Antiproliferative Action of Vitamin D

TIMO YLIKOMI,*‡ ILKKA LAAKSI,* YAN-RU LOU,‡ PAULA MARTIKAINEN,*,‡ SUSANNA MIETTINEN,* PASI PENNANEN,* SAMI PURMONEN,*,‡ HEIMO SYVALA,‡ ANNIIKA VIENONEN,*,‡ AND PENTTI TUOHIMAA*‡

Graduate School of Biomedicine, *Department of Cell Biology, ‡Department of Anatomy, Medical School, and §Drug Discovery Graduate School, 33014 University of Tampere, Finland; †Department of Clinical Chemistry and §Department of Pathology, Centre of Laboratory Medicine, Tampere University Hospital, 33521 Tampere, Finland

I. Introduction
II. The General Mechanism of Vitamin D Action
   A. The Metabolism of Vitamin D
   B. The Genomic Action of Vitamin D; Vitamin D Receptor
III. Antiproliferative Effects of Vitamin D on Cell Growth
   A. Regulation of the Cell Cycle by Vitamin D
   B. Differentiation and Vitamin D
   C. Apoptosis and Vitamin D
IV. The Role of Vitamin D Crosstalk in Growth Factor and Hormone Signaling
   A. Interactions with Growth Factor Signaling
   B. Crosstalk with Nuclear Receptors and Their Ligands
V. Vitamin D and Cancer
   A. Epidemiology
   B. Vitamin D Receptor Polymorphism and Cancer
   C. Mechanisms of Antitumor Effect in Cancer
   D. Clinical Trials
VI. Conclusions and Future Perspectives
References

During the past few years, it has become apparent that vitamin D may play an important role in malignant transformation. Epidemiological studies suggest that low vitamin D serum concentration increases especially the risk of hormone-related cancers. Experimentally, vitamin D suppresses the proliferation of normal and malignant cells and induces differentiation and apoptosis. In the present review we discuss the mechanisms whereby vitamin D regulates cell proliferation and whether it could be used in prevention and treatment of hyperproliferative disorders like cancers.

I. INTRODUCTION

The earliest discovered functions of vitamin D are its important roles in the bone mineralization, absorption, and utilization of calcium. With the discovery of the nuclear receptor for vitamin D came the surprising finding that this receptor could be found in many, probably all, tissues and cells not previously appreciated as targets of vitamin D action. This also suggested that the functions of vitamin D might be broader than previously anticipated. The role of vitamin D as a hormone and paracrine and autocrine factor became evident and it was shown that vitamin D is an important regulator of growth, differentiation, and apoptosis in many tissues (Norman et al., 1980; E. L. Smith et al., 1986; Demay et al., 1992; Suda et al., 1992; Botling et al., 1996). There has thus been much interest in vitamin D in the treatment of many diseases (Bouillon et al., 1995).

The protective action of vitamin D against carcinogenesis became evident on the basis of several epidemiological studies (Doll and Peto, 1981; Hanchette and Schwartz, 1992; Corder et al., 1993). This action is thought to be due to its antiproliferative effect. Our recent epidemiological study (Ahonen et al., 2000a) brought out the following interesting aspects concerning prostate cancer: (1) Vitamin D deficiency may be an especially important cause of cancer in countries having seasonally low ultraviolet irradiation. (2) The prostate cancer risk attributable to vitamin D deficiency is higher among preandropausal than postandropausal men, which suggests that vitamin D acts as an androgen antagonist. (3) Vitamin D probably plays a role in the initiation, promotion and progression of cancer. (4) High serum vitamin D levels delay the development of prostate cancer.

In most normal and cancer cells, vitamin D acts as an antiproliferative factor, but there are exceptions where vitamin D at certain concentrations may stimulate cell proliferation. These effects are believed to be mediated mainly by the nuclear vitamin D receptor (VDR), which is also present in cancer cells, but the exact mechanism whereby vitamin D exerts its antiproliferative action is under intense investigation. It should be noted that local metabolism of vitamin D in the target tissue might play an important role in its antiproliferative action. High expression of the inactivating enzyme, 24-hydroxylase (CYP24), may lead to a complete insensitivity of target cells to vitamin D; CYP24 has therefore been identified as a potent oncogene in breast carcinogenesis (Albertson et al., 2000). In this review, we describe the known mechanisms of the antiproliferative action of vitamin D. We concentrate on genomic action through the VDR, although it is evident that
membrane-mediated mechanisms (through a putative membrane receptor) could equally play an important role.

II. THE GENERAL MECHANISM OF VITAMIN D ACTION

A. THE METABOLISM OF VITAMIN D

There are two different forms of vitamin D: vitamin D₃ and vitamin D₂. Vitamin D₃ is synthesized in humans and animals, whereas vitamin D₂ is the form present in plants. Vitamin D₂ and vitamin D₃ are metabolized in a similar fashion to produce a hormonally active form. Both can be used in the supplementation of animal and human diets (Horst and Reinhardt, 1997). Only the metabolism of vitamin D₃ is discussed in this chapter. Briefly, 7-dehydrocholesterol is photolyzed in the skin to form previtamin D₃, which is transformed to vitamin D₃ and enters the circulation (Holick et al., 1980a). Vitamin D₃ is hydroxylated to 25-hydroxyvitamin D₃ (25OHD₃) in the liver and undergoes another hydroxylation mainly in the kidney to result in 1α,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃], the most active metabolite of vitamin D (Holick, 1995). 25OHD₃ has also been reported to show biological activity, but at a level 500- to 1000-fold lower than 1,25(OH)₂D₃ (Reichel et al., 1989). However, the serum concentration of 25OHD₃ is about 1000-fold that of 1,25(OH)₂D₃. The 24-hydroxylation, which inactivates both 25OHD₃ and 1,25(OH)₂D₃, takes place mainly in the kidney.

7-Dehydrocholesterol is converted to previtamin D₃ in the plasma membrane of skin cells in response to exposure to sunlight (Holick et al., 1980a, 1995), the optimum wavelength of ultraviolet B (UVB) photons for photolysis being between 290 and 315 nm (MacLaughlin et al., 1982; Holick, 1987). Photolabile previtamin D₃ can be isomerized to two biologically inert isomers, lumisterol 3 and tachysterol 3, as a result of continued exposure to sunlight (Holick, 1987). Thus, only a limited amount of 7-dehydrocholesterol accumulates as previtamin D₃ (Holick et al., 1981), which is a thermally unstable cis isomer and is transformed into vitamin D₃ over a period of at least 3 days (Holick et al., 1980b, 1981). Thereafter vitamin D₃ crosses the plasma membrane to enter the extracellular space and is bound to vitamin D-binding protein (DBP) (Holick et al., 1980b) or is isomerized to suprasterol I, suprasterol II, and 5,6-trans-vitamin D₃ by prolonged exposure to sunlight (Holick et al., 1987). Thus, sunlight itself can regulate the synthesis of vitamin D₃, another regulator being melanin, which reduces the amount of previtamin D₃ in that it competes with 7-dehydrocholesterol for UVB
radiation in the skin (Holick, 1987). It has also been noted that UVB radiation is inadequate for the synthesis of previtamin D3 in northern countries during winter (Webb et al., 1988). In addition, sunscreen and clothing prevent the synthesis of previtamin D3 (Alagol et al., 2000).

Vitamin D3 bound to DBP is transported to the liver for 25-hydroxylation. Both microsomal and mitochondrial enzymes have been identified (Masumoto et al., 1988; Axen et al., 1994). Vitamin D3 is taken up during the first pass across the liver (Gascon-Barre and Gamache, 1991) and hydroxylated in hepatocytes (Dueland et al., 1981). There is no evidence for strict regulation of vitamin D3 uptake by vitamin D status (Rojanasathit and Haddad, 1976), the steady-state liver.serum concentration ratio being 0.4–0.6 at physiological levels of vitamin D3 (Gascon-Barre and Gamache, 1991).

1,25(OH)2D3 is synthesized from 25OHD3 mainly in the kidney by the mitochondrial cytochrome P450 enzyme 25OHD3-1α-hydroxylase (CYP27B), which is expressed along the human nephron (Zehnder et al., 1999). Therefore, vitamin D is regarded as a hormone. Studies have also shown extrarenal 1α-hydroxylase activity in lymphocytes (Reichel et al., 1987), alveolar macrophages (Adams and Gacad, 1985), bone, liver, placenta, pancreas, colon, parathyroids, adrenal medulla, cerebellum (Hewison et al., 2000), skin (Horst and Reinhardt, 1997), some prostate carcinoma cell lines, and noncancerous prostatic cells (Schwartz et al., 1998). Therefore, vitamin D functions as an auto- and paracrine factor.

The synthesis of 1,25(OH)2D3 from 25OHD3 by 1α-hydroxylation is strictly controlled. Parathyroid hormone (PTH) treatment has been shown to induce 1α-hydroxylase (Brenza and DeLuca, 2000). On the other hand, calcium, phosphate, and 1,25(OH)2D3 itself are inhibitors of 1α-hydroxylase (Hewison et al., 2000). It seems likely that changes in extracellular calcium constitute the most potent and rapid regulator of 1α-hydroxylase expression. Calcitonin, acidosis, sex steroids, prolactin, growth hormone, glucocorticoids, thyroid hormone, and pregnancy are also potential regulators of 1,25(OH)2D3 synthesis (Henry, 1997).

The 25-hydroxyvitamin D 24-hydroxylase enzyme (CYP24) controls the first step in the inactivation process of 25OHD3 and 1,25(OH)2D3. The enzyme CYP24 is widely distributed throughout the body and is strongly induced by 1,25(OH)2D3. Among target cells shown to express CYP24 after treatment with 1,25(OH)2D3 are prostate cancer (Miller et al., 1995), chondrocyte (Pedrozo et al., 1999a), promyelocytic leukemia (Ishizuka et al., 2000), and osteoblastic cells (Armbrecht et al., 1998). Breakdown of 1,25(OH)2D3 by CYP24 in target cells is likely to modify the growth inhibition effect of 1,25(OH)2D3 and the use of cytochrome P450 enzyme inhibitors renders cells more sensitive to the antiproliferative action of 1,25(OH)2D3 (Zhao et al., 1996; Ly et al., 1999).
For instance, the ability of 1,25(OH)$_2$D$_3$ to inhibit the growth of DU145 cells is substantially increased by liarozole, which blocks 24-hydroxylase activity in these cells (Ly et al., 1999). A recent report indicated that CYP24 may have an oncogenic role in breast cancer (Albertson et al., 2000). It appeared that expression of CYP24 when normalized with that of VDR correlates with the copy number of the CYP24 locus in breast tumors, suggesting that overexpression of CYP24 is due to amplification of the corresponding gene and may thus interfere with control of growth by vitamin D. A summary of the role of vitamin D metabolism involved in the target cell proliferation is given in Fig. 1.

![Diagram of Vitamin D Metabolism](image)

**Fig. 1.** Schematic representation of vitamin D (VD) metabolism. Vitamin D is converted to 25-hydroxyvitamin D (25OHVD) by 25-hydroxylase (CYP27) in liver. 25-Hydroxyvitamin D is hydroxylated in kidney by 1α-hydroxylase (CYP27B), which yields the active form of vitamin D (1,25OHVD). In kidney both hydroxylated forms of vitamin D can be further hydroxylated by 24-hydroxylase, which leads to the inactivation pathway of vitamin D. The physiological importance of 25-hydroxyvitamin D is due to its activation by 1α-hydroxylase and inactivation by 24-hydroxylase in target cells (local metabolism).
B. THE GENOMIC ACTION OF VITAMIN D; VITAMIN D RECEPTOR

Most of the biological actions of 1,25(OH)_{2}D_{3} are exerted via the nuclear vitamin D-receptor-mediated control of target gene transcription. The vitamin D receptor (VDR) is a member of the nuclear steroid and thyroid hormone receptor superfamily and functions as a ligand-inducible transcription factor. It has been clearly demonstrated that the antiproliferative effects of 1,25(OH)_{2}D_{3} are mediated through genomic pathways and therefore require expression of nuclear VDR. Cells which fail to express VDR are not growth-inhibited by 1,25(OH)_{2}D_{3}. For example, the prostatic carcinoma cell line JCA-1 neither expresses VDR nor is affected by 1,25(OH)_{2}D_{3} in growth studies. When stably transfected with VDR cDNA, however, cells are growth-inhibited by 1,25(OH)_{2}D_{3} in a dose-dependent manner (Hedlund et al., 1996a). Similarly, cells expressing VDR in high levels lose their ability to respond to 1,25(OH)_{2}D_{3} when transfected with VDR antisense mRNA, as shown with another prostatic carcinoma cell line, ALVA-31 (Hedlund et al., 1996b). Zhuang et al. (1997) compared VDR content and growth response to 1,25(OH)_{2}D_{3} in four prostatic cancer cell lines: LNCaP, DU145, PC-3, and ALVA-31. The PC-3 and DU145 cells contain low levels of VDR and are poorly growth-inhibited by 1,25(OH)_{2}D_{3}, whereas LNCaP cells have high levels of VDR and are highly sensitive to 1,25(OH)_{2}D_{3} (approximately 55% inhibition). However, ALVA-31 cells contain the highest levels of VDR of the four cell lines, but display only 20% growth inhibition by 1,25(OH)_{2}D_{3}, without any decrease in the general transcriptional activity. It is thus obvious that nuclear VDR is needed for 1,25(OH)_{2}D_{3} growth suppression, but the sensitivity to growth inhibition does not necessarily correlate with VDR content and transcriptional activity.

The absence of functional VDR results in target tissue insensitivity (hereditary vitamin D-resistant rickets; HVDRR) and clinical resistance to 1,25(OH)_{2}D_{3} (Feldman and Malloy, 1990). Hereditary vitamin D-resistant rickets is an autosomal recessive disorder commonly caused by mutations in the VDR-encoding gene resulting in a nonfunctional vitamin D receptor (M. R. Hughes et al., 1988; Ritchie et al., 1989; Sone et al., 1989). The phenotype of VDR knockout mice also shows that functional VDR is essential for mediating functions of 1,25(OH)_{2}D_{3}. Heterozygote mutants appear to be normal, but homozygotes display a phenotype very similar to HVDRR (Y. C. Li et al., 1997; Yoshizawa et al., 1997). After weaning, mice lacking the VDR exhibit growth retardation, impaired bone formation, and uterine hypoplasia with impaired folliculogenesis. These findings thus establish a critical role for
VDR in controlling growth and development in the postweaning stage (Yoshizawa et al., 1997).

The human vitamin D receptor is a nuclear phosphoprotein 427 amino acids in length with a molecular weight of approximately 50 kDa. In addition to the classical target organs of vitamin D (intestine, kidney, and bone), VDR has been localized in a variety of other tissues and cells. VDR, like other nuclear receptors, is a modular protein composed of six distinct segments, designated the A/B, C (DNA-binding domain; DBD), D, and E/F (ligand-binding domain; LBD) domains (for a detailed description of VDR structure see, for example, Haussler et al., 1998). These functional domains of VDR are responsible for such actions as ligand binding, heterodimerization, DNA binding/nuclear localization, and transcriptional activation. As the structure of the receptor would imply, the mechanism by which VDR affects gene transcription and leads to growth inhibition is a complex multistep process (Fig. 2).

Like that of other nuclear receptors, the ligand-binding domain of VDR (E/F domain) is multifunctional: it is involved in dimerization, recruitment of transcription factors, and transcriptional activity. It has been hypothesized that upon ligand binding, VDR undergoes structural changes which enable it to heterodimerize with its partner, retinoid X receptor (RXR). VDR can associate with all three RXR subtypes, α, β, and γ, on vitamin D response elements (VDREs), and the association preference is apparently dependent on gene context (P. N. MacDonald et al., 1993; Kephart et al., 1996). Solomon et al. (1998, 1999) have shown that RXRα becomes phosphorylated through the Ras–Raf–MAPK (mitogen-activated protein kinase) pathway in ras-transformed human keratinocytes, resulting in disruption of VDR:RXR heterodimerization and resistance to the growth inhibition by 1,25(OH)2D3. Studies by Takeshita et al. (2000) suggest that in addition to VDR:RXR heterodimers, VDR homodimers may also be of importance in gene regulation, which is further affected by different coactivator recruitment. It has also been reported that VDR heterodimerizes with thyroid hormone receptor (TR) and retinoic acid receptor (RAR), but these results are somewhat controversial and further work is needed to establish their significance in the regulation of certain VDREs (Schrader et al., 1994a, 1994b; Thompson et al., 1999).

The E/F domain contains a short amphipathic α-helix in extreme C-termini (Danielian et al., 1992). This is termed activation function-2 (AF-2), whereby the receptor regulates the transcription of target genes by recruiting several types of cofactors essential for transcriptional regulation (transactivation/transrepression) (Masuyama et al., 1997). The recruitment of coactivators is a result of the conformational change
Fig. 2. The known genomic mechanisms of 1,25(OH)₂D₃ action which may lead to the control of cell proliferation. Ligand binding causes a conformational change and phosphorylation of VDR that allows it to heterodimerize with RXR. The VDR–RXR heterodimer recruits the DRIP complex (by VDR AF-2) and other cofactors, and binds to the VDRE of the target gene. The VDR–RXR–cofactor complex interacts with transcription factors (X₁–X₇) and the general transcription apparatus to initiate gene transcription. The factors (phosphorylated VDR, RXR, DRIPs) which are important in the control of cell proliferation are shaded.
which receptor undergoes after ligand binding and release of corepressors. Cofactors act as large complexes and many of them are actually chromatin remodelers possessing histone-modifying activities, or act directly on the basal transcription machinery (for review see Rachez and Freedman, 2000). Rachez and coworkers have identified an entire complex of polypeptides which interact with the VDR in a ligand-dependent manner and activate transcription on chromatin templates and in a cell-free transcription assay (Rachez et al., 1998, 1999). The complex is termed DRIP [VDR-interacting proteins; also named TRAP (Fondell et al., 1996)] or ARC (activator-recruited cofactor; Naar et al., 1999) and is recruited to the VDR AF-2 domain by the DRIP205 subunit. The fact that the DRIP complex is identical to ARC, which interacts with several other classes of activators and whose subunits are also shared by other multisubunit cofactors (Naar et al., 1999), suggests that selectivity of transcription control may be dependent on recruitment of different sets of cofactor subunits (Rachez et al., 1999). Thus, the biological activity of the VDR, and ultimately the biological response to the ligand, is determined by the ligand-induced conformational change of the receptor, resulting in selective interactions of VDR with coactivators. Takeyama et al. (1999) showed that the distinct biological responses of vitamin D analogs might be partly explained by the differential affinity of the liganded VDR for coactivator complexes. In another study, C-20 stereoisomers of 1,25(OH)$_2$D$_3$ (20-epi analogs), whose antiproliferative potency is much higher than that of 1,25(OH)$_2$D$_3$, were found to induce VDR/DRIP interaction at concentrations 100-fold lower than that at which 1,25(OH)$_2$D$_3$ induced recruitment of DRIP205 by VDR. It can be concluded that the enhanced biological responses, such as antiproliferation and differentiation of 20-epi analogs, may be due to their influence on the VDR–cofactor (DRIP205) interactions (Yang and Freedman, 1999).

As discussed above, ligand binding has been hypothesized to induce conformational changes in the E/F region of the VDR, which converts the receptor to the active form. Upon such binding, VDR becomes hyperphosphorylated (Jurutka et al., 1993), and heterodimerizes with RXR and other binding partners. These complexes bind strongly to DNA by selectively recognizing the VDREs of promoter regions of target genes. The prototypical VDRE for the vitamin D receptor is composed of two direct imperfect repeats of consensus sequence GGGTCA, interspaced by three nucleotides, denoted DR3. It has been shown that VDR binds to the 3' half-site and its heteropartner RXR to the 5' half-sites of VDRE (Jin and Pike, 1996; Jimenez-Lara and Aranda, 1999). Although dozens of genes are known to be regulated by the 1,25(OH)$_2$D$_3$ hormone, only
a fraction of these have been reported to contain VDREs. VDREs are contained in such positively controlled genes as those for osteocalcin (Kerner et al., 1989), osteopontin (Noda et al., 1990), β3-integrin (Cao et al., 1993), vitamin D-24-OHase (two VDREs) (K. S. Chen and DeLuca, 1995), NPT2 (Taketani et al., 1997), p21 (Liu et al., 1996), and calbindin D28k (Gill and Christakos, 1993). Negative VDREs exist, for example, in the gene for PTH (Demay et al., 1992), PTH-related peptide (Falzon, 1996), type I collagen (Pavlin et al., 1994), and bone sialoprotein (J. J. Li and Sodek, 1993). Recently, Campbell et al. (2000) reported that the antiproliferative effects of 1,25(OH)2D$_3$ on breast and prostate cancer cells are associated with induction of BRCA1 gene expression. Transcriptional activation of BRCA1 was found to be indirectly mediated by the VDR in the 1,25(OH)$_2$D$_3$-sensitive breast cancer cell line MCF-7. The authors conclude that VDR induces factors which regulate BRCA1 gene expression, which, in part, mediates the antiproliferative effects of 1,25(OH)$_2$D$_3$. The proposed genomic mechanisms involved in control of proliferation are given in Fig. 2.

III. ANTIPROLIFERATIVE EFFECTS OF VITAMIN D ON CELL GROWTH

A. REGULATION OF THE CELL CYCLE BY VITAMIN D

The vitamin D [1,25(OH)$_2$D$_3$]-induced antiproliferative action is mediated predominantly through a G1/S phase block of the cell-cycle. 1,25(OH)$_2$D$_3$ regulates many of the cell-cycle regulatory genes and reduces or increases the kinase activities of cyclin-dependent kinases (CDKs) (Fig. 3). This results in a decreased number of cells in the S phase and an accumulation of cells in the G0–G1 phase (Q. M. Wang et al., 1996; Simboli-Campbell et al., 1997; Zhuang and Burnstein, 1998). Decreased phosphorylation of the retinoblastoma protein (pRb) has also been observed in 1,25(OH)$_2$D$_3$-induced G1 arrest (Simboli-Campbell et al., 1997; Zhuang and Burnstein, 1998; Park et al., 2000b). A 1,25(OH)$_2$D$_3$-induced block also at the G2/M checkpoint has been observed in HL60 cells (Godyn et al., 1994). In a recent study, this G2/M block has been shown to be incomplete: Harrison et al. (1999) have demonstrated that 1,25(OH)$_2$D$_3$-treated HL60 cells have decreased p34 (cdc2) levels and kinase activity, which may contribute to G2/M phase block. The ability of some HL60 cells to traverse this block may be the result of increasing levels of other regulators of the G2 traverse, for example, cyclin B1, cdc25C, and CDK7.

The blocking of the G1/S phase by 1,25(OH)$_2$D$_3$ is known to be associated with alteration of protein levels or kinase activity of CDK2, CDK4,
The effect of vitamin D on cell-cycle proteins. 1,25(OH)\textsubscript{2}D\textsubscript{3} regulates several proteins, such as cyclins, cyclin-dependent kinases (CDKs), and cyclin-dependent kinase inhibitors (p21, p27), that are involved in the G1/S phase transition, especially in the phosphorylation (P) of retinoblastoma protein (pRb). Phosphorylation of pRb is cell-cycle-dependent and it plays a key role in the control of cell proliferation. G1, S, G2, and M represent different phases of the cell cycle. Arrows show activation or upregulation. Blunt-ended lines represent inhibition or downregulation. For the role of c-myc see details in the text.

and CDK6. CDK2 kinase activity is decreased in the LNCaP prostate cancer cell line (Zhuang and Burnstein, 1998), and CDK2 and CDK6 activities are decreased in human leukemia HL60 cells (Q. M. Wang et al., 1997) by 1,25(OH)\textsubscript{2}D\textsubscript{3}. The synthetic 1,25(OH)\textsubscript{2}D\textsubscript{3} analog EB1089 lowers protein levels and the kinase activity of CDK2 and CDK6 in HL60 cells (Seol et al., 2000), but in NCI-H929 myeloma cells the protein levels and kinase activity of CDK6 are not changed (Park et al., 2000b). The results concerning the effect of 1,25(OH)\textsubscript{2}D\textsubscript{3} and EB1089 on the regulation of CDK4 protein levels and kinase activities are variable in different cell lines (Q. M. Wang et al., 1997; Park et al., 2000b; Seol et al., 2000).

The primary regulators of CDK activity are cyclins, whose function is mainly controlled by changes in their levels (Morgan, 1995). In primary cells from human colon adenocarcinoma and in human intestinal cells
(Caco2), 1,25(OH)2D3 significantly reduced the basal and epidermal growth factor (EGF)-stimulated expression of cyclin D1 at the mRNA and protein levels (Tong et al., 1999). In human breast cancer MCF-7 cells the cyclin D1 mRNA level decreased rapidly, but protein levels decreased only after 72 h by 1,25(OH)2D3 treatment (Verlinden et al., 1998). Use of the 1,25(OH)2D3 analog EB1089 in NCI-H929 cells decreased the protein levels of cyclin A and cyclin D1, but left the level of cyclin E unchanged, although its associated kinase activity decreased (Park et al., 2000b). Controversially, in a study with HL60 cells the protein levels of cyclin D1 and cyclin E were found to be increased after 48 h of 1,25(OH)2D3 treatment (Q. M. Wang et al., 1996). In a study with U937 human monomyelolytic cells cyclin A, D1, and E protein levels were elevated until 48 h and strongly decreased at 72 h (Rots et al., 1999). It was suspected that a transient proliferative burst and an increase in cyclin expression precedes 1,25(OH)2D3-induced G1 block of these cells.

CDK inhibitors act as negative regulators of growth by causing cells to be arrested in G1 and withdraw from the cell cycle (Grana and Reddy, 1995). Several of these genes (those for p15ink4b, p18ink4c, p21wafl/cip1, and p27kip1) have been found to be 1,25(OH)2D3-regulated (Freedman, 1999). p27kip1 is one of the key regulatory proteins in the cell cycle. It inhibits pRB phosphorylation and arrests cells in G1 by suppressing the activity of several cyclin/CDK complexes (Q. M. Wang et al., 1996, 1997). Upregulation of p27kip1 by 1,25(OH)2D3 has been reported in different human cancer cell lines (Verlinden et al., 1998; Park et al., 2000b; Seol et al., 2000). Putative target proteins for 1,25(OH)2D3-upregulated p27kip1 are CDK2 (Park et al., 2000b) and CDK6 (Q. M. Wang et al., 1997). p21wafl/cip1 is a CDK inhibitor, which is also 1,25(OH)2D3-upregulated in many human cancer cell lines (G. Wu et al., 1997; Seol et al., 2000). Liu et al. (1996) identified a functional VDRE in the p21wafl/cip1 promoter, suggesting a direct regulation of p21wafl/cip1 expression by VDR. p21wafl/cip1 exerts its effect on the cell cycle possibly by inhibition of CDK2— and CDK6-associated kinase activity, which leads to G1 arrest (G. Wu et al., 1997; Seol et al., 2000).

The c-myc protooncogene is a cell-cycle-related protein found to be 1,25(OH)2D3-regulated. C-myc can enhance CDK activity via functional inactivation of the CDK-inhibitors p21wafl/cip1 (Mitchell and El-Deiry, 1999) and p27kip1 (Perez-Roger et al., 1999). In HL60 and U 937 cell lines 1,25(OH)2D3 inhibits proliferation and decreases c-myc gene transcription (Caligo et al., 1996), and in MCF-7 cells 1,25(OH)2D3 and EB1089 were able to reduce the expression of c-myc mRNA (Mathiasen et al., 1993). In a recent study Okano et al. (1999) identified a response
element for the 1,25(OH)$_2$D$_3$ analog 22-oxa-1,25(OH)$_2$D$_3$ in the human c-myc gene. We summarize the factors of cell cycle regulated by vitamin D in Fig. 3.

B. DIFFERENTIATION AND VITAMIN D

1,25(OH)$_2$D$_3$ shows differentiating and growth-controlling activity in a wide range of tissues and cell types. These include keratinocytes (Hosomi et al., 1983), lung cells (Marin et al., 1993), adipocytes (Sato and Hiragun, 1988), activated lymphocytes (Tsoukas et al., 1984), breast (Frappart et al., 1989), prostate (Miller et al., 1992; Skowronski et al., 1993; Esquenet et al., 1996), and colon carcinoma cell lines (Brehier and Thomasset, 1988; X. Zhao and Feldman, 1993) and several leukemia cell lines (Abe et al., 1981; Tanaka et al., 1982). Recent VDR knockout studies also confirm the role of vitamin D in the differentiation of its target organs (Yoshizawa et al., 1997; Y. C. Li et al., 1997, 1998; Amling et al., 1999; Kato et al., 1999; Takeda et al., 1999; Kinuta et al., 2000; Sakai and Demay, 2000).

1,25(OH)$_2$D$_3$ stimulates differentiation of macrophage precursor cells, monocytes. Studies by several groups using both mouse and human cell lines have shown that 1,25(OH)$_2$D$_3$ inhibits the proliferation of these cells and stimulates their differentiation toward a more mature macrophage-like phenotype (Abe et al., 1981; Amento et al., 1984; Koeffler et al., 1984; Mangelsdorf et al., 1984), as reviewed by Hewison and O'Riordan (1997). Monocyte–macrophage differentiation in vitro is associated with an enhanced capacity to synthesize 1,25(OH)$_2$D$_3$, a loss of 24,25(OH)$_2$D$_3$-synthesizing activity, and a decrease in the expression of VDR mRNA and protein; thus an autoregulatory mechanism of monocyte–macrophage generation by 1,25(OH)$_2$D$_3$ may be involved (Kreutz et al., 1993). The human leukemia cell line HL60 may develop toward a different phenotype depending on the activating agent used. 1,25(OH)$_2$D$_3$ induces the formation of monocytes, whereas retinoic acid and 9-cis-retinoic acid induce neutrophil formation (Miyaura et al., 1985; Brown et al., 1994). In the U937 cell line, vitamin D treatment inhibits retinoid-induced cellular differentiation markers, CD23 and CD49f, but in contrast, neither retinoic acid-stimulated, retinoic acid response element (RARE)-mediated transcription nor retinoic acid-induced RAR-β expression is suppressed by vitamin D, suggesting that vitamin D selectively inhibits the retinoic acid-induced differentiation program, but not the RARE-mediated signal (Botling et al., 1996). An autocrine transforming growth factor-β (TGF-β) pathway, activated by vitamin D and retinoids in U937 cells, is involved in the early steps of
the process leading to cell growth arrest and differentiation (Defacque et al., 1999). Protein kinase C (PKC) (Simpson et al., 1998) and TGF-β have been shown to potentiate the vitamin D-induced terminal monocytic differentiation of human leukemic cell lines (Testa et al., 1993). Also, inhibition of p38 MAP kinase activity potentiates 1,25(OH)₂D₃-induced differentiation of HL60 cells (X. Wang et al., 2000).

Osteoblasts possess receptors for 1,25(OH)₂D₃ (Petkovich et al., 1984). Several studies of osteoblastic cells in vitro have shown that 1,25(OH)₂D₃ increases alkaline phosphatase activity (Kurihara et al., 1984) and stimulates the expression of some of the noncollagenous proteins of bone such as osteopontin (Sodek et al., 1995) and the bone-specific calcium-binding protein, osteocalcin (Price and Baukol, 1980). However, concomitant with stimulation of expression of some osteoblast phenotypic markers, there occurs inhibition of others, such as type I collagen (Kream et al., 1986) and bone sialoprotein (Oldberg et al., 1989). Similarly, vitamin D has been reported to reduce type I collagen and alkaline phosphatase mRNA and protein levels in rat organ cultures (Canalis and Lian, 1985) and in rat osteosarcoma cells (Harrison et al., 1989), as reviewed by Aubin and Heersche (1997). In osteoclasts 1,25(OH)₂D₃ stimulates differentiation. In long-term human and primate bone marrow cultures it increases the formation of multinucleated cells with several characteristics of osteoclasts (Roodman et al., 1985; B. R. MacDonald et al., 1987). Both retinoic acid and 1,25(OH)₂D₃ modulate macrophage growth, but in an antagonistic manner. Although retinoic acid strongly promotes macrophage proliferation and impedes multinucleated cell formation, 1,25(OH)₂D₃ inhibits proliferation and alters the kinetics of multinucleated cell formation; thus, proliferative signals induced by retinoic acid can override the signal to differentiate induced by 1,25(OH)₂D₃ (Woods et al., 1995). Recent VDR knockout studies demonstrate VDR-mediated actions of 1,25(OH)₂D₃ in osteoblasts to be essential for osteoclast formation by 1,25(OH)₂D₃, and show that functionally intact osteoclasts can be formed without 1,25(OH)₂D₃ actions under stimulation by other agents such as PTH or interleukin-1α (IL-1α) (Kato et al., 1999; Takeda et al., 1999).

Vitamin D induces keratinocyte differentiation (Hosomi et al., 1983). Human foreskin keratinocytes produce 1,25(OH)₂D₃ and 24,25(OH)₂D₃ from 25OHD₃ in culture. The production of 1,25(OH)₂D₃ by these cells correlates with early events of differentiation such as activity of transglutaminase, the enzyme responsible for crosslinking the proteins of the cornified layer and the levels of a precursor protein, involucrin. The increased production of 24,25(OH)₂D₃, as 1,25(OH)₂D₃ production
declines correlates with the terminal differentiation marker, cornified layer formation (Pillai et al., 1988). 1,25(OH)_{2}D_{3} stimulates the assembly of functional adherent junctions in keratinocytes (Gniadecki et al., 1997). In cultured human keratinocytes insulin attenuates and EGF potentiates 1,25(OH)_{2}D_{3}-induced terminal differentiation (T. C. Chen et al., 1995). Also, all-trans-retinoic acid antagonizes the antiproliferative, prodifferentiating actions of 1,25(OH)_{2}D_{3} in normal human keratinocytes (Gibson et al., 1998). More recently, VDR knockout studies have demonstrated that vitamin D is not critical for keratinocyte growth or differentiation, but impairs the hair cycle of VDR-ablated mice (Sakai and Demay, 2000). By reason of its prodifferentiating and antiproliferating effects, vitamin D and its analog have been used in clinical practice in the management of psoriasis (Kragballe, 1992).

Vitamin D has a function in embryonic muscle growth and maturation. In undifferentiated myoblasts 1,25(OH)_{2}D_{3} increases DNA synthesis and lowers creatine kinase activity, indicating stimulation of cell proliferation and inhibition of myogenesis. In prolonged cultures, when myoblasts elongate and fuse to form differentiated myotubes, 1,25(OH)_{2}D_{3} has promoted myogenesis, as indicated by an inhibition of DNA synthesis and an increase in specific muscle differentiation markers such as creatine kinase activity and myosin expression. Also, increased PKC-\(\alpha\) activity has been observed during 1,25(OH)_{2}D_{3} stimulation of myoblast proliferation, whereas inhibition of PKC activity accompanied the effects of the hormone on myoblast differentiation (Capiati et al., 1999). In the neonatal rat heart 1,25(OH)_{2}D_{3} inhibits cardiac myocyte maturation and acts through a protein kinase-dependent mechanism to maintain cardiac myocytes in an immature state (O'Connell et al., 1995).

The level of vitamin D receptor correlates with the degree of differentiation in human colon cancer cell lines and may serve as a useful biological marker in predicting the clinical outcome in patients. Human colonic tumors express lower vitamin D receptor levels than adjacent normal tissues (Shabahang et al., 1993). In a human colon cancer cell line, HT-29, the brush border hydrolase, maltase, activity increases significantly when undifferentiated cells are exposed to 1,25(OH)_{2}D_{3}, indicating that the hormone can promote differentiation (Brehier and Thomasset, 1988; X. Zhao and Feldman, 1993). It has also been demonstrated that 1,25(OH)_{2}D_{3} stimulates activator protein-1 (AP-1) activation in CaCo-2 cells by a PKC-\(\alpha\)- and Jun N-terminal kinase (JNK)-dependent mechanism, leading to increases in cellular differentiation (A. Chen et al., 1999).
It has been demonstrated that 1,25(OH)2D3 regulates the growth and differentiation of both female and male reproductive organs (Konety et al., 1996; Yoshizawa et al., 1997; Kinuta et al., 2000). VDR knockout studies show that when weaning, female mice lacking a functional vitamin D receptor exhibit uterine hypoplasia, and folliculogenesis is impaired (Yoshizawa et al., 1997; Kinuta et al., 2000). In male mice, decreased sperm counts and sperm motility with histological abnormality of the testis can be observed (Kinuta et al., 2000). In prostate cancer cell lines 1,25(OH)2D3 promotes differentiation (Miller et al., 1992; Skowronski et al., 1993; Esquenet et al., 1996). Although an increasing body of data indicates a role for vitamin D in prostate cancer, little is known as to the role of this hormone in the noncancerous prostate. Examination of the effect of 1,25(OH)2D3 on the growth of noncancerous rat prostates in vivo indicates that in the absence of testosterone, 1,25(OH)2D3 may exert a growth-promoting effect on the prostatic stroma in vivo. In concert with testosterone, it may play an important role in the growth and differentiation of the normal rat prostate (Konety et al., 1996). Studies with a normal epithelial cell line derived from rat dorsal-lateral prostate show that autocrine production of TGF-β may play a significant role in 1,25(OH)2D3-induced prostate differentiation (Danielpour, 1996). In the normal mammary gland 1,25(OH)2D3 affects calcium transport across membranes of functionally differentiated epithelial cells (Mezzetti et al., 1988) and plays a fundamental role in milk protein synthesis (Bhattacharjee et al., 1987). The antiproliferative activity of 1,25(OH)2D3 has been documented in breast carcinoma cell lines and it can also induce differentiation of these cells (Frappart et al., 1989). VDR mRNA has been found to be present in relatively high levels in well-differentiated cells and in low levels in poorly differentiated cells; thus the vitamin D receptor level in breast cancer cells may be used as a marker of differentiation and as a predictor of growth inhibition by 1,25(OH)2D3 (Buras et al., 1994). In rat endometrial cells 1,25(OH)2D3 induces in vivo decidualization (Halhali et al., 1991), and in endometrial carcinoma cell lines 1,25(OH)2D3 treatment has a differentiating effect on cell morphology, cells becoming columnar with pronounced polarity and forming glandlike structures when cultured in collagen gel (Yabushita et al., 1996).

C. APOPTOSIS AND VITAMIN D

In multicellular organisms apoptosis (programmed cell death) is a crucial event in the maintenance of balance between cell proliferation and cell death. Apoptosis is an inherent, highly controlled process
which, in contrast to necrosis, leads to cell death without inflammatory response. From the morphological point of view apoptosis is characterized by cellular shrinkage, chromatin condensation, membrane blebbing, and formation of apoptotic bodies. The complexity of the apoptotic machinery is highlighted by the facts that a wide variety of stimuli can trigger the process and that in this process of apoptosis members of several protein families (e.g., Bcl-2 and caspases) act in concert in variable fashion depending on the nature of proapoptotic stimulus (Adams and Cory, 1998; Thornberry and Lazebnik, 1998). Among identified apoptosis-inducing factors are also vitamin D compounds, which have been shown to induce apoptosis, although not universally, in numerous normal and cancer cell types (Benassi et al., 1997; Campbell et al., 1997; Fife et al., 1997; Danielsson et al., 1998; Nickerson and Huynh, 1999; Diaz et al., 2000).

The most extensively studied model for vitamin D-induced apoptosis is the MCF-7 breast cancer cell line, which exhibits several morphological and biochemical markers of apoptosis after treatment with 1,25(OH)$_2$D$_3$ (James et al., 1996; Simboli-Campbell et al., 1996). Among signs of apoptosis in this cell line are upregulation of clusterin and cathepsin B, which are proteins associated with mammary gland regression. Based on comparison of the effects of 1,25(OH)$_2$D$_3$ and another growth inhibitory factor, tetradecanoylphorbol (TPA), on MCF-7 cells, it has been suggested that cell-cycle arrest is not sufficient to trigger apoptosis, but 1,25(OH)$_2$D$_3$ generates additional signals which lead to the induction of apoptosis in these cells (Narvaez and Welsh, 1997). These signals are apparently not generated in the vitamin D-resistant variant of MCF-7 cells (MCF-7D$_3$Res), despite expression of functional VDR (Narvaez et al., 1996). However, the importance of VDR in programmed cell death has been demonstrated in rat C6 glioma cells by studying a subclone of this cell line which does not express VDR (Davoust et al., 1998). It appeared that the stable transfection of VDR cDNA into this clone restored, albeit not consistently, the capability of 1,25(OH)$_2$D$_3$ to induce cell death.

Although the overall picture of how vitamin D induces apoptosis is unclear, there is evidence that vitamin D may control members of the Bcl-2 family, which have been shown to play one of the major roles in the signaling mechanism of apoptosis. This protein family consists of both antiapoptotic proteins such as Bcl-2 and Bcl-X$_L$ and proapoptotic proteins including Bax, Bak, and Bad (Antonsson and Martinou, 2000). By forming homo- and heterocomplexes, these proteins control apoptosis by regulating mitochondrial permeability and the release of cytochrome c, which leads ultimately to activation of the caspase cascade (see below).
The regulation of balance between antiapoptotic and proapoptotic members of the Bcl-2 family plays a major role in the determination of cell fate in response to some proapoptotic signals (Adams and Cory, 1998). There are a number of studies showing that 1,25(OH)\(_2\)D\(_3\) controls expression of some members of this family. For example, downregulation of the antiapoptotic Bcl-2 protein by 1,25(OH)\(_2\)D\(_3\) is a well-documented phenomenon in MCF-7 cells (Simboli-Campbell et al., 1997; James et al., 1998). Moreover, the capability of 1,25(OH)\(_2\)D\(_3\) to induce apoptosis in MCF-7 cells has been shown to be greatly impaired when Bcl-2 has been overexpressed in these cells. Also, LNcaP prostate cancer cells have been shown to undergo apoptosis after 1,25(OH)\(_2\)D\(_3\) treatment, although there are varying reports concerning the extent of the induced cell death (Fife et al., 1997; Hsieh and Wu, 1997; Blutt et al., 2000). In the study by Blutt et al. (2000), 1,25(OH)\(_2\)D\(_3\) was shown to induce a fivefold increase in the apoptosis of LNcaP cells accompanied by downregulation of Bcl-2 and Bcl-X\(_L\) antiapoptotic proteins. The association of Bcl-2 downregulation with apoptosis was assessed with cells overexpressing the Bcl-2 gene. In these cells, although failing to induce apoptosis, 1,25(OH)\(_2\)D\(_3\) was still able to cause marked growth inhibition. This indicates that in LNcaP cells both cell-cycle arrest and apoptosis are involved in the action of 1,25(OH)\(_2\)D\(_3\). Likewise, neither the capability of 1,25(OH)\(_2\)D\(_3\) to trigger apoptosis nor the downregulation of Bcl-2 in the apoptotic process is straightforward, but displays cell specificity. In HL60 myeloid leukemia cells and in normal human thyrocytes 1,25(OH)\(_2\)D\(_3\) has been shown to protect cells against apoptosis, but in HL60 cells 1,25(OH)\(_2\)D\(_3\) induces downregulation, and in thyrocytes upregulation, of Bcl-2 (Xu et al., 1993; S. H. Wang et al., 1999). Further evidence for the involvement of the Bcl-2 family in the apoptotic mechanism of 1,25(OH)\(_2\)D\(_3\) has recently been gained from colorectal adenoma and carcinoma cells in which 1,25(OH)\(_2\)D\(_3\) was shown to induce apoptosis and consistently increase proapoptotic protein Bak (Diaz et al., 2000).

Besides the Bcl-2 family, caspases also act as crucial effector molecules in apoptosis. Caspases are a family of cysteine proteases comprising initiator caspases, which are responsible for initiating apoptosis events upon activation by proapoptotic signals, and effector caspases, whose activation by initiator caspases (the caspase cascade) causes disassembly of cell structures (Thornberry and Lazebnik, 1998). Recent studies with MCF-7 cells indicate that activation of known caspases may not play a key role in 1,25(OH)\(_2\)D\(_3\)-induced apoptotic signaling. In a study by Mathiasen et al. (1999) inhibition of caspase activity with
the caspase inhibitor CrmA or with inhibitory peptides did not affect the induction of apoptosis by 1,25(OH)2D3. It was also concluded that 1,25(OH)2D3-mediated apoptosis is a tumor suppressor protein p53-independent phenomenon, since 1,25(OH)2D3 induced apoptosis both in MCF-7 cells expressing wild-type p53 protein and in T47D cells, which lack functional p53. In a related study, 1,25(OH)2D3 was shown to induce apoptosis in MCF-7 cells by disruption of the mitochondrial function accompanied by Bax translocation to mitochondria, cytochrome c release, and production of reactive oxygen species (Narvaez and Welsh, 2000). The observed mitochondrial effects were found to be independent of caspase activation and not to take place in an MCF-7 clone which is resistant to 1,25(OH)2D3-mediated apoptosis. However, caspase inhibition was found to prevent some apoptotic events associated with caspase activation downstream of the mitochondria. Thus, commitment of MCF-7 cells to apoptosis by 1,25(OH)2D3 is likely to occur independently of known caspases, although hormone may activate some late effector caspases. On the other hand, Park et al. (2000a) found that in NCI-H929 myeloma cells EB1089 induces apoptosis along with an increase in the activity of the effector caspase 3. In addition, EB1089 caused a decrease in the activity of p44 extracellular signal-related kinase and an increase in the activity of the p38 kinase during apoptosis of these cells, suggesting that these signal transduction pathways may be involved in the apoptotic process.

Finally, there is also evidence that the tumor necrosis factor-α (TNF-α) signaling pathway may contribute to vitamin D-induced apoptosis. In the human HaCat keratinocytes vitamin D compounds induce sphingomyelin hydrolysis with an increase in the amount of cellular ceramide, the known inducer of apoptosis (Hannun, 1996; Geilen et al., 1997). The observed sphingomyelin hydrolysis was found to be mediated by 1,25(OH)2D3-induced expression of TNF-α. On the other hand, in MCF-7 cells the vitamin D analog CB 1093 has been found to enhance cytosolic phospholipase A2 (cPLA2) activation in TNF-α-mediated apoptosis (Pirianov et al., 1999). These two findings may reflect a multiple action of vitamin D in the TNF-α signaling pathway, since the TNF-α effect on sphingomyelin hydrolysis has been shown to be mediated in HL60 cells by arachidonic acid (Jayadev et al., 1994). Interestingly, in U937 human leukemic cells 1,25(OH)2D3 has been shown to inhibit TNF-α-induced apoptosis by interfering with cPLA2 activation, whereas in HL60 cells 1,25(OH)2D3 is suggested to inhibit ceramide-induced apoptosis by activation of sphingosine kinase (Kleuser et al., 1998; Y. L. Wu et al., 1998).
IV. THE ROLE OF VITAMIN D CROSSTALK IN GROWTH FACTOR AND HORMONE SIGNALING

A. INTERACTIONS WITH GROWTH FACTOR SIGNALING

Vitamin D regulates the expression of several growth factors and their receptors and possibly also the availability of the growth factors in tissues. The efficacy of 1,25(OH)₂D₃ in the regulation of cell differentiation and growth might thus depend critically on its ability to regulate specific paracrine–autocrine signaling in tissues. Growth factors like EGF, fibroblast growth factor (FGF), TGF-β, and insulin-like growth factor (IGF) as well as some lymphokines have been shown to be affected by vitamin D and its analogs (Table I).

<table>
<thead>
<tr>
<th>Cytokine/receptor/BP</th>
<th>Cell type</th>
<th>Regulation</th>
<th>Related cellular function</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGF receptor</td>
<td>Colon cancer</td>
<td>↓</td>
<td>Growth inhibition</td>
<td>Tong et al., 1999</td>
</tr>
<tr>
<td></td>
<td>Keratinocytes</td>
<td>↓</td>
<td>Growth inhibition</td>
<td>Boisseau-Garsaud et al., 1996</td>
</tr>
<tr>
<td></td>
<td>Keratinocytes</td>
<td>↑</td>
<td>Growth stimulation</td>
<td>Garach-Jehoshua et al., 1999</td>
</tr>
<tr>
<td>FGF-7</td>
<td>Prostate cancer cells</td>
<td>↑</td>
<td>Growth inhibition</td>
<td>Lyakhovich et al., 2000</td>
</tr>
<tr>
<td>IGF-I</td>
<td>Osteoblasts</td>
<td>↑</td>
<td>Growth inhibition</td>
<td>Scharla et al., 1991</td>
</tr>
<tr>
<td>IGF-I receptor</td>
<td>Breast cancer</td>
<td>↓</td>
<td>Growth inhibition</td>
<td>Xie et al., 1997</td>
</tr>
<tr>
<td>IGFBPs</td>
<td>Osteoblasts?</td>
<td>↑</td>
<td>Growth inhibition</td>
<td>Scharla et al., 1991</td>
</tr>
<tr>
<td></td>
<td>Osteosarcoma cells</td>
<td>↑</td>
<td>Growth inhibition</td>
<td>Nakao et al., 1994</td>
</tr>
<tr>
<td></td>
<td>Breast cancer</td>
<td>↑</td>
<td>Growth inhibition</td>
<td>Rozen et al., 1997</td>
</tr>
<tr>
<td></td>
<td>Prostate cancer</td>
<td>↑</td>
<td>Growth inhibition</td>
<td>Huynh et al., 1998</td>
</tr>
<tr>
<td>Interleukins (-2,-6,-8)</td>
<td>Monocytes</td>
<td>↓</td>
<td>Growth inhibition</td>
<td>Muller et al., 1993</td>
</tr>
<tr>
<td></td>
<td>Keratinocytes</td>
<td>↓</td>
<td></td>
<td>Komine et al., 1999</td>
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<td></td>
<td>Fibroblasts</td>
<td>↓</td>
<td></td>
<td>Srivastava et al., 1994</td>
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<tr>
<td></td>
<td>Chondrocytes</td>
<td>↓</td>
<td>Growth inhibition</td>
<td>Saggese et al., 1993</td>
</tr>
<tr>
<td>Interferon-γ</td>
<td>Monocytes</td>
<td>↓</td>
<td>Growth inhibition</td>
<td>Muller et al., 1993</td>
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<td>TGF-β1</td>
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<td>Growth inhibition</td>
<td>Mercier et al., 1996</td>
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<td>Growth inhibition</td>
<td>G. Wu et al., 1997</td>
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<tr>
<td>TGF-β receptors I and II</td>
<td>Leukemia cells</td>
<td>↑</td>
<td></td>
<td>Jung et al., 1999</td>
</tr>
</tbody>
</table>

*When studied, the effect of vitamin D on cell proliferation is also indicated.*
TGF-β has been shown to reduce the proliferation of many cell types, such as epithelial, leukemic, and bone cells. In breast cancer cells vitamin D inhibits cell proliferation in a dose-dependent manner and the growth inhibition is accompanied by an increase in the expression of TGF-β1. The growth inhibition can be blocked by neutralizing anti-TGF-β antibodies (Mercier et al., 1996). Treatment of bone cells with 1,25(OH)₂D₃ increases TGF-β2 concentrations in human osteoblast cell supernatants in a dose- and time-dependent manner and the increase in TGF-β2 concentrations correlates with an inhibition of osteoblast cell growth. Antibodies directed against TGF-β partially block the inhibition of cellular growth mediated by 1,25(OH)₂D₃ (Y. Wu et al., 1997). With leukemic cells a synergistic action of the TGF-β and vitamin D for cell proliferation has been demonstrated. Clonal growth of HL60 cells has been inhibited in a dose-dependent manner by the vitamin D analog EB1089. Although TGF-β1 alone slightly inhibited proliferation of HL60 cells, the addition of TGF-β1 together with vitamin D analog to the culture elicited a significant synergistic antiproliferative effect in a dose-dependent manner. The antiproliferative effect of EB1089 was partially reversed by TGF-β1-neutralizing antibody (anti-TGF-β1) (Jung et al., 1999).

Vitamin D can influence the expression of TGF-β receptor. In HL60 cells and in osteoblasts EB1089 upregulates the expression of TGF-β receptor type I (TGF-β RI) and type II (TGF-β RII) (Y. Wu et al., 1997; Jung et al., 1999). Vitamin D can also regulate the availability of TGF-β1 for the cells, probably by regulating the synthesis of the latent TGF-β-binding protein. For instance 1,25(OH)₂D₃ also causes a dose-dependent increase in the amount of TGF-β1 protein found in the matrix and a corresponding decrease in TGF-β1 in the media (Pedrozo et al., 1999b).

Vitamin D has also been shown to inhibit the signal transduction pathways of growth factors whose functions are associated with an increase in cell proliferation, like EGF, IGF, and interleukins. Paradoxically, FGF expression is increased in LNCaP prostate cancer cells, although cell proliferation is inhibited (Lyakhovich et al., 2000).

1,25(OH)₂D₃ induces differentiation and inhibits proliferation of bone cells. 1,25(OH)₂D₃ has been shown to inhibit IGF-I release in a dose-dependent manner, in that IGF-I secretion was completely prevented from 6 h onward in 1,25(OH)₂D₃-treated cultures (Scharla et al., 1991). 1,25(OH)₂D₃ treatment also increases the secretion of IGF-binding protein-4 (IGFBP-4) up to 14-fold over 24 h. These events are associated with a decrease in cell proliferation, suggesting that effects of 1,25(OH)₂D₃ on osteoblast proliferation may be mediated in part by decreased levels of IGF-I and increased concentrations of inhibitory
IGFBP-4 (Scharla et al., 1991). Similar upregulation of IGFBPs and downregulation of IGFs by vitamin D and its analogs has been reported in breast cancer, prostate cancer, and osteosarcoma cells. Treatment of these cells with anti-IGF antibody and exogenous recombinant human IGFBP equally inhibited cell proliferation, suggesting that regulation of IGF and its binding protein expression can indeed control cell growth. (Nakao et al., 1994; Rozen et al., 1997; Colston et al., 1998; Huynh et al., 1998), Vitamin D derivatives have also been demonstrated to reduce IGF-I receptor expression in MCF-7 cells, thus limiting responsiveness of MCF-7 cells to the mitogenic effects of IGF-I (Xie et al., 1997). Paradoxically, however, after prolonged treatment with vitamin D (8 days), an upregulation of insulin and IGF-I binding was observed (Vink-van Wijngaarden et al., 1996).

In colon cancer cells 1,25(OH)₂D₃ has been observed to reduce basal cell proliferation by about 50%. When given in combination with EGF, it prevented any rise in proliferation when colon cancer cells were treated with 25 ng/ml EGF, and reduced basal and EGF-stimulated expression of cyclin D1 level in primary cultures as well as in the Caco-2 cell line. This phenomenon was associated with a marked inhibitory effect of 1,25(OH)₂D₃ on EGF receptor expression. Thus the efficacy of 1,25(OH)₂D₃ in the treatment of colon cancer might depend critically on its ability to specifically counteract EGF-stimulated tumor cell growth (Tong et al., 1999).

The effect of vitamin D on keratinocytes seems to depend on the experimental conditions. In vitro, steroid either stimulates or inhibits keratinocyte proliferation, depending on cell culture conditions and vitamin D concentration (Boisseau-Garsaud et al., 1996; Garach-Jehoshua et al., 1999). The growth-inhibitory or growth-stimulatory effects correlated with the stimulation or attenuation of EGF signal transduction pathways, respectively. In conditions where vitamin D inhibited cell growth, it downregulated the expression of EGF receptor (Boisseau-Garsaud et al., 1996), whereas in experiments where vitamin D stimulated keratinocyte growth, cell proliferation could be inhibited by a specific inhibitor of the EGF tyrosine kinase and by EGFR-neutralizing antibody. When stimulating keratinocyte growth 1,25(OH)₂D₃ also caused a marked increase in EGFR expression, which could account for the stimulatory effect of 1,25(OH)₂D₃ on cell proliferation (Garach-Jehoshua et al., 1999).

Vitamin D inhibits the proliferation of mitogen-stimulated leukocytes like T cells and peripheral blood mononuclear cells in a dose-dependent manner (Saggese et al., 1989; Muller et al., 1993). 1,25(OH)₂D₃ treatment in vitro reduces the levels of IL-2 and interferon-γ. This is followed
by a time- and dose-dependent reduction in proliferation. Thus leukocytes may be a direct target for 1,25(OH)₂D₃, resulting in a specific reduction in IL-2 levels and inhibition of proliferation (Muller et al., 1993). Vitamin D and its analogs can inhibit lymphokine secretion also in other cell types besides leukocytes. Interleukin-6 (IL-6) and IL-8 production is inhibited by vitamin D in keratinocytes (Koizumi et al., 1997; Komine et al., 1999), in human fibroblasts (Srivastava et al., 1994), and in chondrocytes (Saggese et al., 1993).

Vitamin D can thus suppress the inflammatory processes by directly affecting the immunocompetent cells as well as inhibiting lymphokine expression by various cells. For example, hyperproliferation of the epidermis and oversecretion of IL-8 by keratinocytes are the characteristic features of psoriasis. The inhibition of IL-8 secretion from keratinocytes by vitamin D₃ could modulate the behavior of immunocompetent cells infiltrating into the skin. Thus vitamin D not only affects keratinocyte proliferation in psoriasis as noted, but can also modulate local immunoreactions. Whether vitamin D could have a therapeutic potential in rheumatoid arthritis through immunomodulatory effects in vivo remains to be studied.

B. CROSSTALK WITH NUCLEAR RECEPTORS AND THEIR LIGANDS

In a variety of cell lines 9-cis-retinoic acid (9cRA) and all-trans-retinoic acid (ATRA) inhibit cell proliferation or induce differentiation and apoptosis in synergy with vitamin D, although the opposite action of these ligands on differentiation has been reported in macrophages and keratinocytes, as mentioned above, and on apoptosis in pancreatic adenocarcinoma cell lines (Pettersson et al., 2000). Combination of 1,25(OH)₂D₃ and 9cRA reduced cell proliferation and induced apoptosis in an additive manner compared to either 1,25(OH)₂D₃ or 9cRA alone in small cell lung carcinoma cell lines (NCI-H82 and NCI-H209) (Guzey et al., 1998). Synergistic growth inhibition and accumulation of cells to the G₁ phase of the cell cycle has been shown in LNCaP cells (Blutt et al., 1997). This inhibition was strongest with a combination of 1,25(OH)₂D₃ and the RXR-specific ligand LG100268, suggesting that VDR/RXR heterodimers are involved in the regulation of proliferation, or that the RXR homodimer regulates some protein which enhances the response to 1,25(OH)₂D₃. Campbell et al. (1998) suggested that expression of RARβ is also needed for synergistic growth inhibition by vitamin D and retinoids or their analogs in prostate cancer cell lines. Studies performed with HL60 cells also suggest synergistic action of vitamin D or its analogs and retinoids on differentiation and apoptosis;
however, effects are complex and dependent on the concentration of the ligands (Bunce et al., 1995; Elstner et al., 1996). In MCF-7 (Saunders et al., 1995) and T-47D cells (Koga and Sutherland, 1991) retinoids increased the growth-inhibitory action of vitamin D. Similarly, MCF-7 cell growth in nude mice was inhibited additively by vitamin D analogs and all-trans-retinoic acid (Koshizuka et al., 1999b). In immunosuppressed mice injected with transformed keratinocytes the tumor-induced angiogenesis was significantly inhibited by retinoids or 1,25(OH)2D3 and the inhibition was synergistic in a combination therapy (Majewski et al., 1993). Regulation of RAR by 1,25(OH)2D3 treatment has been shown in MCF-7 cells, since 1,25(OH)2D3 treatment upregulated RARα, although vitamin D did not affect cell proliferation in this experiment (Schneider et al., 1999).

Glucocorticoids modulate the effects of vitamin D on osteoblast, osteoclast, and adipocyte differentiation, although effects seem to be complex and dependent on cell models and differentiation stage (Raisz and Lukert, 1997). For example, vitamin D induces osteocalcin synthesis in osteosarcoma MG63 cells (Pirskanen et al., 1991) and in Ros 17/2.8 cells (Schepmoes et al., 1991) and this induction is reduced by glucocorticoids. However, in adult human marrow stromal cells and in fetal rat calvaria-derived osteoblasts dexamethasone enhances the induction of osteocalcin expression by vitamin D (Shalhoub et al., 1992). In murine femoral-derived bone marrow stromal cells 1,25(OH)2D3 inhibited the adipogenesis induced by glucocorticoids (Kelly and Gimble, 1998), but in murine Ob 17 preadipocytes the effect on adipose differentiation was biphasic (Lenoir et al., 1996). At low concentrations (0.25 nM or less) 1,25(OH)2D3 stimulated terminal adipose differentiation, but at higher concentrations (>0.25 nM) it inhibited differentiation. In these preadipocytes vitamin D downregulated TR, suggesting that regulation of TR by vitamin D might be involved in the regulation of adipose differentiation. In the adipocyte 3T3-L1 cell line 1,25(OH)2D3 inhibited the differentiation and the expression of PPARγ2 (Hida et al., 1998).

In reproductive tissues the level of sex steroids and the expression of sex steroid receptors have a significant role in controlling cell growth and differentiation. Hence crosstalk between vitamin D and sex steroids has been one aspect of studies concerning regulation of proliferation in normal and malignant cells. It is well known that in breast and breast cancer cells estradiol has growth-stimulatory effects. This estradiol-induced growth stimulation has been shown to be reduced by 1,25(OH)2D3 treatment (Vink-van Wijngaarden et al., 1994). Also, 1,25(OH)2D3 alone has been shown to cause dose-dependent inhibition
of cell growth (Chouvet et al., 1986; Simpson and Arnold, 1986; Eisman et al., 1989). In MCF-7 cells the inhibition of cell growth was associated with decreased estrogen receptor (ER) protein levels, which was held to be due to direct inhibition of ER gene transcription (James et al., 1994; Swami et al., 2000). Furthermore, the growth-inhibitory actions of 1,25(OH)₂D₃ and its analogs correlated strictly with their ability to downregulate ER levels in MCF-7 cells (Swami et al., 2000). Stoica et al. (1999) showed a potential vitamin D response element within the ER promoter. However, 1,25(OH)₂D₃ and its analogs also inhibited proliferation in ER-negative cell lines, suggesting that other signaling routes are also involved (Chouvet et al., 1986; Abe et al., 1991; Nolan et al., 1998). In addition, in ER-positive T-47D cells treatment with 1,25(OH)₂D₃ did not regulate the ER protein level, although it inhibited cell growth (Davoodi et al., 1995). Local regulation of active 17β-estradiol in tissues might be one possible mechanism whereby vitamin D affects cell proliferation.

In keratinocytes antiproliferative doses of 1,25(OH)₂D₃ caused upregulation of 17β-hydroxysteroid dehydrogenase (17βHSD) type II mRNA levels and increased the inactivation of estradiol to estrone (Hughes et al., 1997). Similarly, in HL60 cells 1,25(OH)₂D₃ increased the conversion of estradiol to estrone at differentiation-inducing levels of 1,25(OH)₂D₃ (Mountford et al., 1999). Aromatase P450 (P450AROM) catalyzes biosynthesis of estrogen from C19 steroids. In cultured osteoblasts glucocorticoids induce the expression and enzymatic activity of P450AROM and this induction is enhanced by 1,25(OH)₂D₃ (Tanaka et al., 1996). However, 1,25(OH)₂D₃ alone does not induce aromatase activity. Furthermore, evidence for interaction of VDR and the P450AROM-encoding gene CYP19 has been shown in choriocarcinoma cell line JEG-3 (Sun et al., 1998). In these cells 1,25(OH)₂D₃ induced activity of P450AROM, and RXR/VDR heterodimers were found to bind to an imperfect palindromic response element on the CYP19 gene in response to both receptor ligands. More information on aromatase activity has been gained in studies with VDR-null mutant mice (Kinuta et al., 2000). VDR-null mutant female mice had uterine hypoplasia and impaired folliculogenesis and VDR-null mutant males had histological abnormalities in the testis and decreased sperm counts and motility. Gonads of these mice resembled those of mice with estrogen deficiency, for example, ERα knockout and aromatase gene-deficient mice. Further analysis revealed that VDR-null mutant mice showed decreased P450AROM activity and decreased expression of the aromatase gene, CYP19. When the calcium homeostasis was normalized by a high calcium diet the
level of aromatase activity was increased, as was the expression of the CYP19 gene. These results suggested that vitamin D-maintained calcium homeostasis at least partially regulates estrogen biosynthesis in gonads.

Crosstalk between vitamin D and androgens has been studied in prostate and ovarian cancer cells. Vitamin D has been shown to have an antiproliferative effect on different prostate cancer cell lines; however, the mechanism of action seems to be dependent on the cell line (Skowronski et al., 1993; Peehl et al., 1994; Schwartz et al., 1994). In LNCaP cells, which have high-affinity receptors for androgen, the mechanism was androgen receptor (AR)-dependent (X. Y. Zhao et al., 1999). Treatment with 1,25(OH)2D3 indirectly induced the expression of AR and the antiproliferative effect was blocked by the pure AR antagonist Casodex. In MDA PCa 2a and 2b cells, which have low-affinity receptors for androgen, 1,25(OH)2D3 increased the mRNA level of AR; however, the antiproliferative effect was not blocked by AR antagonist, suggesting an AR-independent mechanism (X. Y. Zhao et al., 2000). Crosstalk between 1,25(OH)2D3 and androgens has also been studied in ovarian cancer cell line OVCAR-3, which is both VDR- and AR-positive and in which both 1,25(OH)2D3 and DHT upregulated VDR and AR expression (Ahonen et al., 2000b). Treatment with 1,25(OH)2D3 inhibited growth and even reduced DHT-induced cell proliferation, suggesting that vitamin D and its analogs might have role in the treatment of ovarian tumors.

V. VITAMIN D AND CANCER

A. EPIDEMIOLOGY

Epidemiological studies have shown a link between low vitamin D levels and the incidence of cancer, suggesting an antitumor role for vitamin D (van Leeuwen and Pols, 1997). The cancers in which the role of vitamin D has been studied include in particular hormone-related cancers like prostate and breast cancer, and colon cancer. In addition to these common cancers, receptors for vitamin D have been detected in many other malignancies including mostly other carcinomas, but also in leukemias and other hematopoietic malignancies as well as in some sarcomas (van Leeuwen and Pols, 1997). The presence of nuclear VDR in these malignancies as well as in the corresponding normal cells supports the concept that these tissues and cells are vitamin D target organs (Feldman et al., 2000).
Schwartz and Hulka were the first to suggest vitamin D deficiency as a risk factor for prostate cancer and it was later estimated that UV exposure accounts for 6% of the variation of prostate cancer mortality (Schwartz and Hulka, 1990; Hanchette and Schwartz, 1992). There seems to be widespread vitamin D deficiency in elderly people, which was suggested to be an important factor in the progression of cancer (Schwartz and Hulka, 1990). However, as has been shown recently, the importance of vitamin D deficiency may be even more critical during the earlier steps of prostate carcinogenesis (Ahonen et al., 2000a). It seems also evident that prostate cancer by itself can have an adverse effect on vitamin D homeostasis and various treatments for prostate cancer may alter circulating 1,25(OH)\textsubscript{2}D\textsubscript{3} levels (Miller, 1998).

Garland et al. showed geographic variation in breast cancer mortality in the United States, supporting the hypothesis involving exposure to solar radiation in the etiology of breast cancer, a hypothesis confirmed by other studies (F. C. Garland et al., 1990; Gorham et al., 1990). However, no differences in vitamin D intake between breast cancer patients and controls was noted in a Canadian study (Simard et al., 1991). A protective effect of 1,25(OH)\textsubscript{2}D\textsubscript{3} for breast cancer in White women was noted (Janowsky et al., 1999), whereas an opposite correlation was noted in Black women, although the number of Black women in the study was small.

First evidence linking vitamin D and colon cancer was derived from death rate analysis of colon cancer in 1980. The death rates tended to increase with increasing latitude and decreasing sunlight (C. F. Garland and Garland, 1980). More direct evidence was obtained from serum measurements of 25OHD\textsubscript{3} and the incidence of colon cancer (C. F. Garland et al., 1989). Niv et al. (1999) demonstrated an inverse correlation between serum levels of the active metabolite of vitamin D and colorectal carcinoma stage. The association of calcium, vitamin D, and colorectal cancer based on existing epidemiological studies was evaluated by Martinez and Willett (1998). The authors concluded that the results for vitamin D suggest that it is inversely associated with colon cancer risk, but additional studies are needed to investigate this relation in more detail.

B. **Vitamin D Receptor Polymorphism and Cancer**

The presence of vitamin D receptor in cancer cells seems to be a prerequisite for vitamin D action. When comparing newly diagnosed breast cancers with relapsed, metastasized disease, Ruggiero et al. (1998) found vitamin D receptor gene polymorphism to be associated
with the metastatic disease. Polymorphism of the VDR gene may influence tumor progression and response to tamoxifen treatment in early-onset (≤37 years) breast cancer (Lundin et al., 1999). Although VDR polymorphism has been associated with prostate cancer in some studies (Taylor et al., 1996; Ingles et al., 1997); no association was found between vitamin D receptor genotype and lethal prostate cancer in the study of Kibel et al. (1998). In human rectal cancer, the vitamin D receptor genotype alone did not influence survival, whereas vitamin D receptor gene BsmI polymorphism was thought to affect the development and prognosis by influencing erbB-2 oncogene expression (Speer et al., 2000).

C. MECHANISMS OF ANTITUMOR EFFECT IN CANCER

So far, most of the experimental data on the antitumor effects and mechanisms of vitamin D action has been obtained from various culture systems in vitro and in animal models in vivo (Miller, 1998; Blutt and Weigel, 1999; Feldman et al., 2000). These antitumor mechanisms include mainly inhibitory effects on cell cycle and proliferation. They include differentiation in many cancer cells and increase in apoptosis in some cancer cell lines. The antitumor effect of vitamin D can be modified by interaction with growth factors and other steroid hormones. High doses of calcitriol inhibit invasion of some cancer cells. Vitamin D may exert an antitumor effect also through an antiangiogenic activity (Majewski et al., 1996). Metastasis of, for example, prostate cancer in the rat Dunning tumor model has been largely inhibited by vitamin D and by its analog EB1089, which did not produce unacceptable hypercalcemia (Lokeshwar et al., 1999). Vitamin D is a potent immuno-suppressor (Lemire, 1997), and this property may have an effect on its antitumor activity. Koren et al. (2000) suggested that 1,25(OH)₂D₃ can act synergistically with anticancer cytokines present in the tumor milieu and that reactive oxygen species have a mediating role in this interaction. The potential antitumor mechanisms of vitamin D in cancer are summarized in Fig. 4.

D. CLINICAL TRIALS

The great majority of causal relationships suggested by epidemiology have not been validated by intervention trials, and therefore caution in interpreting epidemiological findings is warranted (Young and Lee, 1999). The lack of vitamin D intervention studies in humans is largely due to the known toxicity of the active form of vitamin D, calcitriol,
resulting in induction of hypercalcemia. However, D. C. Smith et al. (1999) demonstrated that substantial doses of calcitriol can be administered subcutaneously every other day with tolerable toxicity. The newly developed, less calcemic analogs provide even better tools for treatment trials (Blutt and Weigel, 1999). A phase I study of the effects of vitamin D analog EB1089 in advanced breast and colon cancer showed this analog to be less calcemic than 1,25-dihydroxyvitamin D₃, showing stabilization of the disease in 6 of 36 patients, but with no complete or partial responses (Gulliford et al., 1998). There have also been only limited trials to study the effects of vitamin D or its analogs in prostate cancer in vivo (Feldman et al., 2000), including a small phase II trial of calcitriol in hormone-refractory metastatic carcinoma (Osborn et al., 1995) and a small trial with nonmetastasized prostate cancer in patients with a rising prostate-specific antigen (PSA) as the only measure of recurrent disease (Gross et al., 1998). In spite of the scanty results of these trials, in his review Miller (1998) concludes that the time is ripe to consider the use of vitamin D as a clinical tool in the treatment of prostate cancer, either in the form of chemoprevention trials or in treatment trials, up to hormone-refractory advanced disease.

Treatment experiments in other human tumors and malignancies have been few. They include some trials of treatment of myelodysplastic syndromes with 1,25(OH)₂D₃, aimed at differentiation of the dysplastic myeloid cells, which, without treatment, often lead to leukemia (Koeffler et al., 1985; Richard et al., 1986). These trials were complicated with side-effects of hypercalcemia, and the clinical trials with vitamin D analogs are still in the very beginning stage.

Interaction of chemotherapeutic agents with vitamin D has been shown in the case of cisplatin, which decreases 1,25(OH)₂D₃ levels in the treatment of gynegological cancers (Gao et al., 1993). However, combination of vitamin D or its analogs with other chemotherapeutic agents or with radiation (Sundaram and Gewirtz, 1999) provides new
possibilities in the treatment of cancer. Vitamin D may, for example, sensitize cancer cells for chemotherapy-induced cell death (Q. Wang et al., 2000), and combination treatment may reduce the toxicity of individual agents (Koshizuka et al., 1999a). The role of vitamin D in immunosuppression may also be important in the development of such combination treatments (Lemire, 1997), and in cell culture experiments a synergistic effect of immune cytokines with vitamin D has been shown (Koren et al., 2000).

Although the results from treatment trials with vitamin D and its analogs in cancers have so far been quite modest, the potency of vitamin D treatment has already been shown in the treatment of skin diseases, especially psoriasis. In psoriasis there is an increased proliferation of epidermal stem cells, initiated by lymphokines released from the activated T cells within the skin (Griffiths, 1994). The VDR has been detected in most cell types of the skin, including keratinocytes, Langerhans cells, and activated T cells, which are all involved in psoriasis and serve as natural targets for vitamin D therapy. Development of the effective vitamin D analog calcipotriol and the possibility for local treatment have made psoriasis the best available human in vivo model to study the mechanisms of vitamin D action, many of which are also involved in cancer (Kragbelle, 1997).

Many cancers have a latent period up to 10–20 years, which provides ample time for preventive measures. Urological cancers were found to be especially suitable to the development of chemopreventive agents (Kamat and Lamm, 1999). In case of prostate cancer, in addition to the preventive effect of reduced fat intake, vitamin E, selenium, and soy proteins, a lesser benefit was suggested with intake of vitamins D and C (Kamat and Lamm, 1999; Brawley and Parnes, 2000). The chemoprevention of renal cancer by vitamin D supplementation was also suggested. A possible role for increased dietary calcium and vitamin D in the chemoprevention of breast cancer was noted (Lipkin and Newmark, 1999).

In chemoprevention of colorectal cancer aimed at primary prevention of at-risk subjects or altering the course of precursor lesions or established disease, vitamin D along with nonsteroidal antiinflammatory drugs seem the most promising agents (Langman and Boyle, 1998). Whelan et al. (1999) performed a case–control study to investigate the role of vitamin or calcium supplement in the incidence of adenoma recurrences. They found a protective effect of the use of multivitamins, vitamin E, and calcium supplement, whereas the protective effect of vitamin D alone was insignificant. The results emphasize the complex interactions among various effectors in the protective effect on cancer.
VI. Conclusions and Future Perspectives

Vitamin D has a potent growth-inhibitory function for many cell types with a multitude of mechanisms. It can regulate cell-cycle regulatory proteins, expression, of growth factors and their receptors, nuclear receptor expression, and local metabolism of nuclear receptor ligands (Fig. 5). The growth inhibition aspect is associated with the differentiation-promoting effects of particular cell types. In certain cell types vitamin D can also induce apoptosis or sensitize cells for apoptosis. Thus vitamin D has a wide range of potential therapeutic uses in treating cellular defects characterized by increased cell proliferation and decreased differentiation as in various cancers, immune responses, bone diseases, and psoriasis. Antiinflammatory effects of vitamin D might also be used in treating autoimmune diseases. Epidemiological studies have demonstrated that vitamin D exerts a protective effect against carcinogenesis, suggesting not only that it might be involved in regulating the growth of cancer cells, but that it can also have a role in regulating cancer initiation, promotion, and progression (Ahonen et al., 2000a).

Fig. 5. Schematic representation of factors modifying the antiproliferative action of vitamin D. The effects of the factors together with vitamin D on cell proliferation are indicated as follows: +, increase of proliferation; -, decrease of proliferation. The same symbols in parentheses indicate the effect of vitamin D on the corresponding gene expression.
It is apparent that in the future vitamin D will be widely used in the prevention and treatment of a number of diseases and also in disease prevention. Crosstalk between vitamin D and other signaling systems suggests the possibility of combination therapy. The usefulness of vitamin D is limited by the severe harmful effects arising from its hypercalcemic property. Much effort has therefore been directed to identifying new vitamin D analogs with potent cell-regulatory effects without the calcemic effect. In disease prevention, it is important to adjust vitamin D balance in the case of seasonal vitamin D deficiency and deficient nutritional intake by dietary vitamin D supplementation and by higher UV exposure.

ACKNOWLEDGMENTS

Work in this laboratory was financially supported by grants from the Medical Research Fund of Tampere University Hospital and the Finnish Cancer Foundation.

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ANTIPROLIFERATIVE ACTION OF VITAMIN D

391


ANTIPROLIFERATIVE ACTION OF VITAMIN D


