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Review

Vitamin D and Cancer

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Abstract. *The correlation between decreased morbidity and mortality of cancer and exposure to sunlight is known. The many biological functions of vitamin D that contribute to cancer prevention have only recently begun to be appreciated. Once activated 1,25-dihydroxyvitamin D [1,25(OH)$_2$D$_3$] functions as a potent inhibitor of normal and cancer cellular proliferation. Vitamin D deficiency in mice led to a 60% increase in colon tumor growth, compared to vitamin D-sufficient mice. The ligand binding domain of the Vitamin D receptor was shown to accommodate a class of 1,25(OH)$_2$D$_3$-analogs that possess an additional side-arm. These novel Gemini analogs were evaluated in vitro and in vivo. Select Gemini analogs were 100 times or more effective in inhibiting colon tumor growth in mice, compared to their parent compound. Correcting vitamin D deficiency may decrease the risk of developing colon cancer, while the novel Gemini 1,25(OH)$_2$D$_3$-analogs have the potential for therapeutic application in human colon cancer.*

Historical Perspective on the Role of Sunlight and Vitamin D on Bone Health and Cancer Prevention

More than 100 years before vitamin D was identified, its sun-derived effects on bone health had been reported by Sniadecki in 1822. He suggested that the children living in Warsaw had a high prevalence of rickets due to lack of sun exposure (1). In 1889, Palm reported a similar correlation between sunlight deficiency and rickets. Upon analyzing the prevalence of rickets in London, Manchester, the British Midlands and the Netherlands, compared to China, Mongolia and Tibet, Palm realized that rickets increased with increasing latitude and degree of urbanization (2). It was, however, not until the 1920's that sunlight was widely recognized as having a protective effect against rickets (3,4)

In 1919, Huldschinsky (5) first introduced the idea of using ultraviolet (UV) radiation from a mercury arc lamp as a therapy for rickets in children. Later, Hess and Unger conducted experiments irradiating rats and subsequently infants with a carbon arc lamp (6). They concluded that adequate exposure to UV radiation resulted in a "definite and dependable cure of [rickets]."

Similarly, in 1936 Peller (7) analyzed the relationship between morbidity and mortality of cancer and exposure to radiation. He concluded that exposing the skin to enough UV radiation to induce skin cancer caused a decrease in the incidence of more malignant tumors in organs that were "not at all accessible to treatment." Peller described this method of inducing skin cancer (a more easily treatable cancer) for the sake of preventing the development of more malignant cancers as a novel strategy to decrease cancer mortality. To support his theory of the protective effects of UV radiation against the development of more malignant cancers, Peller and Stephenson proceeded to analyze the incidence of cancer in a population with increased exposure to solar UV radiation, the United States Navy. Compared to age-matched controls, the rate of skin cancer in the US Navy was eight times higher, while the total number of deaths from other cancers was 60% less than the civilian population (8).

In 1941, Apperley also analyzed the incidence and mortality rate of cancer with respect to sun exposure. As a model, he compared total cancer mortality to the percentage of American and Canadian state populations engaged in agriculture. He demonstrated a highly significant correlation between reduced cancer mortality in adults and a career in agriculture. Apperley's analysis led him to conclude that cancer mortality could be reduced "by inducing partial or

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Table I. *Mortality from cancer in cities according to latitude measured between 1908-1912. Modified from Hoffman (9).*

Number of cities	Latitude	Deaths from cancer	Rate (per 100,000)
35	60N-50N	119.374	105.7
48	50N-40N	121.216	92.4
24	40N-30N	37.451	78.1
7	30N-10N	5.696	42.3
4	10N-10S	1.056	40.9
7	10S-30S	3.040	37.7
5	30S-40S	11.048	89.8

complete immunity" by exposing the skin to solar or artificial light. The hypothesis that UV radiation was protective against cancer had been further supported by the earlier work of Hoffman (9). In 1915, he compared cancer mortality in cities according to latitude in the period 1908 and 1912. Hoffman demonstrated (Table I) that cancer mortality increased with increasing distance from the equator.

Solar ultraviolet B (UVB) radiation (290-315 nm) is responsible for vitamin D_3 production in the skin. The solar zenith angle is the angle that the incident solar radiation makes with the axis that runs perpendicular to the earth (10). With increasing latitude (distance from the equator) the zenith angle increases. As a result of an increased zenith angle, solar UV radiation has a longer path length through the UV-absorbing atmosphere. The longer path length reduces the number of photons that reach the earth's surface. Thus, exposure to solar UVB radiation decreases with increasing latitude. Hoffman not only demonstrated that cancer mortality increased with increasing distance from the equator, but he also unknowingly demonstrated that cancer mortality decreased with increasing exposure to solar UVB radiation that may be associated with increased production of vitamin D_3.

More recently, in the 1980's-1990's, Garland and Garland *et al.* (11-14) completed a number of epidemiological studies to evaluate the correlation between cancer, sun exposure and the cutaneous production of vitamin D_3. They demonstrated a strong negative correlation between latitude, sun exposure, vitamin D status and the risk of many different cancers, including colon (11), breast (12), ovarian (13) and melanoma (14). This correlation was further supported by the observation that breast and colon cancer mortality rates increased with increasing UV-absorbing pollution (15), thus preventing the protective solar UVB radiation from reaching the earth's surface.

Furthermore, Grant *et al.* (16, 17) demonstrated that the risk of a total of 13 different cancers was reduced by adequate exposure to solar UVB radiation. Grant calculated that over a span of 24 years, 1970-1994, a total of 566,400 Americans had died prematurely from one of the 13 cancers

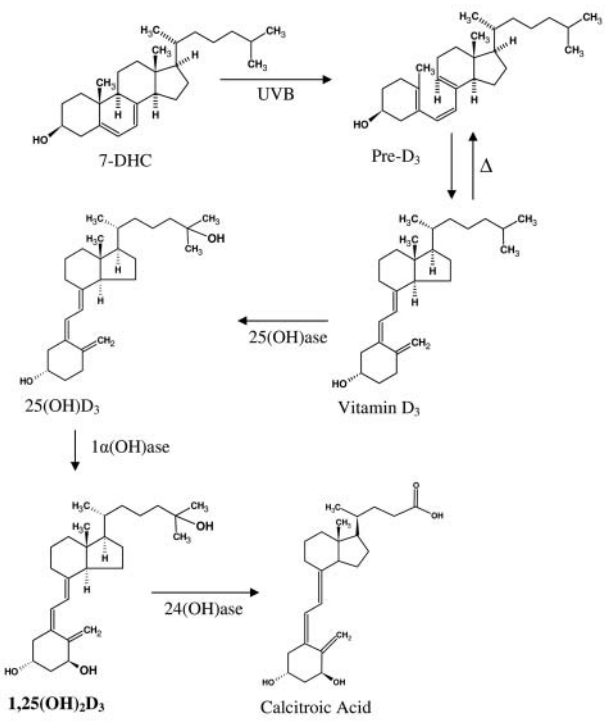

Figure 1. *The molecular structures of the compounds involved in the synthesis and metabolism of 1,25(OH)$_2$D$_3$ from 7-DHC (7-dehydrocholesterol).*

due to inadequate exposure to solar UVB radiation (16). A more recent analysis estimated that currently between 50,000-63,000 Americans and 19,000-25,000 individuals living in the United Kingdom annually die prematurely from cancer due to vitamin D deficiency. It is worth noting that, in the year 2004, the US spent approximately $40-56 billion dollars as a result of the repercussions of vitamin D deficiency (17).

Vitamin D Photosynthesis and Metabolism

The precursor of vitamin D_3, 7-dehydrocholesterol [7-DHC] or provitamin D_3, is present in human skin. When 7-DHC is exposed to UVB radiation, previtamin D_3 is produced. Once formed, the triene system undergoes a spontaneous thermal isomerization to form vitamin D_3. In plants and yeast, the major provitamin D sterol is ergosterol, instead of 7-DHC. Irradiation of ergosterol produces previtamin D_2, which isomerizes to vitamin D_2. Vitamin D_2 has similar biological effects on bone health in humans compared to vitamin D_3 (18), but is only 10-50% as effective as vitamin D_3 in maintaining the circulating levels of 25-hydroxyvitamin D (Figure 1) [25(OH)D] (19, 20).

Once formed in the skin or ingested from the diet, vitamin D (D represents either D_2 or D_3) enters the circulation bound to the vitamin D-binding protein (DBP). DBP transports vitamin D_3 to the liver where it is hydroxylated at C_{25} by

vitamin D_3-25-hydroxylase [25(OH)ase] by four possible enzymes responsible for completion the 25-hydroxylation: CYP27A1, CYP3A4, CYP2R1, CYP2J3 (21). This first hydroxylation produces the major circulating form of vitamin D, 25(OH)D. 25(OH)D is also bound to the DBP and is shuttled to the kidney where it is hydroxylated at C_1 by the cytochrome P450 enzyme 25-hydroxyvitamin D_3-1α-hydroxylase [1α-OHase, CYP27B1] (22). This final hydroxylation results in the activation of 25(OH)D, producing 1,25-dihydroxyvitamin D [$1,25(OH)_2D$]. $1,25(OH)_2D$ exerts its control on calcium homeostasis by maintaining serum calcium levels in the normal range through increased intestinal absorption of calcium and bone calcium resorption (23). Once it has completed its functions, it induces the 25-hydroxyvitamin D-24-hydroxylase (CYP 24). This enzyme initiates the destruction of 1,25(OH)2D by hydroxylating it on C_{24} and C_{23}, which leads to side-arm cleavage at C_{23} to form calcitroic acid (21).

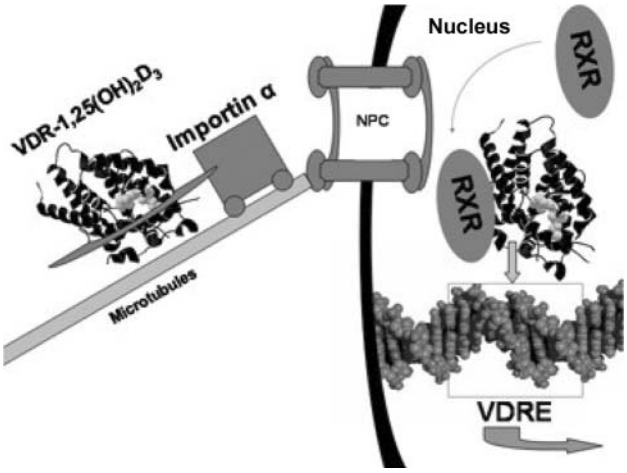

Figure 2. *A pictorial representation of $1,25(OH)_2D_3$ initiating transcription by the VDR-RXR dimer. (VDR)-Vitamin D Receptor, (NPC)- Nuclear Pore Complex, (RXR)-Retinoid X Receptor, (VDRE) – Vitamin D Response Element.*

Nuclear Mechanism of Action

$1,25(OH)_2D_3$ exerts its nuclear effects by binding to its specific nuclear receptor, the vitamin D receptor (VDR). $1,25(OH)_2D_3$ binds to the VDR in the cytoplasm of the cell (Figure 2), causing a conformational change in the protein at helix 12 (Figure 3). This physical change reorients the activation function 2 domain [AF-2] located on helix 12 allowing it to interact with other cytoplasmic proteins and co-activators, and mediates the translocation of the hormone-bound VDR into the nucleus (24). The movement of the VDR into the nucleus is a complex process whereupon binding of $1,25(OH)_2D_3$ to the VDR causes the nuclear receptor to be released from its cytoplasmic docking protein. VDR's hydrophilic nuclear localization sequence (NLS) binds to its NLS receptor, importin α. Through a series of protein-protein interactions, VDR-$1,25(OH)_2D_3$ is shuttled by importin α along the tracks of microtubules to the nuclear pore complex (NPC). The VDR enters the nucleus through the NPC by an energy-dependent process (25). Once in the nucleus, VDR forms a heterodimeric complex with the retinoic acid X receptor (RXR) before binding to its target gene sequence, the vitamin D response element (VDRE) (Figure 2). Once the $1,25(OH)_2D$ -VDR-RXR complex binds to the VDRE, a host of initiation factors including the P160 coactivator proteins, glucocorticoid receptor interacting protein 1 (GRIP-1) and steroid receptor coactivator-1 [SRC-1], DRIP/TRAP complex, histone acetyltransferases (HATs) including CREB binding protein [CBP]/P300, Mothers against decapentaplegic homolog 3 [SMAD3], and Nuclear receptor Coactivator, molecular mass of 62000 kDa, NCoA-62. Interestingly, the NCoA-62 co-activator has also been shown to interact with the v-Ski oncogene, and therefore is also called Ski-interacting protein [SKIP] (26). For an oncogene, v-Ski has the unusual ability to influence cellular proliferation and initiate differentiation (27). These co-activators interact with the VDR and assist in the assembly of the necessary proteins, including RNA polymerase II, required to initiate transcription of the vitamin D-regulated genes. The resulting transcribed mRNA is translated into proteins that act to carry out vitamin D-specific calcemic and non-calcemic functions.

Non-calcemic Functions of Vitamin D

In 1979, it was shown that rats fed a vitamin D_3-deficient diet, followed by an intravenous injection of [^{3}H]-$1,25(OH)_2D_3$, displayed an accumulation of the radioactive hormone in the nuclei of many of the body's tissues (28). This served as the first suggestion that $1,25(OH)_2D_3$ participated in a wide range of physiological functions in addition to its classic role in regulating calcium homeostasis.

It is now known that the VDR exists in more than 30 tissues and innumerable cancer cell lines (4, 29-33). These tissues include brain, breast, colon, intestine, kidney, lung, prostate, skin and stomach and activated T and B lymphocytes (31). In addition to the presence of the VDR in many tissues, it was also demonstrated that many of these same tissues and cancer cell lines expressed the 1α-OHase, rendering them capable of local conversion of $25(OH)D_3$ to the active $1,25(OH)_2D_3$ (32-34). The tissues found to express the 1α-OHase included, among others, skin, prostate, brain, breast, lung, activated macrophages and colon (34-41).

Vitamin D and Cell Growth

Some of the non-classic roles of $1,25(OH)_2D_3$ include its antiproliferative and pro-differentiating effects on normal and malignant cell lines. Originally shown in 1981, the addition of

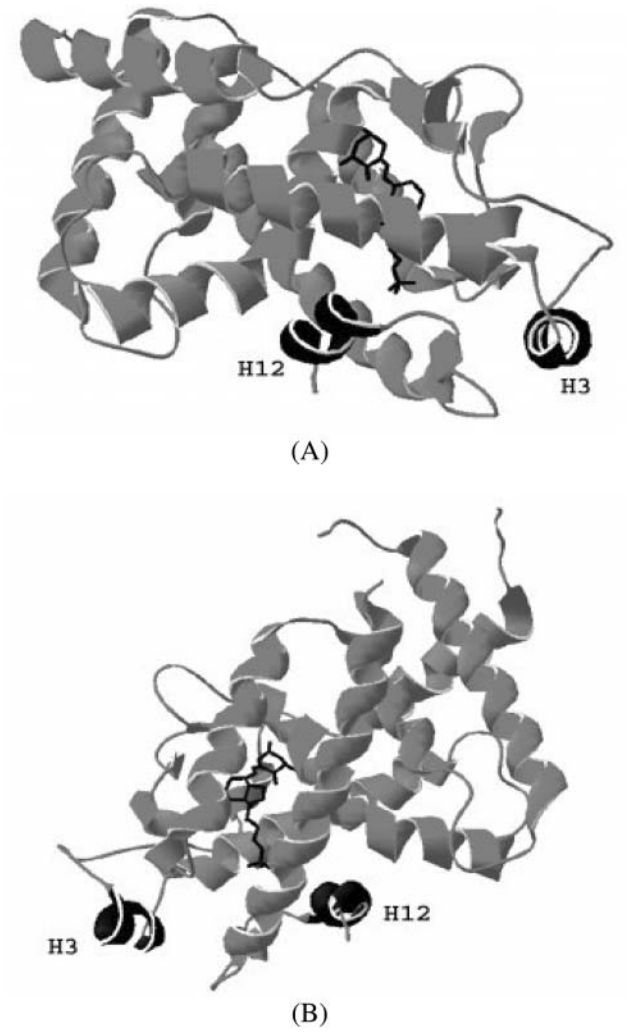

Figure 3. *Pictorial representations of 1,25(OH)$_2$D$_3$ in the ligand binding domain (LBD) of the VDR in three different spatial orientations (A, B and C). Helices 3 (H3) and 12 (H12) are shown in black.*

1,25(OH)$_2$D$_3$ to mouse myeloid leukemia cells served as a potent stimulator of differentiation and inhibitor of cell growth (42). Since then, it has been established that 1,25(OH)$_2$D$_3$ exerts its antiproliferative effect on normal and malignant cell lines that possess the VDR (44, 45). It is believed that 1,25(OH)$_2$D$_3$'s antiproliferative activities are, in part, a result of increasing inhibitors and decreasing activators of cyclin-cyclin-dependent kinase complexes (CDK's) in addition to increasing levels of the cyclin-dependent kinase inhibitors Cip/Kip proteins, p21 and p27 (45). These proteins act to stall the cell cycle at the G1/S check-point, preventing DNA synthesis and, therefore, further cellular growth.

The antiproliferative and pro-differentiating effect of 1,25(OH)$_2$D$_3$ on human and mouse skin cells *in vitro* is well established (46). Anticipating a similar effect of 1,25(OH)$_2$D$_3$ on colon cells, a human colon cancer cell line that expressed the VDR, HT-29, was treated with 1,25(OH)$_2$D$_3$ by Zhao *et al.* in 1993 (47). The addition of 1,25(OH)$_2$D$_3$ successfully inhibited cellular growth in a dose-dependent fashion, and induced the colon cancer cells to differentiate. A wide variety of tumor cell lines including melanoma, leukemia, lung, breast and prostate cancer have been shown to respond to the antiproliferative and pro-differentiating activity of 1,25(OH)$_2$D$_3$ (48).

Aside from antiproliferative, and pro-differentiating role of 1,25(OH)$_2$D$_3$, it has also been demonstrated to be antiangiogenic *in vivo* and *in vitro* (49, 50).

Development of Novel Vitamin D Analogs

Because of the numerous actions of 1,25(OH)$_2$D$_3$, it has been of great interest to characterize the protein responsible for binding this hormone and carrying out its nuclear activities, the VDR.

The VDR has a 48 kDa molecular mass comprising 427 amino acids. The VDR is a member of the superfamily of nuclear receptors for steroids and thyroid hormones (51). Like other members of the superfamily, the DNA binding

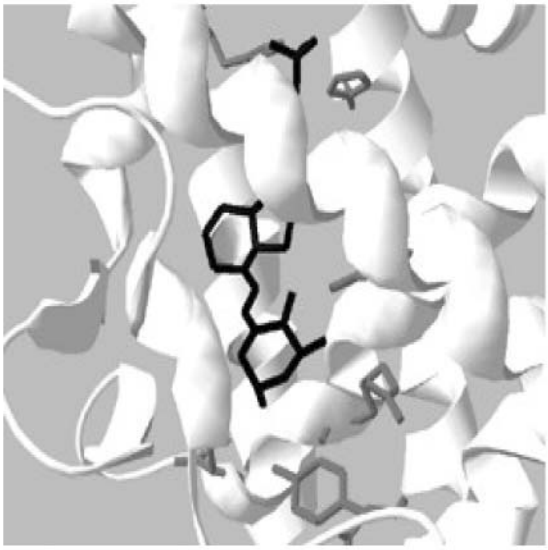

Figure 4. *1,25(OH)$_2$D$_3$ (black structure) in full view in the VDR.*

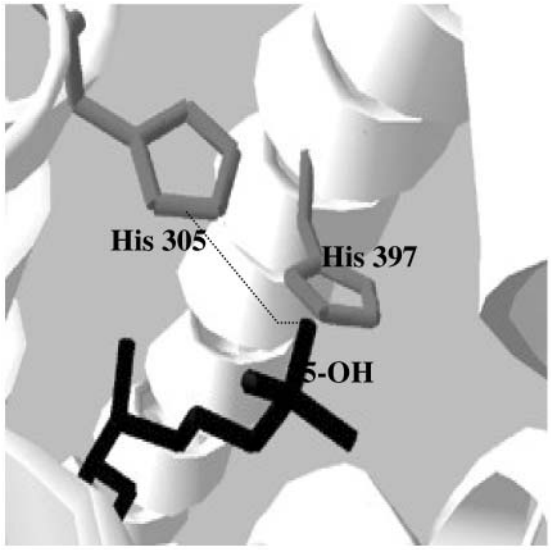

Figure 5. *Intermolecular interactions stabilizing the C_{25}-OH in the ligand binding domain of the VDR.*

domain (DBD) includes an amino acid sequence 70 amino acids long and is rich in polar and positively-charged amino acids including cysteine, lysine and arginine. These chemically reactive amino acids combined with the characteristic zinc-finger binding motif promote highly selective binding of the ligand bound protein to the VDREs (52).

After detailed analysis of the ligand binding domain (LBD) of the VDR, various amino acid residues have been identified as important players in binding 1,25(OH)$_2$D$_3$.

The LBD of the VDR is located at the C-terminus, including amino acids 226-427. The available volume in the LBD is 660 Angstroms^3. 1,25(OH)$_2$D$_3$ acting as a ligand only fills about 381 Angstroms^3, thus occupying only 56% of the available volume (53) (Figure 3).

Using the known amino acid sequence of the VDR, and its crystallized structure, the LBD of the protein has been well characterized (53) (Figure 4). An evaluation of how 1,25(OH)$_2$D$_3$ is oriented within the LBD has provided an important insight into the chemical interactions that are responsible for ligand-receptor binding. These interactions have been essential for the elucidation of the structure-function relationship between the VDR, 1,25(OH)$_2$D$_3$ and the development of super-potent 1,25(OH)$_2$D$_3$ analogs.

Based on the electronegative nature of the 1-, 3- and 25-hydroxyl [1-, 3-, 25-OH] moieties of 1,25(OH)$_2$D$_3$, they are thought to form hydrogen binds within the LBD of the VDR to stabilize 1,25(OH)$_2$D$_3$ in the receptor (54). Through a combination of hydrophobic and electrostatic interactions that occur between the ligand and receptor, the C_1-OH and C_{25}-OH are held in a position that maintains a separation distance of 13 Angstroms (53). The amino acids necessary for

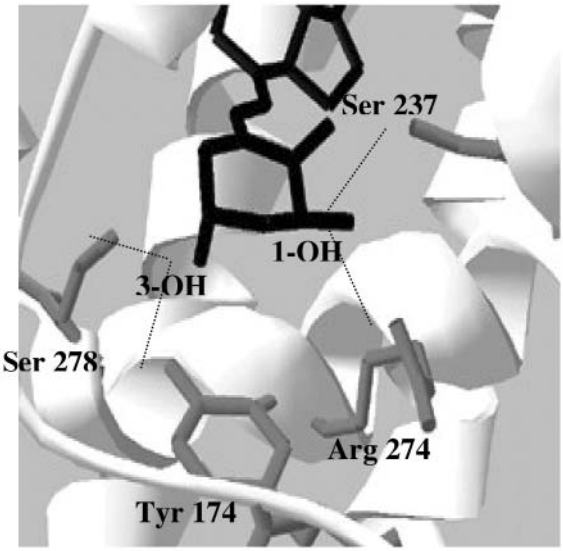

Figure 6. *Intermolecular interactions stabilizing the C_1-OH and C_3-OH in the ligand binding domain of the VDR.*

stabilization of the hydroxyl moieties include His 305 and His 397 that establish hydrogen bonds with C_{25}-OH located at the end of the side-arm (53). The equatorially positioned C_1-OH hydrogen bonds with Ser-237 and Arg-274, while the axially positioned C_3-OH hydrogen bonds with Ser-278 and Tyr 143 (Figures 5, 6) (54). It was also reported that Cys 288 interacts with the C_3-OH of 1,25(OH)$_2$D$_3$, which is essential for aligning the molecule such that the π-electrons of the triene system are aligned with Trp 286 (54). Furthermore,

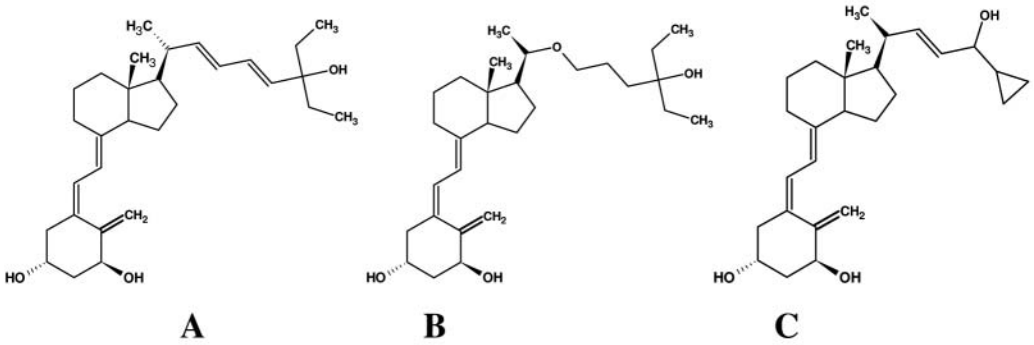

Figure 7. *A) 22E,24E-diene-24,26a,27a-trihomo-1α,25(OH)$_2$D$_3$ [EB-1089], B) 20-epi-22-oxa-26a,27a-bishomo-1α,25(OH)$_2$D$_3$ [KH 1060], C) 1α,24S-dihydroxy-22ene-25,26,27-cyclopropylvitamin D$_3$ [MC 903, Dovonex].*

mutation of Trp 286 to Ala or Phe causes the VDR to lose 90% of its ability to bind to its natural ligand (54).

Other structural components of the VDR that are essential for proper function are the 3 and 12 alpha-helices. Together, these two helices serve as a 3/12 co-activator platform which serve as the site of binding for a set of p160 co-activators including SRC-1 (56) (Figure 3). It was also shown that the correct positioning of helix 10 is essential for binding of RXR and for the recruitment of co-activators (56).

Information regarding the structure-function relationship between the VDR and 1,25(OH)$_2$D$_3$ has become especially valuable in the development of vitamin D analogs aimed at harnessing the antiproliferative and pro-differentiating effects of 1,25(OH)$_2$D$_3$ while minimizing the detrimental calcemic side-effects in treatment of cancer.

Interestingly, and perhaps counter-intuitive, Rochel *et al.* (57) studied the affinities of several potent vitamin D analogs for the VDR. It was assumed that a vitamin D analog with increased potency compared to 1,25(OH)$_2$D$_3$ would be due to the compound's greater binding affinity to the VDR. However, it was shown for the three vitamin D analogs, KH 1060, MC-903 and EB 1089 (Figure 7), that their relative affinities to the VDR were 90, 86 and 83% compared to 1,25(OH)$_2$D$_3$, even though they were more potent.

Vitamin D Sufficiency and Effect of Vitamin D Analogs on Mouse Colon Cancer Growth

Our laboratory evaluated the protective effect of vitamin D sufficiency on colon cancer tumor growth. Using a mouse colon cancer model, the animals were maintained on vitamin D-sufficient or vitamin D-deficient diet for six weeks. After establishing that mice in the vitamin D-deficient group had serum 25(OH)D$_3$-levels ≤5 ng/mL and the vitamin D-sufficient group had levels in the normal range with a mean of 26 ng/mL, the mice received subcutaneous implantation of 10,000 mouse colon cancer cells (MC-26). On day 19, the final day of the study, the vitamin D-deficient mice had a mean tumor volume 60% (mean; $p<0.05$) larger than the vitamin D-sufficient mice. It was thus concluded that adequate dietary vitamin D is protective against colon cancer (Figure 8).

Using the same mouse model, we tested the antitumor effects of a novel class of Gemini vitamin D analogs. Designed by Roche-Bioscience Inc., these compounds have two side-arms, and are hence termed Gemini analogs. Because 1,25(OH)$_2$D$_3$ occupies only 56% of the available volume of the LBD in the VDR, the second side-arm was not only well accommodated, but it could assume one of two equally favored positions (58).

Norman *et al.* (59) compared the binding of the parent Gemini analog and 1,25(OH)$_2$D$_3$ to the VDR and DBP. They demonstrated that the Gemini analog bound the VDR with 38% affinity, compared to 1,25(OH)$_2$D$_3$, whereas the same Gemini analog bound the DBP with only 2.5% relative affinity as compared to 1,25(OH)$_2$D$_3$. A 38% relative binding affinity is higher than what may be expected considering the large increase in volume due to the additional side-arm (Figure 9). When a simple modification was made to 1,25(OH)$_2$D$_3$ and the orientation of C$_1$-OH was changed from α [1α,25(OH)$_2$D$_3$] to β [1β,25(OH)$_2$D$_3$], the resulting relative binding affinity was 0.08% of 1α,25(OH)$_2$D$_3$ (59). Stereoscopic dot maps revealed that the structure of this parent Gemini analog was significantly more flexible, with 2346 different minima conformations, compared to 207 minima conformations of 1,25(OH)$_2$D$_3$. This increased flexibility of the parent Gemini analog may allow it to be more easily accommodated into the LBD, and thus maintain a binding affinity of 38% (59).

Our *in vivo* studies demonstrated that the most potent Gemini analog was at least 1000-fold more effective at inhibiting colon tumor growth compared to mice treated with 1,25(OH)$_2$D$_3$ (10). At a very low dose, 0.002 μg molar equivalents of 1,25(OH)$_2$D$_3$ (E) per mouse, the Gemini analog A (10) reduced the tumor volume by more than 50%, compared to the placebo, while 1,25(OH)$_2$D$_3$ had no

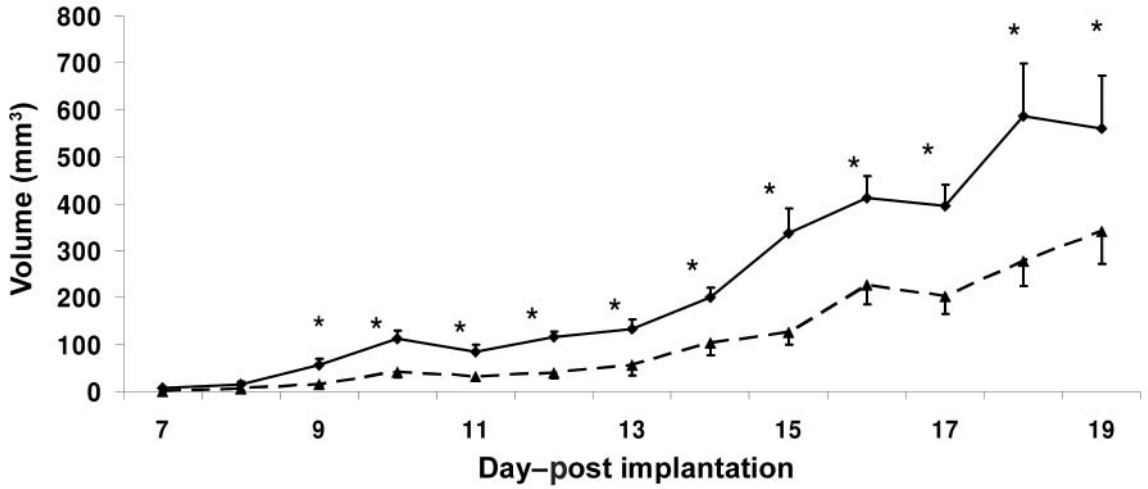

Figure 8. *Effect of vitamin D on mouse colon cancer tumor volume. Balb/c mice fed a vitamin D-deficient normal calcium (0.47% Ca) diet (solid line, black diamonds) or vitamin D-sufficient (500,000 IU/kg diet) and normal calcium (0.47% Ca) diet (dashed line, black triangles). The results are the mean tumor volume in each group on the final day of study, day 20 post-implantation. *p<0.05. Modified from (60).*

significant effect (Figure 10). Mice that received this Gemini analog and were fed a low calcium diet showed no differences in their serum calcium levels.

Conclusion

The maintenance of $25(OH)D_3$ within the healthy range, 30-100 ng/mL (75-250 nmol/L), may be important to protect against the risk of developing up to 13 different cancers, and many other diseases including osteoporosis, heart disease and common autoimmune diseases (17). Veith (61) has suggested that, in order to obtain sufficient 25(OH)D levels, the current recommended adequate intake (AI) of 200 IU for all children and adults 50 years old, 400 IU for adults 50-70 years old, and 600 IU of dietary vitamin D for those 71 years and older is four- to five-fold too low. Even following these AIs, it was recently shown that only 4% of adults ≥51 years old consume the recommended AI (62). Due to the inadequacy of the AI, even by following national guidelines, many people unknowingly continue to consume inadequate dietary vitamin D and, in doing so, continue to be at risk from diseases that may be preventable.

Taking prostate cancer as an example, it has been suggested that the expression of prostatic 1α(OH)ase decreases with age (63). When Barreto *et al.* (64) added the biologically-inert pro-hormone, $25(OH)D_3$, to a culture of primary prostatic epithelial cells with 1α(OH)ase activity, the $25(OH)D_3$ was converted to its active metabolite, $1,25(OH)_2D_3$, and cellular proliferation was inhibited. It has also been suggested that the reduced expression of 1α(OH)ase in prostate cells may be, in part, responsible for the connection between aging and the development of prostate cancer (63). Similarly, Whitlatch *et al.* (65) showed

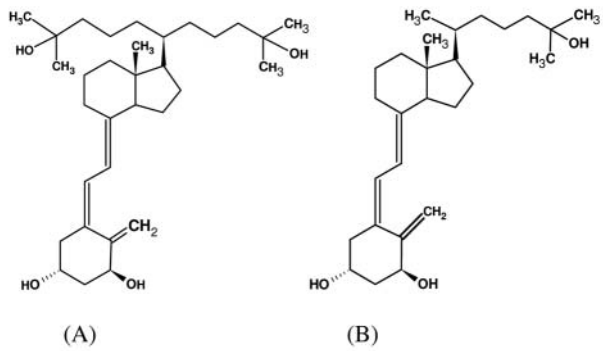

Figure 9. *Structure of the (A) parent Gemini analog: 1α,25-dihydroxy-21-(3-hydroxy-3-methylbutyl)vitamin D_3 and (B) 1α,25(OH)$_2$D$_3$.*

that benign prostatic hyperplasia cells had a 60% reduction in the 1α(OH)ase, while prostate cancer cells from primary cultures had an average of 85% reduction in 1α(OH)ase activity, compared to normal prostate cells. Additionally, the cell line often used as a model for human prostate cancer, LNCaP, was found to have undetectable levels of 1α(OH)ase expression. When these cells were incubated with $25(OH)D_3$, there was no effect on cellular proliferation. When the LNCaP cells were transfected with the 1α(OH)ase cDNA, the cells expressed the 1α(OH)ase enzyme, and enabled them to convert $25(OH)D_3$ to $1,25(OH)_2D_3$. The growth of these 1α(OH)ase-transfected cells were now inhibited by $25(OH)D_3$ (Figure 11).

In the case of colon cancer, $1,25(OH)_2D_3$ has been shown to be important for the growth and development of colon cancer cells. Tangpricha *et al.* (66) showed that highly differentiated human malignant colon cancer tissue had similar levels of 1α(OH)ase compared to the adjacent normal tissue.

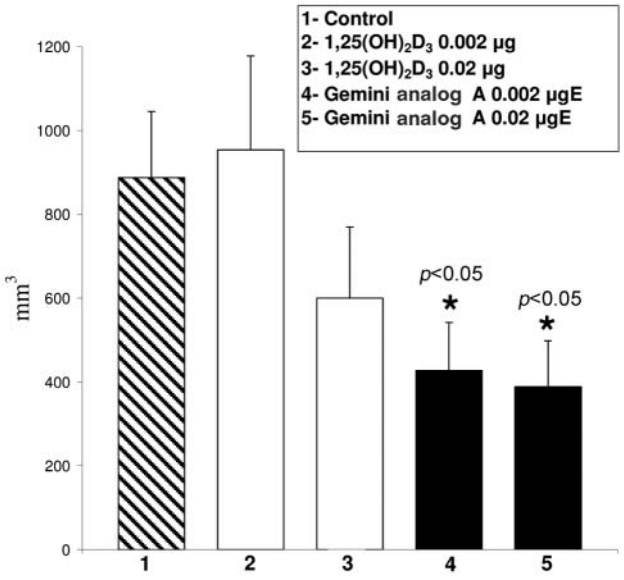

Figure 10. *The effect of 1,25(OH)$_2$D$_3$ (□) and a novel Gemini analog A (■) on colon tumor volume, compared to control (▨). (E) – molar equivalents of 1,25(OH)$_2$D$_3$.*

However, in the less differentiated malignant colon tissue, the expression of 1α(OH)ase was 20- to 30-fold higher than the normal adjacent tissue. Furthermore, 1,25(OH)$_2$D$_3$ is believed to play an important regulatory role in the expression of Id proteins, proteins that inhibit DNA binding and differentiation by, for example, interfering with basic helix-loop-helix transcription factors (69). Id proteins also directly interact with the Retinoblastoma [Rb] pathway. Rb is a tumor suppressor that must be inactivated (hyperphosphorylated) before the cell can pass through the restriction point of the cell cycle, and into the S-phase, or phase of DNA synthesis and cell growth. Thus, direct interaction with the Rb pathway directly exerts a level of control over cellular differentiation, proliferation and apoptosis (67, 68). 1,25(OH)$_2$D$_3$-dependent Id1 increase was shown to induce a more differentiated phenotype, followed by a subsequent up-regualtion in E-cadherin, a transmembrane glycoprotein that functions in cell-cell adhesion and is thought to act as a tumor suppressor protein (69). Loss of E-cadherin is associated with metastasis.

On the other hand, 1,25(OH)$_2$D$_3$ negatively regulates the expression of Id2. When 1,25(OH)$_2$D$_3$ binds to the VDR, the complex sequesters and binds β-catenin. The binding between 1,25(OH)$_2$D$_3$-VDR-β-catenin reduces the β-catenin available to complex with TCF-4 (T-cell factor). The β-catenin/TCF-4 complex has been shown to be one of the main transcriptional activators of Id2 (70). Decreased expression of Id2 results in decreased binding between Id2 and Rb, thus interfering with the Rb-E2F pathway (E2F is a family of DNA-binding transcription factors) and p21, causing the cell to exit the cell cycle and prevent entry into the S-phase and

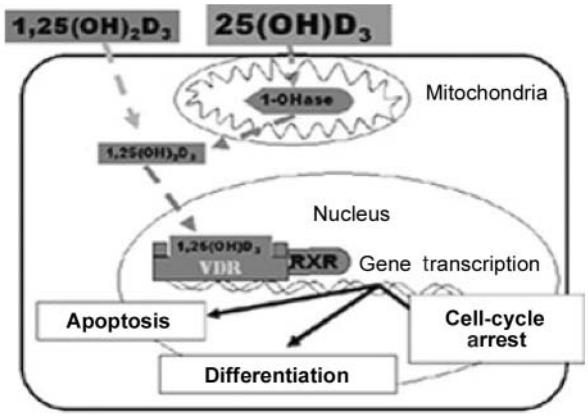

Figure 11. *Local production of 1,25(OH)$_2$D$_3$ and its action on cellular growth and differentiation.*

cell replication (71). It is believed that activated (hypophosphorylated) Rb represses transcription of cell-cycle genes through interaction with E2F transcription factors (71).

The relationship of 1,25(OH)$_2$D$_3$ in controlling prostate and colon cancer growth are just two examples that demonstrate the importance of maintaining healthy 25(OH)D levels to decrease risk of an array of diseases. The data suggest that the local synthesis of 1,25(OH)$_2$D$_3$ plays an important autocrine role in the regulation of cellular growth. Many groups continue to pursue the development of vitamin D analogs that are designed to enhance the antiproliferative and pro-differentiating abilities of 1,25(OH)$_2$D$_3$, without perturbing calcium homeostasis. These analogs could serve as potential candidates for treatment of specific cancers. As the specific mechanisms of action of 1,25(OH)$_2$D$_3$ continue to be elucidated, vitamin D's potential for clinical application in the prevention and treatment of the most common and lethal cancers will become more clear.

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