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The role of vitamin D and retinoids in controlling prostate cancer progression

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Abstract

Prostate cancer is a leading cause of cancer-related deaths in many countries. Premalignant lesions and invasive cancer occur more frequently in the prostate than in any organ other than the skin. Yet, the incidence of clinically detected prostate cancer is much lower than the histopathological incidence. The slow growth of prostate cancer and the low incidence of clinically manifest disease in some geographical locations or racial/ethnic groups suggest that prostate cancer can be controlled, perhaps by dietary factors. Vitamin D and retinoids have emerged as leading candidates both to prevent and to treat prostate cancer. Many of the activities of these compounds, established from epidemiological studies, research with cell culture and animal models, and clinical trials, are consistent with tumor suppressor effects. However, retinoids may have additional tumor enhancer properties that balance or negate anti-cancer activity. This perhaps explains the overall lack of protective effects of vitamin A compounds against prostate cancer found in epidemiological studies, and the minimal efficacy of retinoids in clinical trials to treat prostate cancer. While current efforts focus on developing strategies to use vitamin D compounds to control prostate cancer, the possibility exists that prostate cancer cells may become resistant to tumor suppressor effects of vitamin D. Analyses of experimental model systems show that prostate cancer cells become less sensitive to vitamin D through loss of receptors or signaling molecules that mediate vitamin D's actions, or through changes in metabolic enzymes that synthesize or degrade vitamin D compounds. The potential promise of exploiting vitamin D to control prostate cancer is tempered by the possibility that prostate cancer, perhaps even at early stages, may develop mechanisms to escape tumor suppressor activities of vitamin D and/or retinoids.

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Unique features of prostate cancer

Prostate cancer, perhaps more than any other type of cancer, has features that lend themselves to control of progression. Autopsy studies show that cancer begins to arise in the prostate as early as the 3rd decade of life (Sakr *et al.* 1993), yet most men are not diagnosed with clinically evident prostate cancer until they are in their 60s (Abbas & Scardino 1997). This fact shows that prostate cancer progresses slowly and therefore should offer a wide window of opportunity for control strategies. Another factor relevant to control is the large variation in the incidence of clinical detection and mortality from prostate cancer across countries and among racial or ethnic groups (Hsing *et al.* 2000). For example, an African-American male has about a 15 times greater chance of being diagnosed with prostate cancer than an age-matched Japanese male. However, the incidence of clinically unsuspected

cancer in the prostate does not show such a large variation among different populations (Breslow *et al.* 1977). Overall, men over 50 have about a 40% chance of having cancer in the prostate, regardless of nationality, race or ethnicity. Therefore, the initiation of prostate cancer probably occurs somewhat similarly around the world as far as is known, but progression to a clinically detectable state differs. The discrepancy between the frequency of 'latent' and clinically manifest cancer is presumably due to some variation in factors that control progression.

An examination of the natural history of the histopathogenesis of prostate cancer also shows several points at which progression could be controlled. The initial event in the conversion of normal prostatic epithelium to cancer is considered to be the development of dysplasia, also known as prostatic intraepithelial neoplasia (PIN) (McNeal & Bostwick 1986). Dysplasia occurs very frequently in the prostate and first

appears in men in their 30s (Sakr *et al.* 1993). Invasive prostate cancer has been observed emerging directly from dysplastic lesions (McNeal *et al.* 1991). This, then, is one point of potential control – if progression of normal epithelium to dysplasia could be prevented, then presumably the incidence of invasive prostate cancer would be decreased.

Well-differentiated invasive prostate cancer is classified as grade 3 in the widely used Gleason grading system (Gleason 1977). Cancer at this stage is seemingly curable. In a study of patients at Stanford University who underwent radical prostatectomy to treat prostate cancer, those who had cancer consisting only of grade 3 had a greater than 90% chance of no biochemical recurrence, defined as an increase in serum prostate-specific antigen (PSA), within 5 years of prostatectomy. If, however, grade 4 or 5 (poorly differentiated) cancer was present, then the rate of recurrence was directly proportional to the percentage of grade 4/5 cancer (Stamey *et al.* 1999, 2000). Therefore, another point of control would be prevention of progression of grade 3 cancer (curable) to grade 4 (not curable by surgery). Farther down the line, another point of control would be to prevent the progression of recurrent cancer that is responsive to androgen ablation therapy to androgen-independent cancer (Ahmed & Trump 2002). For the most part, it is only this final stage of prostate cancer that causes death.

Vitamin D compounds, and to a lesser extent retinoids, have emerged as leading candidates to control prostate cancer progression. A variety of review articles have discussed current information regarding the potential ability of vitamin D compounds (Miller 1998, Blutt & Weigel 1999, Konety *et al.* 1999, Feldman *et al.* 2000) or retinoids (Lotan 1996) to prevent or treat prostate cancer. The potential applications of these compounds are based on evidence from epidemiological findings, cell and animal studies, and clinical trials. While many properties of vitamin D compounds and retinoids are consistent with tumor suppressor activity, new findings suggest that prostate cancer cells may develop mechanisms to subvert inhibitory actions of vitamin D or retinoids.

Epidemiology

The first suggestion that vitamin D status might relate to prostate cancer risk came from epidemiological studies. Schwartz and Hulka (1990) calculated that mortality rates from prostate cancer in the US were inversely correlated with latitude and degree of ultraviolet (UV) irradiation. These investigators surmised that vitamin D deficiency was the factor that increased prostate cancer risk in geographical locations with low levels of UV, since UV is the principal factor mediating synthesis of vitamin D. Results from a prospective case-control study of vitamin D metabolite levels in sera collected in the San Francisco Bay area supported this hypothesis (Corder *et al.* 1993). Mean levels of the active circulating metabolite of vitamin D, 1,25-dihydroxyvitamin

D₃ (1,25D), were significantly lower in cases than in controls. An association between serum levels of 1,25D and risk of prostate cancer was not, however, found in all subsequent studies (Braun *et al.* 1995, Gann *et al.* 1996, Nomura *et al.* 1998). It is perhaps noteworthy that while a correlation between prostate cancer risk and serum levels of 1,25D are not consistently noted, some studies instead found that low levels of the vitamin D premetabolite, 25-hydroxyvitamin D₃ (25D), increased risk of prostate cancer diagnosis or aggressiveness (Ahonen *et al.* 2000). This may be related to new information regarding local production of 1,25D from 25D in the prostate, which is discussed later in this article.

Despite the inconsistent findings regarding serum measurements of vitamin D metabolites and prostate cancer risk, additional epidemiological studies add support to the concept that inadequate vitamin D increases prostate cancer risk. Using a measure of solar radiation optimized for vitamin D production, Grant (2002) recently estimated premature cancer mortality in the US due to insufficient solar UV-B radiation (UVB). Approximately 1% of deaths caused by prostate cancer were calculated as premature due to inadequate exposure to UVB. Another reported finding, that high levels of dietary calcium are a significant risk factor for prostate cancer (Giovannucci *et al.* 1998), may be related to vitamin D in that high levels of serum calcium reduce renal production of 1,25D, the major source of circulating hormone.

In contrast to the generally supportive epidemiological findings for the role of vitamin D in diminishing prostate cancer risk, a comprehensive evaluation of the preventive potential of vitamin A concluded that there was little evidence that intake of vitamin A had any substantial preventive effects against any cancer, including cancer of the prostate (Vainio & Rautalahti 1999).

Antiproliferative effects of vitamin D compounds or retinoids on prostate cells

Building on epidemiological studies suggesting that vitamin D deficiency increases the risk of prostate cancer, investigators began to study the effects of vitamin D compounds on prostate cells to establish a mechanistic basis for the epidemiological correlations. It is now well established that vitamin D compounds inhibit the growth of normal prostatic epithelial cells, primary cultures of prostate cancer cells, and many prostate cancer cell lines (reviewed in Miller 1998, Blutt & Weigel 1999, Konety *et al.* 1999, Feldman *et al.* 2000). Growth suppression occurs via the induction of cell cycle arrest and/or apoptosis. Molecules involved in the signaling pathways initiated by vitamin D are not yet well-defined but are being elucidated. The cell cycle inhibitor p21^{WAF1/CIP1} is sometimes but not always required for antiproliferative activity of vitamin D on prostate cells (Zhuang & Burnstein 1998, Moffatt *et al.* 2001), and the insulin-like

growth factor binding protein-3 (IGFBP-3) has been implicated in some types of prostate cells as a mediator of 1,25D action (Boyle *et al.* 2001, Sprenger *et al.* 2001). Studies with androgen-responsive prostate cancer cells show cross-talk between androgen and vitamin D signaling pathways, with upregulation of the androgen receptor by vitamin D mediating many of vitamin D's actions in these cells (Zhao *et al.* 1999, 2000).

Similarly, retinoids also have antiproliferative effects on normal prostatic epithelial cells and primary cultures of prostate cancer cells (Peehl *et al.* 1993, Igawa *et al.* 1994, Liang *et al.* 1999, Pasquali *et al.* 1999) as well as on prostate cancer cell lines (Fong *et al.* 1993, de Vos *et al.* 1997, Pili *et al.* 2001). Like 1,25D, 9-cis-retinoic acid also upregulates the androgen receptor in some prostate cancer cell lines (Zhao *et al.* 1999). In addition to the actions of vitamin D compounds or retinoids as single agents, there are many examples of additive or synergistic antiproliferative effects of these agents in combination on prostate cells (Peehl *et al.* 1995, Blutt *et al.* 1997, Elstner *et al.* 1999). These interactions between vitamin D compounds and retinoids are, at least partially, mediated at the receptor level, as discussed below.

Vitamin D and retinoids as differentiation agents

Several lines of evidence also suggest differentiating effects of vitamin D compounds or retinoids on prostate cells, in addition to cell cycle arrest or apoptosis. Levels of keratins 8 and 18, expressed in the differentiated secretory cells of the luminal prostatic epithelium, are increased by retinoic acid in cultured prostatic epithelial cells (Peehl *et al.* 1993). Upregulation of tissue transglutaminase by retinoic acid in primary cultures of normal and malignant prostatic cells is also consistent with differentiating effects (Pasquali *et al.* 1999). PSA, another differentiation marker of prostatic epithelial cells, is increased in androgen-responsive prostate cancer cells by vitamin D compounds (Zhao *et al.* 1999) or retinoids (Fong *et al.* 1993). Further evidence of differentiation activity of vitamin D was noted in animal studies in which the epithelium of rats maintained on a vitamin D-supplemented diet exhibited greater histological differentiation than that of vitamin D-deficient rats (Konety *et al.* 1996).

Antimetastatic activity of vitamin D and retinoids

In addition to antiproliferative activities, vitamin D compounds and retinoids have properties consistent with antimetastatic behavior. Treatment of several androgen-independent prostate cancer cell lines *in vitro* decreased invasion, adhesion and migration to laminin, probably mediated by decreased expression of $\alpha_6\beta_4$ integrins (Sung & Feldman 2000) and matrix metalloproteinases (MMP) -2 and

-9 (Schwartz *et al.* 1997). These *in vitro* effects of vitamin D may be linked to antimetastatic activity observed in a xenograft model of rat prostate cancer (Lokeshwar *et al.* 1999). Retinoids have also been shown to reduce adhesion, motility, invasion and expression of proteinases, particularly plasminogen activator, by prostate cells (Dahiya *et al.* 1994, Kim *et al.* 1995, Webber & Waghray 1995).

Activities of retinoids that are inconsistent with tumor suppressor activity

While the majority of effects of vitamin D compounds and retinoids on prostate cells are growth inhibitory, it is important to note that not all effects of retinoids on prostate cells are consistent with tumor suppressor activity. Antiproliferative activity of certain retinoids is dose-dependent, and in fact low doses may actually stimulate rather than inhibit growth of prostatic cells (Fong *et al.* 1993, Peehl *et al.* 1993, Jones *et al.* 1997). Other dose-dependent effects of retinoids were observed in organ cultures, in which retinoids either inhibited or increased androgen-induced prostate growth and branching morphogenesis (Aboseif *et al.* 1997). Some studies showed that retinoids may decrease, rather than increase, expression of the differentiation marker, PSA (Dahiya *et al.* 1994, Hsieh & Wu 1997). In other studies, retinoids increased rather than decreased proteinases associated with cellular invasion (Liu & Rabbani 1995). Furthermore, potent growth inhibitory activity of antagonists rather than agonists of retinoic acid receptors on prostate cancer cell lines as well as primary cultures of prostate cancer cells were reported (Hammond *et al.* 2001). These divergent activities of retinoids, some of which could be construed as tumor promoting rather than suppressive, may be relevant to the previously mentioned inability to demonstrate an association between consumption of vitamin A and decreased prostate cancer risk in epidemiological studies (Vainio & Rautalahti 1999).

Chemopreventive activity of vitamin D and retinoids

No completely satisfactory model system exists to test chemopreventive activity of experimental compounds against prostate cancer, but several avenues have been explored. In one approach, investigators fed rats a 'Western diet' with high fat and low calcium and vitamin D (Xue *et al.* 1999). These animals exhibited hyperproliferation of the epithelium of the dorsal prostate. Increased levels of calcium and vitamin D in the diet blocked this hyperproliferation. If hyperproliferation is considered to promote tumorigenesis, then this experiment provides evidence for potential antitumor activity of calcium and/or vitamin D. Another model used to test chemopreventive activity of vitamin D was the G γ /T-15

transgenic mouse model of androgen-independent prostate cancer. In these mice, the vitamin D analog, EB 1089, did not alter the onset of tumors driven by the expression of SV₄₀-antigen in the basal cells of the prostatic epithelium, but did slow tumor growth (Perez-Stable *et al.* 2002).

Chemopreventive activity of retinoids in animal models of prostate cancer has also been shown. Dietary 9-cis-retinoic acid reduced the incidence of prostate cancer in rats treated with androgen and a carcinogen (McCormick *et al.* 1999), and decreased the development of PIN in Noble rats, which spontaneously develop prostate cancer (Christov *et al.* 2002).

Also relevant to chemoprevention strategies are the synergistic activities that vitamin D shows with other putative chemopreventive agents. For example, 1,25D synergistically inhibited the growth of human prostatic epithelial cells with genistein (Rao *et al.* 2002), the component in soy believed to have chemopreventive properties (Moyad 1999).

Clinical activity

The first clinical trial to evaluate the efficacy of vitamin D in treating prostate cancer was a small Phase II trial with 13 patients with hormone-refractory cancer (Osborn *et al.* 1995). No objective responses were seen in response to 1,25D, although serum PSA declined in some individuals. In another small study, seven patients with early recurrent cancer following radical prostatectomy or radiation therapy were treated with 1,25D (Gross *et al.* 1998). In all seven patients, the doubling-time of the rise in serum PSA declined during treatment, suggesting that 1,25D slowed tumor growth (using serum PSA as a surrogate marker for cancer volume). In a recent Phase I trial of an analog of vitamin D, 1-hydroxyvitamin D₂ (Hectoral), two of the patients with hormone-refractory disease had an objective response to Hectoral (> 50% reduction in serum PSA levels) (Liu *et al.* 2002). In all of these trials, hypercalciuria or hypercalcemia was the limiting toxicity. However, results of a recent Phase I trial show that pulse dosing of 1,25D permits substantial dose escalation with minimal toxicity (Beer *et al.* 2001). Other strategies to overcome toxicity yet retain therapeutic efficacy include development of less calcemic analogs of vitamin D, use of 1,25D at low levels in combination with other agents, or treatment with the vitamin D precursor, 25D. The latter approach relies on the conversion of 25D to the active metabolite, 1,25D, through enzymatic activity in the prostate, as discussed below.

Clinical efficacy of retinoids appears less promising than that of vitamin D compounds. Several retinoids have been tested in clinical trials against prostate cancer, but generally with minimal or no efficacy (Trump *et al.* 1997, Culine *et al.* 1999, Kelly *et al.* 2000, Shalev *et al.* 2000). Whether this is due to inherent therapeutic inactivity or to limitations due to toxicity needs to be further explored.

Prostate cancer cells may become less or non-responsive to vitamin D or retinoids

Many prostate cancer cells retain a normal response to 1,25D. For instance, the majority of cells cultured from primary adenocarcinomas of the prostate respond in a similar manner as normal cells to 1,25D (Peehl *et al.* 1994). Similarly, many established prostate cancer cell lines are responsive to 1,25D. Even cell lines representative of advanced prostate cancer, such as androgen-independent cell lines derived from the androgen-dependent cell line LNCaP, still are growth inhibited by vitamin D compounds (Perez-Stable *et al.* 2002, Yang *et al.* 2002). Treatment with 1,25D blocked formation of metastases in an experimental prostate cancer model (Lokeshwar *et al.* 1999), showing that vitamin D can effectively control even late stages of prostate cancer progression. However, some prostate cancer cell lines (Miller *et al.* 1995) and even a primary culture of prostate cancer cells that we recently identified (unpublished results) are resistant to growth inhibition by 1,25D. The existence of these unresponsive cell cultures raises the possibility that resistance to vitamin D may develop during progression of prostate cancer.

Vitamin D and retinoid receptors

Response of cells to 1,25D or retinoids is controlled at several levels. One is by the number and affinity of receptors. Both the vitamin D receptor (VDR) and retinoid receptors (RXRs and RARs) are members of the nuclear hormone receptor superfamily (Mangelsdorf *et al.* 1995). On the basis of ligand binding assays and Scatchard analyses, primary cultures of normal prostatic epithelial cells from the peripheral zone have about 35 fmole VDR per milligram protein with a K_d of approximately 10^{-10} M (Peehl *et al.* 1994). Stromal cells cultured from the prostate have considerably fewer VDRs (Peehl *et al.* 1994), suggesting that epithelial cells are the primary target of vitamin D in the prostate. Ligand binding by VDRs has been measured in tissues, confirming that the prostate is a target organ of 1,25D.

The number of VDRs in primary cultures of prostatic cancer cells spans a fairly large range, but overall does not differ significantly from normal cells (Peehl *et al.* 1994). Some prostate cancer cell lines, however, have very low levels of VDR. Growth inhibition of these cancer cell lines by 1,25D is attenuated. Restoration of VDR to normal levels generally makes these cells responsive to growth inhibition by 1,25D, showing that response of cells to 1,25D depends, at least in part, on levels of VDR (Hedlund *et al.* 1996, Zhuang *et al.* 1997). Whether VDR may be absent or present at abnormally low levels in prostate cancer tissues is not known. Localization of VDR by immunohistochemistry in whole tissues can be problematic, and although a few studies

have shown that VDRs are present in the nuclei of epithelial cells in normal and malignant prostatic tissues (Kivineva *et al.* 1998, Krill *et al.* 2001), no full scale investigation of VDRs in prostatic adenocarcinomas has been published. However, one study suggested that VDR levels may decrease in the prostate after age 60 (Krill *et al.* 2001), causing speculation regarding a possible link between decreased VDR and increased incidence of prostate cancer with age. Studies of other types of cancers showed that loss of VDR had prognostic significance in colon cancer (Evans *et al.* 1998), so further evaluation of VDR in prostate cancer would be worthwhile.

Retinoid receptors are also lost in some prostate cancer cell lines. Depending on the type of receptor that is absent, cells can still retain responsiveness to certain types of retinoids with affinity for remaining receptors. For example, the prostate cancer cell line DU 145 is resistant to all trans-retinoic acid, but responsive to an RAR γ -selective retinoid (Lu *et al.* 1999). DU 145 and PC-3 cells lack RAR β and therefore do not respond to RAR β -selective retinoids unless RAR β is restored (Campbell *et al.* 1998). Aberrant methylation of RAR β DNA was found in a significant number of prostate cancer specimens (Nakayama *et al.* 2001) and immunohistochemical labeling showed that RAR β and RXR β were diminished in prostate cancer compared with normal epithelium (Kikugawa *et al.* 2000). Therefore, resistance to retinoids may indeed develop in prostate cancers through loss or decreased expression of receptors.

Many of the retinoid receptors function as heterodimers with RXR, as does VDR (Kliwer *et al.* 1992). Alterations in RXR in cancer cells would therefore affect both vitamin D and retinoid signaling pathways. Degradation of RXRs has been shown to influence the sensitivity of cells to the antiproliferative effects of 1,25D (Prufer *et al.* 2002). The development of dysplasia in the prostates of mice with conditional disruption of RXR α (Huang *et al.* 2002) illustrates the potential impact that loss of retinoid receptors might have on development of premalignant precursors of cancer.

Alterations in molecular targets of vitamin D or retinoids

Abnormal activity or expression of molecules involved in signaling pathways triggered by 1,25D or retinoids may also attenuate cellular response to these factors. The retinoblastoma tumor suppressor gene (pRb) and the cell cycle inhibitor p21^{WAF-1/CIP-1} are implicated in growth-inhibitory activity of 1,25D. Cancer cells that have deleted or mutated pRb or p21^{WAF-1/CIP-1} are often less sensitive to 1,25D (Zhuang & Burnstein 1998). Expression of pRb (Mack *et al.* 1998) and p21^{WAF-1/CIP-1} (Cheng *et al.* 2000) is variable among prostate cancers, suggesting that cancers with low expression of these genes may not respond optimally to the growth suppressive effects of 1,25D.

In some prostate cancer cell lines, growth inhibition by 1,25D occurs not only due to cell cycle arrest but also due to induction of apoptosis. Genetic changes that occur in prostate cancer may circumvent this tumor suppressive activity of 1,25D. The prostate cancer cell line LNCaP, for instance, undergoes apoptosis when treated with 1,25D in conjunction with decreased expression of the anti-apoptotic protein bcl-2 (Blutt *et al.* 2000). LNCaP cells engineered to overexpress bcl-2, however, no longer died when treated with 1,25D (Blutt *et al.* 2000). Overexpression of bcl-2 may also inhibit induction of apoptosis in prostate cancer cells by retinoic acid (Gao *et al.* 1999). Overexpression of bcl-2 is common in advanced prostate cancer, especially when androgen-independent (McDonnell *et al.* 1992), and 1,25D or retinoids might therefore be less effective in controlling these cancers.

Oncogenic changes alter response to vitamin D or retinoids

As discussed previously, VDR acts as a heterodimer with RXR, mediating cross-talk between the vitamin D and retinoid signaling pathways. This interaction of VDR with RXR is related to synergistic or additive activity of 1,25D and retinoids on prostatic and other types of cells (Peehl *et al.* 1995, Zhao *et al.* 1999). Disruption of this heterodimer has an effect on both pathways. RXR degradation can cause resistance to both vitamin D and retinoids (Prufer *et al.* 2002). Solomon *et al.* (1999) showed that transformation of keratinocytes with a *ras* oncogene disrupted VDR/RXR dimerization and made keratinocytes unresponsive to growth inhibition by 1,25D. These investigators further demonstrated that disruption of the heterodimer was due to *ras* activation of mitogen activated protein kinase (MAPK), which phosphorylated RXR α and prevented binding of RXR α to VDR. Cancers with oncogenic *ras*, which includes a small subset of prostate cancers (Konishi *et al.* 1997), might therefore be resistant to 1,25D.

Other oncogenic changes alter cellular responses to vitamin D compounds and/or retinoids. Prostatic epithelial cells immortalized with SV₄₀ T-antigen became unresponsive to growth inhibition by 1,25D, despite retention of VDR (Gross *et al.* 1996). Loss of response to retinoic acid accompanied conversion of SV₄₀-immortalized prostatic epithelial cells to malignancy by introduction of a *ras* oncogene (Peehl *et al.* 1999).

Metabolism of vitamin D and retinoids

Another element controlling activity of vitamin D or retinoids is metabolism. The P450 enzyme 25-hydroxyvitamin D-24-hydroxylase (24-OHase) initiates the degradation of 1,25D to inactive compounds. Basal levels of 24-OHase in cells are usually quite low, but are quickly upregulated by treatment with 1,25D. DU 145, a prostate cancer cell line

derived from a metastasis, exhibits exceptionally high levels of 24-OHase in response to 1,25D, such that this cell line is very unresponsive to growth inhibition by 1,25D. Treatment of DU 145 cells with liarozole, an inhibitor of P450 enzymes, blocks activity of 24-OHase and restores sensitivity of DU 145 cells to 1,25D (Ly *et al.* 1999). Whether abnormally high induction of 24-OHase, causing rapid degradation of 1,25D and therefore loss of activity, occurs in prostate cancer tissues is unknown.

Regulation of activity of vitamin D and retinoids at the prereceptor level

The major site of synthesis of 1,25D is the kidney. In proximal tubule cells, the enzyme vitamin D 1 α -hydroxylase (1 α -OHase) adds an hydroxyl group to 25D, converting it to 1,25D. Only recently were extrarenal sites of production of 1,25D recognized. The prostate is one of these sites, and Schwartz *et al.* (1998) showed that primary cultures of normal prostatic epithelial cells had 1 α -OHase activity and synthesized 1,25D when given 25D. Conversion of 25D to the active metabolite, 1,25D, by the action of 1 α -OHase resulted in growth inhibition of normal prostatic epithelial cells by 25D (Barreto *et al.* 2000). Thus, local synthesis of 1,25D in the prostate is presumed to be of biological significance and perhaps is more relevant to the control of prostate cancer than are circulating levels of 1,25D. This may explain why low serum levels of 25D, but not 1,25D, were found to correlate with increased risk of prostate cancer in some epidemiological studies. If local production of 1,25D is more important to antitumor activity of vitamin D in the prostate than systemic production, then the availability of circulating levels of the precursor, 25D, would be more significant than circulating levels of 1,25D.

We verified that normal prostatic epithelial cells have 1 α -OHase activity but found that 1 α -OHase activity was significantly reduced in primary cultures of prostate cancer cells (Hsu *et al.* 2001) as it is in the prostate cancer cell lines LNCaP, PC-3 and DU 145 (Schwartz *et al.* 1998, Hsu *et al.* 2001). Decreased activity of 1 α -OHase in primary cultures of cancer cells and in cell lines was confirmed by Whitlatch *et al.* (2002). Insufficient activity of 1 α -OHase in cancer cells has significant consequences with regard to local tumor suppressor effects of vitamin D. While treatment of normal cells with 25D resulted in growth inhibition equivalent to that induced by 1,25D, 25D was much less growth inhibiting than 1,25D on cancer cells (Hsu *et al.* 2001). This differential effect of 25D on normal versus cancer cells is due to the ability of normal but not cancer cells to convert the precursor 25D to the active metabolite, 1,25D, by the action of 1 α -OHase. Indeed, Whitlatch *et al.* (2002) showed that restoration of 1 α -OHase activity in LNCaP cells restored the ability of 25D to inhibit growth. If tumor suppressive effects of vitamin D in the prostate are more dependent on locally

produced 1,25D than on circulating levels of 1,25D, then loss of 1 α -OHase activity could contribute to the ability of prostate cancer cells to escape growth control mechanisms of vitamin D.

It is noteworthy that retinoid metabolism is also abnormal in prostate cancer cells. The enzyme lecithin:retinol acyltransferase (LRAT) converts retinol to retinyl esters, the major storage form of vitamin A within cells. LRAT is greatly reduced in many types of cancer cell lines, including many derived from the prostate (Guo *et al.* 2002). When cultures of normal prostatic epithelial cells were analyzed, all expressed significant LRAT activity. In contrast, primary cultures from adenocarcinomas, like prostate cancer cell lines, had very low levels of LRAT (Guo *et al.* 2002). In the absence of LRAT, storage of internal retinol as retinyl esters is insufficient, and cells lack a pool of precursors to convert to the active metabolite, retinoic acid. This finding in cultured cells may explain the previous report that prostate cancer has up to eight times less retinoic acid than normal prostatic tissues (Pasquali *et al.* 1996), perhaps because of insufficient stores of retinyl esters. Prostate cancer cells may therefore escape the tumor suppressor effects of retinoids in the same way that they appear to escape growth control of vitamin D, by losing the enzymes responsible for local production of active metabolites.

Conclusions

The potential ability of vitamin D and, to a lesser extent, retinoids, to control prostate cancer is based on an extensive and growing body of scientific evidence. Given the correlation between vitamin D deficiency and prostate cancer risk, and the fact that age is the strongest risk factor known for prostate cancer, it is noteworthy that hypovitaminosis D is epidemic among the elderly (Lips 2001). The effects of aging on vitamin D status are due to insufficient exposure to UV, diets insufficient in vitamin D, and decreased activity of enzymes that synthesize active metabolites of vitamin D (Halloran & Portale 1997). It is interesting to consider the effect that restoring vitamin D to normal levels in older men might have on the incidence of clinically detected prostate cancer.

The promise of vitamin D and/or retinoids to control prostate cancer is tempered by the sobering realization that an early event in the progression of prostate cancer may be to eliminate two major growth inhibitory/differentiating/tumor suppressor pathways by loss of enzymes that create the active metabolites of these compounds. If this is validated, then using premetabolites of vitamin D or retinoids to control prostate cancer would be less effective than using active compounds. Additional changes that may occur at later stages in prostate cancer development, such as loss of vitamin D or retinoid receptors and alterations in molecules involved in the signaling pathways of these compounds,

could make cancer cells less responsive even to active metabolites. Although such changes have been found in experimental models, whether these alterations occur in prostate cancer tissues and at what frequencies needs to be determined.

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