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Vitamin D and Prostate Cancer

Review

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Prostate Cancer

Prostate cancer is the most commonly diagnosed cancer and the second leading cause of cancer deaths among American men. It arises primarily from the epithelial secretory cells of the peripheral prostate gland; generally occurs as a multifocal disease within the prostate gland; and then preferentially metastasizes to lymph nodes, bone, lung, and brain. Localized disease can be treated relatively successfully by radical prostatectomy (Catalona and Smith, 1998). In contrast, treatments for metastatic prostate cancer, including androgen ablation, are initially effective, but the majority of patients relapse with androgen independent prostate cancer (Denis, 1998; Leewansangtong and Crawford, 1998). The incidence of prostate cancer and the lack of good, long-term treatments for metastatic disease highlight the need for new chemopreventive and chemotherapeutic treatments. As discussed below, active vitamin D metabolites and analogs of the active form of vitamin D, 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$), are candidates for these treatments.

Vitamin D

Vitamin D is best known for its actions in regulating calcium levels and bone remodeling (reviewed in Brown et al, 1999), but recent studies highlight a role for vitamin D in the growth and differentiation of various cell types. It is synthesized in the epidermis by the conversion of its precursor, 7-dehydrocholesterol, into vitamin D_3, a reaction catalyzed by the ultraviolet rays of sunlight. Subsequent hydroxylation reactions in the liver and kidney produce $1,25(OH)_2D_3$ (Holick, 1984). Levels of $1,25(OH)_2D_3$ are tightly regulated as excess $1,25(OH)_2D_3$ is inactivated by the enzyme 24-hydroxylase (Horst and Reinhardt, 1997). Vitamin D can also be obtained from natural dietary sources such as fatty fish, fish liver oil, and eggs (Chapuy and Meunier, 1997) or from fortified sources such as milk, milk products, and butter (Halloran and Portale, 1997). However, for most people, dietary sources contribute a negligible amount of required vitamin D_3 compared to that derived from sunlight exposure (Holick, 1984).

Link Between Prostate Cancer Risk Factors and Sunlight

Three of the risk factors associated with the development of prostate cancer are age, race, and residence in northern latitudes. Prostate cancer incidence increases with increasing age; interestingly, analyses of autopsy cases show that a large proportion of men who die of other causes also have clinically undiagnosed prostate cancer (Holund, 1980). African American men have nearly twice the risk of developing prostate cancer as Caucasian men (Schwartz and Hulka, 1990). Moreover, African American men generally present with prostate cancer at a younger age and with more advanced disease than Caucasian men (Brawn et al, 1993). Asian men have the lowest risk among these three groups, although the risk for Asian immigrants in the United States increases significantly, suggesting an environmental component in prostate cancer risk (Cook et al, 1999). Finally, epidemiological studies have shown that residence in northern latitudes of the United States increases the risk of developing prostate cancer (Hanchette and Schwartz, 1992).

In 1990, Schwartz and Hulka proposed that these prostate cancer risk factors could be linked to vitamin D deficiency through either reduced sunlight exposure or impaired ability to convert 7-dehydrocholesterol into vitamin D_3. Older men are both less efficient in cutaneous production of vitamin D_3 and may not receive as much sunlight exposure as younger men (MacLaughlin and Holick, 1985). The increased melanin content of darker skin absorbs the ultraviolet light necessary for vitamin D_3 synthesis (Clemens et al, 1982), suggesting a correlation with race. Finally, residence in northern latitudes is linked to decreased exposure to sunlight and, consequently, to a reduction in vitamin D_3 production. In support of the vitamin D deficiency hypothesis, in the United States the rates of prostate cancer mortality vary inversely with exposure to ultraviolet light (Hanchette and Schwartz, 1992). In addition, Corder et al (1993) reported that risk for developing palpable prostate tumors of higher Gleason score decreased in patients with higher serum levels of $1,25(OH)_2D_3$, especially in those men with low serum levels of 25-hydroxyvitiamin D_3 ($25[OH]D_3$), although other studies have not found a correlation (Braun et al, 1995; Gann et al, 1996). One recent study, in which 19 000 ini-

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tially disease-free men were followed over a period of 13 years, revealed that men with low initial serum levels of $25(OH)D_3$ were at greater risk for earlier onset of prostate cancer; their tumors also tended to be more aggressive (Ahonen et al, 2000). Serum levels of $25(OH)D_3$ are more stable than $1,25(OH)_2D_3$ levels (Lissner et al, 1981) and may be more reflective of overall vitamin D status. In conclusion, several risk factors for prostate cancer are associated with a reduction in vitamin D_3 synthesis, which would in turn result in lower levels of the active metabolite, $1,25(OH)_2D_3$. Thus, $1,25(OH)_2D_3$ supplementation may be useful in the prevention of prostate cancer, its treatment, or both.

A Role for Vitamin D Receptor Polymorphisms in Prostate Cancer?

The link between prostate cancer and $1,25(OH)_2D_3$ suggests that there may be a connection between naturally occurring polymorphisms in the protein that mediates $1,25(OH)_2D_3$ action, the vitamin D receptor (VDR), and prostate cancer risk. Polymorphisms in the VDR gene have been linked to the development of osteoporosis (reviewed in Eisman, 1999). Several polymorphisms in the VDR gene have been identified, including a *Bsm*I restriction length polymorphism (RFLP) in intron 8 (Ingles et al, 1998), a *Taq*I RFLP in exon 9 (Taylor et al, 1996), a microsatellite polymorphism in the 3′ untranslated region (Ingles et al, 1997), and a *Fok*I RFLP located in exon 2 (Gross et al, 1996a). The *Bsm*I RFLP, the *Taq*I RFLP, and the microsatellite polymorphism do not alter the coding sequence of the VDR. However, the *Fok*I RFLP generates a protein with 3 additional amino acids at the N terminus. Reports on whether these VDR polymorphisms play a role in prostate cancer are inconclusive. One study suggested that men homozygous for the tt *Taq*I RFLP, which correlates with higher circulating levels of $1,25(OH)_2D_3$ than either the Tt or TT alleles, had a lower risk of developing prostate cancer that would require radical prostatectomy (Taylor et al, 1996). Additional studies have suggested a link between certain polymorphisms and prostate cancer risk (Ingles et al, 1997, 1998; Ma et al, 1998; Correa-Cerro et al, 1999; Habuchi et al, 2000). However, others find no link between polymorphisms in VDR and prostate cancer (Kibel et al, 1998; Watanabe et al, 1999; Blazer et al, 2000). Whether there is truly a link between these different VDR polymorphisms and risk of prostate cancer or whether the link is with a nearby gene warrants further investigation.

Vitamin D Action

Effects of $1,25(OH)_2D_3$ are mediated through the VDR, a member of the nuclear receptor superfamily that includes receptors for steroids (androgen, progesterone, glucocorticoid, and estrogen) as well as for thyroid hormone and retinoids (Mangelsdorf et al, 1995). VDR and other family members are ligand activated transcription factors. $1,25(OH)_2D_3$ passively diffuses into target cells, binds to VDR, and activates target genes containing one or more vitamin D response elements (VDREs) within their promoters. Among the known target genes are osteocalcin, osteopontin, calbindin, 24-hydroxylase, and p21 (reviewed in Jones et al, 1998). VDR functions as a heterodimer with the receptor for 9-*cis* retinoic acid, retinoid X receptor (RXR; MacDonald et al, 1993). Thus, target genes of $1,25(OH)_2D_3$ are transcriptionally activated by heterodimers of VDR and RXR and the transcriptional activities of this complex may in some cases be influenced by 9-*cis* retinoic acid (MacDonald et al, 1993). In addition to its ability to transactivate target genes via the VDR, $1,25(OH)_2D_3$ also has rapid effects in certain cell types that are not caused by changes in gene expression. These include changes in levels of phosphoinositides, increases in intracellular calcium, stimulation of protein kinase C activity, and elevation of cyclic guanosine monophosphate levels; these responses may be mediated by a membrane receptor for $1,25(OH)_2D_3$ that is structurally unrelated to VDR (Nemere et al, 1994; Brown et al, 1999).

Vitamin D and Other Cancers

$1,25(OH)_2D_3$ inhibits the growth and induces cellular differentiation of diverse cell types. For example, $1,25(OH)_2D_3$ induces differentiation of leukemia cells into macrophages (Abe et al, 1981; Bar-Shavit et al, 1983). Further studies in vitro showed that $1,25(OH)_2D_3$ exhibits antiproliferative effects in diverse types of cancer cells including breast (Colston et al, 1989), bladder (Konety et al, 2001), and colon (Thomas et al, 1992). More importantly, $1,25(OH)_2D_3$ and its analogs inhibit the growth of tumors in various cancer models (Eisman et al, 1987; Colston et al, 1992; VanWeelden et al, 1998; Konety et al, 2001). Clearly, $1,25(OH)_2D_3$ has widespread growth inhibitory effects and potential as a therapeutic agent for many different types of cancer.

Vitamin D and Prostate Cancer

Most studies of $1,25(OH)_2D_3$ action in prostate cancer have utilized human prostate cancer cell lines. The most widely studied cell lines are the LNCaP, PC-3, and DU145 cells. Of these, only the LNCaP cells express androgen receptor (AR) and are androgen dependent (ie, they do not grow in castrated hosts) in vivo (reviewed in Blutt and Weigel, 1999). Two new AR expressing lines, MDA PCA 2a and 2b, which are androgen independent in vivo while retaining AR expression, have been established recently from a bone metastasis (Navone et al, 1997). All of these cell lines express VDR (Skowronski et al, 1993; Zhao et al, 2000) as do primary cultures of

prostate cancer cells (Peehl et al, 1994). With the exception of the DU145 cells, treatment of each of these prostate cancer cell lines with $1,25(OH)_2D_3$ is growth inhibitory (Skowronski et al, 1993; Zhao et al, 2000). The extent of this growth inhibition differs; after 6 days of treatment, the LNCaP and MDA PCA 2b cells are extensively inhibited by 10 nM $1,25(OH)_2D_3$ (approximately 40% and 25% of control, respectively [Zhao et al, 2000]), whereas the MDA PCA 2a and PC-3 cells are somewhat less growth inhibited (70% and 60% of control, respectively [Skowronski et al, 1993; Zhao et al, 2000]). $1,25(OH)_2D_3$ also increases expression of prostate specific antigen (PSA), a marker of prostate epithelial cell differentiation, in both the LNCaP and MDA PCA cell lines (Hsieh et al, 1996; Zhao et al, 1997, 2000).

Others have examined the response of prostate cancer cells to $1,25(OH)_2D_3$ using a clonal expansion or an invasion assay. $1,25(OH)_2D_3$ irreversibly inhibits the clonal expansion of primary prostate cancer cells after as little as 2 hours of treatment (Peehl et al, 1994). $1,25(OH)_2D_3$ also inhibits the clonal expansion of LNCaP and PC-3 cells, but, similar to findings in growth assays, DU145 cells were resistant to the effects of $1,25(OH)_2D_3$ (de Vos et al, 1997). Although DU145 cells are not inhibited in growth and clonal expansion assays, their ability to invade Amgel was significantly hindered by a 72-hour treatment with $1,25(OH)_2D_3$ (Schwartz et al, 1997), indicating that $1,25(OH)_2D_3$ may inhibit the ability of the DU145 cells to metastasize.

Vitamin D and Normal Prostate

The effects of $1,25(OH)_2D_3$ on the normal prostate have not been fully determined. However, Peehl et al (1994) showed that primary cultures of epithelial cells and prostatic fibroblasts (stroma) were growth inhibited by $1,25(OH)_2D_3$. Other studies suggest that although normal prostate epithelial cells are growth inhibited, the prostatic stromal cells proliferate in response to $1,25(OH)_2D_3$ in the absence of serum (Krill et al, 1999). There have been few in vivo studies examining the effect of $1,25(OH)_2D_3$ on the normal prostate. In castrated rats supplemented with testosterone, there was no effect of $1,25(OH)_2D_3$ treatment on prostate weight (Krill et al, 1999). However, in the absence of testosterone, treatment of castrated rats with $1,25(OH)_2D_3$ caused an increase in prostate weight due to growth stimulation of the prostatic stromal cells, although the epithelial cells are growth inhibited under these conditions (Konety et al, 1996; Krill et al, 1999).

Requirement for VDR

That the growth inhibitory effects of $1,25(OH)_2D_3$ require the nuclear VDR has been demonstrated in two ways. First, the VDR negative JCA-1 human prostate cancer cell line, whose growth is not inhibited by $1,25(OH)_2D_3$, became responsive upon stable transfection with a plasmid expressing VDR (Hedlund et al, 1996a). Second, reduction of VDR expression levels using an antisense strategy disrupts the growth inhibitory effects of $1,25(OH)_2D_3$ in ALVA-31 cells (Hedlund et al, 1996b). Whereas the growth inhibitory effects of $1,25(OH)_2D_3$ are mediated by the VDR, expression of VDR is not sufficient because both DU145 and TSU-Pr1 cell lines express functional VDR, yet neither is growth inhibited by $1,25(OH)_2D_3$ (Skowronski et al, 1993; Miller et al, 1995).

Vitamin D Analogs

Despite the effectiveness of $1,25(OH)_2D_3$ in inhibiting the growth of prostate cancer cells, clinical use of high levels of $1,25(OH)_2D_3$ is precluded due to unacceptable elevation of serum calcium levels (Holick, 1984). Thus, much effort has been expended designing analogs of $1,25(OH)_2D_3$ that retain the antiproliferative effects of $1,25(OH)_2D_3$, but are less calcemic when administered in vivo. The properties of the analogs that make them less calcemic are not fully understood. However, alterations in binding to vitamin D binding protein, changes in extracellular half-life, cellular uptake, metabolism, and changes in the induced conformation of the VDR/RXR heterodimer have been suggested (Bouillon et al, 1995; Jones et al, 1998). One of the most widely studied $1,25(OH)_2D_3$ 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, or EB1089. EB1089 effectively inhibits the growth of LNCaP cells, exhibits greater potency than $1,25(OH)_2D_3$ (Skowronski et al, 1995), and is only 50% as calcemic in vivo (Hansen and Maenpaa, 1997; Kissmeyer et al, 1997). Other analogs, including 16-diene analogs (Schwartz et al, 1994; Hedlund et al, 1997), 1,25 dihydroxy-16-ene-23-yne-vitamin D_3 (Schwartz et al, 1995), 19-nor-hexafluoride D_3 analogs (Campbell et al, 1997), 19-nor-26,27-bishomo-vitamin D_3 analogs (Kubota et al, 1998), 20-cyclopropyl-cholecalciferol vitamin D_3 (Koike et al, 1999), 5,6-trans-16-ene-vitamin D_3 (Hisatake et al, 1999), and some nonsecosteroidal analogs (Boehm et al, 1999) also inhibit the growth of prostate cancer cells in vitro. Interestingly, some analogs inhibit the growth of the $1,25(OH)_2D_3$ resistant DU145 cells (Schwartz et al, 1994; Campbell et al, 1997; Kubota et al, 1998; Koike et al, 1999), and it has been suggested that DU145 resistance to $1,25(OH)_2D_3$ is due to the rapid metabolism of $1,25(OH)_2D_3$ by 24-hydroxylase in these cells (Ly et al, 1999).

Another approach to reducing hypercalcemic effects is to use $25(OH)D_3$, the immediate precursor to $1,25(OH)_2D_3$, which is also less calcemic in vivo than $1,25(OH)_2D_3$. This approach relies on findings demonstrating that PC-3, DU145, and primary cultures of normal prostate cells possess 1α hydroxylase activity, allow-

ing conversion of 25(OH)D_3 into 1,25(OH)$_2D_3$ intracellularly (Schwartz et al, 1998). Use of 25(OH)D_3 in vitro inhibits the growth of primary prostatic epithelial cells with similar potency to 1,25(OH)$_2D_3$ (Barreto et al, 2000). In summary, less calcemic analogs of 1,25(OH)$_2D_3$ and 25(OH)D_3 inhibit the growth of prostate cancer cells, making them attractive therapeutic agents for prostate cancer.

Vitamin D and In Vivo Studies

Several in vivo studies have been conducted to test a variety of analogs in rodent prostate cancer models with mixed success. The differences between the models precludes identification of optimal analogs at this time. The efficacy of 1,25(OH)$_2D_3$ or two of its analogs has been tested in the Dunning rat prostate adenocarcinoma model (Getzenberg et al, 1997; Lokeshwar et al, 1999). In the first study, 1,25(OH)$_2D_3$ and an analog, Ro25-6760, decreased tumor volume and reduced the incidence of lung metastases (Getzenberg et al, 1997). In a second study, 1,25(OH)$_2D_3$ and EB1089 treatment resulted in smaller tumors compared with control animals and also reduced lung metastases (Lokeshwar et al, 1999). However, the animals in both of these studies developed hypercalcemia and suffered significant body weight loss. Schwartz et al (1995) used a xenograft model of human PC-3 cells in nude mice and showed that a 1,25(OH)$_2D_3$ analog (1,25-dihydroxy-16-ene-23-yne-cholecalciferol) slows tumor growth compared with control animals without increasing serum calcium. Finally, EB1089 was shown to inhibit tumor growth without induction of hypercalcemia or causing weight loss in an LNCaP xenograft model for prostate cancer (Blutt et al, 2000b), demonstrating that growth inhibitory effects can occur in the absence of hypercalcemia. The results of these studies suggest that 1,25(OH)$_2D_3$ analogs have the potential to inhibit tumor growth in vivo.

Mechanism of Growth Inhibition by 1,25(OH)$_2D_3$ in Prostate Cancer Cells

Studies to date suggest that 1,25(OH)$_2D_3$ inhibits growth of prostate cancer cells through a number of mechanisms, including changes in cell cycle progression, increases in apoptosis, and alterations in the IGF growth factor axis.

Cell Cycle—In agreement with findings in leukemic and breast cancer cells (Studzinski et al, 1985; Djulbegovic et al, 1986; Eisman et al, 1989), treatment of LNCaP cells with 1,25(OH)$_2D_3$ causes the cells to accumulate in the G_0/G_1 phase of the cell cycle (Blutt et al, 1997). To distinguish between cells in G_0 and G_1, Blutt et al measured Ki67 expression, a nuclear antigen expressed only in cycling cells (Gerdes et al, 1983), and found that very few treated LNCaP cells express Ki67, consistent with cells exiting the cell cycle (Blutt et al, 2000a). This ability of 1,25(OH)$_2D_3$ to induce quiescence may be responsible for the long-lasting effects of 1,25(OH)$_2D_3$ in these cells.

The immediate targets of 1,25(OH)$_2D_3$ action in the cell cycle are unknown. During the G_1 phase, the decision whether or not to progress through the cell cycle is made, a decision controlled by the retinoblastoma (Rb) gene product. When active, Rb binds and inactivates E2F, a transcription factor critical for progression to the S phase. Cyclin/cyclin-dependent kinase complexes (cyclin/cdk) inactivate Rb by phosphorylation and the cyclin/cdk complex is negatively regulated by cdk inhibitor proteins such as p21 (Lundberg and Weinberg, 1999). Zhuang and Burnstein (1998) demonstrated that the cell cycle accumulation induced in LNCaP cells by 1,25(OH)$_2D_3$ involved up-regulation of the p21 cdk inhibitor and a reduction in cdk2 activity; moreover, Rb is hypophosphorylated (active) and E2F has reduced transcriptional activity in the treated cells. Regulation of p21 may be indirect, because a recent study in U937 myelomonocytic cells suggests that 1,25(OH)$_2D_3$ regulates expression of HOXA10, one of the homeobox transcription factor family members, which in turn directly increases expression of p21 (Bromleigh and Freedman, 2000). Whether regulation of p21 transcription is mediated by the VDR or HOXA10 or a combination of both transcription factors in prostate cancer cells requires further study. Of interest is the finding that DU145 cells, which lack functional Rb (Bookstein et al, 1990), are not growth inhibited by 1,25(OH)$_2D_3$ (Skowronski et al, 1993). Furthermore, abrogation of Rb function using the SV40 large T antigen also compromises the ability of 1,25(OH)$_2D_3$ to inhibit the growth of prostate cancer cells (Gross et al, 1996b). Thus, Rb may be a requirement for 1,25(OH)$_2D_3$ mediated growth inhibition in prostate cancer cells.

Apoptosis—Because 1,25(OH)$_2D_3$ induces apoptosis in some breast cancer cell lines (Welsh, 1994; Simboli-Campbell et al, 1996), the role of apoptosis in prostate cancer cells has also been examined. 1,25(OH)$_2D_3$ appears to induce apoptosis in LNCaP cells (Fife et al, 1997; Hsieh and Wu, 1997; Blutt et al, 2000a); however, the extent reported varies from none (Zhuang and Burnstein, 1998), to a small population of the cells (10%; Blutt et al, 2000a), up to 100% of the cell population (Fife et al, 1997). Blutt et al (2000a) found that apoptosis induced by 1,25(OH)$_2D_3$ is accompanied by a decrease in the expression of 2 antiapoptotic proteins, Bcl-2 and Bcl-X_L. Bcl-2 overexpression substantially reduces LNCaP cell responsiveness to 1,25(OH)$_2D_3$ and blocks the induction of apoptosis by 1,25(OH)$_2D_3$ (Blutt et al, 2000a). The overall contribution of apoptosis to the reduction in cell number is uncertain at this point, but is only one of the effects of 1,25(OH)$_2D_3$. Neither LNCaP-Bcl-2 nor PC-3 cells undergo apoptosis in response to 1,25(OH)$_2D_3$, yet both lines are growth inhibited, albeit to a lesser extent

than the LNCaP cells (Campbell et al, 1997; Blutt et al, 2000a). Further studies will be required to elucidate specific pathways utilized by $1,25(OH)_2D_3$ in order to trigger cell death pathways and to determine the relative importance of apoptosis in its ability to inhibit the growth of prostate cancer cells.

Role of Androgens and Growth Factors in $1,25(OH)_2D_3$ Action

Androgens—Androgen ablation is often used in the treatment of metastatic prostate cancer; however, patients generally relapse with androgen independent disease (Denis, 1998; Leewansangtong and Crawford, 1998). Because of the importance of androgens in prostate cancer, it is important to elucidate any interactions between androgens and $1,25(OH)_2D_3$ in prostate cancer cells. In media supplemented with androgen-depleted charcoal-stripped serum (CSS), LNCaP cells grow extremely slowly and $1,25(OH)_2D_3$ does not inhibit their growth, but it promotes differentiation as measured by PSA secretion (Miller et al, 1992). When dihydrotestosterone is added back to CSS-containing media, the cells grow faster and the antiproliferative effects of $1,25(OH)_2D_3$ are restored (Zhao et al, 1997). Consistent with these findings, $1,25(OH)_2D_3$ inhibits the growth of LNCaP cells in media supplemented with fetal calf serum (FCS) that contains endogenous androgens (Skowronski et al, 1993). Moreover, the growth inhibitory effects of $1,25(OH)_2D_3$ in LNCaP cells grown in media containing FBS are attenuated by the addition of Casodex, an antiandrogen (Zhao et al, 1997). Thus, the growth inhibitory effects of $1,25(OH)_2D_3$ in LNCaP cells may be androgen receptor–dependent; the mechanism of this androgen dependence is mediated, at least in part, by an indirect up-regulation of AR protein by $1,25(OH)_2D_3$ (Zhao et al, 1997).

Findings in the MDA PCA 2a and 2b cells suggest that unlike the LNCaP cells, growth inhibition by $1,25(OH)_2D_3$ is androgen independent because Casodex does not block the inhibitory effects of $1,25(OH)_2D_3$ in FBS-supplemented media (Zhao et al, 2000). Thus, it seems that $1,25(OH)_2D_3$ regulation of prostate cell growth is androgen dependent in the LNCaP cells, but androgen independent in the MDA PCA cell lines. Furthermore, $1,25(OH)_2D_3$ inhibits the growth of the AR negative PC-3 cells, demonstrating that in most cellular contexts, $1,25(OH)_2D_3$ inhibits growth by an androgen independent mechanism.

Growth Factors—There is evidence that the insulin-like growth factor (IGF) axis plays a role in prostate growth and elevated serum IGF-I levels have been found in men with prostate cancer (Chan et al, 1998; Wolk et al, 1998; Kaplan et al, 1999; Stattin et al, 2000). The contribution of elevated IGF-I levels to prostate cancer growth is not yet known. The IGFs are normally secreted by the stromal cells and stimulate the growth of the overlying epithelial cells; however, many prostate cancer cell lines secrete their own IGFs (reviewed in Russell et al, 1998). Epithelial-produced IGF binding proteins (IGFBPs) regulate bioavailability of the IGFs, and there is evidence in prostate cancer cell lines that $1,25(OH)_2D_3$ antagonizes the actions of the IGFs by increasing expression of IGFBPs, including IGFBPs-3 and 6 (Drivdahl et al, 1995; Huynh et al, 1998; Boyle et al, 2001). Regulation of IGFBP-3 appears to be required for $1,25(OH)_2D_3$-dependent inhibition of LNCaP cells grown in defined medium and for up-regulation of p21; interestingly, both antisense oligonucleotides and anti-IGFBP-3 antibodies negated $1,25(OH)_2D_3$ effects both on growth and on up-regulation of p21 protein levels (Boyle et al, 2001). Therefore, $1,25(OH)_2D_3$ actions in prostate cancer may also involve abrogation of IGF-stimulated growth of the epithelial cells.

Vitamin D and Angiogenesis and Metastasis

In addition to the effects of $1,25(OH)_2D_3$ on tumor cell growth, $1,25(OH)_2D_3$ may also act in vivo by reducing angiogenesis within the tumor or by reducing the ability of the tumor cells to metastasize.

Angiogenesis—In order for a tumor to thrive, it must have the ability to stimulate invasion by a blood supply to provide nutrition and oxygen for the rapidly dividing tumor cells (Folkman, 1992), a process known as angiogenesis. $1,25(OH)_2D_3$ inhibits endothelial cell growth, sprouting, elongation, and the ability to form networks in vitro due to induction of apoptosis in the sprouting endothelial cells; it also reduces the number of blood vessels in xenograft breast carcinoma tumors in vivo (Mantell et al, 2000). Furthermore, $1,25(OH)_2D_3$ in combination with 9-*cis* retinoic acid synergistically inhibits angiogenesis in tumors of various origins (Majewski et al, 1996), suggesting that combination therapies would be effective in inhibiting tumor growth. Obviously, further studies on $1,25(OH)_2D_3$ and its ability to inhibit angiogenesis are warranted.

Metastasis—There is a limited amount of evidence suggesting that $1,25(OH)_2D_3$ inhibits metastasis. First, in vitro studies showed that a $1,25(OH)_2D_3$ analog (1,25-dihydroxy-16-ene-23-yne-cholecalciferol) inhibits the invasion of DU145 cells in an Amgel assay (Schwartz et al, 1997). Second, $1,25(OH)_2D_3$ reduces invasion, adhesion, and migration to laminin, a basement membrane protein, in vitro via down-regulation of two laminin receptors, the $\alpha 6$ and $\beta 4$ integrins, in the PC-3 and DU145 cells (Sung and Feldman, 2000). Finally, $1,25(OH)_2D_3$ and one of its analogs (Ro25–6760) reduces the size and number of metastases derived from Dunning prostate tumors in vivo (Getzenberg et al, 1997). Thus, these few studies suggest that $1,25(OH)_2D_3$ not only inhibits the

growth of prostate cancer cells, but may also reduce the ability of the cells to metastasize.

Clinical Studies—Although the effects of $1,25(OH)_2D_3$ have not been tested widely, a recent clinical study tested the potential effects of $1,25(OH)_2D_3$ in 7 men whose disease had failed either radical prostatectomy or radiation therapy, and who had increasing serum PSA levels (Gross et al, 1998). Treatments were given in escalating doses from 0.5 μg/day to 2.5 μg/day. Six of the 7 patients showed significant decreases in the rate of increase of serum PSA values, and, interestingly, 1 patient exhibited a drop in serum PSA. Some of the patients had stabilization of their serum PSA values for more than a year. However, all of the subjects also developed hypercalciuria. Thus, whereas $1,25(OH)_2D_3$ may have some effects on tumor growth in vivo, less calcemic analogs such as EB1089 ought to be tested to try to eliminate the calcemic effects of $1,25(OH)_2D_3$ treatment.

$1,25(OH)_2D_3$ in Combination Therapies for Prostate Cancer

$1,25(OH)_2D_3$ is also promising as a treatment in combination with other agents. EB1089 in combination with other treatments including radiation therapy and tamoxifen further decreases breast cancer cell growth compared with either treatment alone (Vink-van Wijngaarden et al, 1994; Sundaram and Gewirtz, 1999). A number of in vitro studies in prostate cancer cells suggest that combination treatments may also enhance $1,25(OH)_2D_3$ effects in vivo.

One obvious candidate for combination therapy in prostate cancer is 9-*cis* retinoic acid, because VDR heterodimerizes with RXR, and addition of the ligands in combination may have differing effects on prostate cell growth than either ligand alone. At high concentrations, addition of 9-*cis* retinoic acid inhibits the growth of LNCaP cells and induces cellular differentiation as measured by expression of PSA (Esquenet et al, 1996). In LNCaP cells, cotreatment of $1,25(OH)_2D_3$ and low levels of 9-*cis* retinoic acid are more growth inhibitory than addition of $1,25(OH)_2D_3$ alone (Blutt et al, 1997). In fact, administering 9-*cis* retinoic acid and $1,25(OH)_2D_3$ in combination reduces the amount of $1,25(OH)_2D_3$ needed to inhibit the growth of LNCaP cells. These data suggest a potential use of 9-*cis* retinoic acid in combination therapies with EB1089 to inhibit the growth of prostate cancer in vivo.

Platinum agents such as cisplatin have been tested in clinical trials as a treatment for prostate cancer and other cancers with little success (Qazi and Khandekar, 1983; Trump et al, 1990). However, in LNCaP cells, platinum agents (cisplatin or carboplatin) are growth inhibitory (Moffatt et al, 1999) and addition of both $1,25(OH)_2D_3$ and either of these platinum drugs results in greater growth inhibition of the cells than either added alone (Moffatt et al, 1999). Therefore, combinations of $1,25(OH)_2D_3$ and platinum agents may be of benefit in the treatment of prostate cancer.

$1,25(OH)_2D_3$ is inactivated by the enzyme 25-hydroxyvitamin D 24-hydroxylase (24-hydroxylase), which is also a transcriptional target of VDR (Horst and Reinhardt, 1997). Induction of 24-hydroxylase results in rapid $1,25(OH)_2D_3$ metabolism, which may reduce its ability to inhibit cancer cell growth in vivo. Ly et al (1999) demonstrated that use of liarozole, a nonspecific P450 enzyme inhibitor (24-hydroxylase falls into this enzyme class), inhibits the activity of 24-hydroxylase and increases the half-life of $1,25(OH)_2D_3$ in DU145 cells. Furthermore, use of combinations of liarozole and $1,25(OH)_2D_3$ inhibited the growth of DU145 cells, whereas neither agent added alone had any effect on cell growth (Ly et al, 1999). These promising results suggest that combinations of liarozole and $1,25(OH)_2D_3$ may boost the growth inhibitory effects of $1,25(OH)_2D_3$ in prostate tumors. Because liarozole is not a specific 24-hydroxylase inhibitor, the observed effects may be due to inhibition of other enzymes in addition to 24-hydroxylase. Further studies are necessary to determine whether liarozole and $1,25(OH)_2D_3$ will be a useful combination therapy in vivo.

Summary

The original hypothesis of Schwartz and Hulka (1990) proposing that vitamin D deficiency may be a risk factor for prostate cancer has triggered many studies. Epidemiological studies have supported this hypothesis with findings that sunlight exposure is inversely proportional to prostate cancer mortality and that prostate cancer risk is greater in men with lower levels of vitamin D (Hanchette and Schwartz, 1992; Corder et al, 1993; Ahonen et al, 2000). Prostate cancer cells express receptors for $1,25(OH)_2D_3$ and some cell lines are growth inhibited when treated with $1,25(OH)_2D_3$ (reviewed in Blutt and Weigel, 1999). The mechanism of action of these growth inhibitory effects of $1,25(OH)_2D_3$ in LNCaP cells involves G_1 accumulation, induction of quiescence, and an increase in apoptosis of the cancer cells (Blutt et al, 1997, 2000a; Zhuang and Burnstein, 1998). In vivo, $1,25(OH)_2D_3$ and its analogs slow tumor growth and hinder metastasis of prostate tumors in rodent models (Schwartz et al, 1995; Getzenberg et al, 1997; Lokeshwar et al, 1999; Blutt et al, 2000b), and $1,25(OH)_2D_3$ may have clinically relevant effects (Gross et al, 1998). More work is required to elucidate the mechanism of $1,25(OH)_2D_3$ action in prostate cancer cells and to identify optimal $1,25(OH)_2D_3$ analogs in a search for compounds with a better separation of growth inhibitory effects from hypercalcemic effects.

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