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**Review**

# Vitamin D and periodontal disease

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**Abstract: 1,25-Dihydroxyvitamin $D_3$ [1,25$(OH)_2D_3$; 1,25-dihydroxycholecalciferol or calcitriol] is the active form of vitamin $D_3$, a lipid-soluble vitamin that plays a role in calcium and bone metabolism. Recently, vitamin $D_3$ has been shown to function in cancer prevention, immunity and cardiovascular regulation. 1,25$(OH)_2D_3$ exhibits physiological and pharmacological effects by activating the vitamin D receptor (VDR), a transcription factor of the nuclear receptor superfamily. 1,25$(OH)_2D_3$ plays a role in maintaining oral health through its effects on bone and mineral metabolism and innate immunity, and several VDR gene polymorphisms have been reported to be associated with periodontal disease. VDR ligands should prove to be useful in the treatment and prevention of periodontal disease. (J Oral Sci 51, 11-20, 2009)**

Keywords: vitamin D; vitamin D receptor; nuclear receptor; infection; innate immunity; periodontal disease.

## Introduction

Vitamin D plays a role in various physiological processes, including bone and calcium metabolism, cellular growth and differentiation, immunity and cardiovascular function (1,2). Vitamin D is a secosteroid, in which the B ring of the canonical steroid structure is ruptured, and is synthesized from 7-dehydrocholesterol, an intermediate metabolite in cholesterol synthesis, or derived from dietary sources (3). Ultraviolet irradiation in sunlight-exposed skin induces a photochemical reaction of 7-dehydrocholesterol to produce the secosteroid vitamin $D_3$ (cholecalciferol) (Fig. 1A). Vitamin $D_3$ is hydroxylated at the 25-position by the hepatic vitamin $D_3$-hydroxylases, sterol 27-hydroxylase (CYP27A1) and vitamin D 25-hydroxylase (CYP2R1), to yield 25-hydroxyvitamin $D_3$ (25-hydroxycholecalciferol), the major form of vitamin D in the circulation (4). The 25-hydroxyvitamin $D_3$ is further hydroxylated in the 1a-position by 25-hydroxyvitamin D 1α-hydroxylase (CYP27B1). This reaction is tightly regulated and occurs exclusively in the kidney to yield the active metabolite, 1,25-dihydroxyvitamin $D_3$ [1,25$(OH)_2D_3$; 1,25-dihydroxy-cholecalciferol or calcitriol]. Dietary vitamin $D_2$ (ergocalciferol) and vitamin $D_3$ undergo the same activation process, involving 25-hydroxylation in the liver and subsequent 1α-hydroxylatoin in the kidney, and are converted to the active metabolites, 1,25$(OH)_2D_3$ and 1,25$(OH)_2D_2$, respectively (3). These molecules bind to the vitamin D receptor (VDR), a nuclear receptor that is highly expressed in the target organs of calcium homeostasis, such as the intestine, bone, kidney and parathyroid glands (5). Recent epidemiological data and animal studies using VDR-null mice provide evidence for a role of vitamin D in preventing cancer, infection and cardiovascular disease as well as calcium and bone disorders (1,2,6,7) (Fig. 1B). This review focuses on the function of vitamin D in oral health.

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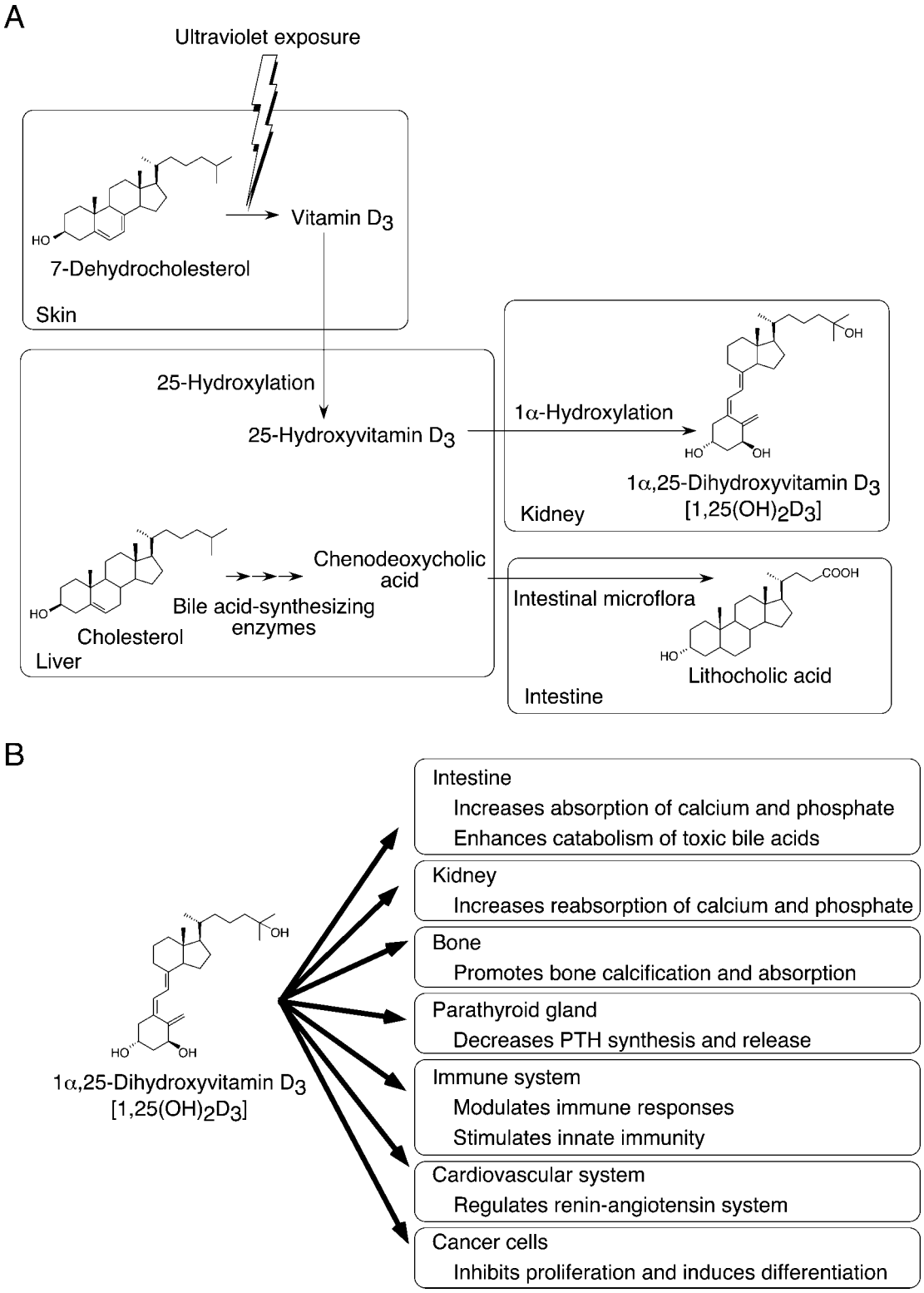


Fig. 1 Synthesis of natural VDR ligands and effects of $1,25(OH)_2D_3$ on target cells.
(A) $1,25(OH)_2D_3$ is the active form of vitamin $D_3$ that activates VDR. 7-Dehydrocholesterol is converted to vitamin $D_3$ by ultraviolet-induced photochemical reaction, and then activated to $1,25(OH)_2D_3$ by 25-hydroxylation in liver and 1α-hydroxylation in kidney. Cholesterol is metabolized to bile acids, such as chenodeoxycholic acid, in the liver. The primary bile acid chenodeoxycholic acid is converted to the secondary bile acid lithocholic acid, which is another natural VDR ligand. (B) $1,25(OH)_2D_3$ exhibits physiological and pharmacological effects by activating VDR in target cells. The physiological role of lithocholic acid remains unknown.

## Transactivation of VDR

VDR belongs to the nuclear receptor superfamily of transcription factor (8). Forty-eight human nuclear receptors have been identified and are classified into three groups on the basis of their ligand-binding characteristics. Steroid hormone receptors, which act as homodimers and mediate endocrine signals, are the first group and include the estrogen, progesterone, androgen, glucocorticoid and mineralocorticoid receptors. The second group are metabolic sensors and were initially identified as orphan receptors (9). Fatty acids, bile acids, oxysterols, and xenobiotics are ligands for this class of receptors. These metabolite-sensing receptors form heterodimers with retinoid X receptors (RXRs). The third group of orphan receptors have no known physiological ligands and may be regulated by ligand-independent mechanisms including phosphorylation. VDR responds to both an endocrine signal, $1,25(OH)_2D_3$, and metabolites, such as lithocholic acid (Fig. 1A), indicating that VDR has dual functions as an endocrine receptor and a metabolic sensor (2). The organization of the human VDR protein, as in other nuclear receptors, has been divided into five regions (A-E) (Fig. 2A). The C region contains a DNA-binding domain with two zinc fingers and is the domain with strongest sequence homology among the member of the superfamily. The C-terminal ligand-binding domain (E region) forms a heterodimerization interface and contains a ligand-dependent transactivation domain called the activation function 2 (AF2). The N-terminal A/B region contains a ligand-independent transactivation domain called the AF1. AF1 domains play a role in tissue specific function of steroid hormone receptors, and the AF1 function of VDR may be limited because of its short A/B region (5).

VDR forms a heterodimer with RXR and is not permissive to RXR ligand activation (10). VDR is localized in both the cytosol and nucleus and accumulates in the nucleus in response to $1,25(OH)_2D_3$ binding (11). The VDR-RXR heterodimer binds preferentially to a DNA response element that consists of a two hexanucleotide (AGGTCA or a related sequence) direct repeat motif separated by three nucleotides (DR3) (5) (Fig. 2B). The DR3 VDR-binding element has been identified in the regulatory regions of many target genes, including 25-hydroxyvitamin D 24-hydroxylase (CYP24A1), calbindin $D_{9k}$, cathelicidin antimicrobial peptide (CAMP) and transient receptor potential vanilloid type 6 (TRPV6). An everted repeat of the hexanucleotide motif separated by six nucleotides (ER6) is another VDR-binding element that regulates expression of the human CYP3A4 gene (12). Mutations in the zinc fingers of the VDR DNA-binding domain cause hereditary vitamin D-resistant rickets (HVDRR) due to deficient target gene induction (5).

Nuclear receptors, including VDR, undergo a conformational change in the cofactor binding site and AF2 domain upon ligand binding, a structural rearrangement that results in the dynamic exchange of cofactor complexes (13). In the absence of ligand, corepressors bind to the AF2 surface, composed of portions of helix 3, loop 3-4, helices 4/5, and helix 11. Ligand binding alters the AF2 surface by repositioning helix 12 (Fig. 2B), reduces the affinity for corepressors, and increases the affinity for coactivator recruitment, allowing nuclear receptors to induce the transcription of specific genes. Cofactor complexes have been classified into three functional categories (14). Members of the first cofactor complex class regulate transcription directly via interactions with general transcription factors and RNA polymerase II. Members of the second cofactor complex class modify histone tails by acetylation or deacetylation. The third class of complexes is involved in ATP-dependent dynamic chromatin remodeling. Ligand-bound VDR is not only involved in transactivation but in some contexts can also mediate transrepression (15). Dynamic and coordinated interaction of cofactor complexes and VDR is required for efficient regulation of transcription.

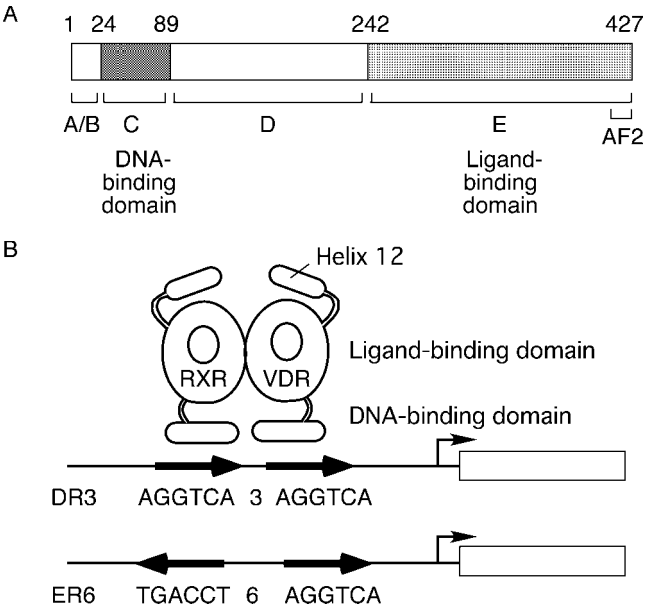


Fig. 2 VDR is a nuclear receptor mediating vitamin D signal. (A) Human VDR consists of 427 amino acids (GenBank accession no. NP_000367).
(B) The VDR-RXR heterodimer binds to DR3 and ER6 elements in the promoter region of target genes. Helix 12 plays an important role in ligand-dependent activation by forming a cofactor interface.

## Mineral and bone metabolism

$1,25(OH)_2D_3$ plays an important role in maintaining calcium and phosphate levels in blood by stimulating intestinal absorption, bone resorption, and renal reabsorption (5) (Fig. 1B). Vitamin D deficiency causes insufficient absorption of dietary calcium and phosphate and results in increased secretion of parathyroid hormone to mobilize bone calcium stores, leading to rickets and osteomalacia. VDR mutations have been identified in HVDRR (16), and VDR-null mice have a similar phenotype to HVDRR patients, including rickets, hypocalcemia, hypophosphatemia, elevated serum $1,25(OH)_2D_3$, and hyperparathyroidism (17). As in HVDRR patients, a high calcium diet prevents rickets and hyperparathyroidism in VDR-null mice (18). These findings indicate that the abnormal bone mineralization associated with vitamin D deficiency and HVDRR are secondary to impaired intestinal calcium absorption. Ligand-activated VDR induces the expression of genes involved in calcium metabolism, such as calbindin $D_{9k}$, TRPV6 and TRPV5 (19). Calbindin $D_{9k}$ is an intracellular calcium transfer protein, and TRPV6 and TRPV5 are epithelial calcium channels. Although calbindin $D_{9k}$ was considered to be an important mediator of vitamin $D_3$ signaling in renal and intestinal calcium absorption, mice lacking calbindin $D_{9k}$ demonstrate that calbindin $D_{9k}$ is not required for calcium homeostasis (20). TRPV6 is expressed in kidney and intestine, while TRPV5 expression is restricted to the kidney (19). Knockout mouse studies demonstrated that TRPV6 is necessary for intestinal calcium absorption and plays an important role in maintaining blood calcium levels (21). Mice lacking Trpv5 have diminished renal calcium reabsorption, resulting in severe hypercalciuria (22). Through a compensatory increase in intestinal calcium absorption mediated by elevated serum vitamin $D_3$ levels and intestinal Trpv6 expression, serum calcium levels are maintained. $1,25(OH)_2D_3$ enhances dietary phosphate absorption by unknown mechanisms. Fibroblast growth factor 23 (FGF23) has been identified as a factor that reduces serum phosphate and $1,25(OH)_2D_3$ levels by suppressing CYP27B1 expression and increasing CYP24A1 expression (23). Since VDR-null mice show normal skeletal development and their phenotype of rickets is rescued by normalization of serum calcium and phosphate levels, the effect of vitamin D deficiency on bone is mediated by dysregulated mineral homeostasis (24).

Activation of VDR by pharmacological doses of $1,25(OH)_2D_3$ directly regulates osteoblasts by inducing the bone-remodeling proteins osteocalcin and osteopontin (5), and up-regulates the receptor activator of NF-κB ligand (RANKL), a paracrine signal for osteoclastogenesis (25). Transplantation of VDR-null bone to wild-type animals increases bone volume and density, indicating that VDR is involved in increased bone resorption or decreased bone formation (26). RANKL is a membrane-bound cytokine that binds to its receptor, RANK, which is expressed on osteoclast precursors, and activates osteoclast differentiation (27). VDR-mediated induction of osteoblast RANKL may account for enhanced bone resorption. Chondrocyte-specific VDR-ablated mice have reduced RANKL expression and delayed osteoclastogenesis (28). These mice also show reduced circulating levels of FGF23 and elevated serum phosphate levels. Since FGF23 is not expressed in chondrocytes, VDR induces an unknown chondrocyte-derived factor that up-regulates FGF23 expression in osteoblasts. Thus, VDR regulates bone homeostasis through actions in osteoblasts and chondrocytes as well as through mineral metabolism.

Osteoporosis is a common metabolic disease characterized by the loss of both the organic and mineral contents of bone, resulting in increased bone fragility and fracture. Osteoporosis and periodontal disease share several risk factors and bidirectional relationships between osteoporosis and periodontal disease have been proposed (29). Osteoporosis results in decreased bone mineral density throughout the body, including the maxilla and the mandible. The lowered density in the jawbones leads to increased alveolar porosity, an altered trabecular pattern and more rapid alveolar bone resorption following invasion by periodontal pathogens. Periodontal infection increases the systemic release of proinflammatory cytokines, which accelerate systemic bone resorption. Vitamin D deficiency is a risk factor for osteoporotic fractures (30), and treatment of osteoporotic women with $1,25(OH)_2D_3$ increases bone mineral density and decreases the incidence of vertebral compression fractures (31). As discussed in the following sections, $1,25(OH)_2D_3$ suppresses proinflammatory responses and enhances innate immunity. Therefore, VDR ligands should be clinically useful in the treatment of osteoporosis-associated periodontal disease.

## Cancer and leukemia

$1,25(OH)_2D_3$ has been demonstrated to inhibit the proliferation and induce differentiation of various types of malignant cells, including prostate, breast, colon, skin, and brain cancers, as well as myeloid leukemia cells *in vitro* (2). Epidemiological studies show an inverse relationship between mortality due to prostate, breast and colon cancers and sunlight exposure (6). Particularly in oral/pharyngeal cancer, low 25-hydroxyvitamin $D_3$ has been associated with increased cancer incidence, suggesting anticancer activity of $1,25(OH)_2D_3$ (6,32). More than 20 years ago,

$1,25(OH)_2D_3$ was discovered to induce the differentiation of murine and human leukemia cells (33). Treatment with $1,25(OH)_2D_3$ or 1α-hydroxyvitamin $D_3$, which is rapidly metabolized to $1,25(OH)_2D_3$, prolongs survival in leukemia mice (34). VDR ligands induce the expression of cyclin-dependent kinase inhibitors, $p21^{CIP1/WAF1}$ and $p27^{KIP1}$, which may contribute to G1 cell cycle arrest of malignant cells (1). Although the anticancer mechanisms of $1,25(OH)_2D_3$ remain to be elucidated, VDR ligands that retain efficient growth-inhibitory activity but have low calcemic activity should be promising anticancer drugs.

## Immune disorders and infection

VDR is widely expressed in immune cells such as antigen-presenting cells, natural killer cells, T cells, and B cells, and $1,25(OH)_2D_3$ has potent immunomodulatory effects (1). The immune effects of $1,25(OH)_2D_3$ are principally mediated through actions on dendritic cells. Alloreactive T cell activation and dendritic cell maturation are inhibited by $1,25(OH)_2D_3$. Hypertrophy due to increased mature dendritic cells in the lymph nodes of VDR deficient mice indicates that $1,25(OH)_2D_3$ modulates antigen-specific immune responses *in vivo* (35). $1,25(OH)_2D_3$ also has an effect on naïve $CD4^+$ T cells to enhance Th2 cell development (36). Therapeutic effects of $1,25(OH)_2D_3$ have been demonstrated in models of several immune diseases, including multiple sclerosis, rheumatoid arthritis, inflammatory bowel diseases, systemic lupus erythematosus, and transplant rejection (1,2).

Host-derived antimicrobial peptides participate in the innate immunity defense against mucosal infection (37). VDR activated by $1,25(OH)_2D_3$ induces the expression of CAMP and β-defensin 4 and kills *Mycobacterium tuberculosis* in macrophages (38). Toll-like receptor activation by bacteria-derived lipopeptide up-regulates expression of VDR and CYP27B1 in macrophages, a mechanism that further enhances target gene induction. Reduced levels of 25-hydroxyvitamin $D_3$ in African Americans correlate with inefficient expression of CAMP mRNA and increased susceptibility to microbial infections. CAMP is widely expressed and secreted by keratinocytes and epidermal glands, and CAMP-deficient mice are susceptible to necrotic skin infection (39). β-Defensins exhibit antimicrobial activity against oral microbes including periodontitis-related bacteria such as *Actinobacilus actinomycetemcomitans*, *Porphyromonas gingivalis*, and *Fusobacterium nucleatum*, *Candia*, and papilloma virus (40). HVDRR patients suffer from frequent dental abscesses, providing further evidence that vitamin D plays a role in oral innate immunity (41).

## VDR polymorphisms and periodontal disease

Along with the loss-of-function VDR mutations responsible for HVDRR (16), associations of several VDR restriction fragment length polymorphisms (RFLPs) with several diseases, including secondary hyperparathyroidism in renal failure, osteoporosis, cancer, nephrolithiasis, diabetes, and periodontal disease, have been reported (42-44). The RFLPs *Bsm*I, *Tru*9I, *Taq*I, *Eco*RV and *Apa*I are located between exons 8 and 9 and may influence mRNA stability (45) (Fig. 3A). The RFLP *Fok*I creates a start codon in exon 2, resulting in an alternative start site. An association between the *Taq*I RFLP (Fig. 3B) and periodontitis has been reported (46-48). An association between the less frequent t allele and localized early onset periodontitis (aggressive

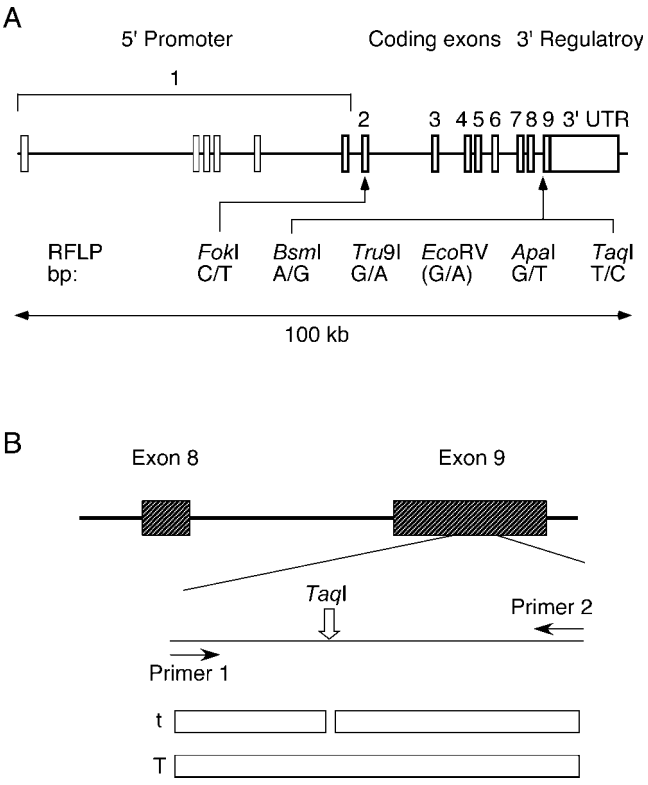


Fig. 3 Structure of the human VDR gene and VDR polymorphisms.
(A) RFLP sites *Bsm*I, *Tru*9I, *Eco*RV, *Apa*I and *Taq*I are located between exon 8 and exon 9. *Fok*I RFLP site is in exon 2. *Fok*I and *Taq*I are in the coding sequence. (B) The *Taq*I RFLP is formed by a single base transition (T→C) at codon 352 in exon 9 of the VDR gene that creates a *Taq*I restriction site. The alleles that result from this change are designated "t" (*Taq*I site present) or "T" (*Taq*I site absent). Digestion of polymerase chain reaction fragments with *Taq*I results in different fragments for the t alleles and the T alleles (54).

Table 1 VDR polymorphisms and periodontitis

| Authors, published year (reference) | Subjects | Country | Polymorphism | Association |
|---|---|---|---|---|
| Henning et al., 1999 (49) | 69 EOP patients (including 20 LEOP) and 72 controls | UK | *Taq*I | LEOP: less frequent t allele |
| Yoshihara et al., 2001 (53) | 42 GEOP patients, 52 AP patients and 55 healthy controls | Japan | *Bsm*I | GEOP: VDR (B non-carrier) and FcgRIIIb (NA2 carrier) combination |
| Sun et al., 2002 (46) | 24 AP patients and 37 EOP patients and 39 healthy controls | China | *Taq*I | EOP: Tt genotype and t allele |
| Tachi et al., 2003 (48) | 74 CP patients and 94 healthy controls | Japan | *Taq*I | CP: TT genotype |
| de Brito Junior et al., 2004 (54) | 69 CP patients and 44 healthy controls | Brazil | *Taq*I, *Bsm*I | CP: TB haplotype and TB/tb heterozygous haplotype |
| Brett et al., 2005 (47) | 51 AgP patients, 57 CP patients, and 100 healthy controls | UK | *Taq*I | CP: TT genotype |
| Park et al., 2006 (55) | 93 GAgP patients and 143 healthy controls | Korea | *Fok*I | GAgP: **C/*C* genotype |
| Meng et al., 2007 (44) | 90 AgP patients and 91 healthy controls | China | *Taq*I | AgP (female): Tt genotype |

AP, adult periodontitis; AgP, aggressive periodontitis; CP, chronic periodontitis; EOP; early onset periodontitis; FcgRIIIb, immunoglobulin-Fcg receptor IIIb; GAgP, generalized aggressive periodontitis; GEOP, generalized early onset periodontitis; LEOP, localized early onset periodontitis

periodontitis) in Caucasian subjects was reported (49) (Table 1). The TT genotype and the T allele are associated with chronic periodontitis in Japanese and Caucasian subjects (47,48), while the tt genotype and t allele are associated with early onset periodontitis (aggressive periodontitis) in Chinese subjects (46). A strong association between Chinese female patients with aggressive periodontitis and the Tt genotype is suggested (44). While the tt genotype is associated with less occurrence of tuberculosis and chronic hepatitis B virus infection (50), the tt genotype and t allele are associated with decreases in bone mineral density and the incidence of osteoporosis (51,52). These finding suggest that the *Taq*I RFLP is associated with both immune function and bone metabolism. Ethnic differences and different mechanisms in pathogenesis between aggressive periodontitis and chronic periodontitis may influence the results of *Taq*I RFLP analysis. The *Bsm*I RFLP in combination with other RFLPs are associated with early onset periodontitis (aggressive periodontitis) and chronic periodontitis (53,54). The *Fok*I RFLP that results in the short VDR protein was demonstrated to increase a risk of generalized aggressive periodontitis in Korean (55). The *Apa*I, *Bsm*I, and *Fok*I RFLPs have been reported to confer elevated risk of severe chronic periodontitis in Japanese men (56). These RFLPs are associated with bone and mineral diseases, and the *Taq*I and *Fok*I RFLPs are associated with increased cancer risk, such as prostate and breast malignancy (42). Further studies are required to elucidate the functional relevance of VDR RFLPs and disease pathogenesis. An inverse association between serum 25-hydroxyvitamin $D_3$ concentrations and periodontal disease has been reported (57). These findings indicate that $1,25(OH)_2D_3$ plays a role in prevention of periodontal disease and that hypomorphic VDR alleles and reduced levels of $1,25(OH)_2D_3$ may be associated with periodontal disease.

## Therapeutic application of VDR ligands

As discussed above, VDR ligands are promising drugs candidates in the treatment of bone and mineral disorders, cancers and leukemia, autoimmune diseases and infection, including periodontal disease. Clinical studies have shown that vitamin D deficiency is associated with elevated risk of cardiovascular disease (7,58). Ligand-activated VDR suppresses renin expression and VDR-null mice develop cardiovascular disease, such as hypertension and cardiac hypertrophy, due to dysregulation of the renin-angiotensin system (59,60). VDR acts as a metabolic sensor for

secondary bile acids, such as lithocholic acid, and induces the expression of genes involved in the metabolism and excretion of toxic bile acids (2,61) (Fig. 1). These findings suggest that VDR-targeted therapies can be applied to cardiovascular disease and cholesterol/bile acid metabolism-related disorders. Insufficient clearance of periodontopathic bacteria and subsequent bone destruction are suggested to cause aggressive periodontitis (43). VDR ligands stimulate innate immunity by inducing anti-microbial peptides and have bone anabolic effects (1,38), suggesting that VDR ligands can be applied for prevention of aggressive periodontitis. A dysregulated release of proinflammatory cytokines by monocytes/macrophages and lymphocytes is considered to induce chronic periodontitis (43). Since $1,25(OH)_2D_3$ has potent immunomodulatory effects, including inhibition of proinflammatory cytokine release (1,62), VDR ligands may be effective in the treatment of chronic periodontitis. Experimental and epidemiological studies suggest that VDR ligands are also useful in the prevention and treatment of oropharyngeal cancer (1,6). Currently, adverse effects, especially hypercalcemia, limit the clinical application of $1,25(OH)_2D_3$ and its derivatives to bone and mineral disorders and psoriasis, a chronic skin disorder characterized by keratinocyte hyperproliferation and inflammatory infiltration of the epidermis and dermis (2). Combined dosing of $1,25(OH)_2D_3$ with other drugs is one approach to overcome the adverse effects (63,64). The development of tissue-selective or function-selective VDR modulators with low calcemic activity provides another approach (65,66). Although topical application of VDR ligands, as in the treatment for psoriasis, may allow for the treatment of periodontal disease without inducing systemic adverse effects, further pharmacological and clinical studies are required.

## Conclusion

In addition to its well-known activity in preventing rickets and osteomalacia, $1,25(OH)_2D_3$ has been shown to have important anticancer, immune modulatory, and innate immune effects, through VDR activation. The $1,25(OH)_2D_3$-VDR system plays a role in oral homeostasis and its dysfunction may lead to periodontal disease. Vitamin D research should make important contributions to advancing oral medicine.

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