

# The Emerging Role of Vitamin D in Oral Epithelial Pathology: A Review

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## Abstract

Carcinogenesis is a multifactorial disease which involves environmental and genetic factors as a predisposing cause that bring biochemical imbalance resulting in the genomic aberrations at the cellular levels. Vitamin D (1,25(OH)<sub>2</sub>D<sub>3</sub>) mediates pleiotropic effects on various molecular pathways that is extensively known in physiologic but elusive in pathological state. Studies suggest that Vitamin D acts through its receptors like VDR/ RXR to form ligands which affects transcription of wide spectrum of genes involved in calcium and phosphate homeostasis as well as cell division and differentiation. It is these latter actions due to which use of Vitamin D is gaining attention in cancer cells as an anti-proliferative agent. Although anti-proliferative role of vitamin D has been realized in the treatment of psoriasis and in parathyroid cell hyperplasia associated with secondary hyperparathyroidism, but its role in premalignant and malignant oral lesions are still in premature state. Immunotherapeutic modalities focus on the expression of the receptors and its ligands to assess the prognosis of the disease which depends on the local and distant spread of neoplastic cells. Though Vitamin D is known to suppress cancers related to breast, lung, bladder, prostate, colorectal and some cancers of oral cavity but chemoresistance and postoperative recurrence are the major challenges to prevent mortality in cancer patients. The present review focuses on the physiologic, epigenetic and molecular interactions of Vitamin D causing lesions affecting the oral cavity.

**Keywords:** Vitamin D; Carcinogenesis; Oral Lesions; Chemoresistance

## Introduction

The head and neck cancer is a major area of concern worldwide due to high morbidity and mortality rate. The pathogenesis suggests that neoplastically altered cell acquire self-renewal capacity owing to multiple level mutations causing disruption of molecular pathways, thereby causing low response to treatment and relatively high recurrence rate of cancer cells. Despite recent advances in treatment modalities, yet overall survival rate of head and neck cancers has been 5 years in approximately 50% patients [1]. This has led to an increased

effort to identify agents that can effectively prevent and improve the efficacy of treatment by regulating proliferation and differentiation of neoplastic cells. One among these aberrations is alterations of Vitamin D levels and its Receptor (VDR) expression in various carcinomas like prostate, breast, colorectal, gastric, head and neck etc.

Vitamin D has been long known as an important regulator of various physiological functions in the human body. It aids in maintaining bone metabolism by regulating calcium and phosphate homeostasis. Its ability to control cell differentiation and proliferation in G1 phase reflects the pleiotropic action of Vitamin D on different cell lineage including neoplastic cells [2].

It is primarily obtained through the diet or conversion of 7-dehydrocholesterol in the skin by ultraviolet-B radiation. Vitamin D and its metabolites are usually hydrophobic which depends on ligand for its transport and storage in adipose tissue. Through the process of hydroxylation, it gets converted to 25-hydroxyvitamin D in liver and then to 1,25-dihydroxyvitamin D in the kidney under the influence of CYP27A1 and CYP27B1 respectively [3]. The amount of 1,25-dihydroxyvitamin D is tightly regulated by parathyroid gland for its role in mineral homeostasis which is expressed by binding to VDR in the nucleus of target cells [4]. The conformational changes in the activated receptor induce or repress gene transcriptions by binding ligand bound VDR to regulatory sites on DNA to form VDR/ RXR heterodimer. Further, formation of Vitamin D Response Element (VDRE) in the nucleus enhances expression of growth factors, cellular differentiation, immunity and repair. In oral cavity, Vitamin D imbalance causes diseases of auto immune nature including recurrent aphthous stomatitis, Behçet syndrome, Systemic lupus erythematosus, Sjögren syndrome and cancers like squamous cell carcinoma [5,6]. It is therefore evident that modification in VDR influence early cellular proliferation and differentiation but the exact mechanism is still unknown. This article attempts to

review and present the effects of Vitamin D-VDR complex in oral premalignant lesions and malignant lesions of oral cavity.

## Molecular pathogenesis

Biological response in the cells occur through a steroid hormone like mechanism involving VDR that specifically regulates the transcription of vitamin D-dependent genes coding for proteins to regulate cellular events, such as intestinal calcium transport and cell division. In the classic steroid model, activated vitamin D<sub>3</sub> enters the nucleus to bind tightly with VDR. Ligand binding protein promotes conformational change in the AF-2 domain of the COOH terminus of the VDR to allow recruitment of positive transcription factors and/or repression of transcriptional inhibitory factors that leads to increased rate of gene transcription [7].

The use of Vitamin D and its analogues may cause hypercalcemia which limits the use of the drug at relatively high concentrations for long duration to achieve desired outcomes. Clinical availability of Vitamin D is also related to good systemic bioavailability, potent anticancer or chemopreventive activity, with low-calcemic effects to maintain cellular homeostasis.

## Protective role of Vitamin D

Vitamin D has distinctive functions in physiological as well as in pathological state of human body. Locally high concentrations of 1,25-(OH)<sub>2</sub>D<sub>3</sub> in sites like skin, prostate and breast have shown altered patterns of gene expression as a protective response which inhibit cell division and promote differentiation of specific cell lines.

## Anti-apoptotic properties

Vitamin D and its analog modulate key mediators of apoptosis in many cancer cell and normal cell in specific manner mainly by inhibiting the anti-apoptotic proteins and/or by stimulating the pro-apoptotic proteins. It regulates gene transcription by forming heterodimer with VDR/ RXR to co-activate vitamin D response elements (VDRE) in the promoter region of target genes. Inecalcitol, 14-epi analogs of Vitamin D induce mRNA expression of p27 act in dose-dependent manner and suppress the expression of amphiregulin, p21 and cyclin D1 indicating its role in both cell cycle arrest and apoptosis. Ma Y et al. observed that activation of the extrinsic pathway by caspase 8/10 and caspase 3 promote apoptosis in SCC cell lines with the reduction of anti-apoptotic proteins like cellular inhibitor of apoptosis protein-1 (c-IAP1) and X-linked inhibitor of apoptosis protein (XIAP) levels [8].

In contrast, Kizildag S and Diaz et al. showed that Vitamin D stimulates apoptosis by downregulating the expression of B-cell lymphoma protein 2 (BCL2), Bcl-2-associated X (BAX) and p21 mRNA in many colon cancer cell lines and chronic myeloid leukemia cell lines [9,10]. Vitamin D is also known to induce apoptosis by mechanisms other than the action on the BCL2 protein family by suppressing insulin-like growth factor

1 (IGF1) signaling and activating forkhead box O3 in breast and kidney cancer cells respectively. Vitamin D and Inecalcitol showed dependence on VDR-mediated transcription of CYP24A1 promoter to express apoptotic influence on known VDR target genes. Ma Y et al. observed stronger anti-proliferative effects and anti-clonogenic capacity in vitro in the presence of enhanced VDR expression while no effect on cell cycle progression or apoptosis was evident in Vitamin D resistant SCC cell lines that expressed very low levels of VDR in vivo suggesting importance of VDR expression to exhibit broad spectrum antitumor activity [8].

## Tumor suppressive properties

Several crosstalks occur to strictly regulate the interaction between cancer cells, host immune cells and tumor microenvironment to create protective network that downstream the proliferating tumor cells. Studies have shown that vitamin D has impending role in inhibiting tumor development by regulating inflammatory processes like cytokines, prostaglandins, MAP kinase phosphatase 5 (MKP5), the nuclear factor kappa B (NF-κB) pathway by regulating effects on cytokines including IL6, IL 8 and IL10 [11,12]. Interleukins are pro-tumorigenic cytokine that may inhibit tumor necrosis factor-related apoptosis and induce COX 2 promoting tumor growth and angiogenesis. Resolution of inflammation is a key event to control tumorigenesis regulated by IL 8 and 10. The effect of Calcitriol on colon cancer cells has shown marked anti-inflammatory effects with suppression of IL 8 by regulating levels of CD14 depending ERK1/2 [13].

Hypoxia inducible factor-1 (HIF-1) belongs to basic helix-loop helix family get activated in the inflammatory and hypoxic environment [14,15]. In the presence of oxygen, HIF-1 α subunits get hydroxylated by three prolyl hydroxylases (PHD1–3) on proline residues which later binds to the Von Hippel-Lindau (VHL) for proteasomal degradation. While, under the hypoxic state, HIF-1α may not be hydroxylated by PHDs, resulting in its stabilization, that in-turn promotes favorable inflammatory microenvironment for disease progression [16,17]. On the contrary, inflammatory response is also dependent on Lipopolysaccharide (LPS), which is able to promote HIF-1α levels for transcription of cytokines such as interleukin-1 beta (IL-1β) and interferon gamma (IFNγ) in immune cells, by increasing succinate levels in macrophages and activating nuclear factor-κB (NF-κB) pathway in myeloid cells [18]. Previous studies observed that vitamin D may block immune responses by regulating T cell differentiation and cytokines secretion in HOKs. Ge et al. reported inhibitory role of Vitamin D on immune cells in the presence of LPS by regulating HIF-1α expression to suppress IFNγ and IL-1β productions in HOKs [19].

The inhibitory effects of Vitamin D on LPS induced HIF-1α via NF-κB pathway in HOKs suggest gain-of-function assays with HIF-1α overexpression following IKKβ transfection, suggesting activated NF-κB pathway induces HIF-1α production. Vitamin D blocked IKKβ-induced HIF-1α expression implies vitamin D suppresses HIF-1α in a NF-κB-dependent fashion. Reports also suggest that VDR may act as a transcription factor to induce VHL transcripts as VHL levels were increased robustly with increasing amounts of VDR expressed in HOKs, influencing HIF-

1 $\alpha$  protein expression. Hence, VDR acts as an important regulator in LPS-induced inflammatory response in oral keratinocytes by impeding NF- $\kappa$ B pathway [19].

### Invasion and Metastasis

Fate and Prognosis of the disease is influenced by size, site, stage and type of tumor growth. The outcome is also dependent on molecular expression like Lipocalin 2 (LCN2), a member of lipocalin superfamily, known to release stress protein in various inflammatory conditions and related carcinoma. The direct mechanism of involvement of LCN-2 is not yet evident but it might suppress HIF-1 $\alpha$  mediated CAIX expression following hypoxic exposure by promoting epithelial to mesenchymal transition in oral tissues. Studies suggest that LCN2 can inhibit EMT by downregulation of the transcriptional repressor of E-cadherin, Twist 1 in liver cancer. Lin CW et al. observed similar finding suggesting that LCN2 suppress HIF-1 $\alpha$  mediated Twist expression in OSCC, thereby controlling metastasis [20]. Lim R et al. noted the suppressive ability of LCN 2 in ovarian and liver cancers by negatively modulating the EMT process[21]. LCN 2 may act as a co-repressor to Vitamin D in certain cell lines, affecting the binding of RPS3 and NF- $\kappa$ B pathway. Huang et al. suggested that LCN2 is a potential regulator of vitamin D, therefore patient should be screened for LCN2 expression to get maximum benefit during chemotherapy[22].

Other associated factors required in the progression of cancer are vascular endothelial growth factors and matrix metalloproteins which are reported to increase in many cancers. Several in vitro and in vivo studies suggest that vitamin D control angiogenesis by downregulating proangiogenic factors like Hypoxia inducible factor 1 and its response proteins by suppressing Vascular Endothelial Growth Factor (VEGF). [23,24]. Masuda S. et al suggested that Vitamin D also aids in downregulating cell invasion-associated proteases like matrix metalloproteinases 2 and 9, serine proteinases and expression of  $\alpha$ 6 and  $\beta$ 4 integrins, both of which are receptors for laminin [25].

### Immune mediated function

The presence of VDR in proliferating immune cells and the ability to metabolize vitamin D have unraveled role of Vitamin D beyond the well-established anti-rachitic factor. Innate and adaptive immunity pathways are controlled directly or indirectly by action of vitamin D on cells through intracrine, autocrine, and/or paracrine manner [26]. Upregulation of the VDR is a common finding in pathogen-induced activation of toll-like-receptors on human monocytes and macrophages during an innate immune response. Certain microbes tend to reduce its recognition by dysregulating the VDR which slowdown innate immune responses. Studies suggest that Epstein-Barr virus (EBV), Mycobacterium leprae, Mycobacterium tuberculosis downregulate expression of VDR which allows pathogens to accumulate in body tissues [27]. The weakened innate defense further causes susceptibility to additional stress on immune system to enhance its chance for survival. In contrary, the immune system overcome the dysregulated response by clearing pathogens through the production of the antimicrobial peptide, cathelicidin which possesses antiviral, antibacterial, and antifungal activity [5,6].

Vitamin D is reported to act on two major groups of epidermal Anti-Microbial Peptides (AMPs), Cathelicidin and Defensin. Wang et al. showed that treatment of 1,25(OH)2D3 leads to robust induction of cathelicidin in neutrophils, monocytes, human keratinocytes and cells of head and neck squamous carcinoma[28]. Cathelicidin is a direct transcriptional target of vitamin D, which is induced by binding of 1,25(OH)2D3-VDR complex to the VDRE in the promoter gene. While, expression of defensin-2 may increase moderately in cells of head and neck squamous carcinoma and in primary cultures of adult keratinocyte. However, studies observed that 1,25(OH)2D3-VDR complex requires NF- $\kappa$ B to co-stimulate with the presence of IL-1[28,29].

Exposure to infectious agents signals Antigen Presenting Cells (APC) stimulate adaptive immunity by the release of T and B lymphocytes. With various studies, it is evident that on one side, CD4+ and CD8+ T cells help to protect the host from undesirable bacterial and fungal stimuli by acting as mediator responsible for initiating auto immune responses. On the other hand, Treg (regulatory T cells), member of FOXP3+ family is known for suppressing massive effector T cell activation by producing interleukins (IL10). The overall effect causes improved resistance of host towards auto immune disease. Vitamin D directly influence FOXP3+ Treg cells in regulating the ratio between CD4+ and CD8+ T Cells and Treg cells to prevent any un-toward body's reaction to inflammatory responses as in auto immune diseases where the harmony between the T Helper cell (Th cells) and Treg cell population is disrupted [30,31]. Vitamin D not only enhances IL-10 secretion by CD4+ T cells but also regulate the normal development and function of invariant NKT cells playing an eminent role in autoimmunity [32]. In recent days, Vitamin D is being used to treat autoimmune disease like SLE, sarcoidosis, Sjogren's syndrome and type 1 diabetes due to its effects on reducing anti DNA antibodies. Abou-RayaA et al. mentioned that vitamin D intake (2000 IU/day for 12 months) in SLE patients improved the inflammatory and hemostatic marker levels and reduced disease activity [33]. Terrier B et al. also mentioned the role of Vitamin D to expand Treg cells to reduce the frequency of expression of Th1 cells, Th17 cells, memory B cells, and auto-antibodies [34].

### Oral lichen Planus

OLP is an autoimmune disease affecting the oral mucosa, skin, nails and genital mucosa characterized by the presence of inflammatory mediators like cytokines which interact between keratinocytes and T lymphocytes. Erosive form of the lesion has maximum tendency for malignant transformation in about 1-2% patients. It is postulated that CD8+ T cells along with mast cells and dendritic cells disrupt the physical integrity of the epithelium [35]. Additionally, studies suggest that bacteria break the epithelial barrier of the mucosa and enhance penetration of harmful substances resulting in aberrant infiltration of T cells. Multiple colonies of bacteria have been demonstrated within lamina propria and epithelium suggesting positive co-relation that may cause immune reactions resulting in apoptosis of basal cells [36]. Mucosal barrier breakdown, epithelial layer thickness

reduction and homeostasis dysfunction are the underlying features of the disease that accelerate invasion of bacteria and antigen to trigger production of inflammatory cells like CD4+ and CD8+ T cells in lamina propria of OLP patients [37,38]. JD et al. observed that VDR levels were 50% lower were associated with increase immunoreactivity in diseased mucosal samples of OLP [39].

Deficiency of Vitamin D favors LPS induced VDR down-regulation which enhances PUMA levels, thereby causing epithelial discontinuity and epithelial cells apoptosis. LPS induced TNF  $\alpha$  expression through miR-346 plays suppressive effect on the expression of VDR in keratinocytes through LPS-TNF $\alpha$ -miR-346 signaling pathway. Vitamin D blocked LPS stimulated PUMA and active caspase 3 inductions by regulation of apoptosis due to impediment of NF-KB activation. Thus, Vitamin D plays a protective role in the maintaining the homeostasis of the epithelial barrier to overcome bacterial challenge, further restricting the development and progression of OLP [39, 40].

Gupta J et al. reported improvement with vitamin D by observing pre and post treatment subjective and objective symptoms in erosive lichen planus patients. He observed treatment outcomes was maximum in patient's treated with combination of topical steroids, vitamin D supplements, and psychological counseling though no satisfactory results could be obtained solely with Vitamin D substitutes [41].

### **Leukoplakia/ Epithelial Dysplasia**

Leukoplakia is a clinical descriptive term for a white lesion of the oral mucosa that represents histologic findings of a wide variety of conditions ranging from epithelial hyperkeratosis, hyperplasia, dysplasia and carcinoma. Studies suggest that 43% of dysplastic lesion has propensity to undergo malignant transformation due to proliferation of basal and suprabasal cells of epithelium. Use of tobacco and allied products are known extrinsic factors to incite the disease. The chemicals generated from smoking tobacco may influence vitamin D metabolism and function, yet studies show conflicting data on compounds that are directly involved in carcinogenesis [42]. Afzal S et al. reported lower plasma 25-hydroxyvitamin D [25(OH)D] levels are prone to higher risk of tobacco-related epithelial dysplasia [43]. The dysplastic cell acquires survival advantage by multiple mutations of random cell lines leading to its clonal selection by evading check points. Grimm M et al. observed the expression of VDR was significantly higher in dysplastic epithelium as compared to OSCC tissue samples that may be due to a feedback loop coupled with defective VDR expression in the tumor cells [44]. However, Reichrath J et al. proposed that inflammatory cytokines expressed by tumor-infiltrating leukocytes in oral premalignant lesion may be responsible to up-regulate VDR expression in epithelial cells. Previous studies report that normal keratinocytes could synthesize biologically active calcitriol and it is speculated that if this functional property is retained by dysplastic epithelial cells may increase content of VDR on the mRNA and protein levels [45].

Correction of Vitamin D serum levels by natural vitamin or

synthetic vitamin D compounds have demonstrated induction of apoptosis supporting the emerging role of Vitamin D signaling in pathophysiology of oral keratinocyte. Cessation of habit along with correction of Vitamin D status may be useful in chemoprevention of such lesions. However, study conducted by Yuan et al. suggests that vitamin D deficiency alone may not be the only factor to alter oral epithelial homeostasis [46]. Vitamin D in VDR + cells may also be useful in sensitizing cells to cause apoptosis. Current treatment therapy aims to target the mutated cell in basal and parabasal layer to either induce apoptosis and / or repair the cellular defects.

### **Squamous cell carcinoma**

Carcinoma is a complex multifactorial disease derived from cells of basal and suprabasal layer of epithelium that has capacity to cause initiation, progression and metastasis of neoplastic cells. Genetic alterations like point mutations, amplifications, re-arrangements, and deletions downregulates expression of epithelial differentiation and promote tumorigenesis. The known evidence suggest that growth arrest, cellular senescence and tumor suppression are maintained through strict monitoring at each level. Yuan et al. suggested that VDR expression in the entire thickness of epithelium could be due to the deregulation of VDR expression in OSCC with significant increase in number of immature epithelial cells in S phase of cell cycle [46]. The proliferation of epithelial cell is maintained through a member of the epidermal growth factor family of peptide growth factors known as Amphiregulin. Vitamin D and EB1089 treatment has shown effectiveness by inducing Amphiregulin levels to suppress proliferation of epithelial cells. Increased formation of GADD45 $\alpha$ -PCNA complexes with Vitamin D and EB1089 also aids to repair DNA at G1/S phase of cell cycle to achieve genomic stability [47].

Antiproliferative and pro-differentiating properties of vitamin D inhibits cell growth of normal and tumor cells by hampering the transition from the G1 to the S phase of the cell cycle. It further activates tumor suppressors like P21 to promote epithelial differentiation and maturation. Akutsu et al. observed that Vitamin D and its analog reduce tumor growth by its repressive effect on p21waf1/cip1 transcripts and protein in vitro and in vivo of murine SCC lines. Microarray experiments revealed EB1089-regulated genes control range of cellular processes such as cell cycle progression, cell adhesion, extracellular matrix composition, inter and intracellular signaling, G protein coupled receptor function, intracellular redox balance and steroid metabolism [47,49]. Vitamin D promotes apoptosis with selective downregulation of IAP (inhibitor of apoptosis proteins) and activation of caspase 8/10 and 3 pathway. Two IAP family members, c-IAP1 and XIAP resulted in strong inhibition of the expression at both mRNA and protein level with inecalcitol treatment in a dose-dependent manner. Ma Y et al. observed the effectiveness of inecalcitol showing significant improvement following treatment by viewing expression of Ki 67. He also mentioned the role of both inecalcitol and 1,25D3 to promote apoptosis through the activation of the extrinsic pathway mediated by caspase 8/10 and caspase 3 pathway using substrate-based caspase activity assays [8,47].

VEGF is a potent angiogenic factor essential for vascularization and progression of tumor cells with higher rate of disease recurrence and a shorter disease-free interval. VEGF expression is suppressed in MCF-7 and MBA-MD231 breast cancer or LNCaP prostate cancer cells. On the contrary, it is evident that Vitamin D and its analogs induce expression of VEGF in SCC cell lines, thereby associated with relapse and poor prognosis in SCC of the oral cavity. Therefore, function of Vitamin D is highly complex and may depend on cell specificity which determines its inhibitory or stimulatory effects. At present, further studies are still under research to estimate the effect of Vitamin D at molecular level following treatment [47].

### Epigenetics of resistance in treatment

Resistance of disease to treatment is still a major challenge for biomedical science involving unknown facets. In many tumors, high VDR expression levels show longer survival and lower recurrence rate while downregulation of VDR is associated with adverse prognosis. Peng H et al. observed lower VDR expression in esophageal carcinoma associated with poor prognosis [49]. In contrary, study conducted by Huang et al. did not observe any significant changes during treatment with cisplatin and Vitamin D on patients with positive VDR expression, suggesting that there might be more factors involved other than VDR expression alone, regulating the complex network of tumorigenesis [22].

Lack of ligand specific sensitivity due to intrinsic and acquired chemoresistance results in low / no response after repeated rounds of chemotherapy, affecting 30% patients. It is observed that usually VDR expression is either absent or silenced in such diseases, blocking the classical NF- $\kappa$ B pathway in solid tumors leading to shorter disease free interval. Current target for treatment include sensitization of VDR/ RXR to enhance drug localization [22].

Presence of mutated cancer cell can also lead to treatment failures as a result of conformational de-arrangement of co-activator complexes that bind VDR to either DRIP or p160/SRC. Proliferation and differentiation of basal cells cause sharp decline in DRIP levels and increases binding with p160/SRC. Bikle D et al. observed lack of differentiation in poorly differentiated esophageal squamous cell carcinoma (ESCC), due to failure of DRIP -SRC complex formation indicating that VDR performs an inhibitory function in the development of ESCC [50].

Vitamin D inhibits NF- $\kappa$ B activation by suppressing LCN2, which downregulate anti-apoptotic genes like FLIP, c-IAP1/2 and XIAP. Huang et al reported that administration of vitamin D with cisplatin enhanced the sensitivity of OSCC and reduced its resistance on repeated cycle by regulating the expression of LCN2. Vitamin D reversed the aberrant methylation caused by cisplatin and lowered the levels of LCN2. Though Vitamin D has shown no direct effect on tumor cells but it may act synergistically with chemotherapeutic agents like cisplatin to inhibit abnormal cellular proliferation [22].

### Miscellaneous

In breast cancer, Vitamin D has shown to inhibit tumor progression in both estrogen receptor positive and estrogen receptor negative tumor cells. The effect of EB 1089 and 22-Oxa-1,25(OH) $_2$ D $_3$  in vitro and in vivo has shown increased survival time, inhibition of metastasis and neovascularization resulting in overall reduction in tumor progression [51,52].

In prostate cancer, Vitamin D inhibits the proliferation of both androgen-dependent and androgen-independent prostate cancer cells. EB 1089 has shown inhibition of lung metastasis without severe weight loss. The newer nonsteroidal analogues like LG190119 inhibited LNCaP prostate cancer xenograft tumor growth without causing hypercalcemia [53,54].

In Colon cancer, Vitamin D analogues like Ro24-5531 reduce proliferation of colon cancer cells in vitro and vivo by reducing azoxymethane-induced crypt cell hyperproliferation, aberrant crypt foci development with inhibition of spontaneous metastasis [55,56].

In Acute myeloid leukemia, Vitamin D analogues is potentially effective for initiation of terminal of differentiation of neutrophilic promyelocytes in human AML cell lines like HL-60, by regulating the monocyte-macrophage pathway [48].

### Future Perspectives

Extensive research is being conducted to know the vast usefulness of the molecule, though promising results are seen in combination treatment with cytotoxic drugs but its effectiveness in treating epithelial premalignant and malignant lesion is still under question. It is evident that VDR polymorphism plays a crucial role in tumorigenesis of various cancer types by affecting absorption, metabolism and the cellular response to vitamin D. Resistance to 1 $\alpha$ ,25(OH) $_2$ D $_3$  in the presence of normal levels of functional VDR is another challenge that affect treatment outcomes. Validation of treatment to therapy must be viewed using latest biomarkers to overcome undue exposure in resistant cases. Research should consider studying VDR polymorphisms to unravel the understanding of vitamin D pathway which could provide evidence for its chemotherapeutic role to prevent cancer development. The search for new vitamin D analogs with potential clinical usefulness must include molecules with good systemic bioavailability, potent anticancer/chemopreventive activity, and low-calcemic effects. The current review observed that there have been many epidemiological studies of breast, prostate and colorectal cancer but lacks reports on lung, esophageal and oral carcinomas. More clinical studies are needed in this direction for treating epithelial malignancies using vitamin D and its analog as a targeted epigenetic approach to therapy.

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