Vitamin D's role in cell proliferation and differentiation

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Vitamin D has pleiotropic effects that go beyond its traditional role in calcium homeostasis. Hundreds of genes with vitamin D receptor response elements directly or indirectly influence cell cycling and proliferation, differentiation, and apoptosis. Vitamin D compounds also have effects on cell function that are nongenomic. The noncalcemic actions of vitamin D influence normal and pathological cell growth, carcinogenesis, immune function, and cardiovascular physiology. This review examines many of the various mechanisms by which vitamin D alters cellular growth and differentiation and explores cell-specific factors that influence responsiveness to vitamin D.

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INTRODUCTION

The finding of the vitamin D receptor (VDR) in many cell types in addition to the classic target tissues of small intestine, kidney, and bone that participate in the homeostasis of calcium and other minerals has led to the realization that vitamin D has many diverse biological functions. Vitamin D receptor response elements (VDREs) have been found in several hundred genes that are involved in a multitude of fundamental cellular processes. Underlying many of the noncalcemic actions of vitamin D, including effects on carcinogenesis, immune function, autoimmune diseases, and cardiovascular disorders, is the ability of vitamin D secosteroids to inhibit cellular proliferation. Vitamin D compounds have been demonstrated, in highly cell-specific manners, to alter cellular proliferation through multiple mechanisms, most prominently via effects on cell cycle progression, apoptosis, and differentiation. In many cases, the ligand-activated VDR directly enhances or suppresses the transcription of key genes regulating cell growth. In other situations, vitamin D somewhat indirectly influences the cell cycle, apoptosis, and/or differentiation by interacting with other important transcriptional regulators or cell signaling systems.

Important effects of vitamin D secosteroids on growth have been demonstrated in normal cells, in pathologic situations characterized by benign hyperplasia, and in numerous cancers and cancer-derived cell lines.

EFFECTS OF VITAMIN D ON NORMAL CELL PROLIFERATION AND DIFFERENTIATION

Studies in VDR knockout mice have revealed that vitamin D/VDR signaling plays an important role in controlling the growth of normal tissues. VDR knockout mice have accelerated breast lobuloalveolar development and premature casein expression during pregnancy and demonstrated delayed involution after lactation. VDR knockouts also show increased epithelial proliferation in the descending colon, assessed by proliferating cell nuclear antigen activity. Heterozygote VDR+/- mice have levels of colonic proliferation that are intermediate between the knockouts and wild-type animals. Vitamin D receptor knockout mice also have abnormal epidermal differentiation, with reduced levels of involucrin, profilaggrin, and loricrin, as well as loss of keratohyalin granules. The addition of calcitriol to normal mouse hematopoietic cells in vitro resulted in differentiation to a...
Vitamin D deficiency results in hyperplasia of the parathyroid glands, and administration of vitamin D secosteroids inhibits parathyroid cell growth. 1,25-dihydroxyvitamin D and several vitamin D analogues are effective in the treatment of parathyroid hyperplasia and secondary hyperparathyroidism associated with chronic renal insufficiency.13,14 A key factor in parathyroid hyperplasia is increased expression of transforming growth factor-alpha (TGFα) and its receptor, the epidermal growth factor receptor (EGFR). Calcitriol inhibits parathyroid cell growth by diminishing the expression of both TGFα and EGFR, increasing the expression of p21waf1 and p27kip1 (inhibitors of cell cycle progression that are discussed in detail below), and downregulating the membrane and nuclear growth signals induced by ligand-activated EGFR.15,16 Induction of the calcium sensing receptor (CaSR) by vitamin D secosteroids plays a key role in sensitizing parathyroid cells to growth inhibition by calcium.13

Calcitriol and several vitamin D analogues improve the hyperproliferative skin condition psoriasis, and are approved for topical and systemic treatment of this disorder.1,3,17,18 The antipsoriatic properties of vitamin D secosteroids may be due to their effects in decreasing proliferation and promoting differentiation of keratinocytes, as well as immunomodulatory actions.2 Expression of several factors involved in proliferation, such as the EGFR, c-myc proto-oncogene, and keratin 16 are downregulated in keratinocytes by vitamin D secosteroids.2 Proteins associated with maturation of the epidermis and formation of a cornified envelope, such as involucrin and transglutaminase, are increased by calcitriol.2,9 Induction of the CaSR and phospholipase C and increased activity of the AP-1 transcriptional complex appear to be mechanisms by which vitamin D compounds induce keratinocyte differentiation.9

A vitamin D analogue also decreased prostate growth in an animal model of androgen-stimulated benign prostatic hyperplasia, and calcitriol inhibited proliferation of primary cultures of cells from hyperplastic prostate glands.10,19

**EFFECT OF VITAMIN D SECOSTEROIDS ON GROWTH OF CANCERS AND CANCER-DERIVED CELL LINES**

Numerous epidemiological studies have suggested that poor vitamin D nutritional status, due to limited sun exposure and poor dietary intake, is a risk factor for various malignancies. These include many of the most prevalent human cancers, including breast, colon, and prostate cancers.1,2,20 Investigations using a wide variety of experimental animal models of cancer have shown that carcinogenesis is enhanced in the setting of vitamin D deficiency, and can be ameliorated by treatment with vitamin D metabolites and analogues.1,2,20 Beneficial effects of vitamin D have been observed in experimental cancers induced by various chemical carcinogens or genetic mutations, and in studies using human cancer cells implanted in nude mice.1,2,20,21 The anti-cancer effects of vitamin D are reviewed in greater detail by Tuohimaa in this supplement, but they appear to involve several important mechanisms, including alterations in cell growth, angiogenesis, tumor invasion and metastatic potential, and immune surveillance.2,20 Numerous studies have demonstrated that vitamin D secosteroids inhibit the growth of many cancer-derived cell lines in vitro, by inhibiting progression through the cell cycle, inducing apoptosis, and driving the cells to a more differentiated phenotype. We will now consider illustrative examples of some of the most important pathways by which vitamin D secosteroids influence these fundamental cellular processes. It must be emphasized that the mechanisms of these anti-growth effects are quite variable in different cell types, and even in different cell lines derived from the same type of cancer.

**CELL CYCLE REGULATION BY VITAMIN D SECOSTEROIDS**

The cell cycle is comprised of multiple phases: gap 0 (G0), gap 1 (G1), synthesis (S), gap 2 (G2), and mitosis (M). The formation of specific cyclin-dependent kinase (Cdk) and cyclin complexes regulates transition through the phases. Vitamin D compounds have been demonstrated to inhibit progression through the cell cycle in many cell systems. The most commonly reported effect has been to cause an arrest at the G0/1 to S transition. For progression from G1 to S, the D-type cyclins complex with Cdk4 or...
Calcitriol and other vitamin D secosteroids have been shown to induce p21waf1 in many cell types. The importance of p21waf1 in the antiproliferative action of vitamin in prostate cancer cell lines was demonstrated in studies performed by Moffatt et al. This group found that 1,25-dihydroxyvitamin D induced both p21waf1 mRNA and protein in several prostate cancer cell lines, including ALVA-31 cells. Stable transfection of ALVA-31 cells with a p21waf1 antisense construct abolished the anti-growth effect of 1,25-dihydroxyvitamin D. Prostate cancer cell lines, such as TSU-Pr1 and JCA-1, whose growth was not altered by calcitriol, failed to show induction of p21waf1 by 1,25-dihydroxyvitamin D. Transfection of these cells with a sense p21waf1 cDNA resulted in growth inhibition. Induction of p21waf1 by vitamin D secosteroids occurs via several different mechanisms in a cell-type-specific manner. The promoter of p21waf1 contains a consensus VDRE, and direct transcriptional regulation of these cells with a sense p21waf1 cDNA resulted in growth inhibition. Induction of p21waf1 by calcitriol or vitamin D secosteroids also influence the synthesis and stability of the Cdk inhibitor p27kip1. The p27kip1 promoter does not contain a VDRE, but interactions have been demonstrated among the VDR, Sp1, and NF-Y transcription factors at the p27 promoter. In addition, calcitriol diminishes degradation of p27kip1 through several mechanisms, including induction of PTEN (a phosphatase that dephosphorylates p27, preventing proteosomal degradation), and reduction of Cdk2 activity and Skip 2 abundance. Vitamin D compounds have also been reported to alter other cell cycle regulatory proteins, such as INK4 proteins, p53, and p21-activated kinase 1, in various cell types. In myeloid leukemia cells, calcitriol enhanced expression of HOXA10, a homeobox protein that causes G1 arrest. In colon cancer cells, vitamin D inactivated p70 S6 kinase, a protein essential for the G1-S phase transition. Sutton et al. recently reported that 1,25-dihydroxyvitamin D induced MN1 in osteoblastic cells, resulting in decreased cell proliferation due to slowing the entry into S-phase.

Calcitriol decreases cyclin D1 abundance and/or activity via several different mechanisms in different cell types. For example, vitamin D inhibits TGFα/EGFR transactivation of cyclin D1, and induces C/EBPβ expression, a suppressor of cyclin D1. Vitamin D compounds alter the levels and or activities of other cyclins and Cdk's in a cell-specific manner. In some cells, calcitriol affects later stages of cell cycle progression. In several cell types, 1,25-dihydroxyvitamin D induces GADD45α, a cell cycle regulator and DNA-damage repair protein, and causes arrest at G2/M.

**EFFECTS OF VITAMIN SECOSTEROIDS ON APOPTOSIS**

Induction of apoptosis also plays a key role in growth inhibition by vitamin D secosteroids. In many cell types, calcitriol or vitamin D analogues alters the content of various bcl-2 family apoptotic regulatory proteins. Vitamin D affects the levels of pro-apoptotic (bax, bak) and/or anti-apoptotic (bcl-2, bcl-XL) proteins, thereby tipping the balance toward apoptosis rather than cell survival. For example, Diaz et al. found that calcitriol and the vitamin D analogue EB1089 induced apoptosis in several colon cancer cell lines, associated in some cases with decreased expression of the anti-apoptotic protein bcl-2 and in others with increased levels of the pro-apoptotic factor bak. Stimulation of apoptosis was not dependent on p53. In retinoblastoma cells, calcitriol induced reciprocal changes in anti-apoptotic bcl-2 and pro-apoptotic bak. Effects of vitamin D compounds on the subcellular distribution of regulatory proteins between the cytosol and mitochondria, cytochrome c release, and mitochondrial membrane potential have been reported. Vitamin D secosteroids activate initiator

Cdk6 to form an active kinase that partially phosphorylates the retinoblastoma (Rb) family of proteins. This initial phosphorylation of Rb partially inactivates Rb, resulting in release of histone deacetylases and induction of transcription of certain genes, including the E-type cyclins. These cyclins bind to and activate Cdk2, which further phosphorylates Rb and other substrates. In the hypophosphorylated state, Rb inhibits growth by sequestering the E2F transcription factor. Hyperphosphorylation of Rb releases E2F that can then activate transcription of many genes necessary for DNA replication, mitosis, and control of later cell cycles' phase transition. A-type cyclins bind to Cdk2 to phosphorylate new substrates during the S phase of the cell cycle. Cyclin-dependent kinase inhibitors including p21waf1, p27kip1, INK4 proteins, and others regulate the formation of active cyclin-Cdk complexes.

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and effector caspases via several different mechanisms, and in some cases also induce apoptosis by a caspase-independent mechanism. Vitamin D compounds increase intracellular calcium that activates the pro-apoptotic proteases microcalpain and caspase 12. Other reported mechanisms for an anti-apoptotic effect of vitamin D metabolites and analogues include down-regulation of IGF receptors, upregulation of MAP kinase, activation of sphingomyelin-ceramide – ganglioside GD3 signaling, reduced expression of Akt kinase, and altered TNFα-mediated apoptosis.3,6

In some cell systems, 1,25-dihydroxyvitamin D may exert an anti-apoptotic effect. For example, a recent study in ovarian cancer cells showed that calcitriol inhibited apoptosis mediated by death receptors but stimulated other pro-apoptotic pathways.37

**Differentiation**

In the 25 years since the seminal observation by Abe et al.,36 that 1,25-dihydroxyvitamin D caused the differentiation of cultured mouse myeloid leukemia cells, calcitriol and vitamin D analogues have been demonstrated to induce differentiation of numerous types of benign and malignant cells. Calcitriol directs the differentiation of leukemia cells to a monocyte/macrophage phenotype, a process that is dependent on the induction of p21waf1.39 Work conducted at our laboratory and others has shown that vitamin D compounds stimulate the maturation of an apical microvillus membrane and the expression of brush border membrane enzymes in cultured colon cancer cells.40 Microarray analysis of vitamin D analogue treatment of squamous cell carcinoma cells found that treatment suppressed expression of markers of cancer progression (N-cadherin, squamous cell carcinoma antigen, tenasin-C, and tumor antigen L6) and induced expression of genes associated with epithelial differentiation (cystatin MI, protease M, type 13 collagen, and desmogleine).34

Extensive studies have demonstrated multiple mechanisms by which 1,25-dihydroxyvitamin D induces differentiation of keratinocytes.2,41 Calcitriol plays a key role in the expression of the CaSR that is central to the regulation of keratinocyte differentiation by calcium.42 In addition, 1,25-dihydroxyvitamin D induces phospholipase C, and in some studies has been shown to acutely stimulate phosphoinositide turnover, resulting in increased intracellular IP3, diacylglycerol, and intracellular calcium levels.42 These changes, along with activation of AP-1 transcription factors lead to induction of multiple genes involved in differentiation, including involucrin, loricin, transglutaminase, and others.43 Transcriptional profiling of calcitriol-treated keratinocytes has demonstrated induction or suppression of numerous genes involved in various stages of keratinocyte differentiation, formation of cornified layers, and desquamation.43 As keratinocytes differentiate, changes in the expression and function of VDR coactivators have been observed, likely contributing to the temporal sequence of vitamin D-mediated gene expression during differentiation.44

In many cell systems, vitamin D secosteroids cause both decreased proliferation and induction of a more differentiated phenotype. It must be emphasized, however, that these events do not always occur together; in some cases, vitamin D compounds decrease growth without stimulating differentiation, whereas in other situations, they induce differentiation without altering cellular growth.

**Interactions with other transcription factors and cell signaling systems**

Vitamin D and the VDR have been demonstrated to interact with other transcription factors and cell signaling systems that play important roles in the regulation of cellular growth, as in the following illustrative examples.

**Androgen receptor.** In LNCaP prostate cancer cells, the effect of 1,25-dihydroxyvitamin D on growth is androgen-dependent and can be blocked by the anti-androgen casodex.45 This may be explained, in part, by upregulation of the androgen receptor by calcitriol.46 Similar cross-talk between androgen receptors and the VDR have also been found in ovarian and breast cancer cells. It should be noted, however, that calcitriol and vitamin D analogues also have antiproliferative effects in androgen-insensitive prostate cancer cells, demonstrating alternative modes of action.47

**Estrogen receptor.** Calcitriol and vitamin D analogues have been demonstrated to downregulate the estrogen receptor in several breast cancer cell lines and to affect activation of gene transcription by the estrogen receptor.36,48,49 Growth inhibition of estrogen-independent breast cancer cells lines by vitamin D secosteroids has also been observed.36

**Insulin-like growth factor.** Vitamin D secosteroids block the mitogenic effect of insulin-like growth factor I (IGF I) in several cell types, including breast cancer cells.36 Vitamin D compounds alter levels of the IGF receptor and various IGF binding proteins that modulate the availability of free cytokine for interaction with the receptor. Vitamin D secosteroids induce IGFBPs 3 and 5 in breast cancer cells,3,36 and calcitriol and transforming growth factor β (TGFβ) act synergistically to increase IGFBP-3 secretion in human bone marrow stroma.50 As described
above, in LNCaP prostate cancer cells, calcitriol induced IGFBP-3, which is an effect required for stimulation of p21waf1 expression and growth inhibition by the secosteroid.25

Transforming growth factor\(\beta\) (TGF\(\beta\)). In many cell types, including normal colonic cells, TGF\(\beta\) inhibits cell proliferation by regulating cell cycle progression and inducing apoptosis. Studies performed by Chen et al.51 in our laboratory examined interactions between calcitriol and TGF\(\beta\)1 in CaCo-2 cells. TGF\(\beta\)1 is secreted by CaCo-2 cells in a latent, biologically inactive form. Insulin-like growth factor II receptors (IGF-IIR) bind latent TGF\(\beta\)1 and facilitate its activation. Chen et al.51 found that 1,25-dihydroxyvitamin D caused an increase in the production of active TGF\(\beta\)1 by CaCo-2 cells, but did not change total TGF\(\beta\)1 secretion. IGF-IIR mRNA and protein were increased by calcitriol, while mannose-6-P, an inhibitor of the IGF-IIR, blocked latent TGF\(\beta\)1 activation by 1,25-dihydroxyvitamin D. Calcitriol decreased growth of CaCo-2 cells, an effect that could be partially blocked by mannose-6-P or an anti-TGF\(\beta\) antibody, indicating that TGF\(\beta\)1 mediates, in part, the antiproliferative effect of 1,25-dihydroxyvitamin D. Ca-Co-2 cells are resistant to the antiproliferative action of TGF\(\beta\)1 in the absence of identified mutations in the TGF\(\beta\)1 signaling. Coadministration of 1,25-dihydroxyvitamin D\(\delta\) sensitized Ca-Co-2 cells to growth inhibition by TGF\(\beta\)1. Calcitriol increased TGF\(\beta\) receptor type I mRNA and protein. Studies using CaCo-2 cells transfected with an antisense TGF\(\beta\)-RI oligonucleotide showed that this receptor was necessary for the antiproliferative effects of calcitriol and TGF\(\beta\)1.

In other cell types, 1,25-dihydroxyvitamin D has been shown to influence various aspects of TGF\(\beta\) signaling. Calcitriol has been found to increase TGF\(\beta\) secretion by breast cancer cells and keratinocytes, and to increase expression of TGF\(\beta\) receptors in breast cancer and HL-60 cells.36,38,39 Transcriptional complexes have been observed between SMAD proteins, downstream effectors of TGF\(\beta\), and the VDR.35

Epidermal growth factor receptor. In addition to the effects on hyperplastic parathyroid cells discussed above, interactions between vitamin D and the EGFR have been found in several types of cancer cells. Tong et al.50 found that calcitriol decreased EGFR abundance on both the apical and basolateral cell surface of CaCo-2 cells and attenuated the growth response induced by EGF. In turn, treatment of these cells with EGF resulted in a reduction of VDR mRNA and protein. Bareis et al.57 however, demonstrated that the interaction between EGF and vitamin D is quite complex, as EGF treatment increased VDR expression in highly differentiated, slowly dividing colon cancer cell lines, but decreased expression in poorly differentiated, highly proliferative lines. Variable effects of 1,25-dihydroxyvitamin D on EGFR have also been reported in different studies of breast cancer cell lines, with some finding that calcitriol decreased EGFR whereas others showed increased EGFR levels.36,38,39 Calcitriol has been reported to alter intracellular EGF processing in BT-20 breast cancer cells.60

Wnt/β-catenin/TCF signaling. Disordered Wnt/β-catenin/TCF signaling plays a key role in the pathogenesis of colorectal and other cancers. Studies performed by Palmer et al.61 in colon cancer cells demonstrated that 1,25-dihydroxyvitamin D\(\delta\) and vitamin D analogues induced expression of E-cadherin, promoting translocation of β-catenin from the nucleus to the cell membrane. Moreover, 1,25-dihydroxyvitamin D\(\delta\) rapidly increased VDR binding to β-catenin, blocking interaction with TCF-4. Consequently, calcitriol inhibited β-catenin-TCF/LEF-1 transcriptional activity, reducing the mRNA levels of c-myc, Tcf-1, CD44, and PPARδ.

Determinants of cellular responsiveness to the antiproliferative action of calcitriol and analogues

Different cells vary greatly in their sensitivity to inhibition of growth by 1,25-dihydroxyvitamin D and its analogues, even cells originating from the same tissue, such as various prostate cancer lines. Multiple factors appear to determine responsiveness to vitamin D.

Vitamin D receptor. Several studies have demonstrated a correlation between the cellular level of the VDR and sensitivity to growth inhibition by vitamin D secosteroids. Hedlund et al.62 reported that JCA-1 prostate cancer cells that expressed little VDR were insensitive to growth inhibition by calcitriol. Transfection of these cells with the VDR resulted in dose-dependent growth inhibition by 1,25-dihydroxyvitamin D\(\delta\). Transfection of ALVA-31 cells with an antisense VDR construct reduced sensitivity to 1,25-dihydroxyvitamin D.53 Our laboratory has shown that stable transfection of CaCo-2 cells with a full-length human VDR cDNA, results in greater inhibition of growth and induction of p21waf1 by calcitriol or the analogue F6-D3. In colorectal and other cancers, decreased VDR expression has been found in advanced neoplasms, suggesting that loss of VDR may contribute to cancer progression.6 VDR polymorphisms may also influence responsiveness to calcitriol. A Fok1 polymorphism causes expression of a three amino acid shorter VDR that has higher activity to suppress growth of peripheral blood mononuclear cells.64

1-Hydroxylase activity. In addition to expressing the VDR, numerous cells, such as prostate, breast, colon, lung,
and parathyroid cells, keratinocytes, monocytes, β cells of the pancreas, and others, express the vitamin D 1α-hydroxylase, permitting local synthesis of the active hormonal form of the vitamin. Several observations are consistent with an autocrine mechanism for an antiproliferative action of vitamin D. Low concentrations of 25-hydroxyvitamin D, insufficient to activate the VDR, have been shown to suppress growth of cells that express the 1-hydroxylase.65 In contrast, cells lacking the 1-hydroxylase fail to demonstrate the antiproliferative action of 25-hydroxyvitamin D.66

24-Hydroxylase activity. The 24-hydroxylase enzyme that catalyzes the initial step in the degradation of 1,25-dihydroxyvitamin D is also active in many cell types. It is of interest that the chromosomal region 20q13.2, which contains the cyp24 gene, is amplified in certain human cancers.67 In prostate and colon cancer cell lines, growth inhibition by calcitriol was inversely proportional to 24-hydroxylase activity.68 Combination of calcitriol with drugs that inhibit the 24-hydroxylase is being explored as an approach to enhance antiproliferative and anticancer effects.

Other metabolic pathways. In some cells, the 3β-hydroxyl group of 1,25-dihydroxyvitamin D can be epimerized forming the 3α compound.69 1,25-dihydroxy-3 epi-vitamin D has significant biological activity and is catabolized more slowly than calcitriol, perhaps leading to a prolonged duration of action. 3-epi vitamin D compounds have been reported to have enhanced activity as pro-apoptotic agents.70

Thus, alterations in the levels of the VDR, 1-hydroxylase, 24-hydroxylase, and other metabolic pathways in different cell types offer opportunities for autocrine or paracrine regulation of specific cellular responses to 1,25-dihydroxyvitamin D. Cytokines and growth factors may affect 1,25-dihydroxyvitamin D synthesis and/or metabolism in a cell-specific manner, thereby regulating the concentration of the active form of the vitamin in the local cellular environment and the responsiveness to vitamin D secosteroids. Although most of the effects of 1,25-dihydroxyvitamin D on cellular proliferation in experimental systems have required concentrations of the secosteroid that are much higher than the circulating serum level, it is entirely possible that these high concentrations can be achieved in local cellular environments.

Intracellular trafficking of vitamin D. In renal tubular cells, the protein megalin is responsible for 25-hydroxyvitamin D uptake, and intracellular vitamin D binding proteins direct 25-hydroxyvitamin D to the mitochondria for 1-hydroxylation.71,72 These proteins also affect the association of 1,25-dihydroxvitamin D with the VDR.73 Other cell types express megalin, and it is possible that they also contain intracellular binding proteins that specifically target vitamin D for activation, degradation, or association with the VDR. Factors that influence the levels of megalin or intracellular vitamin D binding proteins could, therefore, potentially alter the cell-specific responses to vitamin D.

Nongenomic actions of vitamin D secosteroids. Calcitriol and vitamin D analogues produce cellular responses, including alterations in cytosolic calcium, phosphoinositide turnover, protein kinase C subcellular distribution and activity, activation of MAP kinases, etc., that are too rapid to be caused by liganded VDR-mediated changes in gene expression.74 Annexin II and Erp57, a protein thiol-dependent reductase, have been suggested as potential membrane receptors that mediate the rapid, nongenomic actions of vitamin D.75,76 Some, but not all, studies examining the rapid cellular responses to 1,25-dihydroxyvitamin D in cells isolated from VDR knockout mice have suggested that the VDR is needed for the nongenomic responses.76,77 The VDR has been identified in caveolae-enriched membrane fractions, and some have suggested that the VDR is needed for expression of genes involved in the nongenomic responses.78 It is also an intriguing, but unproven, hypothesis that phosphorylation of the VDR by rapidly activated kinases could modify VDR activity.

Although most studies have indicated that VDR-mediated effects are responsible for the antiproliferative actions of vitamin D secosteroids, some observations are consistent with a role for VDR-independent mechanisms. For example, recent studies in 1-hydroxylase and VDR knockout mice have suggested that 1,25-dihydroxyvitamin D regulates parathyroid growth by a VDR-independent mechanism.79 Normalization of the serum calcium level by feeding a high-calcium diet to a 1-hydroxylase-null mouse does not suppress parathyroid hyperplasia, but correction of the serum calcium effectively decreases parathyroid growth in VDR knockout mice with high calcitriol levels. Very high doses of calcitriol have been demonstrated to decrease growth and induce apoptosis in breast epithelial cells from vitamin D knockout mice.80

Role of nuclear coregulator molecules. Nuclear coactivators act synergistically with the VDR to promote gene transcription.1 Coactivators SRC-1 and CBP/p300 have histone acetyl transferase activity that unfolds and exposes the DNA. This is followed by recruitment of the DRIP-TRAP complex that interacts with the VDR to favor assembly of the pre-initiation complex. Transcriptional repression by the VDR involves corepressors that
are histone deacetylases. Variations in the cellular content of these coactivator and corepressor molecules may determine responsiveness to vitamin D secosteroids. Banwell et al.81 reported that aggressive cell lines that were not responsive to 1,25-dihydroxyvitamin D$_3$ had elevated nuclear corepressor levels. Calcitriol-insensitive PC-3 and DU 145 prostate cancer cells had elevated levels of the corepressor SMRT, and MDA-MD-231 cells had increased repressor NcoR1 mRNA. Combination treatment of cancer cell lines with calcitriol and a histidine deacetylase inhibitor resulted in synergistic growth suppression.81 Competition between the VDR and other receptor systems for common nuclear transcriptional modulators could potentially affect vitamin D-VDR-mediated gene transcription.

CONCLUSION

Vitamin D secosteroids alter the growth and differentiation of numerous normal and pathological cell types. The antiproliferative effects of vitamin D compounds are the basis for important therapies such as the systemic and topical treatment of psoriasis and the suppression of parathyroid hyperplasia in chronic renal failure. Vitamin D secosteroids have been demonstrated in numerous experimental animals to inhibit the growth of many types of cancers, and exciting studies are in progress to explore the use of vitamin D compounds in the treatment of human malignancies.

Several important mechanisms appear to be responsible for the antiproliferative effect of vitamin D. Most prominent has been the slowing of cell cycle progression induced by vitamin D, typically due to inhibition of advancement from the G1 to the S phase of the cell cycle. Multiple effects of vitamin D on key regulators of this step of the cell cycle, including p21waf 1, p27kip1, cyclin D1, and others, have been observed in many cell types, and involve diverse actions on gene transcription and protein stability. Vitamin D compounds also induce apoptosis of many cell types by affecting the levels of caspases, regulatory proteins, and cell signaling systems. Differentiation of many cell types is stimulated by vitamin D through induction of arrays of genes and stimulation of signal transduction pathways. Antiproliferative effects of vitamin D compounds are often, but not always, linked to promotion of cellular differentiation.

Vitamin D and the VDR have important interactions with other transcriptional regulators and cell signaling systems, including androgen receptors, estrogen receptors, IGF, TGFβ, β-catenin, and others, to control cell growth and differentiation. Many factors influence cellular responsiveness to vitamin D, including VDR content, 1-hydroxylase and 24-hydroxylase activities, nongenomic actions of vitamin D, levels of nuclear coregulatory molecules, and others. Further understanding of the cell-specific mechanisms by which vitamin D affects growth and differentiation will permit more targeted use of vitamin D compounds and effective combination with other therapeutic agents. Vitamin D analogues that are particularly potent in regulating cell growth and differentiation, but which avoid toxicity by having lesser effects on calcium metabolism, have great promise as novel treatments for cancer and other diseases involving unregulated cell growth.

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