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# VITAMIN D IN THE PREVENTION, DEVELOPMENT AND THERAPY OF ONCOLOGICAL DISEASES

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## Abstract

Vitamin D, traditionally known as a fat-soluble essential vitamin, is a precursor of a powerful steroid hormone that regulates a broad spectrum of physiological processes. In addition to its fundamental role in bone metabolism, epidemiological, preclinical and cellular researches in recent decades have revealed that vitamin D can play a considerable role in the prevention of some pathologies, including extra-skeletal ones, such as neoplasms. Vitamin D, as a prohormone, undergoes first hepatic and subsequently renal metabolism to produce a biologically active metabolite, calcitriol or 1α,25-dihydroxyvitamin D or (1,25 (OH)2D), which binds the vitamin D receptor by regulating the expression of several genes involved in bone metabolism and other biological functions. Furthermore, recent studies have revealed that vitamin D can be also metabolized and activated through a non-canonical metabolic pathway catalyzed by CYP11A1, the gene encoding the cholesterol side chain cleavage enzyme or P450scc. The metabolites of vitamin D deriving from the CYP11A1 enzyme have shown antiproliferative and anti-inflammatory activities and are able to promote the differentiation process on neoplastic cells in comparable way or better than calcitriol, thus contributing to its tumor preventive effect. Clinical data have demonstrated that vitamin D has anticancer activity against prostate, colon, and breast cancers. Several molecular mechanisms of vitamin D involved in tumor etiopathogenesis have been proposed that have not yet been fully clarified. Vitamin D may play a key role in preventing the early stage of the neoplastic process by exerting anti-inflammatory, antioxidant defenses and inducing enzymes responsible for repairing DNA damage and could also be involved in mechanisms of inhibition of cell proliferation, induction of cell differentiation, and cell death. In addition, some studies indicate various mechanisms through which vitamin D can quantitatively and qualitatively influence the intestinal microbiota, strongly linked to chronic inflammatory bowel diseases and the development of colon cancer. However, the metabolism and functions of vitamin D are dysregulated in some neoplasms which therefore develop resistance to the antiproliferative effect of vitamin D, and this promotes tumor development and progression. In this review, studies regarding vitamin D in relation to its activity in cancer have been summarized, as long as the metabolic pathways described for vitamin D.

**Keywords:** *vitamin D, prohormone, tumor, neoplasm*

## Introduction

Vitamin D is a fat-soluble vitamin obtainable from diet, as well as a powerful secosteroid prohormone produced in the skin through solar ultraviolet B (UVB) irradiation (wavelength ranging between 290 and 320 nm). Vitamin D is metabolized to calcidiol in the liver through a 25-hydroxylation followed by alpha-hydroxylation in the kidney that leads to the biologically active form, calcitriol, which binds to the vitamin D receptor (VDR) and fulfills its various physiological functions. The fundamental role of vitamin D is the regulation of calcium and phosphorus metabolism, essential for bone remodeling [1]. However, researches in recent decades suggested that low sunlight exposure and vitamin D deficiency are also associated with an increased risk of extra-skeletal diseases such as neoplasms [2,3].

The first observation of an inverse correlation between exposure to sunlight and the global incidence of oncological diseases was published about 80 years ago [4]. Then, in 1980 and 1992, the first epidemiological studies on groups of American individuals were reported, which correlated a reduced exposure to sunlight of subjects in North America compared to those in South America to the high risk of the onset of prostate and colon cancer, suggesting the possible chemopreventive effect of vitamin D. This correlation with vitamin D was deduced from the fundamental chelostep evidence of its activation process, occured by photolysis reaction of 7-dehydrocholesterol determined by UVB in the skin [5,6]. Several successive epidemiological studies revealed an inverse correlation between serum levels of calcitriol and the high risk of incidence of oncological diseases [7-9]. In addition, experimental data supported the chemopreventive effect of vitamin D [10]. Vitamin D deficiency was shown to contribute to the onset and progression of some types of carcinomas, suggesting that maintaining adequate plasma concentrations of this vitamin could be useful as a chemoprevention factor. In addition, new molecules similar to vitamin D were studied that may structurally and functionally overcome its hypercalcemic effect thus limiting its clinical application [11]. Some in vitro studies reported that cancer cells develop different resistance mechanisms that reduce cellular calcitriol levels, counteracting its antiproliferative effects [12]. The understanding of how the metabolism and signaling of vitamin D are altered in the neoplastic process would help the development of effective therapeutic strategies in overcoming the establishment of such resistance mechanisms [13-15]. Furthermore, the nutritional role of vitamin D has been studied during the COVID-19 pandemic [16,17]. In this review, the metabolic pathways of vitamin D and its interesting chemopreventive effects will be described.

## Primary metabolic pathway of vitamin D

Calcitriol, the biologically active metabolite of vitamin D, is a powerful inducer of calbindin, a high affinity calcium-binding protein expressed in the intestine that is thought to play an important role in the active transport of calcium across the enterocyte [18]. In addition, calcitriol regulates the function and expression of calcium transporters in the intestinal epithelium, ECaC and ICaC, thus leading to an increase in calcium absorption. The vitamin D receptor (VDR) regulates the expression of numerous genes of the bone matrix. In particular, calcitriol, as well as parathyroid hormone (PTH), induces the expression of the RANK ligand (RANKL) in osteoblasts by stimulating the differentiation and activity of osteoclasts. In particular, the RANKL factor is a member of the family of tumor necrosis factors expressed on the surface of stromal fibroblasts and osteoblasts. Circulating calcitriol reduces the plasma concentration of PTH, produced by the parathyroid glands, both directly, that is, by inhibiting the activity of these glands, and indirectly by favoring the increase in plasmatic calcium. The primary function of vitamin D is therefore the maintenance of calcium-phosphorus homeostasis, through their intestinal and renal absorption [19]. When vitamin D levels are inadequate, 10-15% of dietary calcium is absorbed by the small intestine, whereas adequate vitamin D levels bring to intestinal calcium absorption of approximately 30-40%, therefore more effective [20]. In cases of vitamin D deficiency, calcium absorption is insufficient and the human body responds with an increasing production and release of PTH into the circulation. This hormone, in turn, restores calcium by increasing its renal reabsorption and bone

mobilization of calcium by stimulating the production of calcitriol. In addition, the levels of calcidiol and calcitriol are strictly regulated by the enzyme 25 (OH) D 24-hydroxylase or (24-hydroxylase) or (CYP24A1), a primary enzyme that inactivates vitamin D expressed in the kidney. CYP24A1 catalyzes hydroxylation at C-23 and C-24 in both calcidiol and calcitriol. The CYP24A1 pathway produces biologically inactive calcitroic acid which is than excreted in the bile.

In the non-genomic pathway, calcitriol binds the VDR receptor in the membrane, identified as a membrane-associated fast-reacting steroid-binding protein (1,25D-MARRS); this interaction induces variations in cellular signaling pathways, such as those in which calcium and MAPK are involved, *i.e.* mitogen-activated protein kinases that catalyze the phosphorylation of amino acids serine or threonine of different proteins, resulting in modifications of the expression of target genes [21].

It is noteworthy that, calcitriol, as a hormone, tightly regulates vitamin D metabolism through a negative feedback mechanism [22]. Interestingly, the vitamin D inactivating enzyme, CYP24A1, is among the transcriptional targets of the calcitriol-VDR-RXR complex. The promoter region of the CYP24A1 gene contains two vitamin D response elements (VDREs), one at approximately 150-bp and the other at approximately 250-bp upstream of the transcription initiation site, resulting in strong induction of the CYP24A1 gene by calcitriol [23]. Although the increase in serum levels of calcitriol can induce its own degradation by inducing the expression of the enzyme CYP24A1, on the other hand PTH can support the levels of calcitriol by inducing the degradation of the mRNA of this inactivating enzyme through the activation of the cAMP-PKA pathway [24]. The elevated calcium levels resulting from increased calcitriol synthesis can negatively regulate PTH secretion through binding to CaSRs on the surface of the parathyroid gland by implementing a negative feedback mechanism.

## Alternative metabolic pathway of vitamin D

Recently, an alternative pathway in the metabolism of vitamin D has been reported via the CYP11A1 enzyme, also known as the cholesterol side chain cleavage enzyme (P450scc). Originally, the CYP11A1 enzyme was known to catalyze the hydroxylation of cholesterol in the C-22 and C-20 position followed by the cleavage of the bond between C-20 and C-22 to generate pregnenolone, a common precursor of steroid hormones. However, CYP11A1, expressed in peripheral tissues such as the skin and the gastrointestinal tract, appears to emerge as a new enzyme that metabolizes vitamin D, which becomes an alternative substrate to cholesterol [25]. It consists of hydroxylation predominantly in the C-20 or C-22 position, with no side chain cleavage. Overall, it has been estimated that these metabolites could improve defense mechanisms against DNA damage induced by environmental agents and cellular oxidative stress [26]. Therefore, the pleiotropic effects of vitamin D can be attributed not only to the calcitriol-VDR pathway, but also to those of vitamin D metabolites deriving from the action of the CYP11A1 enzyme. This alternative pathway can produce more than 21 vitamin D hydroxymetabolites [27,28].

## New receptors for vitamin D metabolites

It is interesting to note that the metabolites formed by the CYP11A1 enzyme, such as 20(OH)D and 20,23(OH)D, have been shown to act as partial agonists of the VDR receptor [29]. It has been observed that these metabolites, when present in concentrations able to activate the VDR receptor, do not induce the effects of hypercalcaemia or the expression of CYP24A1, an effect that may be observed in response to treatment with calcitriol [30]. In addition to the nuclear receptor VDR, the α and γ isoforms of orphan retinoid receptors (RORα and RORγ), members of the nuclear receptor family of ligand-dependent transcription factors, act as novel receptors for vitamin D metabolites deriving from the metabolism of CYP11A1 [25]. ROR play a key role in the regulation of many physiological processes, including immune and metabolic pathways, and are therefore implicated in oncological, autoimmune and metabolic syndrome diseases [22]. Interestingly, vitamin D metabolites synthesized in the CYP11A1 pathway act as inverse agonists of the RORα and RORγ receptors, thereby inhibiting their transcriptional activity. It would appear that the metabolites 20(OH)D and 20,23(OH)D can suppress the transcription of the

target genes of RORα and RORγ respectively in neoplastic cells.

## Anti-inflammatory effects of vitamin D in tumorigenesis

The beginning of a neoplastic process introduces, in normal cells, irreversible mutations in oncogenes and tumor suppressor genes that control cell proliferation, thus inducing the transformation of these cells which begin to follow an autonomous reproduction program. Vitamin D plays a key role in preventing the onset of the neoplastic process by exerting anti-inflammatory, antioxidant defenses and inducing enzymes responsible for DNA damage repair [3]. Several strategies and new drugs for the treatment of tumors are reported in the literature [31-35]. Chronic inflammation is one of the main factors contributing to the initiation of the neoplastic process [36]. In vivo studies showed that calcitriol reduce the expression of various enzymes involved in the inflammatory process. Firstly, calcitriol is involved in the prostaglandin pathway, the main mediators of the inflammatory response, by inhibiting the expression of cyclooxygenase-2 (COX-2) receptors and prostaglandin receptors, also favoring the indirect degradation of these mediators [37]. Secondly, vitamin D can suppress the p38 protein kinase (MAPK) mediated signaling pathway. In both normal prostate epithelial cells and prostate cancer cells, calcitriol inhibits the production of proinflammatory cytokines such as interleukin-6 (IL-6) by inducing the expression of MAPK phosphatase-5 (MKP-5) which prevents phosphorylation and activation of p38 MAPK. Furthermore, calcitriol is responsible for inhibiting the production IL-6 and tumor necrosis factor (TNF-α) through the induction of MKP-5 in human monocytes and macrophages murine [38]. In fibroblasts, calcitriol increases the protein stability of IκBα, thus preventing its phosphorylation and thus inhibiting the nuclear translocation of the p65 subunit of NFκB [39].

Finally, vitamin D can regulate the interaction between immune and cancer cells in order to inhibit the production of proinflammatory cytokines. Co-culture experiments using peripheral blood mononuclear cells (PBMCs) and colon cancer cells revealed that vitamin D treatment significantly reduced the production of proinflammatory cytokines such as TNF-α, IL-6 and, to a lesser extent, IL-10, supporting the anti-inflammatory effects of vitamin D in the tumor microenvironment [40].

## Antioxidant effects of vitamin D and DNA damage repair mechanisms

Free oxygen radicals (reactive oxide species, ROS) play a key role in the tumorigenesis process as they promote DNA damage, favoring the onset of transformed cells [41]. Therefore, the maintenance of antioxidant defense systems is crucial in preventing the development of the neoplastic process [42,43]. Calcitriol has been demonstrated to be an excellent candidate in the protection from DNA damage induced by oxidative stress as it is able to promote antioxidant defenses [44]. Calcitriol seems to induce the expression of numerous enzymes of the antioxidant defense system in humans [10].

The treatment with calcitriol of human prostatic epithelial (RWPE-1), benign prostatic epithelial (BPH-1) and ovarian cancer (OVCAR3) cell lines increases intracellular glucose-6-phosphate levels dehydrogenase (G6PDH), an enzyme that regulates the intracellular levels of glutathione, the tripeptide that protects human cells from oxidative stress [45-47]. This effect therefore protects cells from apoptosis induced by hydrogen peroxide ($H_2O_2$). Vitamin D-mediated protection from oxidative stress may also be indirectly due to the induction of nuclear erythroid factor (NFE2L2), a transcription factor that controls the gene expression of several antioxidant defense enzymes such as glutathione peroxidase-3 (GPX-3), heme oxygenase-1 (HMOX-1) and aldo-keto reductase 1C2 (AKR1C2) [46]. In vitro studies showed that vitamin D increases the expression of genes involved in DNA damage repair such as the tumor suppressor gene p53, proliferating cell nuclear antigen (PCNA), breast receptor cancer A1 (BRCA1) in breast cancer cells and also ataxia mutated telangiectasia (ATM), recombinant DNA repair protein (RAD50) in PEC cell lines and finally growth arrest and DNA damage-inducible α (GADD45α) in squamous cell carcinoma (SCC) and ovarian cancer cells [48-51]. Vitamin D, in breast cancer [52], can also prevent the degradation of p53-binding protein 1 (53BP1) mediated by the cysteine proteinase Cathepsin L, a lysosomal

endopeptidase [53]. Therefore, vitamin D could be essential for preventing genetic mutations that favor the onset of the neoplastic process thanks to its anti-inflammatory, antioxidant and DNA damage repair functions. Another possible mechanism by which vitamin D can stop cell proliferation is the inhibition of several functions mediated by the Wnt/β-catenin signaling pathway. Some in vitro observations show that in colon cancer cell lines, calcitriol can block β-catenin-mediated transcriptional regulation by hindering the formation of the TCF4-β-catenin transcription complex [54]. Vitamin D has also been shown to inhibit telomerase activity by reducing the expression of telomerase reverse transcriptase (TERT) via miR-49892 in ovarian cancer cells and to induce the expression of cytokine transforming growth factor β (TGFβ), as well as its receptors, leading to inhibition of cell growth [55].

**Vitamin D and apoptosis**

Induction of apoptosis is another important mechanism by which vitamin D appears to exert its chemopreventive effects [56].

## Vitamin D and autophagy

Autophagy or autophagocytosis is a catabolic process by which cells degrade their own cytosolic macromolecules and intracellular components using lysosomal enzymes. This process plays a key role in the regulation of important biological processes such as cell growth, development and homeostasis, maintaining a balance between synthesis, degradation and subsequent recycling of cellular products**.** Although autophagy is generally considered a survival strategy and also a mechanism that protects cells from conditions of increased stress such as lack of energy reserves and oxidative stress, it can be also modulated to cause cancer cell death [57]. Therefore, unlike apoptosis, autophagy, in response to stressful stimuli, can contribute to both cell survival and cell death [58]. Many food components such as selenium, resveratrol, curcumin and vitamin D itself have been reported to promote autophagy [59]. The first evidence regarding the role of calcitriol in promoting autophagy was reported in a study dating back to 1999 [60]. The authors showed that calcitriol and its two analogues, EB1089 or seocalcitol and CB1093, induced growth arrest in MCF-7 breast cancer cells expressing the p53 tumor suppressor gene and in the p53-free T47D breast cancer cell line.

## Effects of vitamin D on breast cancer cells in vitro and in vivo

In vitro and in vivo studies carried out to evaluate the effects of calcitriol and its semisynthetic analogues on the proliferation and malignant progression of breast cancer cells showed that the different vitamin D analogues of the VDR receptor are equally effective in inhibiting the growth of ER (+) breast cancer cell lines such as MCF-7, T-47-D, ZR-75-1, SKBR-3 and in ER (-) breast cancer cell lines such as BT-20, MDA-MB435, MDA-MB-231 and SUM-159PT [61-63]. These data were in agreement with clinical observations describing the therapeutic benefit of vitamin D and its analogues in ER (+) and ER (-) breast cancer [63-66]. In postmenopausal women, when ovarian estrogen production ceases, the local estrogen synthesized in the breast microenvironment leads to the growth of cancer cells which overexpress the ER (+) estrogen receptor. By inhibiting both estrogen synthesis, through selective suppression of aromatase enzyme expression in breast adipose tissue, and signaling through downregulation of ERα in breast cancer cells, calcitriol may have a therapeutic benefit in prevention or treatment of postmenopausal ER (+) breast cancer [67,68]. Although the exact mechanisms by which vitamin D can exert its inhibitory activity on the growth of breast cancer cells are not fully understood, in vitro observations indicate that this molecule can influence the proliferation of cancer cells by causing cell cycle arrest. in the G0/G1 phase, promoting apoptosis and inhibiting tumor angiogenesis [55,69,70].

## Synthetic analogues of vitamin D derived from 19-nor-1α, 25 (OH) 2D3

The molecules derived from 19-nor-vitamin D are in turn synthetic analogues of vitamin D in which the methylene group on the C-19 of the A ring is replaced with two hydrogen atoms [71]. MART-10 (19-nor 2α-(3-hydroxypropyl)-1α, 25 (OH) 2D3) and MART-11 (19nor-2β-3-hydroxypropyl-1α, 25 (OH) 2D3) are two new synthetic analogues of C2-substituted 19-nor-vitamin D having negligible effects on plasma calcium levels and appearing to be effective in the

prevention and treatment of prostate cancer [72]. Inecalcitol and Paricalcitol are two emerging analogues of 19-nor-1α, 25 (OH) 2D3 with antiproliferative properties.

Inecalcitol (19-nor-14-epi 23-ene-1,25 dihydroxyvitamin D3) is a new analogue of 19-nor-vitamin D which differs from calcitriol by epimerization of C14, deletion of C19 and introduction of a triple side chain bond. This analog appears to be less likely to induce hypercalcemia while remaining a potent stimulant of the VDR receptor [73]. Inecalcitol has been shown to suppress both in vitro and in vivo the proliferation of human prostate cancer LNCaP cell lines and that of breast cancer SCC cell lines [74,75]. The antiproliferative activity of inecalcitol, at least in SCC cell lines, appears to be the consequence of the arrest of the cell cycle in the G0/G1 transition phase and of the triggering of the apoptosis cascade through the induction of the intrinsic pathway of apoptosis. with activation of the pathway of caspases 8/10 and 3 and the inhibition of the expression of c-IAP1 and XIAP, proteins inhibiting apoptosis. It has also been shown that inecalcitol represses the expression of the gene encoding cyclin D1 and cyclin C and induces the gene expression of p21 and p27 more efficiently than calcitriol [74]. Inecalcitol was used in clinical studies in combination with the classic anticancer docetaxel [76]. Results from the phase II study in castration-resistant prostate cancer showed that this association gave a better PSA response than docetaxel alone. These data provide support for further evaluation of inecalcitol in the treatment of prostate cancer.

Paricalcitol (19-nor-1α, 25-dihydroxyvitamin D2) is another synthetic analogue of calcitriol, the active form of the Vitamine D. A clinical study has shown that paricalcitol, in combination with taxane-based chemotherapy, appears to be safe and feasible and may have clinical benefit for women with metastatic breast cancer [77].

## Conclusions

In conclusion, epidemiological observations suggest that "sufficient" levels of vitamin D, *i.e.* higher than 30 ng/ml of calcidiol, could offer protection against some oncological diseases. An average level of vitamin D of about 17 ng/ml is considered inadequate and may be responsible of the onset of skeletal pathologies; in addition, it might be a predisposing factor for oncological diseases. On the other hand, many experimental studies provide evidence on the antiproliferative, anti-inflammatory and pro-differentiation effects of vitamin D both in vitro on human tumor cell lines and in vivo on tumor-bearing animals. Therefore, the use of vitamin D or its semisynthetic analogues in tumor therapy could provide effective chemopreventive effects.The possible mechanisms by which vitamin D mediates these effects have only been partially identified. The preclinical data obtained till now are very interesting and the epidemiological data are encouraging. Future clinical investigations could better define the clinical role of vitamin D and its analogues in the prevention and treatment of cancer. Another relevant feature of the "vitamin D/carcinoma paradigm" that needs further investigation is its relation to tumor resistance as this phenomenon can influence the synthesis of the active vitamin D metabolites and, consequently, its potential preventive and therapeutic activity. Therefore, studies directed towards the search for new molecules that can circumvent tumor resistance to vitamin D analogues may be desired.

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