

The pleiotropic actions of vitamin D

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Summary

General knowledge of the role of vitamin D₃ in human physiology has been shaped by its discovery as a preventive agent of nutritional rickets, a defect in bone development due to inadequate uptake of dietary calcium. Studies on the function of the hormonal form of vitamin D₃, 1 α ,25-dihydroxyvitamin D₃, have been greatly accelerated by the molecular cloning and structural analysis of the vitamin D₃ receptor, which is a ligand-activated regulator of gene transcription. Molecular genetic techniques including genomics have helped reveal that 1 α ,25-dihydroxyvitamin D₃ can control more than calcium homeostasis. It has widespread effects on cellular differentiation and proliferation, and can modulate immune responsiveness, and central nervous system function. Moreover, accumulating epidemiological and molecular evidence suggests that 1 α ,25-dihydroxyvitamin D₃ acts as a chemopreventive agent against several malignancies including cancers of the prostate and colon. Here, we survey the most-recent findings and discuss their implications for the potential therapeutic uses of vitamin D analogues. *BioEssays* 26:21–28, 2004. © 2003 Wiley Periodicals, Inc.

Introduction

Vitamin D has been widely known for decades for its primary physiological role in regulating calcium homeostasis. However, accumulating evidence from epidemiological, animal, cellular, biochemical and, most recently, molecular genetic studies has revealed new, more subtle actions of vitamin D. Vitamin D can regulate the proliferation and differentiation of a wide variety of cell types, which has led to the analysis of the potential therapeutic uses of its synthetic analogues as anti-cancer agents, and as modulators of immune and nervous system function. These lines of investigation have been accelerated by two recent developments: the determination of the crystal structure of the vitamin D receptor and the use of large-scale gene expression profiling with microarrays to identify the molecular genetic events underlying vitamin D action. Here, we will provide a brief overview of the discovery of vitamin D and focus on the impacts of recent experimental and technological advances on the potential uses of its analogues in cancer therapy and prevention, in the treatment of autoimmune disorders, and as neuroprotective agents.

A brief history of vitamin D

We know now that vitamin D is not a true vitamin as it can be produced in adequate amounts by moderate exposure of skin to solar ultraviolet B rays. However, it was discovered in parallel lines of experimentation into the causes of rickets, which arises from insufficient uptake of dietary calcium, and is characterized by weakened, deformed bones, muscle spasms and seizures. Rickets became rampant in the growing polluted cities of 18th century Europe. While cod liver oil was discovered as a rich source of an antirachitic activity in 1827, its use did not catch on because of a lack of understanding of the importance of micronutrients. However, by the end of the century scientists were searching for dietary components, spurred on by observations that scurvy (lack of vitamin C) and beriberi (lack of vitamin B1) could be prevented by an appropriate diet. In 1822, a Polish physician, observing that rickets was rare in unpolluted rural areas, experimented with children and concluded that sunbathing cured rickets. By 1919, German researchers showed that artificially produced ultraviolet light could cure rickets. A connection between diet and photochemistry was drawn when feeding ultraviolet light-irradiated skin to rats was shown to be antirachitic, whereas unirradiated skin had no effect. In 1923, Goldblatt and Soames

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Funding agency: The Canadian Institutes of Health Research.

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DOI 10.1002/bies.10368

Published online in Wiley InterScience (www.interscience.wiley.com).

Abbreviations: 1,25(OH)₂D₃, 1 α ,25-dihydroxyvitamin D₃; AF-2, activating function-2; APC, antigen presenting cells; CDK, cyclin-dependent kinase; CNS, central nervous system; CYP, cytochrome P450; DBD, DNA-binding domain; DR, direct repeat; EAE, experimental autoimmune encephalomyelitis; ER, everted repeat; HNSCC, head and neck squamous cell carcinoma; IFN γ , interferon γ ; IL, interleukin; IP, inverted palindrome; LBD, ligand-binding domain; R274, Arginine 274; ROS, reactive oxygen species; RXR, retinoid X receptor; Th1, T helper 1; VDR, vitamin D receptor; VDRE, vitamin D response element.

showed that vitamin D activity could be produced by irradiation of 7-dehydrocholesterol with sunlight or ultraviolet light.⁽¹⁾

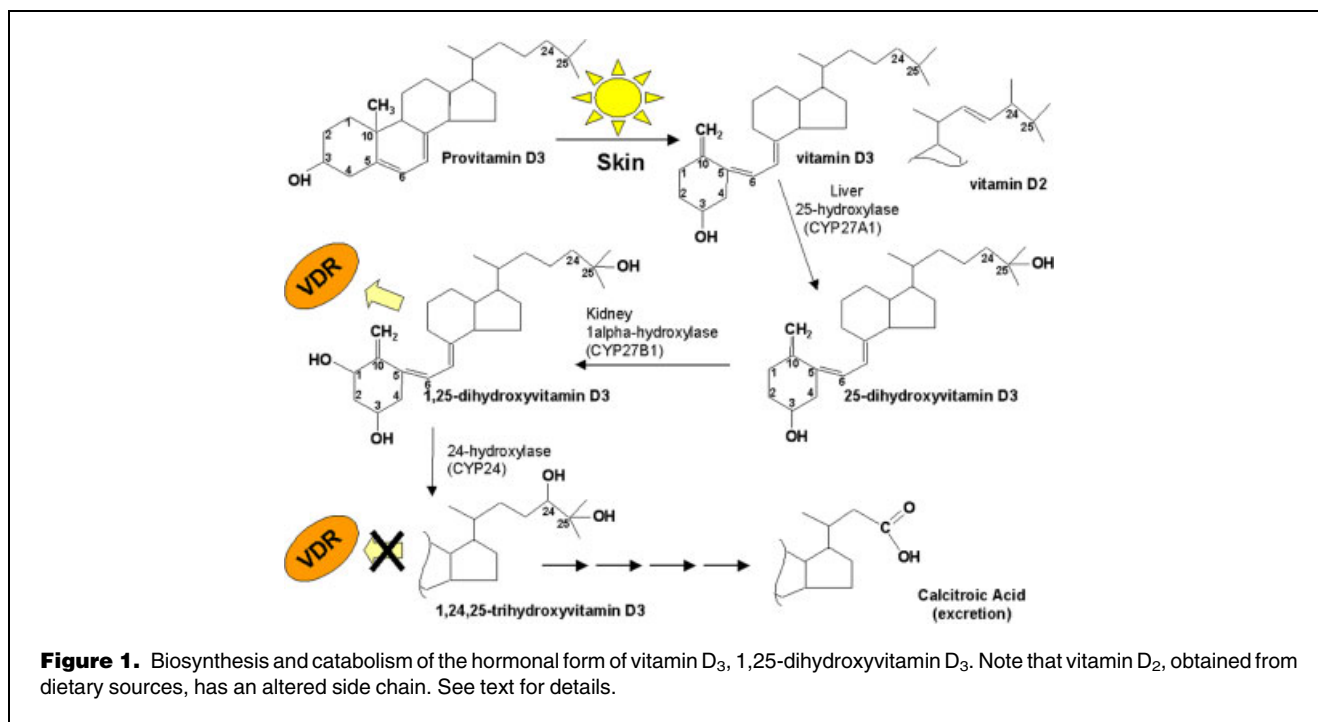
The structure of vitamin D₃ produced from irradiated 7-dehydrocholesterol was determined in 1936, and the anti-rachitic component of cod liver oil was shown to be structurally identical. Vitamin D₃ is steroidal, more specifically a secosteroid (Fig. 1). Note that vitamin D₃ derived from 7-dehydrocholesterol is distinguished from related vitamin D₂ (Fig. 1), obtained from some dietary sources.⁽²⁾ The preparation of radiolabeled compound culminated in 1971 with the establishment that the biologically active form of vitamin D₃ is produced by hepatic 25-hydroxylation, followed by 1 α -hydroxylation, primarily in the kidney. By 1975, the presence of the vitamin D receptor (VDR) was confirmed in the nuclei of cells incubated with radiolabelled hormonal 1 α ,25-dihydroxyvitamin D₃ [1 α ,25(OH)₂D₃].^(1,3) Vitamin D compounds are catabolized by 24-hydroxylation catalyzed by CYP24. 1 α ,24,25-trihydroxyvitamin D₃ (Fig. 1) is 10 times less potent than 1 α ,25(OH)₂D₃,⁽⁴⁾ and further oxidation leads to progressive loss of biological activity and production of water-soluble calcitroic acid, which is excreted.⁽²⁾

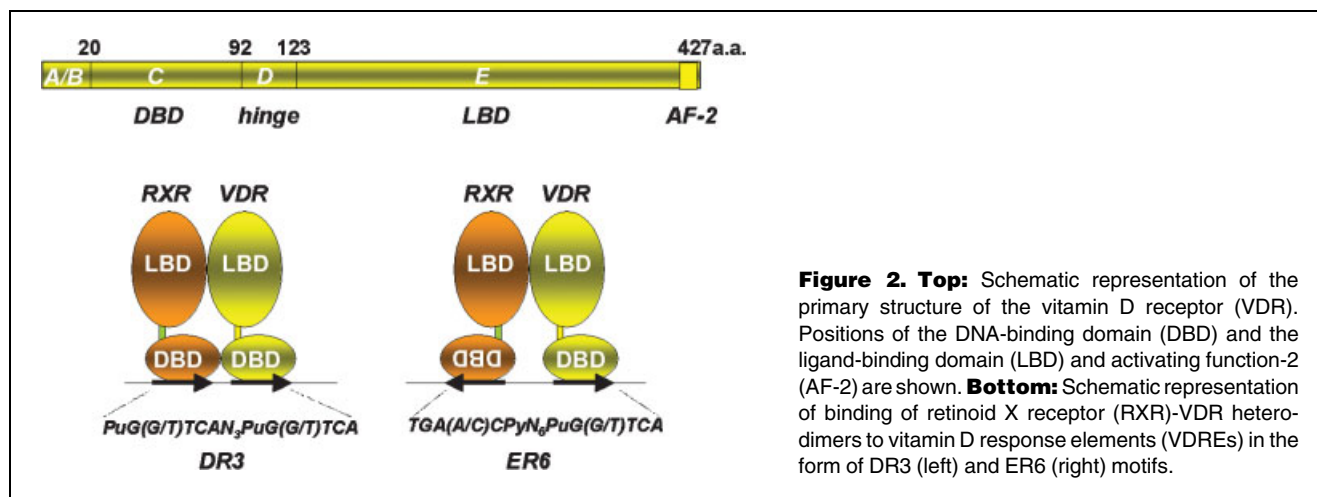
The vitamin D receptor—a hormone-regulated transcription factor

The cDNA encoding the human vitamin D receptor (VDR) was cloned in 1988,⁽⁵⁾ and confirmed that, similar to other steroid receptors, it is a member of the nuclear receptor family. Nuclear receptors are ligand-activated regulators of gene transcription⁽⁶⁾ with a conserved domain structure (Fig. 2). The

highly conserved DNA-binding domain (DBD) contains two zinc fingers that form a single structural domain containing an α -helical reading head that controls specific DNA sequence recognition. The VDR ligand-binding domain (LBD) not only binds ligand but also contains a ligand-regulated C-terminal AF-2 domain (activating function-2) that is essential for its capacity to activate transcription. Similar to several nuclear receptors, the VDR functions as a heterodimer with members of the retinoid X receptor (RXR) family of receptors. Strong interactions between VDR and RXR LBDs are essential for ligand-dependent dimerization and high-affinity DNA binding (Fig. 2).

Nuclear receptors regulate target gene transcription by ligand-controlled recruitment of several accessory proteins known collectively as coregulators. Coregulators are essential for the histone modifications, chromatin remodeling and recruitment of RNA polymerase and ancillary factors necessary for initiation of transcription. A detailed discussion of coregulator recruitment and function is beyond the scope of this review. Readers are referred to reviews on coregulators in general by McKenna and O'Malley,⁽⁷⁾ and those interacting with the ligand-bound VDR in particular by Rachez and Freedman.⁽⁸⁾ Nuclear receptors regulate transcription in part by binding specific DNA sequences called hormone response elements, which are composed of tandem hexameric motifs and normally located in the 5'-flanking region of target genes.⁽⁹⁾ Vitamin D response elements (VDREs) are composed of tandem motifs with the consensus PuG(G/T)TCA often arranged as direct repeats separated by three base pairs



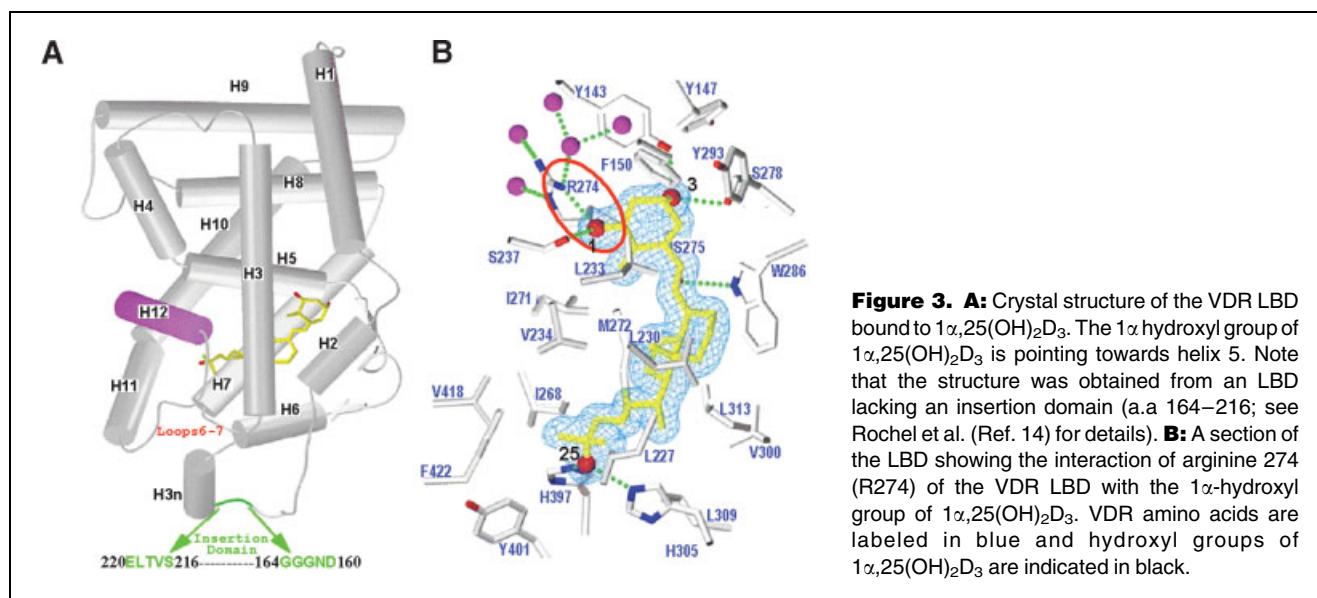


(DR3-type). Inverted palindromes with motifs spaced by nine nucleotides (IP9-type) have also been described.⁽¹⁰⁾ IP elements (toes pointing out) were so-named to distinguish them from palindromic response elements of steroid receptors (toes-in symmetry). VDRE nomenclature leaves some room for confusion, as elements defined as everted repeats with 6 bp spacing (ER6) (Fig. 2), with the same symmetry as IP9 elements, have been described in genes encoding members of the cytochrome P450 family.^(11–13)

The crystal structure of the VDR ligand-binding domain and design of synthetic ligands

The VDR LBD is composed of a series of α -helices (Fig. 3A), and is globally similar to those of other nuclear receptors. In the structure determined in the presence of $1\alpha,25(\text{OH})_2\text{D}_3$ ⁽¹⁴⁾ helix

12 (H12), which corresponds to the ligand-dependent AF-2 domain, is in the “active” conformation suitable for recruitment of coregulatory proteins required for activation of transcription. The structure also provides critical molecular details of the interactions controlling high-affinity ligand binding,⁽¹⁴⁾ and a molecular basis for the disruptive effects of VDR mutations linked to human vitamin D-resistant rickets (hVDRR).⁽¹⁵⁾ For example, mutations of arginine 274 (R274), which forms a critical hydrogen bond with the 1α -hydroxyl group of $1\alpha,25(\text{OH})_2\text{D}_3$ (Fig. 3B), strongly disrupt ligand binding. Remarkably, rational drug design techniques and the VDR crystal structure have led to development of synthetic ligands that have greatly increased affinity for R274 mutants and thereby restore receptor function.^(16–19) These approaches hold promise for the development of compounds to reverse the



symptoms of some cases of hVDRR in particular, and for the restoration of function to specific mutants of other nuclear receptors associated with genetic diseases.

The VDR LBD crystal structure will also be essential for optimization of novel synthetic $1\alpha,25(\text{OH})_2\text{D}_3$ analogues that target a number of indications. The calcemic activity of $1\alpha,25(\text{OH})_2\text{D}_3$ has limited its use in treatment of conditions not related to mineral ion homeostasis. Analogue development has therefore been driven by the desire for compounds with the therapeutic potential of $1\alpha,25(\text{OH})_2\text{D}_3$ but lacking its calcemic activity.^(20,21) Numerous secosteroid analogues with different ring and side-chain modifications have been synthesized, and many display reduced calcemic activity.⁽²¹⁾ More recent efforts have focused on the development of non-secosteroidal compounds identified from combinatorial chemistry libraries and high-throughput screening (e.g. Ref. 17). As detailed below, it is likely that some of these analogues will find use in the prevention and treatment of cancer.

$1\alpha,25(\text{OH})_2\text{D}_3$ and its analogues are antiproliferative and induce cellular differentiation

Over the last few decades, evidence has been gathering that $1\alpha,25(\text{OH})_2\text{D}_3$ has anticancer properties in several tissues. Epidemiological data provide a strong correlation between the prevalence of certain cancers and exposure to sunlight, consistent with chemopreventive effects of $1\alpha,25(\text{OH})_2\text{D}_3$, particularly in prostate and colon cancers.⁽²¹⁾ Exposure to sunlight remains an important source of vitamin D, as many people in northern countries become deficient in circulating $25(\text{OH})\text{D}_3$ during winter, and therefore deficient in $1\alpha,25(\text{OH})_2\text{D}_3$ synthesized in peripheral tissues.⁽²²⁾ Animal studies have provided evidence of chemopreventive actions of $1\alpha,25(\text{OH})_2\text{D}_3$ analogues in models of colon, hamster cheek pouch, hepatocellular, gastrointestinal and skin carcinogenesis.^(23–28) Chemoprevention likely arises in part from the capacity of $1\alpha,25(\text{OH})_2\text{D}_3$ to regulate cellular differentiation and proliferation.⁽²⁹⁾ The potent growth inhibitory effects of $1\alpha,25(\text{OH})_2\text{D}_3$ analogues on cells in culture^(30–34) and in xenograft models of cancer^(35–39) coupled with their low calcemic activity make them potential agents for cancer therapy. Among the most widely studied analogues has been the secosteroidal compound EB1089.⁽⁴⁰⁾ EB1089 treatment reduced tumour growth by 80% in the absence of hypercalcemia in a mouse model of head and neck squamous cell carcinoma (HNSCC), whereas $1\alpha,25(\text{OH})_2\text{D}_3$ induced hypercalcemia and had a lesser inhibitory effect on tumour growth.⁽³⁹⁾ Similar antitumour effects of EB1089 were observed in xenograft models of breast and prostate cancer.^(37,38)

It is unlikely that regulation of a single gene controls the antiproliferative effects of $1\alpha,25(\text{OH})_2\text{D}_3$. Growth inhibition has been associated with several factors, including enhanced transforming growth factor- β signaling,⁽²⁹⁾ and to cell-specific

induction of cyclin-dependent kinase (CDK) inhibitors p21^{WAF1/CIP1} and p27^{KIP1} at both transcriptional and post-transcriptional levels.^(29,32,39,41–43) Increased levels of p27^{KIP1} in $1\alpha,25(\text{OH})_2\text{D}_3$ -treated HNSCC cells were associated with reduced protein turnover rather than enhanced gene transcription.⁽⁴³⁾ $1\alpha,25(\text{OH})_2\text{D}_3$ treatment repressed expression of CKS1 and p45^{SKP2}, components of the SCF^{SKP2} ubiquitin ligase that regulates p27^{KIP1} turnover.⁽⁴³⁾ The reduction in p45^{SKP2} levels is noteworthy because its overexpression is associated with a poor prognosis in HNSCC.^(44,45)

$1\alpha,25(\text{OH})_2\text{D}_3$ and its analogues induce differentiation of both primary cultures of non-malignant cells and cancer cells alike.^(27,41,46–48) Liu et al.⁽⁴¹⁾ showed that $1\alpha,25(\text{OH})_2\text{D}_3$ treatment of leukemic U937 cells induced their differentiation along a monocytic pathway, and that differentiation was driven by the strongly induced expression of the CDK inhibitor p21^{WAF1/CIP1}. Microarray studies of gene expression profiles in cancer cells have underlined the capacity of $1\alpha,25(\text{OH})_2\text{D}_3$ analogues to drive malignant cells to a more differentiated state. EB1089 treatment of HNSCC cells⁽⁴⁸⁾ repressed expression of several markers of cancer progression (e.g. N-cadherin, squamous cell carcinoma antigen, tenascin C, tumour antigen L6) and induced expression of several genes associated with epithelial cell differentiation (cystatin M, protease M, type XIII collagen, desmoglein 3). N-cadherin overexpression in HNSCC cells is consistent with the phenomenon of “cadherin switching” in cancer. Reversal of N-cadherin overexpression in HNSCC is associated with restoration of an epithelial phenotype.^(49,50)

Molecular evidence for the chemopreventive action of $1\alpha,25(\text{OH})_2\text{D}_3$

Microarray studies have provided insights into the molecular events underlying the chemopreventive effects of $1\alpha,25(\text{OH})_2\text{D}_3$ (Table 1). Gene expression profiling revealed that EB1089 treatment induced the growth-arrest and DNA-damage gene (GADD45 α) in HNSCC cells in culture,^(34,39) and in tumour xenografts of a mouse model of HNSCC.⁽³⁹⁾ Induction of GADD45 α expression was recently observed in studies of the antineoplastic effects of $1\alpha,25(\text{OH})_2\text{D}_3$ in insulinoma cells.⁽⁵¹⁾ Ablation of the GADD45 α gene in mice disrupts normal DNA repair and maintenance of global genomic stability.⁽⁵²⁾ This suggests that treatment with $1\alpha,25(\text{OH})_2\text{D}_3$ or its analogues has genoprotective effects; i.e. they protect the genome against accumulation of mutations that underlie cellular transformation and cancer progression.

This notion is supported by observations that EB1089 induces expression of several genes controlling redox balance in HNSCC, including glucose-6-phosphate dehydrogenase, which lies at the head of the pentose phosphate shunt, a source of reducing equivalents, glutathione peroxidase and thioredoxin reductase.⁽⁴⁸⁾ The enzymatic activities encoded by these genes are also induced in treated cells (our unpublished results). Induction of thioredoxin reductase activity

Table 1. Target genes underlying chemopreventive effects of $1\alpha,25(\text{OH})_2\text{D}_3$

Gene	Function	Fold change
Redox balance		
Glucose-6-phosphate dehydrogenase	Pentose-phosphate shunt/NADPH	5.8
Glutathione peroxidase	Peroxide scavenger/protection against oxidative stress	2.8
HtrA	Well conserved prokaryote homologue has antioxidant properties	6
Selenoprotein P	Heparin binding protein with antioxidant properties	4.1
Thioredoxin reductase	Reduced thioredoxin	3
VDUP1	Regulates thioredoxin/antiproliferative	8.2
Xenobiotic metabolism		
nrf2	Induces expression of phase II detoxifying enzymes	2.6
cyp3A7 (P450HFLa)	Xenobiotic metabolism	40
cyp3A4	Xenobiotic metabolism	N/A (Refs. 11–13)
cyp2B6	Xenobiotic metabolism	N/A (Ref. 12)
cyp2B9	Xenobiotic metabolism	N/A (Ref. 12)
DNA repair/genomic stability		
gadd45 α	Maintenance of global genomic stability	N/A (Refs. 33,36,47)

has also been observed in $1\alpha,25(\text{OH})_2$ -treated prostate and breast carcinoma cells.^(53,54) These results are consistent with the observation that treatment of leukemic cells reduces intracellular levels of reactive oxygen species (ROS).⁽⁵⁵⁾ The protective effects of $1\alpha,25(\text{OH})_2\text{D}_3$ against oxidative DNA damage may represent a physiological feedback loop to the photochemical synthesis of vitamin D in skin by ultraviolet light, which is a DNA damaging agent and an inducer of (ROS).⁽⁵⁶⁾ Indeed, direct photoprotective effects of $1\alpha,25(\text{OH})_2\text{D}_3$ were observed in UV-irradiated keratinocytes in vitro and in mouse skin, and were linked to increased expression of free radical scavenging metallothionein.⁽⁵⁷⁾

$1\alpha,25(\text{OH})_2\text{D}_3$ also stimulated expression of the gene encoding the NRF2 transcription factor.⁽⁵⁸⁾ NRF2 is induced by a number of chemopreventive agents, and in turn stimulates expression of several phase II detoxifying enzymes. Ablation of the nrf2 gene in mice rendered them more sensitive to carcinogenesis and eliminated the beneficial effects of chemopreventive agents.⁽⁵⁸⁾ An enhancement of xenobiotic metabolism by $1\alpha,25(\text{OH})_2\text{D}_3$ is also consistent with its direct induction of several genes encoding members of the cytochrome P450 family of oxidative enzymes.^(11–13)

The immuno-modulatory effects of $1\alpha,25(\text{OH})_2\text{D}_3$

The VDR is expressed in most cells of the immune system, including T lymphocytes, and antigen-presenting cells (APC) such as macrophages and dendritic cells.^(59–62) Growing evidence indicates that $1\alpha,25(\text{OH})_2\text{D}_3$ is a modulator of immune system function, consistent with its capacity to control cellular differentiation. One study of mice in which the VDR gene had been ablated concluded that altered immune responses were an indirect consequence of VDR disruption because they could be restored by normalization of calcium homeostasis.⁽⁶³⁾

However, another study revealed abnormal development of pro-inflammatory T helper 1 (Th1) cell development in VDR knockout mice.⁽⁶⁴⁾ Moreover, mice rendered $1\alpha,25(\text{OH})_2\text{D}_3$ deficient by knockout of the gene encoding 25-hydroxyvitamin D3 1α -hydroxylase were deficient in peripheral T lymphocytes.⁽⁶⁵⁾

$1,25(\text{OH})_2\text{D}_3$ signaling can regulate T cell activity both directly and indirectly through modulation of APC function.^(61,62) It inhibits dendritic cell maturation, which is critical for T-cell-mediated immune responses.^(66–68) Treatment reduces expression of the cytokine interleukin-12 (IL-12), whose signaling is critical for Th1 maturation. Moreover, $1\alpha,25(\text{OH})_2\text{D}_3$ directly represses the transcription of genes encoding Th1-associated cytokines such as IL-2 and interferon γ (IFN γ). For example, the hormone-bound VDR inhibits the activity of the NF-AT transcription factor, an activator of the IL-2 gene promoter.^(69,70)

More insights into the effects of $1,25(\text{OH})_2\text{D}_3$ have come from microarray studies in HNSCC cells derived from epithelial keratinocytes.⁽⁴⁸⁾ Keratinocytes are considered to be an integral part of the immune system of the skin.⁽⁷¹⁾ EB1089 downregulated IFN γ -regulated genes encoding 9-27, 1-8D, interferon-inducible 56K protein, the T cell chemokine IP-10, and the chemokine RANTES. IFN γ signaling and overexpression of IP-10 underlie the inflammatory reactions in psoriasis.⁽⁷¹⁾ $1,25(\text{OH})_2\text{D}_3$ also strongly induced expression of T1/ST2, a member of the interleukin-1 receptor family. Gene ablation studies have revealed that T1/ST2 signaling is essential for normal T helper 2, Th2, cell differentiation.⁽⁷³⁾ These results are consistent with EB1089 stimulating Th2 responses, and inhibiting a number of genes associated with proinflammatory Th1 responses.

These findings help provide a molecular basis for the therapeutic potential of $1\alpha,25(\text{OH})_2\text{D}_3$ analogues in treatment

Th1-stimulated autoimmune diseases. Indeed, in mice, $1\alpha,25(\text{OH})_2\text{D}_3$ can prevent systemic lupus erythematosus, experimental autoimmune encephalomyelitis (EAE), collagen-induced arthritis, inflammatory bowel disease and autoimmune diabetes.⁽⁶²⁾ For example, treatment of mice with myelin basic protein induces EAE, a multiple sclerosis-like disease whose progression is driven by activated T cells. Dietary $1\alpha,25(\text{OH})_2\text{D}_3$ prevented the onset of EAE and the progression of established disease.^(74,75) The most firmly established clinical use of $1\alpha,25(\text{OH})_2\text{D}_3$ analogues is in the treatment of the Th1-driven chronic inflammatory skin disease psoriasis, which affects 2% of the population. $1\alpha,25(\text{OH})_2\text{D}_3$ analogues account for 50% of all drugs used to treat mild to moderate disease. Analogues are used topically, and one of the most thoroughly tested is the secosteroidal compound calcipotriol,^(76–78) which is effective either alone or when administered in combination with anti-inflammatory steroids.⁽⁷⁹⁾

Neuroprotective effects of $1\alpha,25(\text{OH})_2\text{D}_3$ in the central nervous system

While $1\alpha,25(\text{OH})_2\text{D}_3$ can protect against progression of neurodegenerative disorders such as EAE through its effects on the immune system, recent evidence suggests that it can act directly on the central nervous system (CNS) itself. The VDR is widely expressed throughout the CNS,⁽⁸⁰⁾ and is a strong inducer of nerve growth factor expression.⁽⁸¹⁾ Several studies have suggested that $1\alpha,25(\text{OH})_2\text{D}_3$ has neuroprotective effects. In vivo experiments in rodents have shown that $1\alpha,25(\text{OH})_2\text{D}_3$ retards age-related decreases in hippocampal neuronal density,⁽⁸²⁾ and protects against neuronal cell death in a rodent model of stroke.⁽⁸³⁾ Moreover, $1\alpha,25(\text{OH})_2\text{D}_3$ can act directly on primary cultures of rat hippocampal neurons to inhibit expression of markers associated with neuronal aging.⁽⁸⁴⁾ Part of the neuroprotective effects of $1\alpha,25(\text{OH})_2\text{D}_3$ in the CNS may also lie in its capacity to protect cells from ROS. Studies in cultured rat neurons showed that $1\alpha,25(\text{OH})_2\text{D}_3$ protected against the neurotoxic effects of agents that caused oxidative damage by increasing intracellular levels of glutathione,⁽⁸⁵⁾ consistent with its effects on redox balance observed in cancer cells (see Table 1).

Conclusions

Studies on vitamin D action have come a long way since its discovery as an antirachitic agent. The broad expression pattern of the VDR, and the widespread effects of its hormone on cellular differentiation and proliferation have opened up a number of new fields of investigation for basic researchers interested in $1\alpha,25(\text{OH})_2\text{D}_3$ function as well as those more concerned with the therapeutic potential of its synthetic analogues. Much remains to be done to fully understand the potential of $1\alpha,25(\text{OH})_2\text{D}_3$ analogues as chemopreventive agents against cancer and neuronal aging, as well as their potential in combating a number of autoimmune disorders. However, results to date auger well for the future.

Acknowledgments

We thank Dr. Jean-Marie Wurtz, IGBMC, Illkirch, France for help with Figure 3.

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