Mechanisms of the Anti-Cancer and Anti-Inflammatory Actions of Vitamin D

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Abstract

Calcitriol, the hormonally active form of vitamin D, is being evaluated in clinical trials as an anti-cancer agent. Calcitriol exerts multiple anti-proliferative, pro-apoptotic, and pro-differentiating actions on various malignant cells and retards tumor growth in animal models of cancer. Calcitriol also exhibits several anti-inflammatory effects including suppression of prostaglandin (PG) action, inhibition of p38 stress kinase signaling, and the subsequent production of pro-inflammatory cytokines and inhibition of NF-κB signaling. Calcitriol also decreases the expression of aromatase, the enzyme that catalyzes estrogen synthesis in breast cancer, both by a direct transcriptional repression and indirectly by reducing PGs, which are major stimulators of aromatase transcription. Other important effects include the suppression of tumor angiogenesis, invasion, and metastasis. These calcitriol actions provide a basis for its potential use in cancer therapy and chemoprevention. We summarize the status of trials involving calcitriol and its analogs, used alone or in combination with known anti-cancer agents.

1. INTRODUCTION

Vitamin D is the major regulator of calcium homeostasis in the body and is critical for the normal mineralization of bone (1). The most biologically active vitamin D metabolite, calcitriol (1α,25-dihydroxyvitamin D_3), is produced by sequential hydroxylations of the precursor vitamin D_3 (cholecalciferol) in the liver and the kidneys. Calcitriol acts similarly to classical steroid hormones by binding to the vitamin D receptor (VDR) and by functioning via both genomic and nongenomic pathways to regulate target gene expression. The traditional actions of calcitriol include enhancing calcium and phosphate absorption from the intestine to maintain normal concentrations in the circulation and providing adequate amounts of these minerals to the bone-forming site to allow mineralization of bone to proceed normally (1). However, it has become evident that calcitriol has many additional effects including anti-proliferative, pro-differentiating, anti-inflammatory, and immunomodulatory activities that implicate this hormone in a wide array of actions unrelated to bone or mineral metabolism (1–6). This review focuses on the implications of these calcitriol actions for cancer and inflammation and discusses the potential utility of calcitriol in cancer therapy. Because this field is growing, we focus on cancers of the breast, prostate, and colon, where most of the anti-cancer actions of vitamin D have been studied. Because many classical actions of vitamin D to inhibit cancer growth have been discussed (7–11), we emphasize the newer anti-inflammatory actions that are starting to gain recognition. The possible efficacy of vitamin D in the prevention and treatment of diseases in addition to cancer has been reviewed recently in several publications (1–3, 5, 6, 12, 13).

2. VITAMIN D METABOLISM

Vitamin D is more than a micronutrient and not technically a vitamin; rather, it is the essential precursor to the potent steroid hormone calcitriol. Dietary vitamin D exists in two forms: vitamin D_3 (cholecalciferol), which is present in animal sources, and vitamin D_2 (ergocalciferol), which is present in plant sources. (When no subscript is present, D_2 and/or D_3 is indicated.) However, the energy of sunlight (ultraviolet B rays) synthesizes vitamin D in the skin from the precursor 7-dehydrocholesterol, which is converted to the secosteroid vitamin D_3. Whether derived from the diet or synthesized in the skin, vitamin D is first hydroxylated in the liver to form the circulating prohormone 25-hydroxy vitamin D [25(OH)D] by the enzyme 25-hydroxylase (CYP27A1) (1) (**Figure 1**). Conversion of 25(OH)D to calcitriol is subsequently accomplished in the kidneys in a tightly controlled enzymatic step catalyzed by 1α-hydroxylase (CYP27B1) (1). However, many extrarenal tissues also express 1α-hydroxylase that is not as tightly regulated as the renal enzyme. In these sites, the concentration of circulating 25(OH)D, which serves as substrate, determines the production rate of calcitriol (dashed line in **Figure 1**), which then acts in these tissues in a paracrine manner (14).

3. VITAMIN D AND CANCER

3.1. Epidemiology

Epidemiological studies have suggested both increased incidence rates and elevated mortality rates in several cancers in geographical regions or in populations that are exposed to less solar ultraviolet B (UV-B) radiation (15). Sunlight is considered a surrogate for vitamin D levels, and the potential anti-cancer benefit in regions exposed to high sunlight is attributed to vitamin D production because UV light is essential for the cutaneous synthesis of vitamin D (16, 17). The sunlight–vitamin D hypothesis has been proposed for several cancers (18), including colorectal cancer (CRC) (16), prostate cancer (PCa) (17), and breast cancer (BCa) (19). Some studies report

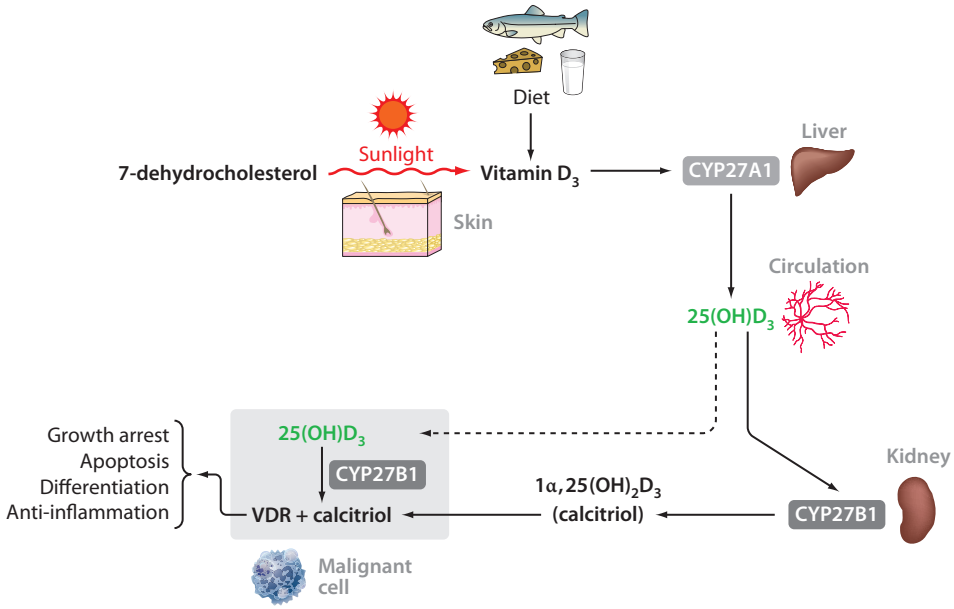

Figure 1

Vitamin D metabolism. Vitamin D_3 (cholecalciferol) is derived from the diet or synthesized in the skin via the energy of sunlight (ultraviolet B rays) from the precursor 7-dehydrocholesterol. Vitamin D_3 is first hydroxylated in the liver to form the circulating prohormone 25-hydroxy vitamin D_3 [25(OH)D_3] by the enzyme 25-hydroxylase (CYP27A1) and probably also by other enzymes (e.g., CYP2R1). Conversion of 25(OH)D to calcitriol is subsequently accomplished in the kidneys in a tightly controlled enzymatic step catalyzed by 1α-hydroxylase (CYP27B1). However, many extrarenal cells—including malignant cells—also express 1α-hydroxylase that is not as tightly regulated as the renal enzyme. In these sites, the concentration of circulating 25(OH)D_3, which serves as substrate, determines the production rate of calcitriol (*dashed line*), which elicits anti-proliferative effects by binding to the vitamin D receptor (VDR) and acting in an autocrine/paracrine manner.

an inverse association between cancer risk and circulating levels of 25(OH)D, which reflect both sun exposure and dietary vitamin D intake (18). The evidence is strongest for CRC: Both circulating 25(OH)D levels and vitamin D intake are inversely associated with colorectal adenoma as well as CRC incidence and recurrence (20, 21). In addition, in CRC patients, higher prediagnosis plasma 25(OH)D levels were associated with a significant improvement in overall survival (22). A recent reanalysis of data from the Women's Health Initiative (WHI) randomized trial concluded that estrogen therapy concurrent with calcium and vitamin D supplementation increased the risk of developing CRC. In the women concurrently assigned to placebo arms (no estrogen) of the estrogen trials, the calcium and vitamin D supplementation was beneficial in reducing the risk of CRC (23). The evidence for an increased risk of PCa in vitamin D–deficient populations is somewhat weaker; some studies suggest an inverse correlation between serum 25(OH)D levels and PCa risk (24), whereas others do not support such a correlation (25). A recent analysis of epidemiological data concluded that a serum 25(OH)D level of approximately 52 ng ml^{-1} (130 nmol $liter^{-1}$) was associated with a 50% reduction in the incidence of BCa (19). Several studies have also examined, with inconsistent results, the association between polymorphisms in the *VDR* gene and the risk for colon and prostate cancers, but some studies suggest a poorer prognosis for certain single-nucleotide polymorphisms in the *VDR* gene (20, 26–28).

3.2. Vitamin D–Metabolizing Enzymes as Regulators of the Anti-Cancer Effects of Calcitriol

3.2.1. Role of 1α-hydroxylase. Extrarenal 1α-hydroxylase, the presence of which has been demonstrated in several sites, contributes to the local production of calcitriol within these tissues (14). In some cancers such as parathyroid carcinomas, the expression levels and activity of 1α-hydroxylase in the cancer cells are lower than in the normal cells (29). However, in other malignant cells such as CRC and BCa cells, 1α-hydroxylase levels are elevated, at least before the cancers progress to a far-advanced stage (30). Although measurable, 1α-hydroxylase levels in PCa cells appear to be reduced, possibly owing to decreased 1α-hydroxylase promoter activity in these cells (31). These observations suggest that the administration of the precursor 25(OH)D might be an effective chemopreventive strategy in various cancers, including early PCa when 1α-hydroxylase activity would still be high and thus allow intratumor synthesis of calcitriol (32).

3.2.2. Role of 24-hydroxylase. In essentially all target cells, including cancer cells, calcitriol induces the expression of the enzyme 24-hydroxylase (CYP24), which catalyzes the initial step in the conversion of the active molecule calcitriol or the precursor molecule 25(OH)D into less-active metabolites (1). Therefore, the degree of growth-inhibitory response elicited by calcitriol is inversely proportional to the 24-hydroxylase activity in malignant cells (33, 34). PCa cells that have high 24-hydroxylase expression exhibit decreased sensitivity to calcitriol, resulting in a negligible to a very low degree of growth inhibition following calcitriol treatment (35). However, co-addition of inhibitors of 24-hydroxylase—such as liarozole, ketoconazole, or genistein, a soy isoflavone that directly inhibits 24-hydroxylase enzyme activity (36)—renders the cells more responsive to calcitriol (35–37). These observations suggest that combinations of calcitriol with inhibitors of 24-hydroxylase may be a useful strategy in cancer treatment. The combination therapy may also allow the use of calcitriol at lower concentrations and thereby reduce its hypercalcemic side effects. However, the combination approach to increase the biological activities of calcitriol by inhibiting CYP24 would also increase its calcemic activity and the risk of hypercalcemic side effects. Alternatively, structural analogs of calcitriol that resist 24-hydroxylation may be more biologically active and more useful for cancer therapy (5, 38).

4. MECHANISMS OF THE ANTI-CANCER EFFECTS OF CALCITRIOL

Calcitriol exerts anti-proliferative and pro-differentiating effects in many malignant cells and retards tumor growth in animal models of cancer (7–11, 27, 39–47). Several important mechanisms have been implicated in the anti-cancer effects of calcitriol, some of which are discussed in the next sections.

4.1. Regulation of Cell Proliferation and Apoptosis

Calcitriol inhibits the growth of many malignant cells by inducing cell cycle arrest and stimulating apoptosis (7–11, 27, 39–47). The molecular mediators of these calcitriol actions have been well characterized. Calcitriol inhibits the proliferation of PCa cells through cell cycle arrest in the G_1/G_0 phase (9, 10, 48) in a p53-dependent manner (10) by increasing the expression of the cyclin-dependent kinase inhibitors $p21^{Waf/Cip1}$ and $p27^{Kip1}$ (48–50), decreasing cyclin-dependent kinase 2 (CDK2) activity (48), and causing the hyperphosphorylation of the retinoblastoma protein (pRb) (51). Furthermore, calcitriol appears to regulate the nuclear-cytoplasmic trafficking of CDK2 and causes cytoplasmic mislocalization of CDK2 in PCa cells, leading to growth arrest and inhibition

of cell proliferation (52). As the loss of the expression of cell cycle regulators has been associated with a more aggressive cancer phenotype along with decreased prognosis and poorer survival, these observations suggest that calcitriol may be a suitable therapy to inhibit cancer progression. Calcitriol also induces apoptosis in several PCa and BCa cells by mitochondrial disruption and the activation of the intrinsic pathway of apoptosis; it does so by suppressing the expression of anti-apoptotic genes such as *Bcl*$_2$ and increasing the expression of the pro-apoptotic gene *Bax* (11, 40, 53). In BCa cells such as MCF-7, calcitriol-mediated apoptosis also involves calcium release from the endoplasmic reticulum and activation of calpain (40).

4.2. Stimulation of Differentiation

Calcitriol has been shown to induce the differentiation of a number of normal and malignant cells. Studies in various CRC models have demonstrated the tumor-inhibitory and pro-differentiation effects of calcitriol or its analogs both in vitro and in vivo (41, 54, 55). Calcitriol stimulates a variety of immature hematopoietic myeloid cells to differentiate into mature cells, including M-1 mouse myeloid leukemic cells, HL-60 human promyelocytic leukemia cells, U-937 human monocytic cells, and peripheral human monocytes (56). It stimulates myeloid leukemic cell lines to terminally differentiate into monocytes/macrophages (50). In HL-60 cells, the calcitriol-induced response is the stimulation of terminal differentiation into cells that have characteristics of macrophages, and the response appears to be mediated by inhibition of the expression of the *c-myc* oncogene (57). Calcitriol also reduces the expression of *c-myc* in several PCa cells, including androgen-independent PCa cells (58).

4.3. Regulation of Androgen and Estrogen Receptor Signaling

The sex steroid hormones androgens and estrogens drive the growth of PCa and BCa cells, respectively. Androgen receptor (AR) deregulation and aberrant androgen synthesis significantly contribute to the progression of PCa into androgen-independent or castrate-resistant PCa (CRPC) that is not amenable to therapy (59, 60). Interestingly, there is cross talk between calcitriol actions and androgen and estrogen signaling in PCa and BCa cells. Calcitriol upregulates AR expression in LNCaP PCa cells (61, 62). The androgen-inducible, growth-inhibitory gene *AS3/APRIN* is also induced by calcitriol in LNCaP cells (63). The anti-proliferative action of calcitriol in LNCaP cells appears to be androgen dependent as it could be blocked by the AR antagonist casodex (64). However, this is not the case for other PCa cells (65). In general, calcitriol exerts both androgen-dependent and androgen-independent growth-inhibitory effects on PCa cells, and cells of the LNCaP lineage are more responsive to calcitriol than are most other PCa cells (66). In estrogen receptor (ER)-positive BCa cells, calcitriol significantly reduces ER expression and inhibits estrogen stimulation of cell proliferation (67–69). Calcitriol reduces ER expression by a direct transcriptional repression of the *estrogen receptor α* (*ERα*) gene (68, 69). Recent studies show that in BCa cells and in the mammary fat surrounding breast tumors, calcitriol decreases the expression of aromatase, the enzyme that catalyzes estrogen synthesis from androgenic precursors by a transcriptional repression of the *aromatase* gene (70).

4.4. Modulation of Growth-Factor, Oncogene, Tumor-Suppressor, and Transcription-Factor Actions

In many malignant cells, calcitriol modulates growth-factor actions such as upregulation of the expression of the *insulin-like growth factor binding protein-3* (*IGFBP-3*) gene in PCa cells, which

in turn leads to an increase in the expression of *p21*, causing cell cycle arrest (71, 72). Other molecular mechanisms that mediate the anti-proliferative and differentiation-inducing effects of vitamin D compounds in myeloid leukemic cells include the upregulation of homeobox genes such as *Hox A10* and *Hox B4*, the downregulation of Bcl_2, and the modulation of the intracellular kinase pathways p38, mitogen-activated protein kinase (MAPK), extracellular regulated kinase (ERK), and phosphatidylinositol 3-kinase (PI3K) (56). The most common and initial alteration in sporadic CRC is the aberrant activation of the Wnt/β-catenin signaling pathway, and calcitriol inhibits β-catenin transcriptional activity in colon cancer cells by promoting VDR binding to β-catenin, preventing its translocation to the nucleus, and inducing E-cadherin expression (73). Calcitriol also inhibits the Wnt pathway by increasing the expression of genes that encode the extracellular Wnt inhibitors DICKKOPF-1 and DICKKOPF-4 (73). The Snail transcription factor represses VDR expression and thus abolishes the anti-proliferative and differentiation-inducing effects of calcitriol in cultured colon cancer cells (74). Increased Snail expression in human colon tumors is associated with a loss of responsiveness to calcitriol and its analogs; thus it may be used as an indicator of patients who are unlikely to respond to vitamin D therapy (74).

4.5. Anti-Inflammatory Effects

Data are accumulating to support the idea that inflammation contributes to the development and progression of many cancers (75–77). Inflammatory mediators such as cytokines, chemokines, prostaglandins (PGs), and reactive oxygen and nitrogen species enhance tumorigenesis through the activation of multiple signaling pathways in tumor tissue (75–77). Recent research, including observations from our laboratory, suggests that calcitriol exhibits anti-inflammatory actions that may contribute to its beneficial effects in several cancers in addition to the multiple actions described above (78). Studies in PCa and BCa cells reveal that calcitriol exerts important regulatory effects on some of the key molecular pathways involved in inflammation, such as inhibition of PG synthesis and actions, inhibition of stress-activated kinase signaling and the resultant production of inflammatory cytokines, and inhibition of nuclear factor κB (NF-κB) signaling and the production of pro-angiogenic factors. We describe these pro-carcinogenic inflammatory pathways in the following sections and then discuss the molecular mechanisms underlying the anti-inflammatory actions of calcitriol to inhibit these pathways.

4.5.1. Regulation of prostaglandin metabolism and signaling. PGs promote carcinogenesis and play a positive role in the progression of many cancers by stimulating cellular proliferation, inhibiting apoptosis, promoting angiogenesis, and activating carcinogens (79). Extensive data support the idea that cyclooxygenase-2 (COX-2), the enzyme responsible for PG synthesis, is an oncogene and an important molecular target in cancer therapy (80–82).

4.5.1.1. COX-2. Cyclooxygenase (COX)/prostaglandin endoperoxidase synthase is the rate-limiting enzyme that catalyzes the conversion of arachidonic acid to PGs and related eicosanoids. COX exists as two isoforms: COX-1, which is constitutively expressed in many tissues and cell types, and COX-2, which is inducible by a variety of stimuli. *COX-2* is regarded as an immediate-early response gene whose expression is rapidly induced by mitogens, cytokines, tumor promoters, and growth factors (81). Genetic and clinical studies indicate that increased COX-2 expression is one of the key steps in carcinogenesis (83). Long-term use of anti-inflammatory agents such as nonsteroidal anti-inflammatory drugs (NSAIDs), which inhibit COX enzyme activity, has been shown in some studies to be associated with a decrease in death rate from several cancers such as colorectal, stomach, breast, lung, prostate, bladder, and ovarian cancers (84–87).

Several studies (88, 89), but not all (90, 91), suggest a causative and/or stimulatory role for COX-2 in prostate tumorigenesis and demonstrate its overexpression in prostate adenocarcinoma. Appreciable COX-2 expression is seen in areas of proliferative inflammatory atrophy (PIA), which are lesions that have been implicated in prostate carcinogenesis (91). Silencing of COX-2 in metastatic PCa cells induces cell growth arrest and causes morphological changes associated with enhanced differentiation, highlighting the role of COX-2 in prostate carcinogenesis (92). COX-2 expression in prostate biopsy cores and PCa surgical specimens is inversely correlated with disease-free survival (93) and is an independent predictor of recurrence (94). Elevated COX-2 protein levels have been reported in ~40% of invasive breast carcinomas (80). NSAIDs inhibit the development of BCa in a variety of animal models (reviewed in Reference 80). Interestingly, PG signaling stimulates the transcription of the *aromatase* gene (95), and a positive correlation between COX-2 and aromatase expression in human BCa reflects this causal link (96, 97). COX-2 overexpression in BCa correlates with features of aggressive cancer, including larger tumor size, a higher grade, increased proliferation, ER-negative status, and overexpression of the *Her-2/neu* oncogene (98–100). An inverse relationship between COX-2 protein levels and disease-free survival in BCa patients has also been shown (99, 101). Some epidemiological observations reveal a significant reduction in the incidence of CRC among chronic users of NSAIDs (84, 85, 87). A critical link between COX-2 and colorectal tumorigenesis was demonstrated when $Apc^{\Delta 716}$ mutant mice were mated to COX-2 knockout mice, and a dramatic reduction in the number of intestinal polyps was seen in the doubly null progeny compared with COX-2 wild-type (WT) mice (102). COX-2 protein is significantly overexpressed in CRC (82, 103, 104), and increased COX-2 expression correlates with a larger polyp size and progression to invasive carcinoma (105, 106).

Local production of PGs at the tumor sites via the infiltration of inflammatory cells also increases the risk of carcinogenesis and/or cancer progression (91, 104, 107, 108). In CRC, COX-2 expression has been found in the carcinoma cells as well as in infiltrating macrophages within the tumors (109, 110). In other cancers, COX-2 expression has been demonstrated in vascular endothelial cells, fibroblasts, and smooth muscle cells around the cancer (111, 112). PGs generated by COX-2 act in an autocrine and paracrine manner to stimulate cell growth. At the cellular level, both arachidonic acid (the substrate for COX) and the product prostaglandin E_2 (PGE_2) stimulate proliferation by regulating the expression of genes that are involved in growth regulation, including *c-fos* (113). Studies in experimental models of cancer have shown that *COX-2* enhances tumor development and progression by promoting resistance to apoptosis and stimulating angiogenesis and tumor invasion; it is therefore regarded as an oncogene (80–82).

4.5.1.2. 15-PGDH. 15-Hydroxyprostaglandin dehydrogenase (15-PGDH) is the enzyme that catalyzes the conversion of PGs to their corresponding 15-keto derivatives, which exhibit greatly reduced biological activity. The *15-PGDH* gene has been described as an oncogene antagonist in colon cancer by Yan et al. (114). Their studies show that *15-PGDH* is universally expressed in normal colon but is routinely absent or severely reduced in cancer specimens. Most importantly, the stable transfection of a *15-PGDH* expression vector into colon cancer cells greatly reduces the ability of the cells to form tumors and/or slows tumor growth in nude mice, demonstrating that *15-PGDH* functions as a tumor suppressor (114). Another study in mice also demonstrates that *15-PGDH* acts in vivo as a highly potent suppressor of the development of colon neoplasia (115). Low expression of *15-PGDH* and methylation of the *15-PGDH* promoter in 30–40% of primary breast tumors have been reported by Wolf et al. (116). Their studies in BCa cells also demonstrated a suppression of cell proliferation in vitro and decreased tumorigenicity in vivo following the overexpression of *15-PGDH*, thus supporting a tumor-suppressor role for *15-PGDH* in BCa.

4.5.1.3. Prostaglandin receptors. PGE and PGF are the major PGs stimulating the proliferation of PCa cells, and they act by binding to G protein–coupled membrane receptors (prostanoid receptors). The PG receptors EP and FP are expressed in many malignant cells (113, 117) and in most endothelial cells, macrophages, and stromal cells found in the tumor microenvironment. Interaction between PG and its receptors can send positive feedback signals to increase COX-2 mRNA levels (113, 118). Therefore, irrespective of the initial trigger of COX-2 expression, PGs could mediate a wave of COX-2 expression at the tumor sites not only in the cancer cells but also in the surrounding stromal cells, infiltrating macrophages, and endothelial cells, thereby promoting tumor progression.

4.5.1.4. Calcitriol effects on the prostaglandin pathway. Our studies demonstrate that calcitriol regulates the expression of several PG pathway genes in multiple PCa cell lines and primary prostatic epithelial cells established from surgically removed prostate tissue from PCa patients (117) as well as in ER-positive and ER-negative BCa cells (70), as shown in **Figure 2**. Calcitriol significantly decreases the expression of COX-2 and increases that of 15-PGDH in various PCa and BCa cells (70, 117). As a result, calcitriol treatment of these cells decreases the levels of

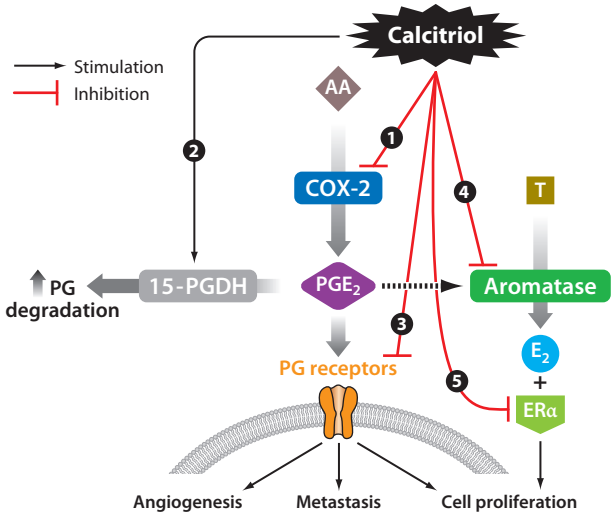

Figure 2

Inhibition of the prostaglandin (PG) pathway and estrogen signaling by calcitriol. Calcitriol inhibits the synthesis and biological actions of pro-inflammatory PGs by three mechanisms: (*1*) suppression of the expression of cyclooxygenase-2 (COX-2), the enzyme that synthesizes PGs, (*2*) upregulation of the expression of 15-hydroxyprostaglandin dehydrogenase (15-PGDH), the enzyme that inactivates PGs, and (*3*) downregulation of the expression of PG receptors that are essential for PG signaling. PGs stimulate proliferation, angiogenesis, and other pro-carcinogenic pathways. In addition, calcitriol also inhibits estrogen synthesis and signaling as follows: (*4*) Calcitriol decreases the expression of aromatase, the enzyme that converts androgenic precursors to estrogens both in the cancerous breast epithelial cells and in the breast adipose fibroblasts surrounding the tumor. It accomplishes this both directly by the transcriptional repression of the aromatase promoter II and indirectly by the reduction of the levels of prostaglandin E_2 (PGE_2), a major stimulator of aromatase transcription via promoter II. (*5*) Calcitriol also downregulates estrogen receptor α (ERα) levels by the direct transcriptional repression of the *ER*α promoter. The downregulation of both the hormone (E_2) and receptor (ERα) levels by calcitriol thus significantly reduces the important proliferative stimulus of estrogens on ER-positive breast cancer (BCa) cells. Other abbreviations: AA, arachidonic acid; E_2, estradiol; T, testosterone. Adapted with permission from Reference 78.

biologically active PGs, thereby reducing the growth stimulation due to PGs. In PCa cells, calcitriol also decreases the expression of the PG receptors EP and FP and inhibits PG-mediated functional responses (117). We postulate that the downregulation of PG receptors by calcitriol inhibits the positive feedback exerted by PGs on COX-2, thereby limiting the wave of COX-2 expression at the tumor sites and slowing tumor progression. These calcitriol actions contribute to the suppression of the proliferative and angiogenic stimuli provided by PGs in malignant cells and constitute important pathways that mediate the anti-inflammatory effects of calcitriol (**Figure 2**). Importantly, combinations of calcitriol with the COX inhibitors NSAIDs exhibit synergistic enhancement of growth inhibition in cultures of PCa cells (117), raising the possibility that these combinations would have utility in the treatment of PCa (see Section 6).

4.5.1.5. Inhibition of the prostaglandin pathway by calcitriol and suppression of estrogen synthesis in breast cancer. The calcitriol-mediated decrease in COX-2 expression in BCa cells is especially interesting, because tumor samples from BCa patients have exhibited a tight coupling between the expression levels of COX-2 and aromatase, the enzyme that catalyzes estrogen synthesis from androgenic precursors (96, 97). Aromatase expression in the breast is critical for the progression of ER-positive BCa in postmenopausal women. Whereas BCa cells express aromatase and have the capacity to synthesize estrogens, aromatase in the normal breast is primarily expressed in the stromal mesenchymal cells of the breast adipose tissue [breast adipose fibroblasts (BAFs) or preadipocytes], where its transcription is driven primarily by the tissue-specific promoter I.4 in normal breast adipose tissue (95). However, in the presence of BCa, the transcription switches from promoter I.4 to predominantly promoter I.3 and promoter II in both the cancerous epithelial cells and the surrounding BAFs (95). Interestingly, calcitriol regulates the expression of aromatase in a tissue-selective manner. Our findings reveal that calcitriol significantly decreases aromatase expression by a direct transcriptional repression of promoter II/I.3 in human BCa cells and a cell culture model of preadipocytes (70) (**Figure 2**). Furthermore, calcitriol decreases aromatase expression in xenografts of human BCa cells established in immunocompromised mice as well as in the mammary adipose tissue surrounding the xenograft tumors in these mice (70). Promoters I.3 and II, which are used predominantly in malignant breast epithelial cells and the BAFs surrounding a breast tumor, are responsive to cyclic adenosine monophosphate (cAMP) (119) and are significantly stimulated by PGE_2 (120, 121). Therefore, calcitriol-mediated reduction in PG levels arising from the suppression of COX-2 expression and induction of 15-PGDH expression in BCa cells provides an important second, indirect mechanism for calcitriol's downregulatory effect on aromatase expression in BCa cells and the tumor-adjacent BAFs (**Figure 2**). By reducing estrogen synthesis and downregulating ERα levels, calcitriol attenuates the mitogenic stimulus of estrogen on BCa cells, causing significant inhibition of BCa cell proliferation (**Figure 2**).

Aromatase inhibitors (AIs), which inhibit the enzymatic activity of aromatase, have become the major therapeutic agents to treat ER-positive BCa and inhibit BCa progression or recurrence in postmenopausal women after primary surgical and/or radiation therapy (122, 123). Combinations of calcitriol with AIs exhibited enhanced growth-inhibitory effects in BCa cell cultures (70). AIs inhibit estrogen synthesis globally and therefore have a detrimental effect at sites such as bone, where normal estrogen function is required for the maintenance of bone homeostasis. COX-2-derived PGs (124) and local estrogen production by aromatase in bone (95) play important roles in bone metabolism and skeletal growth. The development of selective aromatase modulators (SAMs) that inhibit aromatase expression in breast, but allow unimpaired estrogen synthesis at other desirable sites such as bone, would have great utility in BCa therapy (95). We postulate that calcitriol acts as a SAM, decreasing aromatase expression in BCa cells and breast adipose tissue surrounding BCa (70) while increasing aromatase expression in bone cells (70, 125); thus it has

the potential to ameliorate the AI-induced side effect of osteoporosis when used in combination with an AI in BCa patients.

4.5.2. Induction of MAP kinase phosphatase 5 and inhibition of stress-activated kinase signaling. In normal human prostate epithelial cells, calcitriol increases the expression of MAP kinase phosphatase 5 (MKP5), also known as DUSP10 (126). MKP5 is a member of the dual-specificity MKP family of enzymes that dephosphorylate and thereby inactivate MAPKs. MKP5 specifically dephosphorylates p38 MAPK and the stress-activated protein kinase Jun-N-terminal kinase (JNK), leading to their inactivation. Calcitriol increases *MKP5* transcription by binding to and activating the VDR and its subsequent interaction with a vitamin D response element (VDRE) identified in the *MKP5* promoter (127). The calcitriol-mediated increase in MKP5 is seen in cells derived from normal prostate epithelium and primary, localized adenocarcinoma. The action leads to downstream anti-inflammatory responses by causing the dephosphorylation and inactivation of the p38 stress-induced kinase, resulting in a decrease in the production of pro-inflammatory cytokines that sustain and amplify the inflammatory response (128), such as interleukin-6 (IL-6). Pretreatment of prostate epithelial cells with calcitriol significantly attenuates the increase in IL-6 production following treatment of the cells with the pleiotropic cytokine tumor necrosis factor α (TNFα) (127).

IL-6 is a major pro-inflammatory cytokine that participates in inflammation-associated carcinogenesis (129), and it has been implicated in the pathogenesis of several cancers (130, 131). Serum IL-6 levels were significantly elevated and positively correlated to tumor burden in CRC (132), BCa (133), and PCa patients (133), who also exhibited a positive correlation between IL-6 levels and the number of bone metastases (133). IL-6 has been shown to be associated with PCa progression (130). The ability of calcitriol to reduce the production of pro-inflammatory cytokines such as IL-6 by inhibiting p38 signaling (127) demonstrates its significant anti-inflammatory effects in cancer cells. Interestingly, calcitriol upregulation of MKP5 was seen only in primary cells derived from normal prostatic epithelium and primary, localized adenocarcinoma but not in the established PCa cell lines derived from PCa metastasis. We therefore speculate that a loss of MKP5 might occur during PCa progression, as a result of a selective pressure to eliminate the tumor-suppressor activity of MKP5 and/or calcitriol.

4.5.3. Inhibition of nuclear factor κB activation and signaling. NF-κB comprises a family of inducible transcription factors ubiquitously present in all cells. NF-κB transcription factors are important regulators of innate immune responses and inflammation (134). In the basal state, most NF-κB dimers are bound to specific inhibitory proteins named IκB proteins, and pro-inflammatory signals activate NF-κB mainly through IκB kinase (IKK)-dependent phosphorylation and degradation of the inhibitory IκB proteins (134). Free NF-κB then translocates to the nucleus and activates the transcription of pro-inflammatory cytokines, chemokines, and anti-apoptotic factors (134). In contrast to normal cells, many malignant cells have elevated levels of active NF-κB (135). Constitutive activation of NF-κB has been observed in androgen-independent PCa (136, 137). The NF-κB protein RelB is uniquely expressed at high levels in PCa with high Gleason scores (138). NF-κB plays a major role in the control of immune responses and inflammation and promotes malignant behavior by increasing the transcription of the anti-apoptotic gene Bcl_2 (139), cell cycle progression factors such as *c-myc* and cyclin D_1, proteolytic enzymes such as matrix metalloproteinase 9 (MMP-9) and urokinase-type plasminogen activator (uPA), and angiogenic factors such as vascular endothelial growth factor (VEGF) and interleukin-8 (IL-8) (137). IL-8, an angiogenic factor and a downstream target of NF-κB, is also a potent chemotactic factor for neutrophils and is associated with the initiation of the inflammatory response (140).

Calcitriol is known to directly modulate basal and cytokine-induced NF-κB activity in many cells including human lymphocytes (141), fibroblasts (142), and peripheral blood monocytes (143). Calcitriol and its analogs block NF-κB activation by increasing the expression of IκB in peripheral blood mononuclear cells and macrophages (143, 144). A reduction in the levels of the NF-κB inhibitory protein IκBα has been reported in mice lacking the VDR (145). IKKβ-mediated activation of NF-κB contributes to the development of colitis-associated cancer through the activation of anti-apoptotic genes and the production of IL-6 (146). The addition of a VDR antagonist to colon cancer cells upregulates NF-κB activity by decreasing the levels of IκBα, suggesting that VDR agonists suppress NF-κB activation (147). There is considerable evidence for the inhibition of NF-κB signaling by calcitriol in PCa cells. Calcitriol decreases the levels of the angiogenic and pro-inflammatory cytokine IL-8 in immortalized normal human prostate epithelial cell lines (HPr-1 and RWPE-1) and established PCa cell lines (LNCaP, PC-3, and DU145) (148). The suppression of IL-8 by calcitriol appears to result from the inhibition of NF-κB signaling. Calcitriol reduces the nuclear translocation of the NF-κB subunit p65, thereby inhibiting the NF-κB complex from binding to its DNA response element and consequently suppressing the NF-κB stimulation of transcription of downstream targets such as IL-8 (148). Thus calcitriol could delay the progression of PCa by suppressing the expression of angiogenic and pro-inflammatory factors such as VEGF and IL-8. In addition, calcitriol also indirectly inhibits NF-κB signaling by upregulating the expression of IGFBP-3, which has been shown to interfere with NF-κB signaling in PCa cells by suppressing p65 NF-κB protein levels and the phosphorylation of IκBα (149). NF-κB also furnishes PCa cells with an adaptive response to cytotoxicity induced by redox-active therapeutic agents and is implicated in radiation resistance of cancers (150). A recent study shows that calcitriol significantly enhances the sensitivity of PCa cells to ionizing radiation by selectively suppressing radiation-mediated RelB activation (151). Thus calcitriol may serve as an effective agent for sensitizing PCa cells to radiation therapy via suppression of the NF-κB pathway.

4.6. Inhibition of Angiogenesis

Angiogenesis, the process of formation of new blood vessels from existing vasculature, is a crucial step in the continued growth, progression, and metastasis of tumors (152). VEGF is the most potent stimulator of angiogenesis. PGs, as discussed below, are also important pro-angiogenic factors. The initiation of angiogenesis is controlled by local hypoxia, which induces the synthesis of pro-angiogenic factors that activate signaling pathways; this synthesis leads to the structural reorganization of endothelial cells that favors new capillary formation (153). Stimulation of angiogenesis in response to hypoxia is mediated by hypoxia-inducible factor 1 (HIF-1), which directly increases the expression of several pro-angiogenic factors including VEGF (154, 155). Early studies indicated that calcitriol was a potent inhibitor of tumor cell–induced angiogenesis in experimental models (156). Calcitriol inhibits VEGF-induced endothelial cell tube formation in vitro and decreases tumor vascularization in mice that bear xenografts of BCa cells overexpressing VEGF (157). Calcitriol and its analogs also directly inhibit the proliferation of endothelial cells (158, 159), leading to the inhibition of angiogenesis.

At the molecular level, calcitriol exerts its anti-angiogenic effects by regulating the expression of key factors that control angiogenesis. Calcitriol reduces the expression of VEGF in several malignant cells, including PCa cells, through transcriptional repression of HIF-1 (158). Furthermore, as discussed above, calcitriol inhibits malignant cell–induced angiogenesis by suppressing the expression of the pro-angiogenic factor IL-8 in an NF-κB-dependent manner (148). TRAMP (transgenic adenocarcinoma of the mouse prostate)-2 tumors established in VDR knockout mice had enlarged vessels and increased vessel volume compared with the tumors in WT mice, suggesting

an inhibitory role for the VDR and calcitriol in tumor angiogenesis (159). Furthermore, increased expression of pro-angiogenic factors such as HIF-1α, VEGF, angiopoietin-1, and platelet-derived growth factor (PDGF) was also seen in the tumors in the VDR knockout mice (159).

Another important mechanism by which COX-2 promotes tumor progression (in addition to the pathways discussed above) is through the stimulation of angiogenesis; consequently, COX-2 inhibitors have been used to block angiogenesis and tumor proliferation (160). The pro-angiogenic effect of COX-2-generated PGE_2 might arise from its action to increase HIF-1α protein synthesis in cancer cells (161). An analysis of human PCa specimens revealed that increased COX-2 immunostaining was associated with increased infiltration of T lymphocytes and macrophages and increased CD31-marked microvessel density, indicating a positive correlation between COX-2 expression and both inflammation and angiogenesis (108). Pro-inflammatory cytokines released by tumor-adjacent inflammatory cells such as T lymphocytes and macrophages may induce COX-2 in the epithelial cells in prostate atrophic lesions, thus promoting tumor progression (162). Suppression of COX-2 expression by calcitriol therefore provides an important indirect mechanism by which calcitriol inhibits angiogenesis, in addition to its direct suppressive effects on pro-angiogenic factors such as HIF-1 and VEGF. It has been suggested that VEGF induction of p38 and JNK pathways are necessary for COX-2 expression in endothelial cells (163). As discussed above, calcitriol inactivates the p38 pathway by inducing MKP5 expression. Thus MKP5 induction and VEGF suppression by calcitriol could further contribute to its anti-angiogenic effects through p38 inactivation.

MMPs promote angiogenesis by mediating the degradation of the basement membrane of the vascular epithelium and the extracellular matrix, thereby creating a passageway in these barriers for the formation of new capillaries (153). In human PCa cells, calcitriol decreases the expression and activity of MMP-9 while increasing the activity of its counterpart tissue inhibitor of metalloproteinase 1 (TIMP-1), thereby decreasing angiogenesis and the invasive potential of these cells (148). **Figure 3** summarizes the anti-inflammatory and anti-angiogenic effects of calcitriol, along with some of the molecular targets that mediate these effects in malignant cells and in other cell types present at the tumor site.

5. THE ROLE OF ANTI-INFLAMMATORY EFFECTS OF CALCITRIOL IN CANCER CHEMOPREVENTION

As discussed above, current perspectives in cancer biology suggest that inflammation plays a role in the development of PCa. De Marzo et al. (107) have proposed that the PIA lesions in the prostate, which are associated with acute or chronic inflammation, are precursors of prostate intraepithelial neoplasia (PIN) and PCa. The epithelial cells in PIA lesions have been shown to exhibit many molecular signs of stress, including elevated expression of COX-2 (91). Inflammatory bowel disease is associated with the development of CRC (164, 165). Research that demonstrates anti-inflammatory effects of calcitriol (discussed in Section 4.6) in malignant cells and in the infiltrating inflammatory cells at the tumor sites supports the idea that calcitriol may play a role in delaying or preventing the development and/or progression of cancer.

5.1. Prostate Cancer

PCa generally progresses slowly, likely over decades, before symptoms become obvious and diagnosis is made (166). Inflammation in the prostate has been proposed to be an etiological factor in the development of PCa (107). The observed latency in PCa provides a long window of opportunity for intervention by chemopreventive agents. Dietary supplementation of COX-2-selective

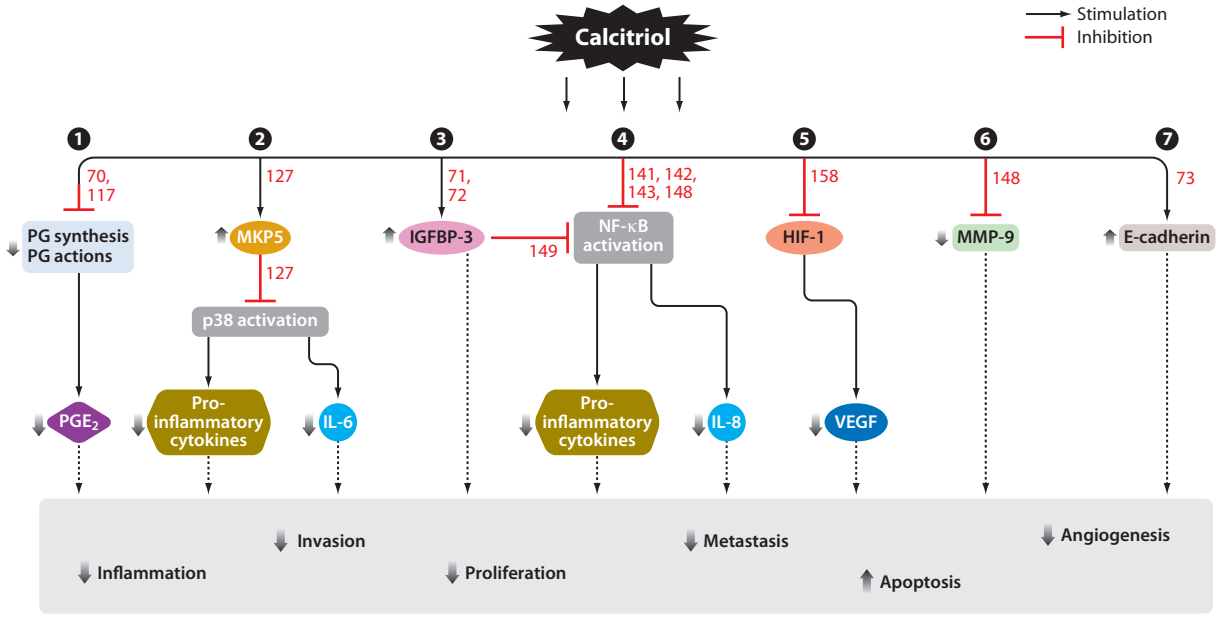

Figure 3

Some of the molecular pathways that mediate the anti-inflammatory actions of calcitriol. Several newly identified calcitriol target genes reveal multiple molecular pathways of anti-inflammatory actions of calcitriol in many cell types. These include (*1*) the inhibition of prostaglandin (PG) synthesis and biological actions; (*2*) the induction of MAP kinase phosphatase 5 (MKP5) expression, subsequent inhibition of p38 stress kinase activation, and production of pro-inflammatory cytokines such as interleukin-6 (IL-6); (*3*) the upregulation of the expression of insulin-like growth factor binding protein-3 (IGFBP-3), which suppresses proliferation and inhibits NF-κB activation; (*4*) the inhibition of nuclear factor κB (NF-κB) signaling, which results in the attenuation of the synthesis of pro-inflammatory cytokines such as interleukin-8 (IL-8); (*5*) the inhibition of tumor angiogenesis due to suppressive effects on the expression of pro-angiogenic factors such as hypoxia-inducible factor 1 (HIF-1), vascular endothelial growth factor (VEGF), and IL-8; and (*6*) the decrease in the expression of matrix metalloproteinase 9 (MMP-9) and (7) the increase in the expression of E-cadherin, leading to the inhibition of invasion and metastasis. Solid lines indicate direct actions of calcitriol, and dotted lines indicate downstream effects of calcitriol. Abbreviation: PGE_2, prostaglandin E_2. Adapted with permission from Reference 78. The numbers in red correspond to the References that describe the pathways shown.

NSAIDs such as celecoxib has been shown to suppress prostate carcinogenesis in the TRAMP model of PCa (167). The use of NSAIDs such as aspirin has been shown to reduce serum prostate-specific antigen (PSA) levels in patients with latent PCa, indicating a beneficial effect of NSAID use (168). Because calcitriol exhibits significant anti-inflammatory effects, we hypothesize that calcitriol has the potential to be useful as a chemopreventive agent in PCa. The efficacy of calcitriol as a chemopreventive agent has been examined in *Nkx3.1; Pten* mutant mice, which recapitulate stages of prostate carcinogenesis from PIN lesions to adenocarcinoma (169). The data reveal that calcitriol significantly reduces the progression of PIN from a low grade to a high grade, thereby inhibiting the development of invasive cancer. Calcitriol is more effective when administered before, rather than subsequent to, the initial occurrence of PIN.

5.2. Breast Cancer

In considering possible pathways of BCa prevention, calcitriol appears to play an important role in the normal development of mammary glands, where it opposes estrogen-driven proliferation and

maintains differentiation (11, 170). Mammary glands in VDR-null mice exhibit accelerated growth and branching during puberty, pregnancy, and lactation as compared with WT mice. Involution of mammary glands after weaning, a process driven by epithelial cell apoptosis, proceeds at a slower rate in VDR-null mice (171). Several studies using various rodent models of chemical carcinogen–induced BCa have concluded that vitamin D exerts a beneficial effect in preventing the development of tumors (reviewed in Reference 170). The calcitriol analog EB1089 inhibits proliferation of human BCa cells and exhibits more potency than calcitriol in inhibiting tumor growth induced by the carcinogen *N*-nitroso-*N*-methylurea (NMU) (40). Welsh and coworkers (171, 172) exposed WT and VDR-null mice to the chemical carcinogen 7,12-dimethylbenz[α]anthracene (DMBA) and observed an increased number of preneoplastic lesions in the mammary glands of VDR-null mice compared with WT mice. The VDR-null mice also developed a variety of skin tumors, demonstrating an increased sensitivity to tumorigenesis. These observations suggest that calcitriol and its analogs might be useful in the chemoprevention of BCa.

5.3. Colon Cancer

Studies in a number of CRC models have demonstrated the tumor-inhibitory and pro-differentiation effects of calcitriol or its analogs (41, 54, 55, 173). A study in the *Apc*^{min} mouse model has demonstrated that both vitamin D and calcium individually exert inhibitory effects on the development of polyps and exhibit a synergistic effect when used together (47). Collectively, these data suggest the need for clinical trials that evaluate calcitriol and its analogs as agents that prevent and/or delay progression in cancer patients with early disease.

6. CLINICAL TRIALS TO ASSESS ANTI-CANCER EFFICACY

Clinical trials have been carried out in patients with several different malignancies to test the effects of (*a*) vitamin D_3 [the dietary supplement that is the precursor to 25(OH)D and calcitriol], (*b*) the active hormone calcitriol, (*c*) calcitriol analogs that have been designed to exhibit decreased calcemic effects while maintaining equal or even increased anti-proliferative activity, and (*d*) combinations of calcitriol with other agents or therapy regimens. In this section, we briefly describe some of these studies.

6.1. Vitamin D_3 (Cholecalciferol) in Cancer Trials

The WHI clinical trial and observational study evaluated the effect of supplementation with calcium and modest doses of vitamin D_3 (400 IU) primarily to prevent hip and other fractures and secondarily to prevent CRC and BCa (174). Initial published results of this study did not find a protective effect of calcium and vitamin D against CRC (175). However, a recent reanalysis of the data concluded that concurrent estrogen therapy modified the effect of calcium and vitamin D supplementation on CRC risk, and in the women assigned to placebo arms of the estrogen trials, the supplementation was beneficial (23).

The presence of 1α-hydroxylase in malignant cells and the paracrine anti-proliferative actions of locally synthesized calcitriol (14) raise the possibility that increasing the circulating concentrations of 25(OH)D by dietary administration of precursor vitamin D_3 (cholecalciferol) will have a potent anti-cancer effect due to the conversion of 25(OH)D to calcitriol within the tumors. In addition, 1α-hydroxylase is present in multiple cell types within the tumor microenvironment, such as tumor-infiltrating macrophages and endothelial cells. Eliciting an anti-cancer effect through treatment with vitamin D_3 and allowing the local production of calcitriol in the tumor site to

generate paracrine actions of calcitriol might be safer than raising circulating calcitriol levels via calcitriol administration, which can be associated with hypercalcemia, hypercalciuria, and the development of renal stones. In a small pilot clinical study, cholecalciferol was given to PCa patients at 2,000 IU per day, and they were monitored prospectively every 2–3 months over a period of 21 months. The results showed a beneficial effect: prolongation of serum PSA doubling time in 14 out of 15 patients (176). Serum PSA is a useful biomarker of prostate cell abundance, and its rate of rise is often used as a surrogate for tumor growth, after primary therapy has removed or irradiated the prostate. An alternate and complementary hypothesis is that very high doses of vitamin D_3 generate increased concentrations of circulating 25(OH)D that achieve high enough levels to directly bind to and activate the VDR (177). In a recent Phase II trial in women with metastatic BCa, Amir et al. (178) administered the very high dose of 10,000 IU daily of vitamin D_3 for 4 months and noted only a small but statistically significant increase in serum calcium and a significant decrease in serum parathyroid hormone. Treatment unmasked two cases of primary hyperparathyroidism but was not associated with direct toxicity. The authors concluded that daily ingestion of vitamin D_3 at 10,000 IU for 4 months appeared to be safe in patients without comorbid conditions that cause hypersensitivity to vitamin D. However, there did not appear to be a significant palliative benefit on the progress of BCa in these far-advanced cases (178).

6.2. Calcitriol in Clinical Trials

Calcitriol is a U.S. Food and Drug Administration (FDA)-approved drug (for other indications), and its therapeutic utility has been evaluated in clinical studies in cancer patients. Many of the clinical trials evaluating calcitriol and its structural analogs have been conducted in PCa patients, and a relatively smaller number of studies have been carried out in patients with other malignancies. A decrease in the rate of rise of serum PSA levels has been observed in PCa patients following modest but supraphysiological daily doses (2–2.5 μg daily) of calcitriol (179, 180), indicating a beneficial effect of calcitriol in slowing the progression of the disease. However, in addition to decreasing or stabilizing the rate of rise of PSA, the objective benefits were small and the risk of renal stones was significant (180). The anti-proliferative effects of calcitriol in cultured cells have been observed at high concentrations. Achieving adequate concentrations in patients runs the risk of causing hypercalcemia and hypercalciuria, which may lead to renal stone formation (180, 181). Other investigations have attempted to realize higher concentrations of calcitriol via the intermittent administration of very high doses; this approach was designed to achieve greater efficacy without toxicity. High doses of calcitriol were given 3 times a week (182), or very high doses were given once weekly (179, 181). This administration schedule apparently elicited calcitriol's anti-proliferative effects and resulted in only transient hypercalcemia and infrequent occurrence of renal stones.

6.3. Calcitriol Analogs

An alternate approach to develop effective treatment with reduced risk of hypercalcemia undertaken by many academic investigators and pharmaceutical companies is to develop calcitriol analogs that exhibit equal or even increased anti-proliferative activity while exhibiting a reduced tendency to cause hypercalcemia. A Phase I trial evaluated the calcitriol analog EB1089 (seocalcitol) in 36 patients with advanced BCa and CRC (183). Although this study did not demonstrate an anti-tumor effect, as determined by a reduction in tumor volume, stabilization of the disease was seen in 6 patients on treatment for more than 3 months. The efficacy and safety of EB1089 was also tested in a study of 22 patients with hepatocellular carcinoma, and partial to complete

remission was seen in 2 of the patients (184). Although benefit was seen in a small percentage of patients, the result is somewhat encouraging because this is a uniformly lethal cancer.

6.4. Calcitriol as a Part of Combination Therapy

Calcitriol is also used in combination therapy with other agents to achieve enhanced anti-cancer effects (182, 185). A Phase II trial in patients with androgen-independent PCa using high-dose oral calcitriol (12 μg daily, 3 times per week) with dexamethasone (4 mg daily, 4 times per week) showed a 50% reduction in PSA in 28% of the patients and no symptomatic hypercalcemia (182). Another Phase II trial tested the combination of calcitriol, dexamethasone, and carboplatin in patients with hormone-refractory PCa and found a PSA response in 13 out of 34 patients (186). The results of the ASCENT I clinical trial in advanced PCa patients, who had failed other therapies, demonstrated that extremely high doses (45 μg) of a formulation of calcitriol (DN-101, Novacea) administered orally once a week along with the usual regimen of the chemotherapy drug taxotere caused a statistically significant improvement in overall survival and time to progression. The findings suggested that calcitriol may enhance the efficacy of active drugs in cancer patients and provide a survival advantage (185). Interestingly, patients in the calcitriol-plus-docetaxel arm showed a statistically significant reduction in the incidence of venous and arterial thrombosis compared with docetaxel alone (187). The ASCENT I trial did not meet its primary endpoint, i.e., a lowering of serum PSA. However, the promising survival results prompted the initiation of a larger Phase III trial (ASCENT II) with survival as an end point. A new, improved docetaxel regimen (dosing every 3 weeks) was used in the control arm of the ASCENT II trial, and this regimen was compared with DN-101 plus the older docetaxel dose regimen (once a week), resulting in an asymmetric study design. Unfortunately, the improved survival due to the combination demonstrated in the ASCENT I trial could not be confirmed in the ASCENT II trial (188). In fact, the trial was prematurely stopped by the data safety monitoring board when an excess number of deaths were noted in the study arm (DN-101 plus old docetaxel regimen) versus the control arm (new docetaxel regimen). After the trial was stopped, further analysis (189) suggested that the increased deaths in the treatment arm compared with the control arm arose not from calcitriol toxicity but instead from better survival in the control arm, whose patients received the new and improved docetaxel regimen.

Another recent study tested the combination of high-dose calcitriol (DN-101) with mitoxantrone and prednisone in patients with metastatic androgen-independent PCa who did not undergo previous chemotherapy. It concluded that calcitriol did not significantly add to the activity of mitoxantrone and prednisone as assessed by the decline in serum PSA levels (190). In a randomized, double-blind, Phase II study in a similar patient population, the addition of daily doses of doxercalciferol (1α-hydroxyvitamin D_2) to weekly docetaxel did not enhance the PSA response rate or survival (191). Another study tested the effect of the combination of weekly high-dose calcitriol and docetaxel in PCa patients whose disease progressed after first-line chemotherapy using docetaxel alone, and it showed that high-dose calcitriol restored the sensitivity to chemotherapy with docetaxel (192).

Preclinical observations in PCa cells (117) prompted a single-arm, open-label Phase II study designed to evaluate the combination of the nonselective NSAID naproxen (375 mg naproxen twice a day) and high-dose calcitriol [45 μg calcitriol (DN-101) orally once a week] in patients with early recurrent PCa (193). The trial was based on the rationale that NSAID inhibition of COX-2 activity and PG synthesis could be synergistically enhanced by coadministration of calcitriol (117). After 21 patients had been enrolled, the trial was prematurely stopped when the FDA put a temporary hold on DN-101 on the basis of the data from the ASCENT II trial described above. The therapy

was well tolerated by most patients. A prolongation of the PSA doubling time was achieved in 75% of the patients, suggesting a beneficial effect of the combination therapy (193, 194).

6.5. Summary of Clinical Trial Data

The results of clinical trials using vitamin D, calcitriol, or various vitamin D analogs in cancer patients have thus far been somewhat disappointing. Epidemiology findings and preclinical data from cell culture and animal models showing substantial anti-cancer effects raised expectations about the benefit of calcitriol therapy in cancer patients, but the modest efficacy seen in the clinical trial data was not as impressive as hoped. The trials in early recurrent disease show stabilization of disease, as evidenced by the rise in serum PSA levels slowing or stopping. However, many of the clinical trials, including the ASCENT trials, have been carried out in patients with far-advanced cancer who have failed multiple therapies. Preclinical observations demonstrating significant inhibitory effects of calcitriol in initial stages of cancer development suggest that calcitriol may be more effective when used in early disease and/or in chemoprevention. However, it would be premature to conclude from the limited number of completed clinical trials that calcitriol has little efficacy in cancer patients.

The following important criteria to achieve optimal efficacy remain to be elucidated: What is the optimum dose or schedule of calcitriol to use? Which form of the drug is most effective with the least toxicity (dietary vitamin D_3, calcitriol itself, or an analog)? When in the course of cancer would it be most effective to administer, and what drug combinations would generate the most benefit? Although calcitriol does induce apoptosis in some cancer cells, its major actions emphasize anti-proliferative, anti-inflammatory, and pro-differentiating effects. Because its actions are more cytostatic than cytolytic, we believe that calcitriol would be most effectively used in chemoprevention or in inhibiting or delaying the progression of cancer in patients with early disease. We believe that, when administered at the highest dose that can be tolerated without side effects and probably in combination with other drugs, vitamin D, calcitriol, or its analogs will augment other therapies and be a useful addition to cancer treatment (195).

7. CONCLUSIONS

Many epidemiologic studies indicate that vitamin D deficiency increases the risk of a variety of cancers and that higher levels of vitamin D are associated with better prognosis and improved outcomes. Extensive research provides evidence for the anti-proliferative, anti-inflammatory, and pro-differentiation effects of calcitriol in cell culture models, and tumor-inhibitory effects in animal models of cancer further support its potential utility in cancer prevention and treatment. Although we have emphasized calcitriol's anti-inflammatory activity in this review because of the relatively new data on this activity, multiple molecular pathways of calcitriol action in cancer cells have been identified; these pathways provide a mechanistic basis for its potential efficacy in cancer. The data suggest that calcitriol has therapeutic and cancer-preventive effects in several malignancies. Although the preclinical data are persuasive and the epidemiologic data are intriguing, no well-designed clinical trial of optimal administration of vitamin D as a cancer therapy has ever been conducted (195). The preclinical data provide considerable rationale for continued development of vitamin D or an analog for cancer therapy. Future clinical trials should be designed using good clinical trial design principles. Such studies may finally provide compelling data that demonstrate whether there is a role for vitamin D or its analogs in cancer prevention or therapy (195).

From an analysis of the available data, we have reached several conclusions. Vitamin D deficiency is a risk factor for a number of cancers, and vitamin D supplementation to increase serum

levels of 25(OH)D appears to exert chemopreventive actions on cancer development. The avoidance of vitamin D deficiency should be an important goal for reducing cancer incidence as well as reducing the risk of other diseases including osteoporosis. The optimum target level of vitamin D supplementation to reduce cancer risk is unclear, but we believe that it will turn out to be higher than the current normal range cut-point of serum 25(OH)D at 30 ng ml^{-1}. Anti-inflammatory activity is an additional important pathway by which calcitriol can exert chemopreventive and/or therapeutic anti-cancer activity. Vitamin D, calcitriol, or its analogs may have the best utility as cancer therapeutic agents when used in cancer patients with early disease and perhaps in combination with other drugs. We believe that calcitriol and its analogs should therefore be further evaluated in clinical trials in patients with early or precancerous disease. Higher concentrations of calcitriol in the circulation may have to be achieved to obtain convincing proof of efficacy, and this may require intermittent therapy or the use of less calcemic analogs. In the case of established cancer, it is reasonable to consider that combination therapy will be required and that vitamin D, calcitriol, or an analog added to other effective therapies will likely increase the benefit of the standard therapy and perhaps reduce some side effects. New clinical trials that treat cancer patients with minimal or early disease with high doses of calcitriol, dietary vitamin D, or new potent analogs as well as combination therapy may finally demonstrate the promise of the benefit of vitamin D in cancer prevention and treatment.

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