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Molecular Aspects of Vitamin D Anticancer Activity

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Environment may influence the development and prevention of cancer. Calcitriol has been associated with calcium homeostasis regulation. Many epidemiological, biochemical, and genetic studies have shown non-classic effects of vitamin D, such as its involvement in the progression of different cancers. Although vitamin D induces cellular arrest, triggers apoptotic pathways, inhibits angiogenesis, and alters cellular adhesion, the precise mechanisms of its action are still not completely established. This article will present a revision about the molecular aspects proposed to be involved in the anticancer action of calcitriol. Adequate levels of vitamin D to prevent cancer development will also be discussed.

Keywords: Vitamin D, Calcitriol, Cancer, Molecular mechanisms

INTRODUCTION

Cellular environment may be the most crucial determinant for a normal cell to become a cancerous one. Some studies indicate that heritable genetic factors may contribute to merely 5% of all cases. Moreover, it has been suggested that 33–66% of cancers may be prevented by dietary factors (1). In this line, epidemiological and preclinical data provide evidence that vitamin D has significant protective effects against cancer (2). In 1990, Garland *et al*. (3) found strong and inverse association (-0.8, $p < .001$) between sunlight and breast cancer mortality, and the same protective effect was described for melanoma, colon, and prostate cancer. Reduced cancer incidence and mortality were due to increased 25-hydroxyvitamin D (25(OH)D) levels, the indicator of vitamin D status (4). Serum 25(OH)D levels above 48 ng/mL are proposed to reduce by half the risk of developing breast cancer (4). Other studies have demonstrated that the hormonal form of vitamin D, 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) or calcitriol, plays a key role regulating many of the cellular mechanisms involved in cancer progress as it can regulate over 60 genes that participate in the control of cell growth and cell cycle, cellular differentiation, apoptosis, immune modulation, and angiogenesis (5). This article will briefly present the broad spectrum of molecular mechanisms proposed to be involved in the anticancer effects of calcitriol.

VITAMIN D SYNTHESIS AND CATABOLISM

Two major forms of vitamin D are D_2 (or ergocalciferol) and D_3 (or cholecalciferol). Vitamin D can be obtained from diet (salmon, tuna, liver, egg yolks, etc.), fortified food, or supplements. As shown in Figure 1, another main source is the conversion of 7-dehydrocholesterol in the skin when it is exposed to sunlight (UV-B light). D_2 and D_3 forms of vitamin D bind to D-binding proteins in the circulatory system and are converted in the liver to 25(OH)D by 25-hydroxylase enzyme. Serum 25(OH)D is the primary circulating form of vitamin D, with longer half-life than calcitriol (days *vs* hours, respectively). When the biological active form is needed to fulfill body needs, 25(OH)D is finally converted in the kidney to the hormonal active form $1,25(OH)_2D_3$ by 1α-hydroxylase (CYP27B1; 6), a process regulated by parathyroid hormone (PTH). The $1,25(OH)_2D_3$ metabolizing enzyme is a 24 hydroxylase (CYP24A1), which limits calcitriol actions by catabolism. These enzymes are found in many tissues where $1,25(OH)_2D_3$ can be either synthesized or degraded. CYP27B1 enzyme is also expressed in normal colon, brain, placenta, pancreas, lymph nodes, and other tissues, thus allowing calcitriol to function in an autocrine and/or paracrine mode. Indeed, local metabolism of $1,25(OH)_2D_3$ has been suggested to be involved in the etiology of several types of human cancer (7).

GENOMIC AND NON-GENOMIC SIGNALS

Calcitriol actions are mediated by its interaction with the vitamin D receptor (VDR), which is present in many cell types including breast, colon, muscle, prostate, brain, etc. In these tissues, $1,25(OH)_2D_3$ also exerts paracrine and/or autocrine effects, maybe explaining the pleiotropic actions of calcitriol described in the literature (8). After entering the cytoplasm, the secosteroid hormone binds to VDR and the receptor associates with its heterodimer partner, the retinoid X receptor (RXR). The complex recruits specific coactivator molecules (9), such as steroid receptor coactivators (1, 2, and 3), histone acetyltransferases (p300, CBP), and the mediator complex subunit 1 (MED1). These molecules

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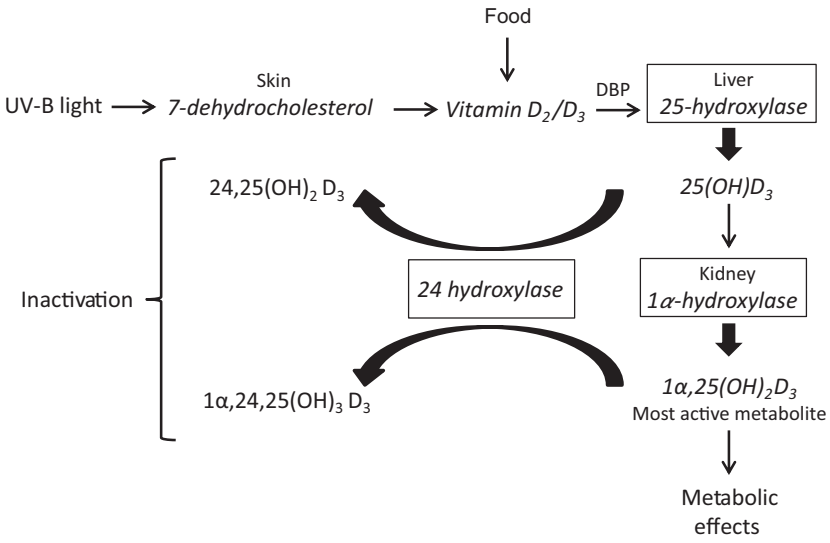

Figure 1. Vitamin D synthesis and catabolism. Pro-vitamin D_3 (7-dehydrocholesterol) is converted to vitamin D_2/D_3 in response to ultraviolet B (UV-B) exposure. Vitamin D_3 binds to vitamin D-binding protein (DBP) in the bloodstream, and is transported to the liver, where D_3 is hydroxylated by liver 25-hydroxylase. 25-hydroxycholecalciferol ($25(OH)D_3$) is then hydroxylated in kidney in position 1 by 1α-hydroxylase and transformed in the active $1\alpha,25(OH)_2D_3$ or calcitriol, which has different metabolic effects. Both metabolites are inactivated to $24,25(OH)D_3$ and $1\alpha,24,25(OH)_2D_3$ by a 24-hydroxylation (24-hydroxylase).

possess intrinsic chromatin-modifying enzymatic activities, and so recruit transcription factors to the promoter. The VDR-RXR complex translocates to the nucleus and binds to hexameric vitamin D response elements (VDREs) in the promoter/enhancer regions of calcitriol target genes. In these regions, they may promote or suppress specific cellular events, and consequently, translate metabolic needs into cellular actions (2). VDR transcriptional activity can also be inhibited by co-repressors, such as the silencing hormone receptor-corepressor, hairless, and Alien (9). Calcitriol modulation of many oncogenes or suppressor genes is described in normal and malignant cells, but only a few of such genes contain VDREs in their promoter regions. This suggests that calcitriol may also indirectly modulate signaling cascades or other unknown non-genomic mechanisms (10). Furthermore, many studies have indicated that $1,25(OH)_2D_3$ induces rapid responses, apparently affecting several signal transduction pathways including phosphoinositide signaling, intracellular calcium movements, adenylyl cyclase, mitogen activated protein kinases (MAPK) function, etc. (11). Recent studies support the idea that non-genomic and genomic effects may integrate in a unique mode of action of nuclear receptor ligands, in which the non-genomic effects constitute signaling pathways required for the effects at the genome level (12). However, the molecular mechanisms underlying the antiproliferative action of calcitriol still remain unclear. While many known genes containing VDREs contribute to calcium homeostasis, others are involved in cellular proliferation or apoptosis. Alternatively, non-genomic pathways are suggested to exert the antiproliferative action of calcitriol. For example, VDR knock-down by siRNA does not affect growth inhibition induced by calcitriol in breast cancer cells, suggesting that not all the effects rely on classical VDR pathway *per se* (13). On the other hand, VDR expression is low in high-grade undifferentiated colon cancers and complete loss of the receptor in knockout mouse results in increased DNA damage and hyperproliferation. In consequence, this may imply the potential importance of VDR for prevention of colon tumorigenesis (14). The state of cellular differentiation may also modify calcitriol-induced effects. The stimulation of CYP24A1 and the calcium channel transient receptor potential vanilloid 6 (TRPV6) is significantly blunted in proliferating Caco-2 cells as compared to differentiated ones. However, in proliferating cells, VDR levels are not lower and the mRNA for the receptor is not less stable. This suggests that either ligand-induced nuclear import of VDR or the movement of the receptor within the nucleus to the VDREs may be reduced in proliferating cells (9). The regulation of intranuclear VDR has not been fully studied yet.

REGULATION OF CELL CYCLE

Transformation of normal to cancer cells involves accumulation of genetic and epigenetic factors that normally control cell division, adhesion, and programmed cell death (PCD). Malignant disease comprises alteration of several oncogenes and loss of tumor repressor genes and/or normal mechanisms of DNA repair. Genes like p21 and p27 regulate the entry and passage through the cell cycle and, consequently, control cell division. In this way, it is possible to stop a damaged cell from multiplying until it can be repaired or eliminated by apoptosis. Other genes, like BRCA 1 or BRCA 2, also regulate the repairing systems (2). If these genes are mutated or inactivated, more mutations may be propagated. Protein products of BRCA genes regulate transcription in the nucleus. Calcitriol has been shown to increase BRCA 1 gene expression in MCF-7 breast cancer cells and the

sensitivity to the antiproliferative effects of the steroid was associated with an ability to modulate BRCA 1 protein (15).

Calcitriol and VDR are required for the control of normal cell cycle (16), as they regulate the expression of cyclins and their dependent kinases (CDKs). Moreover, treatment of human breast cancer cells with $1,25(OH)_2D_3$ increases the expression of CDKN1A and CDKN1B (which encodes p27) and represses cyclin D1 and D3, hence inhibiting CDK activity. Calcitriol blocks the progression to S phase by its interaction with cyclin D and, therefore, the steroid inhibits cell proliferation and directly controls the activity of p21 and p27 proteins (17, 18).

Calcitriol may also have indirect effects on the regulation of cell cycle due to cross-talks with other signaling pathways mediated by growing factors (IGF, EGF), and also upregulates the gene that encodes insulin growth factor binding protein 3 in prostate and colon cancer cells (10). Unfortunately, the effect of calcitriol on the regulation of cell cycle varies from one tumor cell type to another thus impeding a unified hypothesis of the secosteroid mechanism of action.

CALCITRIOL PARTICIPATION IN CELL DIFFERENTIATION AND APOPTOSIS

Apoptosis is a normal part of tissue terminal differentiation and is tightly regulated. In addition to the antiproliferative effects, calcitriol may exert antitumor effects by regulating key mediators of apoptosis. Cancer often shows deregulations of the apoptotic pathways and p53 is one of the defective genes described in this pathology. The p53 gene is a major barrier against cancer progression and its inactivation alters the normal apoptosis mechanisms, as it has been demonstrated that almost 50% of human cancers carry mutations in this gene (18). Calcitriol regulates p53 function and influences other genes affecting apoptosis such as some heat shock proteins (18). In human myeloid leukemia cells, VDR protein has been shown to interact with heat shock protein 90 (Hsp90), which is important for the calcitriol-induced differentiation of these cells (19). However, Stambolsky *et al*. (20) have suggested that mutant p53 can enhance proliferation, survival, and tumorigenicity in mice. High levels of mutant p53 probably enable its interaction with VDR and increases nuclear accumulation of the receptor, which is associated with high histological grade tumor. In this way, calcitriol changes from an apoptotic agent to an anti-apoptotic one.

MAPKs are involved in the regulation of cell growth, differentiation, and proliferation in cancer. They are activated in response to hormonal or stress factors, and JNK and p38 are examples of stress-activated kinases. High levels of ERK and JNK activity have been observed in several cancer lines (21). Moreover, high activities of JNK and c-Jun correlate with development of distant metastases. In murine squamous tumor cells, calcitriol inhibits ERK phosphorylation and promotes MEK cleavage and inactivation by caspases (22). Another report demonstrates that JNK and p38 are activated by calcitriol in MCF-7 breast cancer cells, in which the steroid induces apoptosis. Indeed, JNK and p38 have been previously shown to be activated by calcitriol in colon carcinoma, prostate cancer, and leukemia cells (21).

Calcitriol downregulates mitogenic pathways, and thus promotes apoptosis, by decreasing IGF receptors and stimulating IGF binding proteins (23). The transcription factor activator protein 1 (AP-1) is a key regulator of cell differentiation and apoptosis. This factor binds to the promoter region of c-Jun gene and regulates its expression. Calcitriol rapidly increases c-Jun expression inducing PKC-dependent activation of ERK2 and JNK1. This pathway alters AP-1 transcriptional activity in intestinal cells and modifies alkaline phosphatase activity, a marker of cell differentiation (24). The steroid also induces differentiation in myeloid leukemia cell lines, dependent on the formation of activated VDR and PI3K complexes (25). A link between VDR and the RAS-MAPK or PI3K-AKT pathways has also been suggested. However, the prognostic role of VDR expression or its relationship with PIK3CA or KRAS mutation remains uncertain. Kure *et al*. (26) demonstrated that among 619 colorectal cancers, 38% of the tumors showed VDR overexpression by immunohistochemistry, significantly associated with KRAS mutation (odds ratio, 1.55; 95% confidence interval, 1.11–2.16) and PIK3CA mutation (odds ratio, 2.17; 95% confidence interval, 1.36–3.47).

In breast tumor and leukemia cells, calcitriol downregulates Bcl-2 expression, a pro-survival protein, while in colon and prostate cells, the steroid upregulates the expression of the pro-apoptotic proteins Bax and Bak (10). In addition, calcitriol can directly activate caspases to induce apoptosis. Additionally, calcitriol sensitizes cancer cells to radiation or to other cytotoxic compounds like reactive oxygen species (ROS) and increases mitochondrial membrane damage and cytochrome c (cyt c) release (23). Also, calcitriol-induced attenuation of the PI3K pathway is accomplished in gastric cancer cells inducing the expression of PTEN, a negative regulator of the kinase associated with increased apoptosis (27). In MCF-7 breast cancer cells, vitamin D_3 induces cell growth blockade associated with a significant reduction in Akt phosphorylation (28). Moreover, the combination of calcitriol with AKT inhibitors cooperates to induce growth arrest and senescence in prostate cancer cells (29).

The mechanisms by which calcitriol induces apoptosis may vary depending on the cancer cell type and even within different normal and malignant cell lines. For instance, calcitriol activates caspases in non-malignant MCF-12A cells while in malignant MCF-7 cells there is no caspase activation clearly identified (21). Moreover, induction of apoptosis is not always part of the antiproliferative effects of calcitriol. Sometimes, cells are directed to a more advanced differentiation state by the steroid. This switch may depend on the presence of other factors such as calbindin-D_{28k}, a calcitriol-induced protein. This protein acts as a calcium buffer and/or binds (and inhibits) caspase 3, resulting in a blocking of the apoptosis signal in neuronal cells (30).

Another signaling system reported to be affected by calcitriol is Hedgehog (HH). HH signaling underlies several human tumors, including basal cell carcinoma (31). Recently, vitamin D_3 has been shown to suppress HH signaling in

an *in vitro* model system, comparable to the effects of cyclopamine, a known inhibitor of the HH pathway. These results are specific for vitamin D_3 and seem to be independent of the VDR (31).

CALCITRIOL-INDUCED CALCIUM SIGNALING IN CANCER

Calcium is a central signaling ion that controls cell growth, proliferation, and survival of normal and malignant cells, acting as a modulator of various key enzymes. As Ca^{2+} cannot be degraded as other messengers, the intracellular free calcium levels are tightly regulated by ion channels, exchangers, pumps, binding proteins, and by the release of calcium from the intracellular stores (32). One of the channels is TRPV6, which plays a role in calcium homeostasis affecting intestinal cation absorption, renal excretion, and bone metabolism (33). TRPV6 is found at the luminal side of epithelial cells and functions as a gatekeeper of calcium entry across epithelia (34). Its regulation is controlled by $1,25(OH)_2D_3$, estrogen, and dihydrotestosterone (35, 36). The channel is a key partner in maintaining calcium homeostasis, and also plays an important role in tumor development and progression. It is strongly expressed in advanced stages of prostate cancer, whereas there is very little or no expression in the healthy tissue (37). In prostate cancer cells, TRPV6 plays a role in calcium influx leading to reduced proliferative ability of the cells. The expression of TRPV6 may play a role in tumor development and it is also enhanced in mammary adenocarcinoma tissue, as compared to normal cells (33). Little is known about the regulation of calcium entry mechanisms in breast cells; nevertheless, calcitriol is a TRPV6 stimulator. In T47D breast cancer cells, low concentrations of vitamin D (up to 1 nM) promote cell growth, while increasingly higher concentrations cause the blockade of cell cycle in G_1 phase, reducing cell proliferation (60%) and stimulating apoptosis (7%; 38, 33). Therefore, the regulation of TRPV6 channel would be a therapeutic tool by keeping cancer cells from rapidly growing.

The model of calcium-dependent apoptosis proposes that an apoptotic insult causes the Ca^{2+} release from the ER, determining, in part, the amount of calcium taken up by mitochondria. The modulation of Ca^{2+} handling by pro- or anti-apoptotic proteins is exerted altering the gating properties of specific isoforms of inositol-trisphosphate receptor (IP_3R; 39). When cytosolic calcium levels are high, the mitochondrial membrane potential drives calcium accumulation into the mitochondria. Alternatively, de-energized mitochondria release calcium back to the cytosol by different ways. One of them is the aperture of the mitochondrial permeability transition pore (mPTP), which allows cyt c release in combination with other pro-apoptotic factors. The overall process leads to the activation of executor caspases (39). In the absence of caspases, other proteases such as calpains and cathepsins may contribute to apoptosis (40). Calpain substrates include membrane receptors, transporters, and intracellular enzymes. This mechanism of apoptosis may be especially relevant in tumors lacking caspase-3 activity (40). On the other hand, low levels of cytosolic calcium binding proteins, like calbindin-D_{28k}, may not be sufficient for cell buffering capacity and thus promote apoptosis (41).

The alteration of apoptosis mechanisms may underlie the pathophysiology of proliferative disorders like mammary tumorigenesis. Calcium induces apoptosis and regulates apoptosis pathways evocating changes in Ca^{2+} intracellular concentration ($[Ca^{2+}]_i$; 32). However, the exact mechanism of calcium apoptosis regulation is still debatable. Caspases and calpains are possible targets, as preventing increases in intracellular calcium concentration may be a way to bypass apoptosis (42). Bcl-2 protein inhibits calcium release from ER stores and ionomycin-induced calpain activation promotes decrease of Bcl-2 thereby triggering the intrinsic apoptotic pathway (43). Interestingly, calcium handling is different between normal and cancer breast cells. Calcitriol induces a sustained increase in intracellular calcium triggering apoptosis in human breast carcinoma cells through the activation of calpains, but not in normal mammary cells, due to their different calcium entry regulation and buffering mechanisms (41). Normal breast cells are resistant to calcitriol-induced apoptosis because their cytosolic Ca^{2+} buffering capacity is larger than that from the breast cancer cells (44). Another remarkable characteristic is that calbindin-D_{28k} is expressed in normal breast cells, but not in cancer cells (44). Therefore, calcitriol and its analogs may be useful for cancer treatment. Calcitriol activates calcium entry and depletes ER stores through IP_3R in MCF-7 breast cancer cell (45). Calcitriol-evoked increase in $[Ca^{2+}]_i$ is directly associated with the induction of apoptosis through the activation of Ca^{2+}/calpain-dependent caspase 12. Also, confocal microscopy reveals changes in VDR localization from the cytoplasmic to nuclear compartment in calcitriol-treated breast cancer cells. Thus, VDR translocation may be part of the mechanism of apoptosis (45).

VITAMIN D AND AUTOPHAGIC CELL DEATH

The most studied PCD that counteracts tumor growth is caspase-mediated apoptosis. However, it would be dangerous for the organism to depend on a single executer program to clear potential anomalous cells. Certainly, there is evidence for alternative PCD pathways, in which lysosomes and cathepsins function as executors. The release of enzymes from permeabilized lysosomes may trigger caspase-mediated and -independent PCD. Tumor necrosis factor (TNF) receptor family, p53 protein, or oxidative stress may trigger lysosomal breakdown (46). Autophagy (AP) is an evolutionary conserved lysosomal pathway involved in the turnover of proteins, macromolecules, and whole organelles. The normal function of AP is the recycling of nutrients essential for the maintenance of energy levels and cell survival during metabolic stress. Nonetheless, AP is a process sometimes deregulated in tumors. AP is characterized by an increase in the number of vacuoles surrounded by a double membrane (autophagosomes) that sequester cytoplasm

and organelles to be digested when they fuse to lysosomes. This process is mediated by conserved autophagy-related proteins (Atg proteins) and inhibited by a serine threonine protein kinase known as mTOR, the mammalian target of rapamycin. The exact mechanism by which AP protects from tumorigenesis is still speculative. Autophagic degeneration clearly does not depend on caspase activation and AP can be pharmacologically blocked by inhibition of PI3K or depletion of proteins involved in AP process, as beclin 1, the mammalian homolog of Atg6. Beclin 1 enhances AP through combination with PI3KIII in the initial phase. It has also been reported that beclin 1 is an insufficient tumor suppressor. Besides, there are several beclin 1 negative regulators such as Bcl-2 and Bcl-X_L (46). Cells dying by AP may present some characteristics of apoptosis similar to DNA fragmentation or breaks, despite the lack of caspase activation. Calcium mobilizing agents, like vitamin D analogs, induce a massive increase in the number of autophagosomes in breast cancer cells, accompanied by functional AP that is dependent on the activation of a cytosolic Ca^{2+}/calmodulin-dependent protein kinase kinase β (CAMKKβ). The kinase is necessary for the induction of AMPK, a natural inhibitor of mTOR (47). In this way, mTOR inhibition promotes AP. This caspase-independent cell death pathway may prove to be useful in the treatment of apoptosis-defective chemoresistant cancers, but much more studies will be required.

CALCITRIOL INDUCTION OF ROS GENERATION

Exposure to calcitriol as a single agent induces pro-differentiative effects on almost all cancer cells. However, the steroid also exerts anticancer effects by cooperating with endogenous and therapeutic antitumoral agents, such as TNFα, menadione, etc. These agents share the feature of bringing out ROS generation in their target cells. Intrinsic ROS are produced normally within the cells as natural by-products of aerobic metabolism, and it is well established that basal ROS levels are higher in malignant cells (14). Calcitriol improves the effect on ROS generation in colon cells in a dose-dependent manner, independently of caspase activation (14). On breast cancer cells, calcitriol-induced ROS generation also enhances the antiproliferative activity of other compounds like menadione (48) or L-buthionine-S,R-sulfoximine (BSO; 49). This effect is accompanied by increased oxidative stress, as shown by glutathione (GSH) reduction after cell treatment (48). Calcitriol also increases glucose-6-phosphatase dehydrogenase activity, the rate-limiting enzyme in the generation of NADPH. These findings suggest that calcitriol causes an alteration in the overall cellular redox potential that could translate into alteration of cell proliferation and apoptosis (48, 50). The intensity or duration of ROS release is a potentially determining factor in the biological outcome (51). Low intensity of ROS production may be important in metabolic adaptation while moderate production stimulates signals involved in the inflammatory mediators. High levels of ROS might signal the induction of pathways such as apoptosis or autophagy (51).

CALCITRIOL AND CELL ADHESION MOLECULES

Another important subject in cancer development is the loss of contact inhibition, allowing cells to move. Calcitriol increases the transcription of E-cadherin gene, a transmembrane cell adhesion molecule that maintains cells in a polarized phenotype of classical epithelial cells. Loss of E-cadherin function is a characteristic event during the transition from adenoma to carcinoma, which involves the acquisition of invasive capacity. Calcitriol induces the expression of E-cadherin and promotes cancer cells to change their shape and to become more adhesive, similar to a normal phenotype (52). β-catenin is another cell-adhesion molecule. The steroid promotes its export to the plasma membrane and prevents β-catenin-induced cell proliferation, migration, and invasiveness, hence driving colon carcinoma cells to differentiation (52).

Anomalous activation of the Wnt/β-catenin signaling pathway due to mutations is the most common and initial alteration in colorectal cancer. This pathway is activated in almost 90% of colon cancers. Calcitriol distinctly regulates two genes encoding the extracellular Wnt inhibitors, DICKKOPF-1 and DICKKOPF-4 (DKK-1, DKK-4). Calcitriol increases the expression of DKK-1 RNA and protein, the later acting as a tumor suppressor in human colon cancer cells (53). In contrast, the secosteroid represses DKK-4 transcription by inducing direct VDR binding to its promoter. DKK-4 is a target of the Wnt/β-catenin pathway and is up-regulated in colorectal tumors promoting cell migration and invasion and a proangiogenic phenotype (53). As shown, $1,25(OH)_2D_3$ exerts a complex set of regulatory actions leading to the inhibition of the Wnt/β-catenin pathway in colon cancer cells.

CALCITRIOL AND ANGIOGENESIS

Calcitriol inhibits the proliferation of endothelial cells *in vitro* and reduces angiogenesis *in vivo* as well as the inflammatory process associated with cancer formation (10). The hormone can reduce vascular endothelial growth factor (VEGF) mRNA in tumor cells and also upregulates the potent anti-angiogenic factor thrombospondin 1 in colon tumor cells (10). A significant inhibition of metastases is observed in prostate and lung murine models and these effects may be due, at least in part, to calcitriol anti-angiogenic effects (23). In tumor-derived endothelial cells (TDEC), calcitriol induces G_0/G_1 arrest. In contrast, normal endothelial cells are unresponsive to the steroid. TDEC are more sensitive to calcitriol due to an epigenetic silencing of CYP24A1. The promoter of this gene results to be hypermethylated in two regions located at the 5′ end, while the extent of methylation in these two regions is significantly lower in normal cells (54). The same epigenetic modification is observed in prostate cancer cell lines (55). The antiproliferative effect of calcitriol in tumor cells and TDEC is VDR-dependent, as loss of VDR can lead to abnormal tumor angiogenesis (56). Other authors also proposed that the anti-angiogenic effects of calcitriol are mediated by the hypoxia-inducible factor

(HIF-1) pathway as the steroid reduces the protein expression of HIF-1α subunit and the VEGF in various human cancer cells. 1,25$(OH)_2D_3$ also inhibits HIF-1 transcriptional activity and cell proliferation under hypoxia (57).

ANTI-INFLAMMATORY EFFECTS OF CALCITRIOL

Inflammation contributes to the development and progression of many cancers (58) and recent studies suggest a role of calcitriol as an anti-inflammatory agent. Prostaglandins (PG) promote carcinogenesis by stimulating cell proliferation or inhibiting apoptosis. In this context, cyclooxigenase-2 (COX-2), the enzyme responsible for PG synthesis, is regarded as an oncogene and as an important target for cancer therapy. Different studies have shown that mitogens, cytokines, and tumor promoters highly enhance COX-2 expression. Moreover, COX-2 inhibitors decrease the risk of prostate cancer (59) and the enzyme is overexpressed in invasive breast and colorectal cancer (23). In prostatic epithelial and breast cancer cells, calcitriol decreases the expression of COX-2 and increases the levels of 5-PGDH, the enzyme that catalyzes the conversion of prostaglandins to their derivates with low biological activity. The reduction of PG levels in breast cancer cells also offers a second indirect mechanism for the steroid suppressive effect on aromatase levels, critical for the expression of the estrogen receptor (23). Decreasing both estrogen synthesis and estrogen receptor levels, calcitriol reduces the proliferative effects of estrogen on breast cancer cells.

In prostate cancer cells, calcitriol increases the expression of MAPK phosphatase 5, which inactivates MAPKs and contributes to calcitriol-induced antiproliferative effects on these cells. This action leads to the inactivation of p38 stress-induced kinase responsible for inflammatory responses, such as the production of interleukin-6 (60).

NFκB is a family of inducible transcription factors present in all cells that regulate immune responses and inflammation. The steroid inhibits NFκB signaling, essential for T-helper cell activation, increases regulatory T response necessary for immunosuppression (59), and decreases the levels of pro-inflammatory cytokines such as interleukin-6 (IL-6) and IL-8 (23), and TNF-α (61). Calcitriol has been proposed to inhibit the calcium sensitizing Rho/ROCK and thus regulates IL-8 levels (62). Interestingly, calcitriol can increase anti-inflammatory cytokines levels up-regulating IL-4 (63) and IL-10 synthesis as well as IL-10 receptor expression (64).

Table 1 summarizes the diverse molecular mechanisms proposed to be involved in calcitriol anticancer activity that have been described above.

VDR GENE POLYMORPHISMS AND CANCER

The most frequently studied gene for the evaluation of vitamin D status is VDR. The relevance of VDR gene restriction fragment length polymorphisms for cancer has been investigated in recent years. More than 470 single-nucleotide polymorphisms have been searched in the human VDR gene (65). Apart from the possible role in regulating calcium homeostasis, VDR polymorphism may influence the risk of occurrence and prognosis of cancer. Many studies have focused on Fok1, Bsm1, Taq1, Apa1, and Cdx2 polymorphisms. Unfortunately, these studies offer controversial results (66). Data indicating association of VDR polymorphisms and cancer risk are the strongest for breast cancer (Bsm1, Fok1), prostate cancer (Fok1), and malignant melanoma (Fok1). Data suggesting association with cancer prognosis are the strongest for prostate cancer (Fok1), breast cancer (Bsm1, Taq1), malignant melanoma (Bsm1), and renal cell carcinoma (Taq1; 66). Other gene polymorphisms involved in vitamin D metabolism have been studied, such as CYP27B1

Table 1. Molecular Mechanisms of Calcitriol Anticancer Activity

		Refs
Cell cycle	↓ CDK activity. Blocks the progression to S phase. Cross-talks with signaling pathways of growing factors.	17, 18, 10
Cell differentiation and apoptosis	Regulates p53 and Hsp. Inhibits ERK phosphorylation, stimulates MEK cleavage. ↑ JNK, c-Jun, and p38. ↓ Mitogenic pathways. Induces differentiation in leukemia cells. ↓ Bcl-2, ↑ Bax and Bak. Blocks PI3K (through PTEN induction). ↓ Akt phosphorylation blocking cell growth.	18, 22, 21, 23, 24, 10, 27, 28
Calcium signaling	↑ Ca^{2+} entry inducing TRPV6 activity and depletion of ER Ca^{2+} stores. ↑ $[Ca^{2+}]_i$ triggering apoptosis through calpains and caspase 12.	45, 32, 42, 41
Autophagy	Vitamin D analogs activate CAMKKβ inhibiting mTOR.	47
ROS generation	↑ Antiproliferative activity of MEN and BSO through ↑ ROS. ↑ Glucose-6-phosphatase dehydrogenase activity.	48–50
Cell adhesion molecules	↑ E-cadherin gene transcription and ↑ β-catenin in plasma membrane driving to differentiation. ↑ DKK-1 ↓ DKK-4 (target of Wnt/beta-catenin pathway).	52, 53
Angiogenesis	↓ VEGF gene expression and HIF-1 protein and activity, ↑ thrombospondin 1, ↓ metastases.	10, 57, 23
Anti-inflammatory effects	↓ COX-2 and ↑ 15-PGDH. Inactivation of p38 stress-induced kinase responsible for inflammatory responses (IL-6 production). ↓ NFκB signaling. ↑ T response, ↓ IL-8, IL-6, and TNF-α, ↑ IL-4, IL-10, and IL-10 receptor.	23, 58–64

CDK: cyclin-dependent kinases; Hsp: heat shock protein; ERK: extracellular signal-regulated kinase; MAPK: mitogen-activated protein kinase; JNK: c-Jun N-terminal kinases; PI3K: phosphoinositol 3-kinase; TRPV6: transient receptor potential vanilloid 6; ER: endoplasmic reticulum; CAMKKβ: Ca^{2+}/calmodulin-dependent protein kinase kinase β; mTOR: mammalian target of Rapamycin; ROS: reactive oxygen species; BSO: L-buthionine-S,R-sulfoximine; DKK: Dickkopf-related protein; VEGF: vascular endothelial growth factor; HIF-1: hypoxia-inducible factor 1; COX-2: cyclooxigenase-2; 15-PGDH: 15-hydroxyprostaglandin dehydrogenase; IL: interleukin; NFκB: nuclear factor kappa B; TNF-α: tumor necrosis factor α.

or GC gene (which encodes for vitamin D-binding protein) without any association with cancer risk (66).

Although the study of gene polymorphisms may be useful in the future, the evidence to date is inconclusive. Perhaps, instead of regarding only the effect of few variants among the possible candidate genes alone, the future studies might also consider nutrient or environmental interactions (67). Multiple bioactive food components may influence the response to vitamin D such as calcium, which reduces renal hydroxylation of 25(OH)D. Moreover, total body fat may sequester vitamin D esters. Other dietary nutrigenetic factors to be considered for altering the effect of cholecalciferol are retinol, phytoestrogens, folate, genistein, etc. (67). In summary, although inadequate vitamin D status could be associated with cancer, it is clear that other factors can influence the overall response to vitamin D.

NEW APPROACHES TO IDENTIFY VITAMIN D EFFECTS AGAINST CANCER

To identify genes directly regulated by calcitriol on different types of cancer cells, many microarray studies have been performed. Although it is difficult to compare between different studies due to the different experimental setups, among all proposed vitamin D functions (e.g., induction of apoptosis, cell cycle blocking, etc.), the most common regulated gene described was CYP24A1 (68). However, when the list of genes involved in calcitriol-induced regulation of cancer cells with different microarrays is compared, only a small set of genes is found to be collectively regulated. These findings reinforce the idea that calcitriol acts by different mechanisms depending on the cell type or tissue studied. Microarray studies demonstrate that calcitriol upregulates p21 protein and insulin growth factor binding protein 3 (IGFBP3) and inhibits COX-2 in prostate cancer cells (68). Calcitriol treatment increases JNK and other members of this family in colorectal cancer cells. Also in these cells, the steroid upregulates Gem, a Ras family member involved in the inhibition of voltage-dependent calcium channels, and sorcin, a calcium binding protein. Then, both proteins may promote the inhibition of calcium mediated cell growth (69). The induction of genes involved in oxidative stress, as glutathione peroxidase, has been described in colon cancer cells (68).

Chromatin immunoprecipitation (ChIP) is another technique to study regulatory regions of genes responsive to VDR. Turunen *et al*. (70) have studied calcitriol-induced regulation in human embryonic kidney HEK-293 and in MCF-7 cells. This approach identified four VDREs for CYP24A1 and cyclin C genes, three in the p21 promoter, and two in the CYP27B1 gene, many of them under control of other transcription factors, as p53. The ChIP-on-chip technique (ChIP plus hybridization of the chromatin fragments on microarrays) was also applied and identified as VDR-associated chromatin regions for TRPV6, LPR5, RankL, and p53 in different cancer cell lines (67). However, similar VDREs may be differentially regulated depending on the promoter context. Moreover, the regulation of VDR gene expression may be different across vitamin D target tissues, cell environment, or even in the same type of cells with dissimilar stage of differentiation (71).

Very little research has used the proteomic approach. However, Byrne and Welsh (72) have described the results of an antibody-based proteomic approach to search for proteins involved in the sensitivity or resistance to vitamin D-mediated apoptosis in MCF-7 cells. Over 270 proteins were analyzed and the authors identified 10 regulated ones. Some of them are consistent with the proapoptotic action of calcitriol, but other identified targets are not previously linked to the steroid signaling and so, further studies will be necessary to determine the relevance of these proteins.

ADEQUATE LEVELS OF VITAMIN D TO PROTECT FROM CANCER DEVELOPMENT

The last two decades have provided insight into the molecular mechanisms involved in human cancer. However, we have still limited knowledge regarding the processes that may control resistance toward chemotherapy. Some cancer cell lines are often insensitive to calcitriol or require toxic concentrations. Additionally, epidemiological data demonstrate an inverse relationship between vitamin D and cancer, but the clinical use of the steroid is precluded in anticancer therapy due to the induction of hypercalcemia. Hence, numerous analogs have been synthesized to be used as chemotherapeutic agents with lower calcemic effects. Calcitriol also enhances the cytotoxic effects of gamma radiation, certain antioxidants, and other combinations, e.g., cisplatin or doxorubicin (DNA damaging agents), paclitaxel (microtubule-disrupting agent), etc. It is important to note that sufficient dose and exposure to calcitriol is critical to achieve anticancer effects. Several trials have demonstrated that it is feasible to administer high doses of the steroid through intermittent regime but the anticancer effects are largely disappointing (73). This may be due to the use of calcitriol at much lower doses considering the dose-limiting hypercalcemia.

Paradoxically, some studies have revealed that calcitriol can also promote cell proliferation and survival. This paradox may be explained by calcitriol metabolism due to CYP24A1. Rashid *et al*. (74) have proposed that calcitriol used at 0.1 nM, which approximates to the physiological level of the hormone, significantly enhanced cell growth in breast cancer cells. On the other hand, at pharmacological doses generally used to measure antiproliferative potency (100 nM), cells are acutely sensitive to growth inhibition. The proliferative effect at low doses is completely abolished by ketoconazole, a CYP24A1 inhibitor. The combination of low doses of calcitriol and CYP24A1 inhibitor may enhance the application of $1,25(OH)_2D_3$ as an anticancer agent (74). Another approach suggests the use of the non-toxic $25(OH)D_3$ as a potential chemopreventive agent. The increased expression of CYP27B1 in colon cancer cells treated with the pro-hormone leads to the hypothesis that $25(OH)D_3$ may offer possible therapeutic options in colon cancer or in other cells expressing endogenous 1α-hydroxylase (75).

Thirty years ago, Garland and Garland (76) hypothesized that a poor vitamin D status accounted for the higher

mortality rates of colon cancer. More recently, in a prospective study followed for almost 20 years and evaluating over 47,000 individuals, Giovanucci *et al*. (77) demonstrate a strong association between low levels of predicted 25(OH)D and increased cancer incidence, particularly for cancer in the digestive system. Epidemiological and clinical data sustain the protective role of vitamin D. This nutrient may be useful to improve public health, as nearly all studies worldwide suggest that serum 25(OH)D levels in the population are significantly below healthy values (78, 79). It is necessary to revise recommendations on vitamin D intake that may be optimal for human health. A group of experts suggested that vitamin D intakes in the range 1,000–4,000 IU per day may rise serum values of 25(OH)D to optimal levels (above 80 nmol/L), at least in individuals with limited sun exposure (80). This supplementation was considered without risk of toxicity or adverse effects (78), but is only based on proper calcium homeostasis, whereas the level needed to other health-promoting functions is still unknown and possibly would be higher. Besides, although UV radiation can increase the risk of skin cancer, even short exposures to sunlight (5–15 min between safety hours on arms, legs, and face) may increase vitamin D serum levels, without reddening the skin. However, although calcium and vitamin D are metabolically related, it is still a topic of debate whether they interact in carcinogenesis. Some studies suggest that adequate 25(OH)D levels are associated with reduced risk of recurrent colon adenoma only among subjects receiving calcium supplements (81). Nevertheless, all studies avoid considering the longer "latency time" before the appearance of colorectal cancer. Instead, serum 25(OH)D levels 10–25 years prior to cancer detection may be more important than those determined at diagnosis.

CONCLUDING REMARKS

The effects of calcitriol on different cell types described above have not yet clear markers or patterns about the exact modulation induced by the steroid hormone. The heterogeneity of the response may depend on the cell type (normal or malignant) or even the state of differentiation or VDR expression levels. The effect of calcitriol on proliferation, differentiation, and angiogenesis seems to be mediated through genomic and non-genomic mechanisms. Calcitriol potentiates the antitumor effects of many well-known therapeutic compounds and several clinical trials indicate that the administration of calcitriol or its analogs in a suitable dose to mediate anticancer effects is safe and feasible. However, an extensive analysis of the epidemiological data on vitamin D and cancer has been performed by the World Health Organization (82) and concludes that, although observational studies link low 25(OH)D levels with colorectal adenoma and cancer, the causal global relationship between cancer and vitamin D is still unclear and additional randomized clinical trials are needed until definitive evidence is obtained (67). Meanwhile, it should be necessary to have a restrictive attitude regarding the use of vitamin D supplementation or UV exposure and many considerations should be taken into account. It is possible that vitamin D may be more effective to avoid cancer progression than to retard its appearance. Moreover, perhaps the evaluation of 25(OH)D levels or vitamin D intake should be done early in life and thus, these studies in adults may be not relevant. Nevertheless, the clinical research community is constantly revising guidelines for optimal vitamin D intake and serum 25(OH)D levels to reduce significantly cases of cancer in the future.

ABBREVIATIONS

$1,25(OH)_2D_3$	1,25-dihydroxyvitamin D_3 (or calcitriol)
25(OH)D	25-hydroxyvitamin D
AP	Autophagy
AP-1	Transcription factor activator protein 1
Atg proteins	Autophagy-related proteins
BSO	L-buthionine-S,R-sulfoximine
$[Ca^{2+}]_i$	Intracellular calcium concentration
CAMKKβ	Ca^{2+}/calmodulin-dependent protein kinase kinase β
CDKs	Cyclins dependent kinases
ChIP	Chromatin immunoprecipitation
COX-2	Cyclooxigenase-2
CYP24A1	24 hydroxylase
CYP27B1	1α-hydroxylase
cyt c	Cytochrome c
D_2	Ergocalciferol
D_3	Cholecalciferol
EGF	Epidermal growth factor
GSH	Glutathione
HH	Hedgehog
HIF-1	Hypoxia-inducible factor
Hsp	Heat shock protein
IGF	Insulin growth factor
IGFBP3	Insulin growth factor binding protein 3
IP_3R	Inositol-trisphosphate receptor
MAPKs	Mitogen activated protein kinases
MED1	Mediator complex subunit 1
mPTP	Mitochondrial permeability transition pore
NCoR	Silencing hormone receptor-corepressor
PCD	Programmed cell death
PG	Prostaglandins
PTH	Parathyroid hormone
ROS	Reactive oxygen species
RXR	Retinoid X receptor
TDEC	Tumor-derived endothelial cells
TNF	Tumor necrosis factor
TRPV6	Transient receptor potential vanilloid 6
VDR	Vitamin D receptor
VDREs	Vitamin D response elements
VEGF	Vascular endothelial growth factor

DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the article.

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REFERENCES

1. Lichtenstein P, Holm NV, Verkasalo PK, Iliadou A, Kaprio J, Koskenvuo M, Pukkala E, Skytthe A, Hemminki K. Environmental and heritable factors in the causation of cancer: analyses of cohorts of twins from Sweden, Denmark and Finland. N Engl J Med 2000;343:78–85.
2. Ingraham BA, Bragdon B, Nohe A. Molecular basis of the potential of vitamin D to prevent cancer. Curr Med Res Op 2008;24:139–149.
3. Garland FC, Garland CF, Gorham ED, Young JF. Geographic variation in breast cancer mortality in the United States: a hypothesis involving exposure to solar radiation. Prevent Med 1990;19:612–622.
4. Garland CF, Gorham ED, Mohr SB, Grant WB, Giovannucci EL, Lipkin M, Newmark H, Holick MF, Garland FC. Vitamin D and prevention of breast cancer: pooled analysis. J Steroid Biochem Mol Biol 2007;103:708–711.
5. Schwartz GG, Hall MC, Stindt D, Patton S, Lovato J, Torti FM. Phase I/II study of 19-nor-1alph-25-dihydroxyvitamin D_2 (paracalcitol) in advance, androgen-insensitive prostate cancer. Clin Cancer Res 2005;11:8680–8685.
6. DeLuca HF. Overview of general physiologic features and functions of vitamin D. Am J Clin Nutr 2004;74:494–500.
7. King AN, Beer DG, Christensen PJ, Simpson RU, Ramnath N. The vitamin D/CYP24A1 story in cancer. Anticancer Agents Med Chem 2010;10:213–224.
8. Demay MB. Mechanism of vitamin D receptor action. Ann NY Acad Sci 2006;1068:204–213.
9. Cui M, Klopo A, Jiang Y, Fleet JC. The effect of differentiation on 1,25 dihydroxyvitamin D-mediated gene expression in the enterocyte-like cell line, Caco-2. J Cell Physiol 2009;218:113–121.
10. Deeb KK, Trump DL, Johnson CS. Vitamin D signalling pathways in cancer: potential for anticancer therapeutics. Nature Rev 2007;7:684–700.
11. Mizwicki MT, Norman AW. The vitamin D sterol-vitamin D receptor ensemble model offers unique insights into both genomic and rapid-response signaling. Sci Signal 2009;2:re4.
12. Ordóñez-Morán P, Muñoz A. Nuclear receptors: genomic and non-genomic effects converge. Cell Cycle 2009;8:1675–1680.
13. Costa JL, Eijk P, van de Wiel MA, ten Berge D, Schmitt F, Narvaez CJ, Welsh J, Ylstra B. Anti-proliferative action of vitamin D in MCF7 is still active after siRNA-VDR knock-down. BMC Genomics 2009;10:499–510.
14. Koren R, Wacksberg S, Weitsman GE, Ravid A. Calcitriol sensitizes colon cancer cells to H_2O_2-induced cytotoxicity while inhibiting caspase activation. J Steroid Biochem Mol Biol 2006;101:151–160.
15. Campbell MJ, Gombart AF, Kwok SH, Park S, Koeffler HP. The antiproliferative effects of 1-alpha-25$(OH)_2D_3$ on breast and cancer cells are associated with the induction of BRCA1 gene expression. Oncogene 2000;19:5091–5097.
16. Han SH, Jeon JH, Ju HR, Jung U, Kim KY, Yoo HS, Lee YH, Song KS, Hwang HM, Na YS, Yang Y, Lee KN, Choi I. VDUP1 upregulated by TGF-beta I and 1,25-dihydroxyvitamin D_3 inhibits tumor cell growth by blocking cell-cycle progression. Oncogene 2003;22:4035–4046.
17. Johnson CS, Muindi JR, Hershberger PA, Trump DL. The antitumor efficacy of calcitriol: preclinical studies. Anticancer Res 2006;26:2543–2549.
18. Maruyama R, Aoki F, Toyota M, Sasaki Y, Akashi H, Mita H, Suzuki H, Akino K, Ohe-Toyota M, Maruyama Y, Tatsumi H, Imai K, Shinomura Y, Tokino T. Comparative genome analysis identifies the vitamin D receptor gene as a direct target of p53-mediated transcriptional activation. Cancer Res 2006;66:4574–4583.
19. Marcinkowska E, Gocek E. Heat shock protein 90 interacts with vitamin D receptor in human leukemia cells. J Steroid Biochem Mol Biol 2010;121:114–116.
20. Stambolsky P, Tabach Y, Fontemaggi G, Weisz L, Maor-Aloni R, Siegfried Z, Shiff I, Kogan I, Shay M, Kalo E, Blandino G, Simon I, Oren M, Rotter V. Modulation of the vitamin D_3 response by cancer-associated mutant p53. Cancer Cell 2010;17:273–285.
21. Brosseau CM, Pirianov G, Colston KW. Involvement of stress activated protein kinases (JNK and p38) in 1,25 dihydroxyvitamin D_3-induced breast cell death. Steroids 2010;75:1082–1088.
22. McGuire TF, Trump DL, Johnson CS. Vitamin D_3-induced apoptosis of murine squamous cell carcinoma cells: selective induction of caspase-dependent MEK cleavage and upregulation of MEKKK-1. J Biol Chem 2001;279:53213–53221.
23. Vanoirbeek E, Krishnan A, Eelen G, Verlinden L, Bouillon R, Feldman D, Verstuyf A. The anti-cancer and anti-inflammatory actions of 1,25$(OH)_2D_3$. Best Practice Res Clin Endocrinol Metab 2011;25:593–604.
24. Chen A, Davis BH, Bissonette M, Scaglione-Sewell B, Brasitus TA. 1,25-Dihydroxyvitamin D_3 stimulates activator protein 1-dependent Caco-2 cell differentiation. J Biol Chem 1999;274:35505–35513.
25. Hmama Z, Nandan D, Sly L, Knutson KL, Herrera-Velit P, Reiner NE. 1alpha,25-dihydroxyvitamin D_3-induced myeloid cell differentiation is regulated by a vitamin D receptor-phosphatidylinositol 3-kinase signaling complex. J Exp Med 1999;190:1583–1594.
26. Kure S, Nosho K, Baba Y, Irahara N, Shima K, Ng K, Meyerhardt JA, Giovannucci EL, Fuchs CS, Ogino S. Vitamin D receptor expression is associated with PIK3CA and KRAS mutations in colorectal cancer. Cancer Epidemiol Biomarkers Prev 2009;18:2765–2772.
27. Pan L, Matloob AF, Du J, Pan H, Dong Z, Zhao J, Feng Y, Zhong Y, Huang B, Lu J. Vitamin D stimulates apoptosis in gastric cancer cells in synergy with trichostatin A/sodium butyrate-induced and 5-aza-2'-deoxycytidine-induced PTEN upregulation. FEBS J 2010;277:989–999.
28. Proietti S, Cucina A, D'Anselmi F, Dinicola S, Pasqualato A, Lisi E, Bizzarri M. Melatonin and vitamin D_3 synergistically down-regulate Akt and MDM2 leading to TGFβ-1-dependent growth inhibition of breast cancer cells. J Pineal Res 2010;50:150–158.
29. Axanova LS, Chen YQ, McCoy T, Sui G, Cramer SD. 1,25-dihydroxyvitamin D_3 and PI3K/AKT inhibitors synergistically inhibit growth and induce senescence in prostate cancer cells. Prostate 2010;70:1658–1671.
30. Choi WS, Lee E, Lim J, Oh YJ. Calbindin-D_{28K} prevents drug-induced dopaminergic neuronal death by inhibiting caspase and calpain activity. Biochem Biophys Res Commun 2008;371:127–131.
31. Tang JY, Xiao TZ, Oda Y, Chang KS, Shpall E, Wu A, So PL, Hebert J, Bikle D, Epstein EH Jr. Vitamin D_3 inhibits hedgehog signaling and proliferation in murine basal cell carcinomas. Cancer Prev Res 2011;4:744–751.
32. Berridge MJ, Bootman MD, Lipp P. Calcium: a life and death signal. Nature 1988;395:645–648.
33. Bolanz KA, Hediger MA, Landowski CP. The role of TRPV6 in breast carcinogenesis. Mol Cancer Ther 2008;7:271–279.
34. Inoue J, Choi J, Yoshidomi T, Yashiro T, Sato R. Quercetin enhances VDR activity leading to stimulation of its target gene expression in Caco-2 cells. J Nutr Sci Vitaminol 2010;56:326–330.

35. Song Y, Peng X, Porta A, Takanaga H, Peng JB, Hediger MA, Fleet JC, Christakos S. Calcium transporter 1 and epithelial calcium channel messenger ribonucleic acid are different regulated by 1,25 dihydroxyvitamin D_3 in the intestine and kidney of mice. Endocrinology 2003;144:3885–3994.
36. Van Cromphaut SJ, Rummens K, Stockmans I, Van Herck E, Dijcks FA, Ederveen AG, Carmeliet P, Verhaeghe J, Bouillon R, Carmeliet G. Intestinal calcium transporter genes are regulated by estrogens and the reproductive cycle through vitamin D receptor-independent mechanisms. J Bone Min Res 2003;18:1725–1736.
37. Lehen'kyi V, Flourakis M, Skryma R, Prevarskaya N. TRPV6 channel controls prostate cancer cell proliferation via Ca^{2+}/NFAT-dependent pathways. Oncogen 2007;26:7380–7385.
38. Mathiasen IS, Lademann U, Jäättelä M. Apoptosis induced by vitamin D compounds in breast cancer cells is inhibited by Bcl-2 but does not involve known caspases or p53. Cancer Res 1999;59:4848–4856.
39. Giacomello M, Drago I, Pizzo P, Pozzan T. Mitochondrial Ca^{2+} as a key regulator of cell life and death. Cell Death Differ 2007;14:1267–1274.
40. Sareen D, Darjatmoko SR, Albert DM, Polans AS. Mitochondria, calcium and calpain are key mediators of resveratrol-induced apoptosis in breast cancer. Mol Pharmacol 2007;72:1466–1475.
41. Mathiasen IS, Sergeev IN, Bastholm L, Elling F, Norman AW, Jäättelä M. Calcium and calpain as key mediators of apoptosis-like death induced by vitamin D compounds in breast cancer cells. J Biol Chem 2002;277:30738–30745.
42. Carafoli E, Molinari M. Calpain: a protease in search of a function? Biochem Biophys Res Commun 1998;247:193–203.
43. Gil-Parrado S, Fernandez-Montalvan A, Assfalg-Macnleidt I, Popp O, Bestvater F, Holloschi A, Knoch TA, Auerswald EA, Welsh K, Reed JC, Fritz H, Fuentes-Prior P, Spiess E, Salvesen GS, Machleidt W. Ionomycin-activated calpain triggers apoptosis: a probable role for Bcl-2 family members. J Biol Chem 2002;277:27217–27226.
44. Sergeev IN. Calcium as a mediator of 1,25-dihydroxyvitamin D_3-induced apoptosis. J Steroid Biochem Mol Biol 2004;89–90:419–425.
45. Sergeev IN. Calcium signaling in cancer and vitamin D. J Steroid Biochem Mol Biol 2005;97:145–151.
46. Hoyer-Hansen M, Bastholm L, Mathiasen IS, Elling F, Jäättelä M. Vitamin D analog EB1089 triggers dramatic lysosomal changes and beclin 1-mediated autophagic cell death. Cell Death Differ 2005;12:1297–1309.
47. Hoyer-Hansen M, Schultz Nordbrandt SP, Jäätellä M. Autophagy as a basis for the health-promoting effects of vitamin D. Trends Mol Med 2010;16:295–302.
48. Marchionatti AM, Picotto G, Narvaez C, Welsh J, Tolosa de Talamoni NG. Antiproliferative action of menadione and $1,25(OH)_2D_3$ on breast cancer cells. J Steroid Biochem Mol Biol 2009;113:227–232.
49. Bohl LP, Liaudat AC, Picotto G, *et al.* Buthionine sulfoximine and 1,25-dihydroxyvitamin D induce apoptosis in breast cancer cells via induction of reactive oxygen species. Cancer Investigation (In press).
50. Koren R, Hadari-Naor I, Zuck E, Rotem C, Liberman UA, Ravid A. Vitamin D is prooxidant in breast cancer cells. Cancer Res 2001;51:1439–1444.
51. Finkel T. Signal transduction by mitochondrial oxidants. J Biol Chem 2012;287:4434–4440.
52. Palmer HG, González-Sancho JM, Espada J, Berciano MT, Puig I, Baulida J, Quintanilla M, Cano A, de Herreros AG, Lafarga M, Muñoz A. Vitamin D_3 promotes the differentiation of colon carcinoma cells by the induction of E-cadherin and the inhibition of β-catenin signaling. J Cell Biol 2001;154:369–387.
53. Pendás-Franco N, Aguilera O, Pereira F, González-Sancho JM, Muñoz A. Vitamin D and Wnt/beta-catenin pathway in colon cancer: role and regulation of DICKKOPF genes. Anticancer Res 2008;28:2613–2623.
54. Johnson CS, Chung I, Trump DL. Epigenetic silencing of CYP24 in the tumor microenvironment. J Steroid Biochem Mol Biol 2010;121:338–342.
55. Luo W, Karpf AR, Deeb KK, Muindi JR, Morrison CD, Johnson CS, Trump DL. Epigenetic regulation of vitamin D 24-hydroxylase/CYP24A1 in human prostate cancer. Cancer Res 2010;70:5953–5962.
56. Chung I, Han G, Seshadri M, Gillard BM, Yu WD, Foster BA, Trump DL, Johnson CS. Role of vitamin D receptor in the antiproliferative effects of calcitriol in tumor-derived endothelial cells and tumor angiogenesis in vivo. Cancer Res 2009;69:967–975.
57. Ben-Shoshan M, Amir S, Dang DT, Dang LH, Weisman Y, Mabjeesh NJ. 1alpha,25-dihydroxyvitamin D_3 (calcitriol) inhibits hypoxia-inducible factor-1/vascular endothelial growth factor pathway in human cancer cells. Mol Cancer Ther 2007;6:1433–1439.
58. Colotta F, Allavena P, Sica A, Garlanda C, Mantovani A. Cancer-related inflammation, the seventh hallmark of cancer: links to genetic instability. Carcinogenesis 2009;30:1073–1081.
59. Feet JC, Desmet M, Johnson R, Li Y. Vitamin D and Cancer: a review of molecular mechanisms. Biochem J 2012;441: 61–76.
60. Nonn L, Peng L, Feldman D, Peehl DM. Inhibition of p38 by vitamin D reduces interleukin-6 production in normal prostate cells via mitogen-activated protein kinase phosphatase 5: implications for prostate cancer prevention by vitamin D. Cancer Res 2006;66:4516–4524.
61. Zhu Y, Mahon BD, Froicu M, Cantorna MT. Calcium and 1alpha,25-dihydroxyvitamin D_3 target the TNF-alpha pathway to suppress experimental inflammatory bowel disease. Eur J Immunol 2005;35: 215–224.
62. Hamid AR, Umbas R, Mochtar CA. Recent role of inflammation in prostate diseases: chemoprevention development opportunity. Acta Med Indones-Indones J Intern Med 2001;43:59–65.
63. Cantorna MT, Woodward WD, Hayes CE, DeLuca HF. 1,25-dihydroxyvitamin D3 is a positive regulator for the two anti-encephalitogenic cytokines TGF-beta 1 and IL-4. J Immunol 1998;160:5314–5319.
64. Canning MO, Grotenhuis K, de Wit H, Ruwhof C, Drexhage HA. 1-alpha,25-dihydroxyvitamin D_3 hampers the maturation of fully active immature dendritic cells from monocytes. Eur J Endocrinol 2001;145: 351–357.
65. Rukin NJ, Strange RC. What are the frequency, distribution and functional effects of vitamin D receptor polymorphisms as related to cancer risk? Nutr Rev 2007;65:96–101.
66. Köstner K, Denzer N, Müller CS, Klein R, Tilgen W, Reichrath J. The relevance of vitamin D receptor (VDR) gene polymorphisms for cancer: a review of the literature. Anticancer Res 2009;29:3511–3536.
67. Davis CD, Milner JA. Nutrigenomics, vitamin D and cancer prevention. J Nutrigenet Nutrigenomics 2011;4:1–11.
68. Kriebitzsch C, Verlinden L, Eelen G, Tan BK, Van Camp M, Bouillon R, Verstuyf A. The impact of $1,25(OH)_2D_3$ and its structural analogs on gene expression in cancer cells: a microarray approach. Anticancer Res 2009;29:3471–3484.
69. Ward Y, Kelly K. Gem protein signaling and regulation. Methods Enzymol 2006;407:468–483.
70. Turunen MM, Dunlop TW, Carlberg C, Väisänen S. Selective use of multiple vitamin D response elements underlies the 1,25-dihydroxyvitamin D_3-mediated negative regulation of the human CUP27B1 gene. Nucleic Acid Res 2007;35:2734–2747.
71. Fleet JC. What have genomic and proteomic approaches told us about vitamin D and cancer? Nutr Rev 2007;65:127–130.
72. Byrne B, Welsh J. Identification of novel mediators of vitamin D signaling and $1,25(OH)_2D_3$ resistance in mammary cells. J Steroid Biochem Mol Biol 2007;103:703–707.
73. Ma Y, Trump DL, Johnson CS. Vitamin D in combination cancer treatment. J Cancer 2010;1:101–107.

74. Rashid SF, Mountford JC, Gombart AF, Campbell MJ. 1α,25-dihydroxyvitamin D_3 displays divergent growth effects in both normal and malignant cells. Steroids 2001;66:433–440.
75. Murillo G, Matusiak D, Benyia RV, Mehta RG. Chemopreventive efficacy of 25-hydroxyvitamin D_3 in colon cancer. J Steroid Biochem Mol Biol 2007;103:763–767.
76. Garland CF, Garland FC. Do sunlight and vitamin D reduce the likelihood of colon cancer? J Epidemiol 1980;9:227–231.
77. Giovannucci E, Liu Y, Rimm EB, Hollis BW, Fuchs CS, Stampfer MJ, and Willett WC. Prospective study of predictors of vitamin D status and cancer incidence and mortality in men. J Natl Cancer Inst 2006;98:451–459.
78. Hathcock JN, Shao A, Vieth R, Heaney R. Risk assessment for vitamin D. Am J Clin Nutr 2007;85:6–18.
79. Lappe JM, Travers-Gustafson D, Davies KM, Recker RR, Heaney RP. Vitamin D and calcium supplementation reduces cancer risk: results of a randomized trial. Am J Clin Nutr 2007;85:1586–1591.
80. Suitor CW, Meyers LD. Dietary Reference Intakes Research Synthesis: Workshop Summary. Washington, DC: National Academies Press, 2006.
81. Wactawski-Wende J, Kotchen JM, Anderson GL, Assaf AR, Brunner RL, O'Sullivan MJ, Margolis KL, Ockene JK, Phillips L, Pottern L, Prentice RL, Robbins J, Rohan TE, Sarto GE, Sharma S, Stefanick ML, Van Horn L, Wallace RB, Whitlock E, Bassford T, Beresford SA, Black HR, Bonds DE, Brzyski RG, Caan B, Chlebowski RT, Cochrane B, Garland C, Gass M, Hays J, Heiss G, Hendrix SL, Howard BV, Hsia J, Hubbell FA, Jackson RD, Johnson KC, Judd H, Kooperberg CL, Kuller LH, LaCroix AZ, Lane DS, Langer RD, Lasser NL, Lewis CE, Limacher MC, Manson JE; Women's Health Initiative Investigators. Calcium plus vitamin D supplementation and the risk of colorectal cancer. N Engl J Med 2006;354:2287–2288.
82. IARC. Vitamin D and Cancer: IARC Working Group Reports. Lyon: International Agency for Research on Cancer, 2008.