 1,25D is dependent on the activity of AR. While 1,25D significantly decreased growth of LNCaP cells cultured in media containing fetal bovine serum (and endogenous androgen), it did not inhibit growth of LNCaP cells when they were cultured in media containing charcoal-stripped serum, which lacks androgens (33). Furthermore, the antiproliferative effect of 1,25D in LNCaP cells was blocked by the antiandrogen casodex (33). If AR antagonist reversal of 1,25D action were a typical prostate cancer response, then VDR agonists might not be useful in combination with antiandrogen treatment. However, in the AR-positive MDA PCA 2a and 2b cells, growth inhibition by 1,25D was not affected by the presence of androgens or casodex in either cell line (27). Additionally, LNCaP-104R1

cells, an androgen-independent derivative of the LNCaP cells which still expresses AR, are growth inhibited by 1,25D in the absence of androgens (34). Thus, functional interactions between AR and VDR are observed only in a subset of prostate cancer cells.

Cell Cycle

A decrease in cell growth is often caused by a reduction in cell cycle progression. 1,25D causes a G_0/G_1 arrest in human prostate epithelial cells (HPEC; Ref. 35) and increases the percentage of LNCaP cells that accumulate in the G_0/G_1 phase of the cell cycle (24, 36). 1,25D induces a G_0-like quiescent state in LNCaP cells; expression of Ki-67, a nuclear protein that is expressed in cycling cells, is dramatically decreased in LNCaP cells treated with 1,25D (26, 37). However, 1,25D treatment does not induce G_1 accumulation or decrease expression of Ki-67 in the PC-3 cell line (26).

Progression of cells from G_1 to S phase of the cell cycle is regulated by the retinoblastoma protein (Rb). In its active form, Rb is hypophosphorylated and binds to E2F family members, preventing transcription of genes required for the progression to S phase. Phosphorylation of Rb leads to disruption of Rb-E2F complexes, resulting in E2F-mediated transcription and S phase progression (38). 1,25D increases the level of hypophosphorylated Rb and decreases E2F transcriptional activity in LNCaP cells (36). 1,25D does not significantly inhibit growth of DU145 cells, which lack functional Rb (25, 39). In addition, prostate cancer cells transformed with SV40, which inactivates Rb, are less sensitive to the growth inhibitory effects of 1,25D (39), suggesting a role for Rb in response to 1,25D. The cyclin E-cdk2 protein complex maintains Rb in a hyperphosphorylated state, and kinase activity of this complex can be inhibited by cyclin-dependent kinase inhibitors (cdki), including p21 and p27. 1,25D treatment increases stability of p27 protein, decreases nuclear localization of cdk2, and decreases cdk2 activity in LNCaP cells (36, 40). Up-regulation of p21 appears to be required for 1,25D-induced growth inhibition in ALVA 31 cells, as ALVA 31 cells stably expressing p21 antisense show decreased sensitivity to 1,25D growth inhibition (41). 1,25D also down-regulates c-Myc (26), a protein that acts early in G_1, suggesting that 1,25D acts at multiple points in G_1. The tumor suppressor p53 also regulates cell cycle progression (42). However, inactivation of p53 by overexpression of a dominant negative fragment of p53 in LNCaP cells did not prevent a G_1 arrest (26). However, the cells continue to express Ki-67 and recover quickly from 1,25D treatment (26).

Apoptosis

Inhibition of breast cancer cell growth by 1,25D has been associated not only with cell cycle arrest but also the induction of apoptosis (43–45). Several groups have detected DNA fragmentation, a hallmark of apoptosis, in LNCaP cells treated with 1,25D (26, 37, 46, 47) as well as in 1,25D-treated ALVA 31 cells (48). However, 1,25D does not induce apoptosis in PC-3 cells (26), demonstrating that 1,25D can reduce prostate cancer cell growth independently of apoptosis.

The mechanism(s) by which 1,25D induces apoptosis in prostate cancer cells have not been fully elucidated. However, recent studies suggest that, similar to breast cancer cells (45), 1,25D-induced apoptosis occurs via the intrinsic apoptotic signaling pathway. Activation of the intrinsic apoptotic pathway leads to alterations in mitochondrial membrane permeability that result in the release of cytochrome C into the cytosol and the subsequent activation of downstream caspases, both of which can be blocked by members of the Bcl-2 family of proteins. In LNCaP and ALVA 31 cells, 1,25D down-regulates expression of two antiapoptotic Bcl-2 family members, Bcl-2 and Bcl-X_L (37, 48). Overexpression of Bcl-2 prevents 1,25D-induced apoptosis in both the LNCaP and ALVA 31 cell lines (37, 48). 1,25D also induces apoptosis in LNCaP cells via a p53-independent pathway, as LN56 cells, which contain a dominant negative p53, are as sensitive to 1,25D-induced apoptosis as the parental LNCaP cell line (26).

Growth Factors

Several studies have suggested that alterations in the insulin-like growth factor (IGF) signaling axis are associated with an increased risk for prostate cancer (49–52). This signaling pathway consists not only of the ligands IGF-I and IGF-II and their receptors, but also the IGF binding proteins (IGFBPs), which regulate cell growth by both IGF-dependent and -independent mechanisms (53). Treatment of PC-3 cells with 1,25D or EB1089 decreases expression of IGF-II mRNA (54). In addition, 1,25D induces expression of IGFBP-3 and -6 in human prostate cancer cell lines and primary cultures of prostatic epithelial cells (7, 54–57). In LNCaP cells grown in serum-free medium, up-regulation of IGFBP-3 is required for the growth inhibitory effects of 1,25D; 1,25D-induced growth inhibition is blocked by an IGFBP-3 neutralizing antibody, as well as by IGFBP-3 antisense oligonucleotides (57).

Transforming growth factor beta (TGFβ) inhibits growth of several cell types, including cells derived from the prostate (58, 59). 1,25D activates the TGFβ signaling pathway in PC-3 cells (60), and growth inhibition by 1,25D is blocked by a combination of neutralizing antibodies directed against TGFβ isoforms TGF-β1 and TGF-β2.

Regulation of Additional Gene Products by 1,25D

In addition to the gene products mentioned above, 1,25D also alters expression of other proteins that have been associated with the regulation of tumor growth and progression. In the LNCaP and PC-3 cell lines, 1,25D decreases expression of parathyroid hormone-related protein

(PTHrP), a protein that increases proliferation of human prostate cancer cells (61–63). Expression of the BRCA-1 gene, which is often mutated in familial breast cancer, is induced by 1,25D not only in the MCF-7 breast cancer cell line, but also in LNCaP and PC-3 cells (64). In addition, a recent cDNA microarray study revealed that 1,25D down-regulates expression of fatty acid synthase, a protein that is overexpressed in prostate cancer and has been shown to enhance growth and survival of LNCaP cells (65, 66).

VDR Agonists in Combination with Other Treatments

Although VDR agonists are quite effective in inhibiting prostate cancer cell growth in some cases, they may be even more useful when combined with other treatments. The fact that activation of target genes by 1,25D involves VDR-RXR heterodimers suggests that the growth inhibitory effect of 1,25D may be enhanced in the presence of retinoids. The combination of 9cis retinoic acid (9cisRA) and 1,25D synergistically inhibits growth of the LNCaP cell line (24, 67) but not the PC-3 cells (26).

A limiting factor in the effectiveness of 1,25D is its inactivation by the P450 enzyme 24-hydroxylase. Expression of 24-hydroxylase is induced to varying degrees in prostate cancer cells upon 1,25D treatment (25), and it has been proposed that this elevation in 24-hydroxylase activity underlies the resistance of DU145 cells to 1,25D growth inhibition. Consistent with this, the combination of the P450 inhibitor liarozole fumarate and 1,25D synergistically inhibits DU145 cell growth (68).

1,25D and its analogs also increase the effectiveness of cytotoxic agents currently in use for the treatment of prostate cancer. In LNCaP and DU145 cells, treatment with 1,25D or the vitamin D analog Ro25-6760 increased the growth inhibition produced by suboptimal concentrations of the platinum drugs cisplatin and carboplatin (69). In addition, pretreatment with 1,25D or 19-nor 1α,25-dihyroxy-vitamin D_2 sensitized LNCaP cells to the growth inhibitory effects of ionizing radiation (70). Addition of 1,25D to mitoxantrone/dexamethasone combination therapy led to regression of PC-3 xenograft tumors (71). Because treatment with cytotoxic agents often induces adverse side effects, combination therapies involving vitamin D analogs and suboptimal levels of cytotoxic drugs could be effective at reducing prostate tumor growth while producing fewer of the side effects associated with cytotoxic agents.

Vitamin D Analogs and Inhibition of Tumor Growth

Although 1,25D effectively inhibits prostate cancer growth *in vitro*, the hypercalcemia that is induced by growth inhibitory concentrations of 1,25D limits its use as a therapeutic agent for prostate cancer. Several synthetic vitamin D analogs have been developed that mimic the growth inhibitory effects of 1,25D but are less calcemic. Several of these potently decrease the growth of human prostate cancer cells *in vitro* (10, 72–79). It is presently unclear why these compounds show greater potency and decreased hypercalcemic effect compared with 1,25D. The improved efficacy may be the result of enhanced activation of VDR, decreased affinity for vitamin D binding proteins, and/or decreased degradation of active compound. There are a few studies that suggest that some of the analogs are less effective in inducing VDR action in the intestine (the site of calcium absorption) than in other tissues (80, 81). Interestingly, some of these compounds inhibit growth of DU145 cells, which are resistant to 1,25D (74, 78, 82). In addition, recent studies involving LNCaP and PC-3 xenograft models have indicated that some less calcemic analogs are also effective at decreasing prostate cancer cell growth *in vivo* (78, 83–88). One of the most extensively studied vitamin D analogs is EB1089 (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). *In vitro*, EB1089 is a more potent inhibitor of LNCaP and PC-3 cell growth than 1,25D (24, 54, 72, 74). EB1089 is also an effective inhibitor of tumor growth *in vivo*. EB1089 concentrations ranging from 0.5 to 2.5 μg/kg have been shown to reduce growth of MAT LyLu Dunning prostate tumors in Copenhagen rats, as well as subcutaneous LNCaP xenograft tumors propagated in nude mice, while producing less hypercalcemia than 1,25D (83, 85, 86, 88). In addition, several nonsecosteroidal vitamin D receptor agonists have been developed by Ligand Pharmaceuticals that effectively decrease LNCaP cell growth (89). One such compound, LG190119, also inhibits growth of LNCaP tumors at concentrations that do not induce hypercalcemia (90). In addition to directly reducing growth of prostate cancer cells, there is some evidence in other cancer models that VDR agonists reduce angiogenesis (91, 92). This may be due to direct actions of VDR agonists on endothelial cells preventing blood vessel formation, or to reductions in secretion of angiogenic factors by the tumor cells. Although some types of endothelial cells are inhibited by VDR agonists, others are not (83, 91, 93). Furthermore, there is evidence that VDR agonists reduce metastasis. In the Dunning rat Mat LyLu model of metastatic prostate cancer, treatment with 1,25D, as well as the analogs EB1089 and Ro25-6760, decreases the number of lung tumor metastases (86, 87). For metastases to form, prostate cancer cells must escape the primary tumor and invade through the basement membrane. 1,25D inhibits invasion of the aggressive PC-3 and DU-145 cells (94). In addition, adhesion and migration of PC-3 and DU-145 cells to the basement membrane protein laminin was reduced with 1,25D treatment and was associated with decreased expression of α6 and β4 integrins (94). Collectively, these studies suggest that vitamin D analogs are promising therapeutic and chemopreventive agents for prostate cancer.

Clinical Studies Involving Vitamin D

Few clinical trials have explored the effectiveness of 1,25D on prostate cancer progression in human patients. Gross *et al.* found that daily calcitriol administration to seven men with rising PSA levels following surgery or radiation therapy (95) induced an increase in PSA doubling time in six of the seven patients suggesting reduced disease progression. The development of hypercalciuria limited the amount of 1,25D that could be administered. In a similar study, weekly dosing with 1,25D was better tolerated and led to an increase in median PSA doubling time without the development of hypercalcemia or hypercalciuria (96). In a recent phase I trial involving 25 patients with hormone refractory prostate cancer, the vitamin D analog 1α-hydroxyvitamin D_2 induced a partial response in two men, with disease stabilization in an additional five patients (97). Beer *et al.* have reported that weekly administration of 1,25D, in combination with docetaxel, produced a minimum of 50% reduction in PSA values in 30 out of 37 patients with metastatic cancer, and suggested that this was better than the response in other trials of docetaxel alone (98). Vitamin D analogs may therefore be an effective therapy for the treatment of early, as well as advanced, prostate cancer.

Summary

Since the initial hypothesis suggesting an increased risk of prostate cancer in patients with reduced levels of vitamin D metabolites, studies by a variety of investigators using different models have shown that VDR agonists also have potential as therapeutic agents. 1,25D and its analogs inhibit growth of human prostate cancer cells, as well as normal prostatic epithelial cells, both *in vitro* and *in vivo*. However, prostate cancer cells vary in their sensitivity to the growth inhibitory effects of 1,25D, ranging from extremely sensitive (LNCaP) to resistant (DU145). 1,25D reduces prostate cancer cell growth by multiple mechanisms, which include cell cycle arrest, the induction of apoptosis, and regulation of growth factor signaling. Studies in rodent models show that less calcemic analogs can reduce growth and/or metastasis, suggesting that these compounds would be useful in treating prostate tumors. While the few clinical trials that have been conducted suggest that VDR agonists reduce prostate cancer progression, additional studies are required to define situations where VDR agonists, either alone or in combination with other drugs, would serve as effective therapeutic agents for prostate cancer.

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