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Vitamin D: Classic and Novel Actions

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Abstract
Background: Classically, vitamin D has been implicated in bone health by promoting calcium absorption in the gut and maintenance of serum calcium and phosphate concentrations, as well as by its action on bone growth and reorganization through the action of osteoblasts and osteoclasts cells. However, in the last 2 decades, novel actions of vitamin D have been discovered. The present report summarizes both classic and novel actions of vitamin D. ***Summary:*** 1,25$(OH)_2$ vitamin D, the active metabolite of vitamin D, also known as calcitriol, regulates not only calcium and phosphate homeostasis but also cell proliferation and differentiation, and has a key a role to play in the responses of the immune and nervous systems. Current effects of vitamin D include xenobiotic detoxification, oxidative stress reduction, neuroprotective functions, antimicrobial defense, immunoregulation, anti-inflammatory/anticancer actions, and cardiovascular benefits. The mechanism of action of calcitriol is mediated by the vitamin D receptor, a subfamily of nuclear receptors that act as transcription factors into the target cells after forming a heterodimer with the retinoid X receptor. This kind of receptors has been found in virtually all cell types, which may explain its multiple actions on different tissues. ***Key Messages:*** In addition to classic actions related to mineral homeostasis, vitamin D has novel actions in cell proliferation and differentiation, regulation of the innate and adaptative immune systems, preventive effects on cardiovascular and neurodegenerative diseases, and even antiaging effects.

Introduction

Vitamin D was first characterized like a vitamin in the 20th century and now it is recognized as a prohormone. Two major forms of vitamin D are vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol). Vitamin D_3 is synthesized in the skin of humans and is consumed in the diet via the intake of animal-based foods, mainly fish oils,

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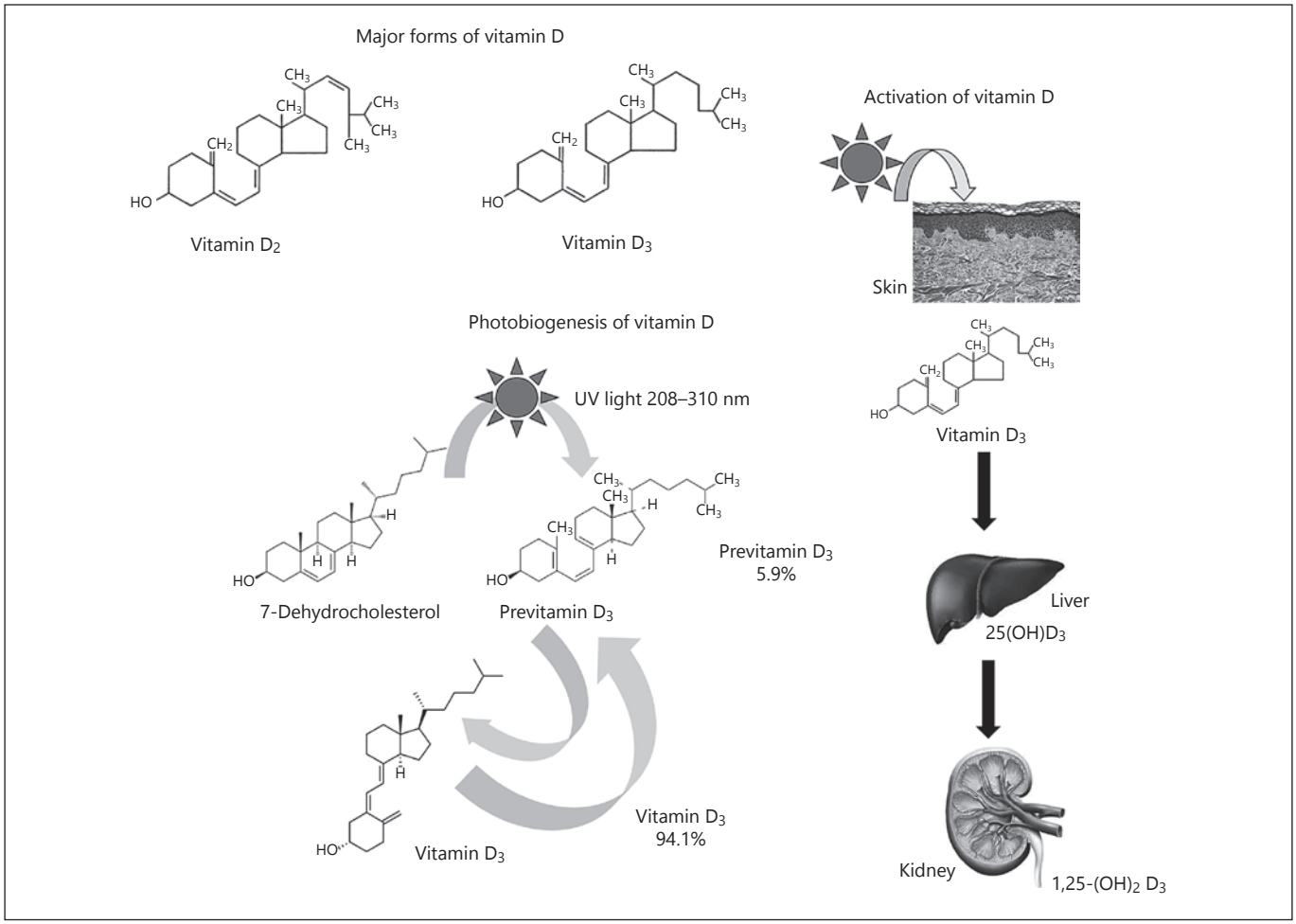

Fig. 1. Vitamin D forms, photobiosynthesis, and activation.

whereas vitamin D_2 is derived from plant sources, is not largely human-made, and added to foods [1]. Vitamins D_2 and D_3 forms differ only in their side chain structure (Fig. 1). The differences do not affect metabolism (i.e., activation), and both forms have the prohormone function.

Vitamin D Absorption and Photobiogenesis

Vitamin D obtained from sun exposure, food, and supplements is biologically inactive (either the vitamin D_2 or D_3) and must undergo activation through 2 consecutive enzymatic hydroxylation reactions occurring in the liver and kidney (Fig. 1).

Dietary vitamin D (either vitamin D_2 or D_3) is usually absorbed at the small intestine with other dietary fats [2]. The presence of fats in the lumen triggers bile acids release, which initiate emulsification and support the formation of lipid-containing micelles, which diffuse into enterocytes [3]. Once absorbed, exogenous vitamin D is packaged into chylomicrons, and thus is transported to the liver. A fraction of the vitamin D contained in the chylomicron can be taken up by adipose tissue and skeletal muscle [4]. Once remnant chylomicrons reach the liver, a specific carrier protein, the vitamin D binding protein (DBP) makes it possible for them to enter the hepatocytes and later facilitates their transport to different tissues that need them. Endogenously, vitamin D_3 can be photosynthesized in the skin.

7-Dehydrocholesterol (provitamin D_3) is converted to the previtamin D_3 form (precalciferol) following its exposure to ultraviolet B (UVB) radiation [1]. Subsequently, it can suffer a thermal isomerization to vitamin D_3 in the epidermis (Fig. 1). Alternatively, previtamin D_3 may be photoconverted to nonactive forms, such as tachysterol

and lumisterol, which may exert different biological activities [5]. The production of vitamin D_3 in the skin is due to the extent and quality of the UVB radiation reaching the dermis as well as the availability of 7-dehydrocholesterol and the characteristics of the skin.

Liver and Renal Metabolism of Vitamin D to the Active Hormonal Form

Once vitamin D enters the circulation through the skin or from the lymph, it is cleared by the liver or storage tissues within a few hours. In the liver, precalciferol is rapidly hydroxylated by the 25-hydroxylase, a cytochrome P450 enzyme, (mainly the CYP2R1), which forms 25-hydroxyvitamin D ($25(OH)_2D$; calcidiol), through an unregulated process [6]. Once synthesized, DBP-bound 25(OH)D is secreted into blood and requires a renal hydroxylation to obtain the active form 1α,25 dihydroxyvitamin D (calcitriol). The average plasma life of 25(OH)D is around 3 weeks; this is what makes serum levels of this 25(OH)D indicative of the body vitamin D storage and status.

When calcitriol is required due to a lack of calcium or phosphate, 25(OH)D is 1α-hydroxylated in the kidney forming the physiologically active form $1,25(OH)_2D$. This reaction is catalyzed by the 25(OH)D 1α-hydroxylase enzyme, which is another CYP450-dependent system (CYP27B1) [7]. This step occurs in the mitochondria of the proximal convoluted tubule cells, and is very tightly regulated by blood calcium and phosphate levels through parathyroid hormone (PTH) and the fibroblast growth factor 23 (FGF-23) [8]. Furthermore, $1,25(OH)_2D$ can act as a suppressor of CYP27B1, although the mechanism is not fully understood. Vitamin D can be stored in the adipose tissue, this accumulation being higher in obese than in normal weight subjects, but this stored vitamin D is not readily available, since it is not released when needed [9].

Inactivation and Excretion of Vitamin D

The CYP450 24-hydroxylase is present in the proximal convoluted tubule cells and in all target cells, expressing the vitamin D specific receptor (VDR). Calcitriol induces its own destruction by stimulating the 24-hydroxylase, which is also responsible for the degradation of its precursor, $25(OH)D_3$. Several oxidation reactions follow this 24-hydroxilation and sometimes the conjugation with glucuronic acid, thereby forming a number of compounds excreted through the bile [6]. The renal excretion is usually very low (<5%). The DBP-vitamin D complex may be filtrated at the glomerulus and specifically re-uptaken in a process mediated by a DBP-specific cubilin-megalin receptor system [10].

Regulation of Vitamin D Metabolism

Regulation of calcitriol depends on the balance between 1α-hydroxylase and 24-hydroxylase activities. Both enzymes are rigorously regulated by serum calcium, calcitriol, and phosphate levels. Under low serum calcium conditions, or low levels of vitamin D, PTH secreted by the parathyroid glands stimulates the synthesis of the 1α-hydroxylase, resulting in the increase of $1,25(OH)_2D$ activation [11]. PTH also inhibits 24-hydroxylase [12], and can induce osteoclast and osteocytes synthesis of the FGF-23, which acts by reducing the expression of renal sodium-phosphate transporters [13]. FGF-23 can also adjust vitamin D homeostasis by suppressing renal expression of 1α-hydroxylase and inducing 24-hydroxylase, thus reducing serum calcitriol levels and subsequently serum calcium under hyperphosphatemia conditions [14].

Classical Action of Vitamin D: Regulation of Calcium and Phosphate Homeostasis.

Calcitriol participates in the regulation of plasma ionized calcium and phosphate levels by acting on their intestinal absorption, renal excretion, and calcium bone mobilization as described below (Fig. 2). When serum calcium levels decrease, PTH secretion is stimulated and activates calcitriol synthesis. Both PHT and calcitriol stimulate calcium renal reabsorption and mobilization from bones (bone resorption).

In contrast, if serum calcium levels rise, PTH secretion drops, leading to a decrease of calcitriol and calcium mobilization. Indeed, if serum calcium levels become too high, the parafollicular cells of the thyroid secrete calcitonin, which block calcium mobilization from the bone and stimulate calcium and phosphorous excretion [15], contribute to keep calcium levels within the normal range.

Calcitriol acts directly on 3 target tissues with the aim of maintaining optimal serum calcium levels. In addition, through VDR, calcitriol suppresses parathyroid gene expression and parathyroid cell proliferation, reinforcing its direct action on increasing serum calcium levels [16].

The first target organ is the intestine (without PTH mediation); here calcitriol stimulates intestinal calcium

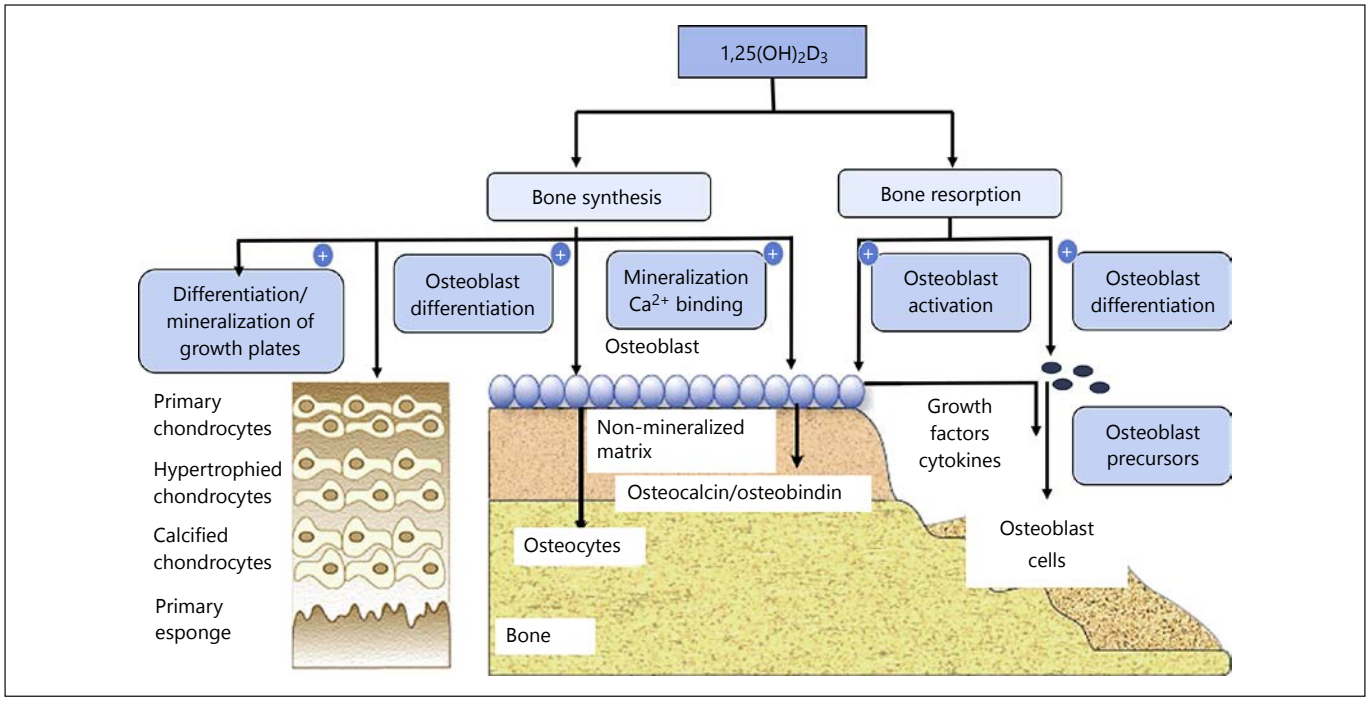

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Fig. 2. Vitamin D classic actions in the bone system.

absorption that depends on its presence in the diet, intestinal solubility, and intestinal absorption capacity, which is the result of the balance between transcellular and paracellular intestinal absorption [2]. When calcium intake is high, paracellular transport will be sufficient [17]. Transcellular transport involves 3 phases: (1) entrance of calcium through specific calcium channels (such as TRPV6) present in membranes of the brush border; (2) intracellular transport mediated by calbindin; and (3) calcium active transport to the blood stream at the basolateral surface mainly mediated by specific carriers [2, 14, 17].

The second organ are the kidneys; calcitriol with PTH encourages the renal distal tubule reabsorption of calcium. Calcitriol influences (1) calcium entrance through the apical membrane; (2) calbamicin-mediated calcium diffusion; and (3) active transport thought the basolateral membrane [18]. Vitamin D inhibits phosphate reabsorption indirectly by increasing FGF-23 osteocytes expression, and directly by inducing α-klotho (FGF-23 co-receptor) [14].

The third target tissue is the bone. Calcitriol mobilizes calcium from bone, a process requiring PTH [19]. When serum calcium levels decrease, PTH-dependent calcitriol activation prompts the formation and VDR-mediated differentiation of osteoclasts. This activation induces the mobilization of calcium from the bone by stimulating the secretion of the receptor activator for nuclear factor kappa-B ligand, which, in turn, is responsible for osteoclastogenesis and bone resorption [20]. At the same time, vitamin D inhibits mineralization through the increase of pyrophosphate levels and osteopontin [21]. Calcitriol promotes bone formation and growth, by activating chondrocyte differentiation, and increasing serum calcium and phosphate levels. Thus, vitamin D deficiency results in inadequate mineralization of the skeleton, and when low vitamin D levels are maintained, bone growth plates cannot be mineralized due to calcium and phosphate depletion [22, 23].

Mechanisms of Action of Vitamin D

The mechanism of action of the calcitriol is mediated by the VDR, which belongs to a subfamily of nuclear receptors that act as transcription factors into the target cells after forming a heterodimer with retinoid X receptor (RXR). Once dimerized, the complex binds to the VDR element, in the promoter regions of target genes or at distant sites, to positively or negatively regulate their expression [24]. As the VDR has been found in virtually all cell types [25], it may explain its multiple actions on different tissues [26].

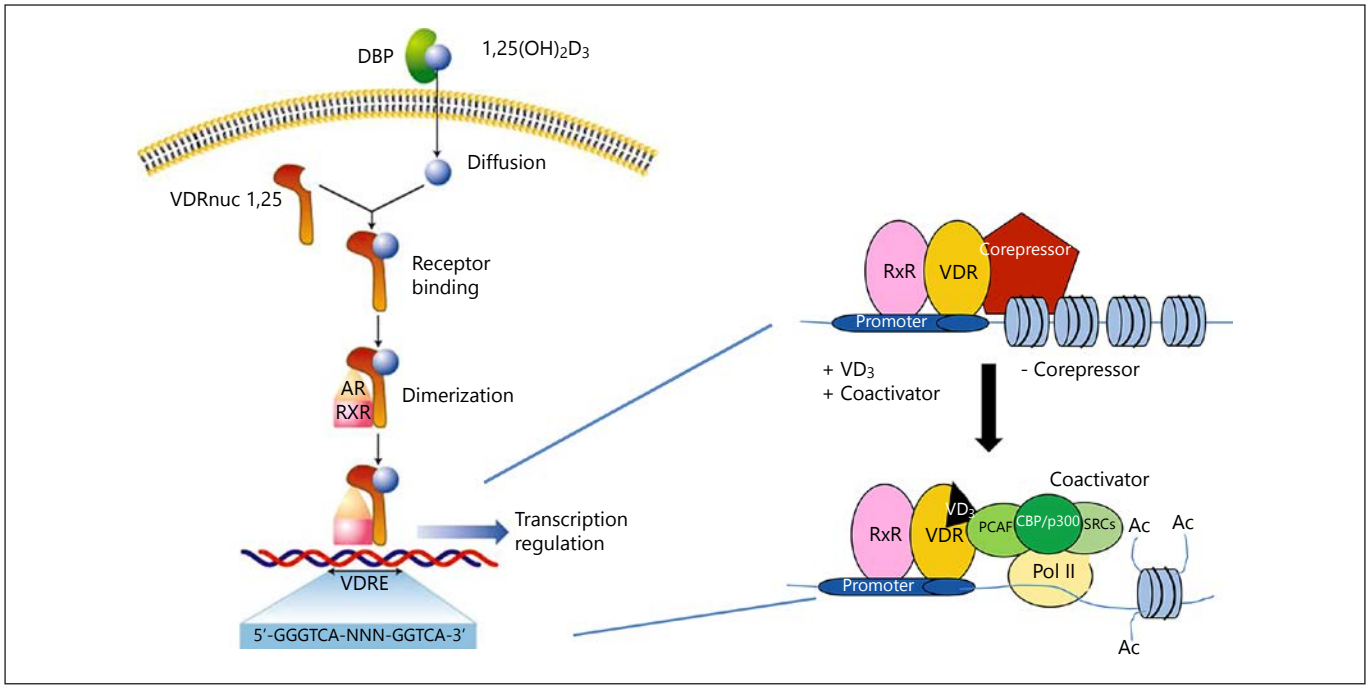

Fig. 3. Molecular mechanism of action of vitamin D. CBP/p300; CREB-binding protein binding protein p300, PCAF; P300/CBP-associated factor, SRC, steroid receptor coactivators.

Besides $1,25(OH)_2D_3$, the VDR-RXR dimer can associate with other molecules as the p160 coactivators family of steroid receptor coactivators 1, 2, and 3, that have histone acetylase (HAT) activity, and are primary coactivators that bind to the AF2 domain of liganded VDR [27]. Members of p160 family recruit proteins as secondary coactivators, such as CBP/p300, which also have HAT activity, resulting in a multi-subunit complex that modifies chromatin and destabilizes histone/DNA interaction [28]. The modification of histones occurs not only by acetylation, but also through methylation [27]. Liganded VDR interacts with basal transcription factors (TFIIB and several TATA DNA box binding protein-associated factors). VDR-intermediated transcription is facilitated by the mediator, a multi-protein complex that functions through the recruitment of RNA polymerase II and promotes the formation of the preinitiation complex [29] (Fig. 3).

There is increasing evidence that specific CAAT enhance binding protein (C/EBP) family members may be key mediators of $1,25(OH)_2D_3$ action. C/EBP is induced by $1,25(OH)_2D_3$ in kidney and osteoblastic cells and cooperates with $1,25(OH)_2D_3$ and VDR in enhancing *Cyp24a1* and *Bglap* genes transcription [30]. C/EBP and VDR cooperate in the transcriptional regulation of the human antimicrobial peptide cathelicidin in lung epithelial cells, and Runx2 and VDR collaborate in the transcriptional regulation of mouse osteopontin in osteoblastic cells [30]. C/EBP, Runx2, and VDR all contribute to the control of matrix metalloproteinase 13 gene transcription [31]. The SWI/SNF complexes contribute to transcriptional activation by VDR. C/EBP recruits the SWI/SNF complex to promote $1,25(OH)_2D_3$ induction of *Cyp24a1* and *Bglap* transcription [32] (Fig. 3).

Low-affinity nutritional VDR ligands including curcumin, polyunsaturated fatty acids, and anthocyanidins initiate VDR signaling, whereas the longevity factors resveratrol and sirtuin 1 potentiate VDR signaling [33]. The result of VDR genomic interactions is the transcription regulation of multiple genes, in many cases far from the *cis* site of VDR binding. However, in a few cases, VDR can exert a regulatory action in the absence of calcitriol.

The overarching principles of $1,25(OH)_2D_3$-mediated gene regulation in target cells are as follows: i) VDR-binding sites are about 2,000–8,000; ii) active transcription unit is the VDR/RXR heterodimer; iii) distal-binding site location is dispersed in cis-regulatory modules (enhancers) across the genome; iv) VDR/RXR-binding site sequence (VDR element) is mediated by classic hexameric half-sites (AGGTCA) separated by 3 base pairs

and repression is mediated by divergent sites; v) DNA mode of binding is predominantly, but not exclusively, $1,25(OH)_2D_3$-dependent; vi) enhancers contain binding sites for multiple transcription factors that facilitate both independent or synergistic interaction; vii) epigenetic enhancers signatures are defined by the dynamically regulated posttranslational histone H3 and H4 modifications and selectively regulated by $1,25(OH)_2D_3$; viii) and VDR-binding sites are highly dynamic, as they change during cell differentiation, maturation, and disease activation and thus have consequential effects on gene expression [34]. Some mutations in the *VDR* affect severely its functionality causing rickets resistant to vitamin D, a rare autosomic recessive disease, also known as type II rickets. Those mutations modify the binding to VDR, the nuclear location of the calcitriol-receptor complex, the binding of the VDR to the *cis* elements, or the binding of VDR to some coactivators.

Novel Actions of Vitamin D

Vitamin D regulates cell proliferation and differentiation and has a key role in the responses of the immune and nervous systems. In fact, observational studies suggest that high serum concentrations of vitamin D protect against cardiovascular disease (CVD), diabetes, and colorectal cancer [35].

Evidence of extraskeletal effects of $1,25(OH)_2D_3$ includes xenobiotic detoxification, oxidative stress reduction, neuroprotective functions, antimicrobial defense, immunoregulation, anti-inflammatory/anticancer actions, and cardiovascular benefits [27]. The first evidence of novel activities of the vitamin D hormone was the demonstration that VDR was present in other tissues like keratinocytes, promyelocytes, monocytes, lymphocytes, ovarian cells, islet cells of the pancreas, and so on. [26].

Vitamin D and Cell Proliferation and Differentiation

Calcitriol and VDR have been shown to control the expression of genes associated with cellular proliferation and differentiation, suggesting a key role in cancer prevention. There is some evidence that vitamin D levels provide a protective status to lower the risk of cancer. Some analyses on publications of colon, breast, prostate, and ovarian cancer revealed that in numerous cases, vitamin D_3 levels correlated with reduced incidence of cancer [36]. Conversely, other studies suggest no or only weak evidence for a link between vitamin D levels and cancer protection, and there are examples where high vitamin D levels may actually increase risk (pancreatic cancer) [37].

Preclinical studies show that calcitriol and its analogs have antitumor effects *in vitro* and *in vivo* through multiple mechanisms including the induction of cell cycle arrest, apoptosis, differentiation, and the suppression of inflammation, angiogenesis, invasion, and metastasis [38].

The first demonstration that vitamin D was related to the terminal differentiation of promyelocytes to monocytes was reported in 1981 [39]. Recently, calcitriol and several structurally related members of the vitamin D class of seco-steroids have demonstrated the ability to regulate the hedgehog (Hh) signaling pathway, responsible of tissue differentiation during embryogenesis and maintenance of stem cell populations in certain adult tissues.

In fact, dysregulation of Hh signaling results in its constitutive activation and uncontrolled cellular proliferation and multiple mechanisms through which aberrantly activated Hh signaling contributes to tumor formation, growth, and metastasis [40]. Cross talk mechanisms between vitamin D/VDR signaling and the Hh pathway have not been well defined; however, evidence suggests that their interactions may play an important physiological role, primarily in proper skin homeostasis and the oncogenic development of basal-cell cancer, which is the most common type of skin cancer. These mechanisms include the transcriptional control of Hh pathway components by VDR as well as the ability of vitamin D ligands to directly modulate Hh pathway target genes [40]. To maintain tight control over calcitriol-mediated differentiation, keratinocytes are capable of expressing all the enzymatic machinery necessary to produce and metabolize calcitriol. The levels of active CYP27A1 and CYP27B1 in keratinocytes are controlled by multiple factors including calcium levels, calcitriol concentration, UVB radiation, and stage of cellular differentiation, suggesting that the levels of calcitriol produced are tightly regulated at multiple stages [41].

Multiple studies have demonstrated the chemo-preventative and chemotherapeutic properties of both calcitriol and VDR in skin. Prolonged UVB radiation damages keratinocyte DNA, primarily through the formation of mutagenic cyclobutane pyrimidine dimers (CPDs). Direct topical administration of calcitriol or its analogues protected against CPD formation and increased CPD clearance [42].

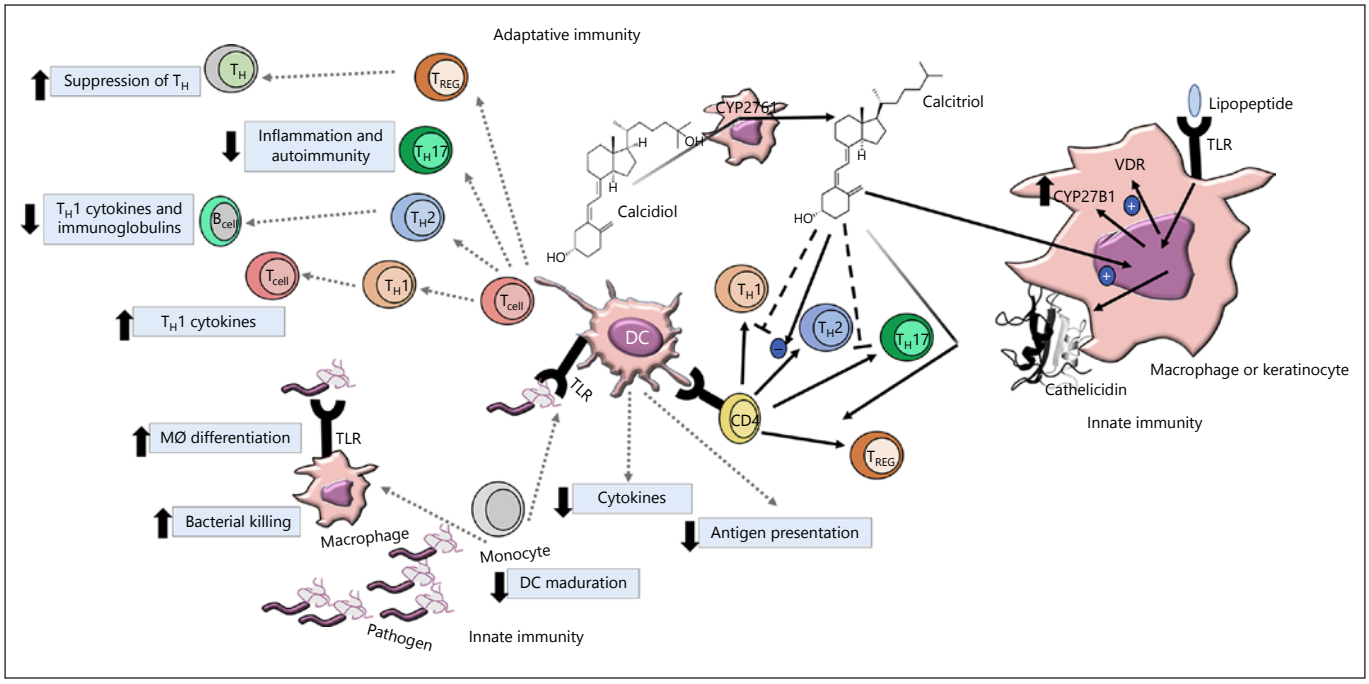

Fig. 4. Vitamin D effects on innate and adaptative immunity. CYP, cytochrome; MØ, macrophage; T_H, T-helper cell; TLR, toll-like receptor; T_{REG}, T regulatory cell, VDR, vitamin D receptor.

Vitamin D and the Immune System

$1,25(OH)_2D_3$ has important immunomodulatory actions, namely, the enhancement of the innate immune system and inhibition of the adaptative immune responses, associated with an increased synthesis of interleukin (IL)-4 by T helper (Th)-2 lymphocytes and the upregulation of regulatory T lymphocytes (T-reg). In fact, different types of immune cells, for example, dendritic cells (DC), macrophages, and T and B lymphocytes express VDR and most of them are able to synthesize calcitriol through an independent regulation pathway responding to a number of proinflammatory agents as bacterial lipopolysaccharide and tumor necrosis factor alpha (TNF-α) [24].

Macrophages-derived cytokines promote Th differentiation to Th0 cells. Later, with the cooperation of some costimulatory exogenous cytokines produced by a number of antigen presenting cells (APC), namely, macrophages and DC, Th0 differentiate to Th1 or Th2 cells, which in turn regulates cell and antibody immune responses. Calcitriol can regulate the immune responses in secondary lymphoid organs as well as in target organs through a number of mechanisms (Fig. 4).

Regulation of the Innate Immune Response by DC and Macrophages

Calcitriol increases the defense capacity of macrophages inducing their differentiation, phagocytic capacity, and antimicrobial activity (increasing the expression of cathelicidins). Moreover, calcitriol inhibits the proliferation of monocytes, and promotes the differentiation of monocytes to macrophages, these effects being mediated by the upregulation of Fc surface cell receptors and by an increase in cell respiration. In addition, calcitriol inhibits DC proliferation, maturation, as well as their immunostimulatory properties leading to the induction of T-reg cells. Consequently, vitamin D deficiency results in a less tolerogenic status to foreign antigens [26, 27].

Inhibition of the Pro-Inflammatory Response of APC

Calcitriol inhibits the expression of APC cytokines, namely, IL-1, IL-6, IL-12, and TNF-α and decreases the expression of a set of major histocompatibility complex class II cell surface proteins in macrophages, and the development of proinflammatory Th1 and Th17 cells, while inducing T-reg and Th2 cells, which in turn downregulate the activity of Th1. Thus, calcitriol inhibits the production of IL-12 and stimulates the production of IL-10, while downregulating the expression of some costimula-

tory molecules, for example, clusters of differentiation (CD) CD40, CD80, and CD86, required for the activation of DC and other APC, leading to Th1 inhibition. Additionally, calcitriol acts directly on T cells inhibiting the secretion of IL-2, a cytokine essential for lymphocyte clonal expansion, and interferon gamma [26, 27].

Calcitriol also inhibits B cell differentiation and antibody production. Additionally, it inhibits the apoptosis of enterocytes and promotes the synthesis of antimicrobial peptides, and reduces the proliferation of keratinocytes in psoriasis, favoring cell differentiation in both cases [26, 27].

Vitamin D and CVD

Experimental studies have established that calcitriol and VDR are critical regulators of the structure and function of the heart. In addition, clinical studies have associated vitamin D deficiency with CVD. Emerging evidence demonstrates that calcitriol is highly involved in CVD-related signaling pathways, particularly the Wnt signaling pathway. Addition of calcitriol to cardiomyocyte cells demonstrated the (i) inhibition of cell proliferation without promoting apoptosis; (ii) decreased expression of genes related to the regulation of the cell cycle; (iii) promotion of the formation of cardiomyotubes; (iv) induced expression of casein kinase-1-α1, a negative regulator of the canonical Wnt signaling pathway; and (v) increased expression of noncanonical Wnt11, which has been recognized to induce cardiac differentiation during embryonic development and in adult cells [43].

Neuroprotective Effects of Vitamin D

Vitamin D metabolites naturally pass through the blood-brain barrier, giving them access to neuronal and glial cells. Therefore, a number of roles for vitamin D have been observed in various neurological/neuromuscular disorders [44]. It has also been proposed that microglia within the central nervous system can generate calcitriol *in situ* and this might represent an antitumor response. Calcitriol can inhibit the synthesis of inducible nitric oxide synthase, leading to upregulation of glutathione; thus it could play a role in neuroprotection or neuromodulation [34].

There is widespread expression of the VDR in the brain of adult rodents, with high levels found in sensory, motor, and limbic systems, suggesting a role for vitamin D throughout life. Expression of functional VDRs within both neurons and glia of the adult hippocampus provide further evidence for vitamin D's importance in the adult central nervous system. In the human brain, both VDR and 1α-hydroxylase, the enzymes required for calcitriol production, have been observed to be in high levels in the substantia nigra, suggesting a potential link between this vitamin and the dopamine neuron population linked with Parkinson's disease [26, 34].

Antiaging Activity of Vitamin D

Many of the health span advantages conferred by $1,25(OH)_2D_3$ are related to its induction of α-klotho, a renal hormone that is an antiaging enzyme/coreceptor that protects against skin atrophy, osteopenia, hyperphosphatemia, endothelial dysfunction, cognitive defects, neurodegenerative disorders, and impaired hearing [33]. Together, $1,25(OH)_2D_3$ and α-klotho maintain the molecular signaling systems that promote growth (p21), development (Wnt), antioxidation (Nrf2/FOXO), and homeostasis (FGF-23) in tissues crucial for normal physiology, while simultaneously guarding against malignancy and degeneration [45]. Hence, VDR liganded to $1,25(OH)_2D_3$ regulate the expression of set of genes related to health span, with the α-klotho target playing a key role in the facilitation of health span by delaying the chronic diseases of aging.

Disclosure Statement

The authors declare no conflicts of interest related to the present article.

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