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BIOCHEMISTRY OF VITAMIN D AND ITS ROLE AGAINST CANCER

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Review Article

ABSTRACT

One of the types of fat soluble vitamins, vitamin D, is a family of compounds consisting of 9-10 Secosteroids. These are classified into five different forms two of which are found naturally- vitamin D_2 (photo chemically synthesized in plants) and D_3 (synthesized in animal and human skin). Sources of such vitamin include dietary components and exposure to sunlight and the functions are carried out through the vitamin's interaction with its receptors in the body as it participates in several mechanisms in the form of different analogs of itself. The aim of this review is to better understand the mechanism of the vitamin D receptors and various analogs of the vitamin D as well as its role against different types of cancers. Receptors of vitamin D have been isolated that are involved in various regulatory processes which extend beyond classic functions of the hormone like vitamin. Several analogs of this vitamin have been discovered throughout the years which have shown to have role against different types of diseases including cardiovascular, autoimmune, inflammatory diseases and most importantly, cancers. Mechanism of anticancer action of the hormone like fat soluble vitamin has also been studied and it has helped to identify a molecular link between the vitamin and prevention and regression of colorectal, prostate, breast and colon cancer as well as multiple myeloma which might lead to the conclusion that supplementation of vitamin D, in the correct amount might have a role of great importance in the prevention of cancer.

Keywords: Vitamin D; receptors; analogs; cancer.

1. INTRODUCTION

1.1 Sources and Classification of the Vitamin-D

Vitamins are organic compounds that are required by living beings in small amounts for their optimum growth and development. These compounds are generally classified into two main groups- water soluble vitamins and fat soluble vitamins. Vitamin D is one of the types of fat soluble vitamins which had been identified in 1919 by Edward Mellanby during his experiments with rickets [1]. "Vitamin D is a family of compounds consisting of 9-10 Secosteroids, which are classified into the following five forms- Ergosterol or Ergocalciferol(D_2), Cholecalciferol (D_3), 22,23-dihydroergocalciferol (D_4), Sitosterol or 24-

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ethylcholecalciferol (D_5) and Stigmasterol (D_6)" [2]. "Only two of these forms, D_2, synthesized in plants, and D_3, synthesized in animal skin in response to sunlight, are found naturally" [3]. "Both of these vitamins are the main sources of the activity of vitamin D and are known as provitamins. Sources of the vitamin include dietary components like fishes which are fatty in nature, fish liver oils, egg yolk etc. As a result, it can be ingested through the consumption of natural foods. Furthermore, because it is created by the body with the help of sunshine, skin exposure to the sun is a primary source of this vitamin. The structure of fat-soluble vitamin D is similar to that of sterols, and it works similarly to a hormone" [4].

1.2 Classic Functions of Vitamin D

"The modulation of bone metabolism and the absorption of calcium and phosphate via the intestines are two most well known effects of vitamin D. With the help of Parathyroid hormone (PTH), it plays a critical role in maintaining serum ionized calcium concentrations" [5]. "The maintenance of a constant concentration of calcium ions in the extracellular fluid is referred to as calcium homeostasis. Vitamin D controls calcium reabsorption in the kidneys' proximal tubule cells, as well as the activity of cells that remodel and form bone. It inhibits the manufacture and secretion of parathyroid hormone in the parathyroid glands, as well as the growth of parathyroid gland cells. Moreover, active vitamin D makes the parathyroid glands more vulnerable to the effects of calcium. Therefore, regulation of calcium involves three tissues- kidneys, bone and intestine and the process is influenced by three hormones namely, PTH, calcitonin and activated form of vitamin D_3which is known as calcitriol. These three hormones operate in concert to maintain the constancy of the plasma calcium ion levels" [6].

Table 1. The overall effects of vitamin D on its various target tissues and cells have been listed in this table (7)

System	Targeted segments	Functional effects
Digestive	Duodenum	Increases intestinal calcium absorption
	Jejunum	Increases intestinal phosphate transport
Musculoskeletal	Osteoblasts (and, in turn, osteoclasts) and chondrocytes	Increases bone formation by bone mineralization and matrix formation, increases osteocalcin and osteopontin.
Renal	Distal tubules (Ca) Proximal Tubules (Phosphates)	Increases reabsorption of calcium and phosphate and detoxification of vitamin D_3
Endocrine	Parathyroid gland (Chief cells)	Decreases the levels of PTH
	Thyroid gland	Decreases TSH (Thyroid Stimulating Hormone
	Pancreatic β-cells	Increases Insulin secretion
Immune	Monocytes/ macrophages and T lymphocytes (helper type 1)	Suppresses γ-interferon and IL-1-6
Central nervous	Dorsal root ganglia (glial cells) and hippocampus	Increases the production of NGF (nerve growth factor), neurotropin 3 and leukemia-inhibitory factor.
Epithelial	Epidermal skin (keratinocyte) Hair follicle	Increases differentiation
	Female reproductive tract	Carries out uterine development
	Mammary Prostate Colon	Decreases cell growth
Others	Diverse cells and cancer cell lines	Decreases cell growth and angiogenesis and increases differentiation and apoptosis

Vitamin D is essential to trigger calcium absorption from the intestine and kidneys. Precursors to vitamin D are formed in the skin and then processed in the liver. The last stage in the conversion of an inactive form to an active for occurs in the proximal tubules of kidney cells. Upon activation, vitamin D triggers calcium absorption from the intestine and proximal tubule hence an increase in the levels of blood calcium. Synthesis of the active form of vitamin D is increased by PTH. Calcitriol acts on the intestine and causes formation of Ca^{++} binding protein, alkaline phosphatase and Ca^{++} stimulated ATPase which results in increased Ca^{++} absorption from the intestine. The summary of overall mechanism of intestinal calcium absorption has been depicted in the Fig.1.

Calcium uptake is promoted by calcitriol, which leads to calcium phosphate deposition in bone osteoblasts. As a result, it is critical for bone development. Because the bone is a major source of calcium and phosphate, calcitriol, in association with PTH, enhances calcium and phosphate mobilization from the bone, resulting in an increase in plasma calcium and phosphate levels. However, a vitamin deficit causes increased PTH secretion, accelerated bone turnover, and resultant loss of bone mineral content. Moreover, suboptimal levels reduce calcium absorption in the intestine and increase urine calcium excretion, resulting in a negative calcium balance in the body. Therefore, it is a necessary steroid hormone for adequate calcium and phosphorus homeostasis, as well as the healthy growth and maintenance of bone. It also has significant homeostatic activities in different systems of the human body including endocrine system, nervous system, epidermal system, and system related to immunity as well, during fetal and adult development and differentiation. "Vitamin D may also have a regulatory role in the rennin-angiotensin-aldosterone pathway by modulating rennin production" [7].

Such specific effects of vitamin D in different target organs or tissues have been summarized in the Table. 1.

2. BIOCHEMISTRY OF VITAMIN D

Vitamin D can be obtained from various sources, including diet and exposure to ultraviolet rays of the sunlight. However, vitamin D from these sources is not biologically active and cannot achieve full hormonal function until it is activated by the body with the help of sunlight and specific enzymes. The synthesis, absorption and transport of this fat soluble, hormone-like vitamin are very briefly discussed here.

2.1 Synthesis

In the synthesis of vitamin D_3, which is of animal origin, first cholesterol is converted into 7-dehydrocholesterol by an enzyme called 7-dehydrocholesterol reductase (DHCR7). UVB radiation from the sun then turns it into Provitamin-D_3 which spontaneously transforms into vitamin D_3 (Cholecalciferol) at body temperature. Cholecalciferol can also be obtained through diet. Metabolic activation of this transformed vitamin D consists of adding two separate hydroxyl groups, one at the carbon 25 which occurs through the liver (25-hydroxy D_3) and the other at the carbon 1 which occurs through the kidneys (1,25-dihydroxy D_3). The final result of this two step hydroxylation is the most biologically active form of the vitamin D which is 1,25-Dihydroxy- vitamin D_3 also known as calcitriol. "Any break in this activation process such as inadequate oral intake, inadequate sunlight, liver disease or kidney disease can lead to active vitamin D deficiency" [8,9].

"Hydroxylation of the circulating 25-hydroxy D_3 to the more active metabolite, $1,25(OH)_2D_3$ or calcitriol in the kidney and cancer cells is carried out by the enzyme cytochrome P450, CYP27B1, which is also known as 1α-hydroxylase or 1α(OH)ase or 1(OH)ase. This is a very efficiently controlled conversion process. Raised levels of parathyroid hormone (PTH) and hypocalcemia play important role in increasing this conversion process" [9, 10].

Although vitamin D can be synthesized in adequate amounts in the skin, large numbers of individuals throughout the world have been found to be deficient in vitamin D due to their inadequate exposure to the UVB rays of the sunlight.

"Another form of vitamin D which is of plant origin is vitamin D_2 (Ergocalciferol). This form can be derived from ergosterol and it functions much like vitamin D_3 (cholecalciferol). However, it is less active than Cholecalciferol" [4].

2.2 Absorption and Transport

"Once activated by the kidney, active vitamin D is fairly unstable and can rapidly degrade. Its degradation begins by CYP24A1, 24-hydroxylase or 24(OH)ase, and produces 1,24,25-trihydroxyvitamin D_3 ($1,24,25D_3$)" [11]. "However, the degradation can be avoided through the protection or attachment to protein based carriers. These protein based carriers can primarily consist of the vitamin D binding protein (DBP) and vitamin D receptor (VDR), two proteins central to the metabolism and mechanism of action of active vitamin D or calcitriol" [5].

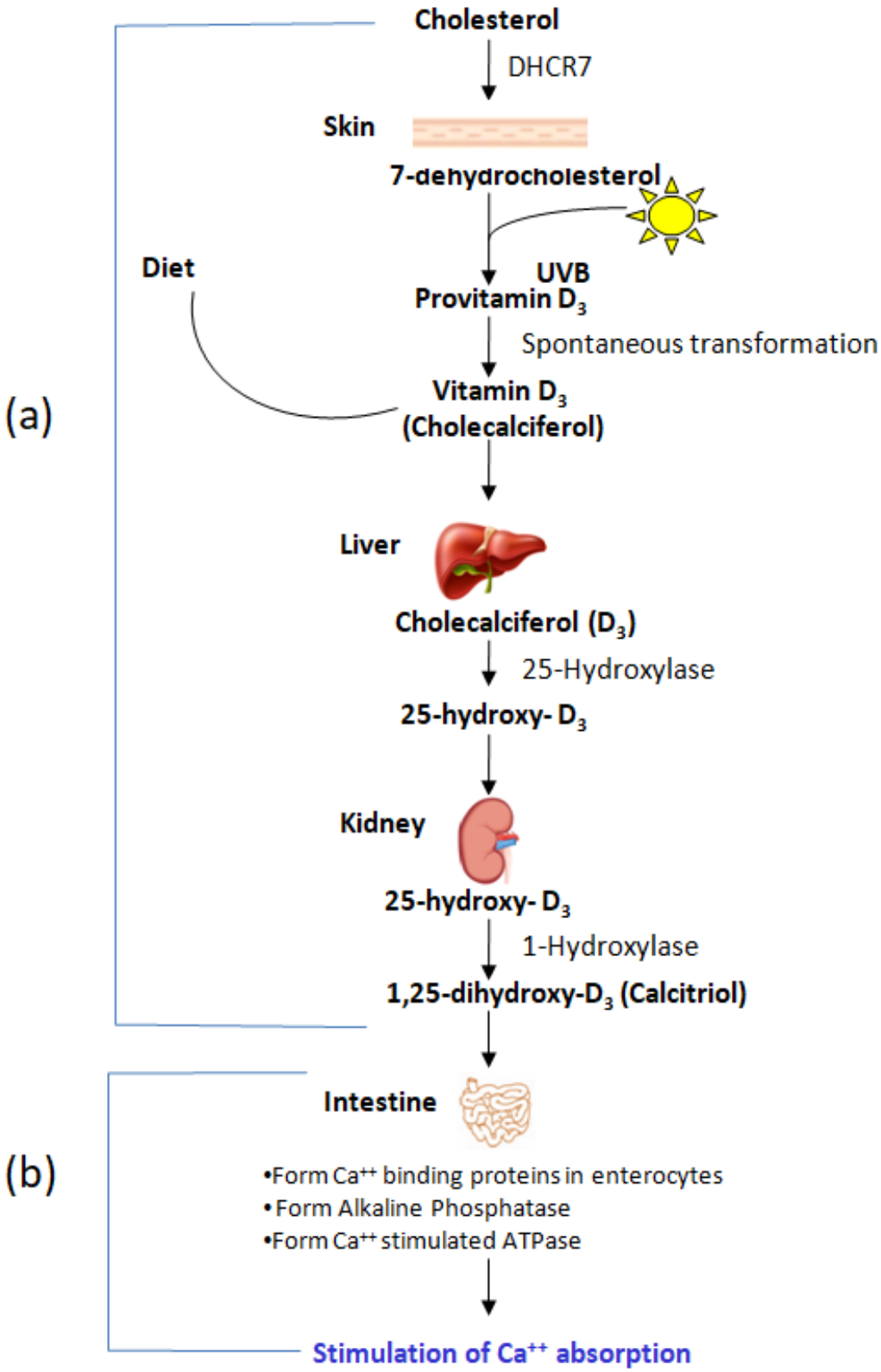

Fig. 1. (a) Schematic representation of the active Vitamin D (Calcitriol) synthesis. (b) Summary of intestinal calcium absorption

The affinity of active vitamin D to its binding protein is one of the many factors that influence its biological activity and half life. The vitamin is primarily transported to its target tissues through these binding proteins. Once it reaches the target cell, active D must be released from the protein as current evidence suggests that it is the unbound fraction or free hormone that has accessibility to target cells. Therefore, the free active D is taken up by the target cells and then it is either rapidly metabolized by these target cells or bound to VDRs. Upon binding with active D, the VDR undergoes conformational changes that allow it to interact with several other transcriptional factors within the nucleus. In order to interact with transcriptional factors, and effect gene transcription, the active D-VDR complex must interact with RXR for the formation of a hetero dimer that can then bind to selective or promoter sites of the target cell DNA. This new complex then recruits various co-activators and/or co-repressors that influence gene expression and alter cellular activity which includes protein synthesis and secretion, cellular proliferation and/or differentiation. "The cell type and location determines the overall cellular response, the number or availability of VDRs and the affinity of active vitamin D towards them" [9].

2.3 Factors Related to Difference in Serum Vitamin D Concentrations

Vitamin D molecules must be activated in order to be physiologically useful. Because vitamin D is activated in the liver and kidney, its efficacy is lowered if one of these organs fails. As a result, patients with chronic liver or kidney disease are more likely to have low levels of either form of the vitamin. Low vitamin D serum concentrations can be caused by a variety of genetic factors, including a lack of active vitamin D production, vitamin D-binding protein abnormalities, and VDR abnormalities. Low vitamin D activity can be caused by any of these problems, but they are uncommon.

It might be possible that the seasons have an impact in this regard as well since the production of vitamin D in human or animal body is regulated by sunlight. During summer, as the body is exposed more into sunlight, vitamin D production can be slightly higher than that of in the winter or autumn or any other seasons. In 1890, British researchers noticed that rickets was almost non-existent in areas near the equator, and this led to the discovery of the link between sunlight and vitamin D synthesis. The findings revealed that the regional incidence of sunlight exposure had a significant impact on the disease's prevalence [12]. "Another study found that naval officers who were exposed to a lot of sunshine had greater incidence of skin cancer but reduced rates of other cancers" [9].

In case of serum vitamin D levels, factors like age and skin color can also be considered. In a study, when compared to a healthy adult, those with dark skin, those who have less exposure to sunlight, and those who are older, all have a lower capacity to produce vitamin D. Furthermore, the amount of vitamin D stored in the body declines with age, especially in people who reside far from the equator during the winter months. It is also established that the capacity of the intestine to absorb vitamin D is reduced by gastrointestinal illnesses such as celiac and Crohn's disease, as well as cystic fibrosis. Similarly, gastrointestinal surgeries, especially bypass procedures, cause vitamin D deficiency. Therefore, while determining a person's vitamin D level, all of the mentioned factors must be considered.

3. VITAMIN D RECEPTOR (VDR) AND VITAMIN D ANALOGS

3.1 Distribution and Functions of VDR

Vitamin D_3 regulates the Vitamin D Receptor (VDR), which is a nuclear receptor (NR) that functions as a transcription factor. It is the first human NR gene to be discovered with mutations. VDR has a molecular mass of 48 kDa and 427 amino acids. It belongs to the nuclear receptor super family for steroid and thyroid hormones (8). "The DNA binding domain (DBD), like other members of the super family, has a 70-amino-acid sequence that is rich in polar and positively-charged amino acids including cysteine, lysine, and arginine. These chemically reactive amino acids, when paired with the zinc finger binding motif, allow the ligand-bound protein to bind to the vitamin D receptor elements (VDREs) in a very selective manner" [8].

VDR coupled calcitriol, along with the retinoid X receptor (RXR) and its ligand (9 cis-retinoic acid) forms heterodimers. These heterodimers occupy certain nucleotide sequences known as vitamin D response elements (VDREs). Thus, VDR is principally responsible for calcitriol's biological effects via genomic processes [13]. "After being translocated into the nucleus, calcitriol can bind to either membrane or cytosolic VDR. When calcitriol binds to VDR, it induces VDR to dimerize with the retinoid X receptor (RXR). The ligand-bound VDR-RXR complex also binds to vitamin D response elements (VDRE) in many regulatory regions of target genes, including the promoters and distal locations" [14]. "The dimer binds to VDRE with the help of numerous additional co-modulators resulting

in positive or negative transcriptional control of gene expression.VDR controls transcription of target genes by attracting chromatin-active co-regulatory complexes" [15].

Various amino acid residues have been identified as essential players in binding calcitriol after extensive investigation of the VDR's ligand binding domain (LBD). The VDR's LBD, which includes amino acids 226-427, is found near the C-terminal. The accessible volume in the LBD is 660 Angstroms^3, however the ligand calcitriol fills only 381 Angstroms^3, filling just 56% of the available volume. The LBD of the protein has been thoroughly described using the known amino acid sequence of the VDR and its crystalline structure. [8].

"Vitamin D receptors have been isolated throughout the body and are engaged in a variety of regulatory processes that go beyond calcium balance and mineral metabolism. They have been discovered to be expressed in all of the vitamin's major target tissues. They can be found in a variety of organs and cells all over the body. In intestinal cells, VDR is involved in immunity, proliferation, differentiation, permeability, and host-microbial interactions" [16]. "The activated form of vitamin D can affect T-cell differentiation, macrophage activation, and cytokine production because VDRs are found on most immune system cell types. VDR is also involved in the control of both innate and adaptive immune responses. Although epithelial VDR expression alone can improve genetic and pharmacological models of colitis, VDR expression in both innate and adaptive immune cells can impact the inflammatory response. VDR signaling reduces inflammation by boosting anti-inflammatory cytokine secretion and regulating the development of to lerogenic dendritic cells (DC) and regulatory T cells" [17].

"The kidneys, intestines, bone, and parathyroid glands are among the most well-known organs linked to vitamin D and calcium homeostasis" [9]. Other than that, VDRs have also been discovered in the pancreas, skeletal muscle, lungs, central nervous system, reproductive organs, and skin, among other tissues and systems. Moreover, they have been found in cardiomyocytes and vascular smooth muscle cells all over the cardiovascular system and scientific study is currently underway to better understand the role of active D in the cardiovascular system. Because of its lower quantity in patients' serum, VDR levels could be used as a diagnostic marker for colorectal cancer [18].

3.2 Vitamin D Analogs

Analogs are referred to such chemical compounds that are structurally similar to one another but there is slight difference in the composition, which has resemblance to the substitution of one atom in the place of another in the structure of an element, or in the presence of a specific functional group. Several such analogs of vitamin D has been identified and isolated throughout the years which are said to form as a result of a difference in the orientation of C1-OH, and they often tend to function specifically in different diseases and disorders in the body [9].

The orientation of calcitriol within the ligand binding domain (LBD) has revealed vital information about the chemical interactions that lead to ligand-receptor binding. The structure-function relationship between the VDR and calcitriol, as well as the generation of super-potent vitamin D analogs, has all been elucidated attributable to these interactions [8]. "According to studies, suppressing cancer cell proliferation needs supraphysiological dosages of calcitriol which can cause in vivo adverse effects such hypercalcemia and hypercalciuria. Approximately in the last 30 years, scientists have created over 3,000 vitamin D analogs with partially selective properties" [19]. The research progress related to some of the vitamin D analogs with the potential to be developed into anticancer agents is very briefly mentioned here in this review.

Several researchers have been able to use vitamin D analogs with different orientations as an effective inhibitor or suppressor in diverse investigations. As a result, a mouse model was employed in a study to assess the anticancer effects of a new family of vitamin D analogs. These analog compounds were designated Gemini analogs because they have two side-arms. Because calcitriol takes up just 56% of the LBD's available space in the VDR, the analog's second side-arm was not only comfortably accommodated, but it could also take one of two equally favored places [8].Scientists compared the binding of the parent Gemini analog and calcitriol to the VDR and DBP and discovered that when compared to calcitriol, the Gemini analog bound the VDR with 38% affinity, whereas the same Gemini analog bound the DBP with only 2.5 percent relative affinity. Given the considerable increase in volume due to the new side-arm, a relative binding affinity of 38 percent is higher than one might predict.

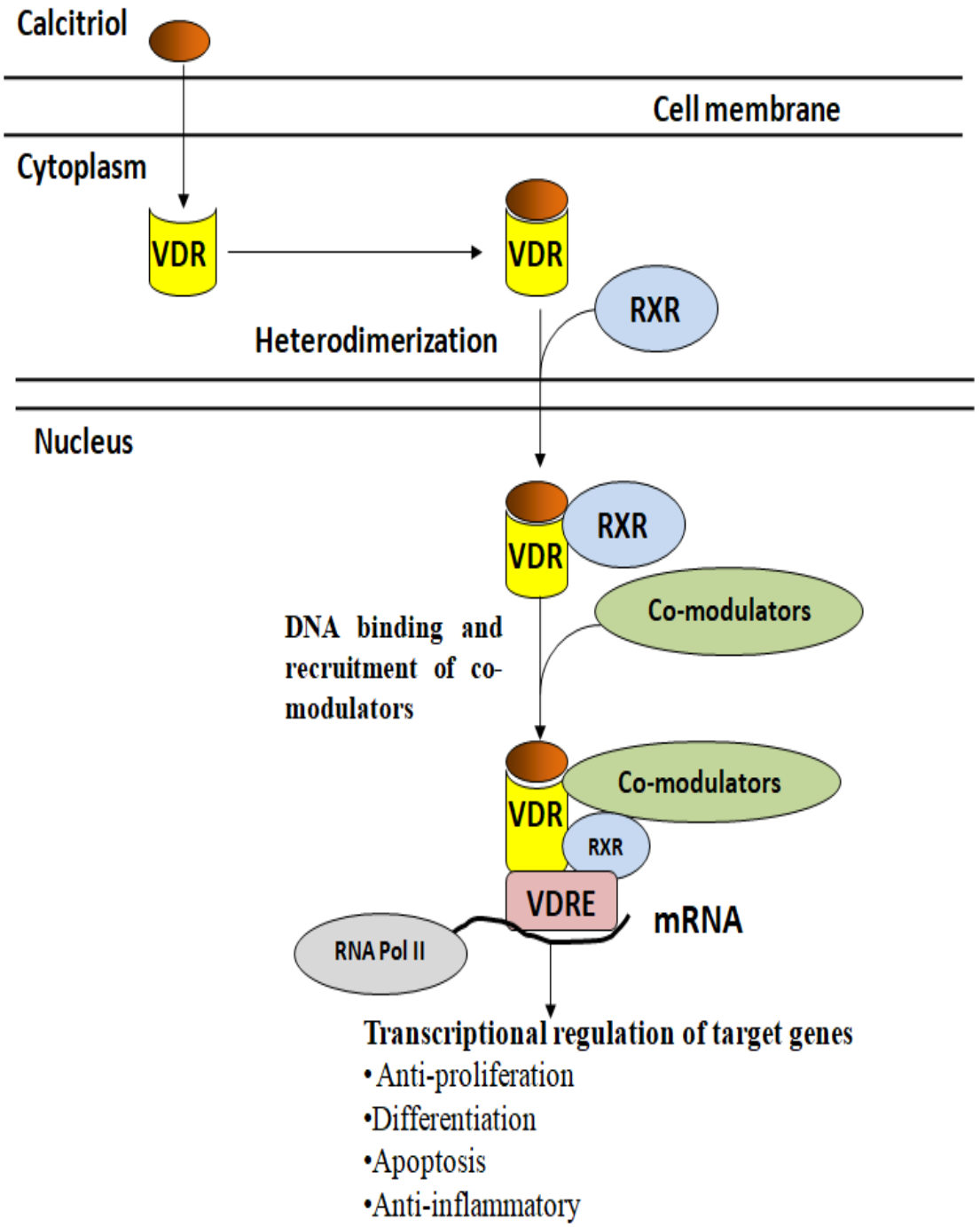

Fig. 2. Genomic mechanism of calcitriol action through VDR

When the orientation of C1-OH was modified from α to β position in calcitriol, the resulting relative binding affinity was 0.08% of the vitamin. The structure of this parent Gemini analog was substantially more flexible, with 2346 distinct minima conformations compared to 207 minima conformations of calcitriol, according to stereoscopic dot maps. The parent Gemini analog's greater flexibility allows it to be more easily accommodated into the LBD, resulting in a 38% binding affinity [20].”In vivo investigations on mice revealed that the most potent Gemini analog was at least 1000-fold more efficient than mice treated with the vitamin calcitriol at suppressing colon tumor growth” [8]. The Gemini analog A reduced tumor volume by more than 50% compared to the placebo at a relatively low dose of 0.002 µg molar equivalents of analog E per animal, while calcitriol had no effect. The serum calcium

levels of mice given this Gemini analog and fed a low calcium diet exhibited no variations.

Several analogs of vitamin D have exhibited substantial anticancer or anti-inflammatory activity in cell cultures and animal models, but only a few have been successfully used to treat cancer or inflammatory conditions. Many researchers are still working on vitamin D analogs that will improve the antiproliferative and pro-differentiating properties of vitamin D without disrupting calcium homeostasis. These analogs could be used to treat certain tumors in the future. The findings show that local active vitamin D production plays an important autocrine function in cellular growth regulation. "Vitamin D's potential for therapeutic application in the prevention and treatment of the most prevalent and fatal malignancies will become clearer as the exact mechanisms of action of this vitamin continue to be explored" [9].

"Analogs of calcitriol that are marketed are- Rocaltrol, Calcijex, Silkis or vectical. Few of the analogs in clinical trials for different diseases and disorders are- Inecalcitol or TX522 (Acute myeloid), Seocalcitol or EB1089 (Advanced breast and colorectal cancer), and 2MD or DP001 (Secondary hyperparathyroidism) etc" [9].

4. ROLE OF VITAMIN D IN DIFFERENT DISEASES OR DISORDERS

"Vitamin D levels in the healthy range of 30-100 ng/mL (75-250 nmol/L) may be crucial in preventing the development of up to 13 different cancers, as well as many other disorders such as osteoporosis, heart disease, and common autoimmune diseases" [8]. "Some autoimmune disorders, such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), diabetes mellitus type 1, multiple sclerosis, inflammatory bowel disease, and Hashimoto's thyroiditis, are linked to vitamin D insufficiency" [29]. Calcitriol has been widely praised for its effects on mineral homeostasis, as well as metabolic illnesses, immunological disorders, and cancer. To stop cancer cells from multiplying, a supra physiological dosage of calcitriol is required. The dose, on the other hand, will cause calcemic side effects such hypercalcemia and hypercalciuria. Although various vitamin D analogs have shown substantial anticancer or anti-inflammatory activity in cell cultures and animal models, only a few have been successfully used to treat cancer or inflammatory illnesses [9].

For a very long time, calcitriol has been known for its role in calcium and phosphate metabolism, which is crucial for bone mineralization. Moreover, evidence suggests that active form of vitamin D has non-classical effects on the cardiovascular, endocrine, and immunological systems. The target genes of vitamin D receptors mediate vitamin D's physiological activities. Multiple cancers and disorders that cause inflammation such as rheumatoid arthritis, multiple sclerosis and inflammatory bowel disease are all linked to vitamin D deficiency [9].

5. VITAMIN D AND CANCER

5.1 Role of Vitamin D in Cancer

Calcitriol is generated not only in the kidney but also in various other bodily tissues with the help of the enzyme 1-OHase which is expressed on them. These include the prostate, colon, and breast tissues. This finding suggests that, in addition to calcium mobilization, vitamin D regulates other body functions such as cellular proliferation and differentiation, immunological function modulation, vascular tone, and influences on renin and insulin production, all of which are unrelated to calcium metabolism [21]. Active vitamin D has anti-proliferative and pro-differentiating effects on normal and malignant cell lines, among other non-traditional activities. Higher vitamin D levels are thought to protect against a variety of malignancies, presumably through genomic and non-genomic effects mediated by the VDR, such as autocrine/ paracrine metabolism of the VDR's ligands. It is a well-known powerful regulator of cell proliferation and differentiation, having latest evidence of an effect on angiogenesis, cell death and tumor invasion, making it a potential cancer-regulating agent [9].

Calcitriol inhibits tumor growth in vitro and in vivo in case of various types of carcinoma, such as murine squamous cell carcinoma (SCC), rat metastatic prostatic adenocarcinoma, human prostatic adenocarcinoma (e.g. Prostate cancer cell line, PC-3and Lymph Node Carcinoma of the Prostate, LNCaP), human breast, colon, pancreatic cancer, leukemia, myeloma, and lymphoma lines [22].Regular sun exposure has been linked to a significant reduction in some cancer death rates as well as overall cancer mortality rates, which could be linked to the body's vitamin D metabolic pathway.

According to some ecological research, UV-rays from the sunlight may protect against female breast cancers, prostate cancer, colorectal cancer and colon cancer. Several studies have also found a link between circulating vitamin D and the prevention of colorectal and prostate cancer [8]."Recent epidemiological findings revealed a clean connection between low levels of vitamin D and an elevated risk of chronic

illnesses of diverse etiologies" [21]. "Many significant chronic diseases, including some deadly malignancies, have been linked to a lack of sun exposure and vitamin D insufficiency. Sun exposure and serum vitamin D content have been demonstrated to have an inverse connection with the risk of acquiring and/or surviving cancer in epidemiological studies" [23]. "The most effective ways to prevent vitamin D deficiency appear to be moderate sun exposure and the usage of vitamin D supplements. In addition to cancer protection, some researchers have discovered that vitamin D causes cancer cells to die in vitro and in vivo" [21].

"Calcitriol was previously shown to be a powerful activator of differentiation and inhibitor of cell proliferation when added to mouse myeloid leukemia cells" [8]. Since then, it has been proven that the active form of vitamin D has an anti-proliferative effect on VDR-positive normal and malignant cell lines.

5.2 Mechanism of Anticancer Action of Vitamin D

The mechanism of vitamin D's anticancer activity at the cellular level is still unknown. However, cells, including cancer cells, have been demonstrated to have unique nuclear vitamin D receptors (VDRs) for the active metabolite calcitriol. These receptors are members of the nuclear receptor superfamily, which also includes steroid/thyroid hormone receptors. Calcitriol binding to VDR causes a conformational shift in the receptor, which activates it and causes it to dimerize with the nuclear retinoic X receptor. In the promoters of target genes, the heterodimer subsequently attaches to VDREs, promoting transcription and causing changes in phosphocalcic metabolism or cell division, differentiation, and death regulation [9, 21]. Calcitriol has been reported to affect the activity of more than 60 genes when linked to VDR, resulting in prodifferentiating, antiproliferative, and antimetastatic effects on cells, as well as cell cycle and angiogenesis impacts [21]. As a result, vitamin D's non-calcium mobilizing effects are linked to cell proliferation, differentiation, apoptosis, and angiogenesis.

Calcitriol's molecular mode of action spans non-genomic responses to longer-term processes that have long-term genomic impacts. Cross talk (unwanted signals in a communication channel) between this metabolite's signaling pathways and those of other growth factors or hormones that together regulate cell proliferation, differentiation, and cell survival is central to the genetic processes of vitamin D activity [7]. Increase in inhibitors and decrease in activators of cyclin-cyclin-dependent kinase complexes (CDK's), as well as increased levels of the cyclin-dependent kinase inhibitors Cip/Kip proteins, p21 and p27, are thought to be responsible for the vitamin's anti-proliferative properties [8]. These proteins stop the cell cycle at the G1/S checkpoint, blocking DNA synthesis and thereby cellular proliferation.

5.3 Genomic and Non-genomic Pathways of Vitamin D Analogs for Cancer Prevention

Both genomic and non-genomic mechanisms are involved in the actions of calcitriol and its analogs. Calcitriol works through pharmacologically separate nuclear receptor-mediated and plasma membrane-initiated processes, as previously mentioned. It interacts with the VDR in the cell nucleus to produce genomic effects, or it interacts with the VDR in the caveolae of the plasma membrane to produce non-genomic effects. VDR is found in more than 30 human organs, and its activation involves over 60 genes in several cell lines. The VDR is expressed by both benign and malignant proliferative cells. Heterodimers are formed by Calcitriol with the retinoid X receptor (RXR) and its ligand when attached to VDR, and these dimers occupy certain nucleotide sequences (VDREs). This complex, in concert with a number of transcription factors, results in the transcription of vitamin D-responsive genes [22].

Non-genomic pathways may be able to work together with the traditional genomic pathway. Non-genomic signaling is fast, doesn't require transcription, and can influence transcription indirectly through cross-talk with other signaling pathways. Some evidence state that the beginning of the non-genomic effects occurs at the plasma membrane which involves a non-classical membrane receptor as well as a novel vitamin D receptor known as $1,25D_3$MARRS (Membrane-Associated, Rapid Response Steroid-binding) (22). Interaction occurs between calcitriol and vitamin D receptor (VDR) to produce genomic or non-genomic effects. VDR is found in the cell nucleus or caveolae of the plasma membrane. Moreover, calcitriol can interact directly with the new receptor $1,25D_3$-MARRS.

The activation of one or more second messenger systems, such as G protein-coupled receptors, phospholipase C, protein kinase C (PKC) or phosphatidylinositol-3-kinase (PI3K), may occur when calcitriol binds to the plasma membrane. More possible results of the interaction are the opening of voltage-gated calcium or chloride channels as well as

the creation of second messengers. Some of these second messengers, particularly RAF/MAPK (RAF-Rapidly Accelerated Fibrosarcoma, MAPK-Mitogen-Activated Protein Kinase), may regulate gene expression by modulating a cross-talk with the nucleus [22].

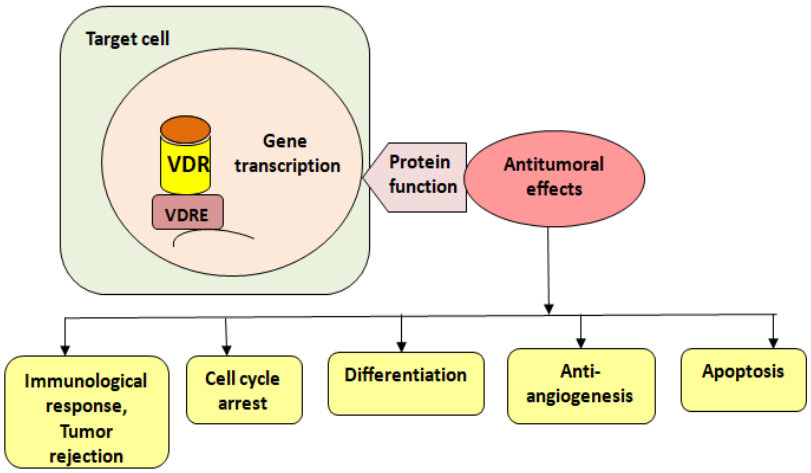

Fig. 3. Schematic representation of the anti-tumoral effects by activated vitamin D receptor (VDR)

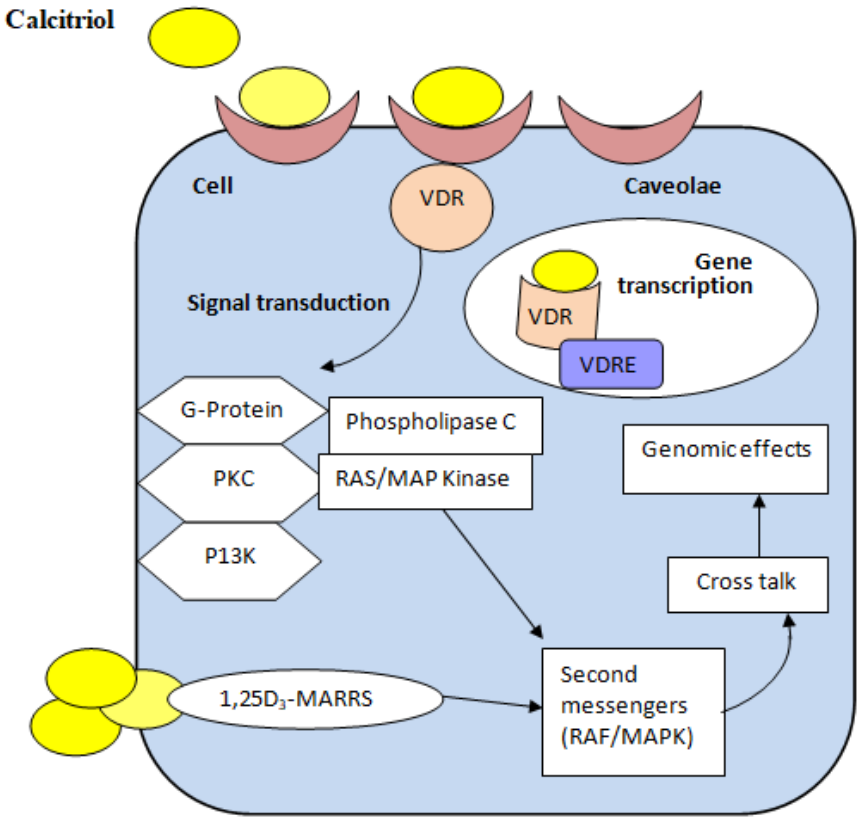

Fig. 4. Calcitriol operates through mechanisms initiated by plasma membrane and mediated by nuclear receptor

VDR (Vitamin D Receptor); **VDRE** (Vitamin D Receptor Elements); **PKC** (Protein kinase C); **PI3K** (Phosphatidylinositol-3-kinase); **RAS/MAP Kinase** (Ras, a family of related proteins that are expressed in all animal cell lineages and organs, originated from "Rat sarcoma virus"; MAP kinase- Mitogen-Activated Protein Kinase); **RAF/MAPK**(RAF-Rapidly Accelerated Fibrosarcoma, MAPK-Mitogen-Activated Protein Kinase); **MARRS**(membrane-associated, rapid response steroid-binding).

5.4 Potential of Vitamin D in Prevention and Regression of Different Types of Cancers

Vitamin D's anticancer properties have been extensively researched in a range of malignancies in vitro and in vivo, with the most promising results discovered in colorectal, breast, and prostate cancers [21].The results of limited clinical trials for cancer prevention or treatment have been unclear. Increased calcium and vitamin D intake was linked to a lower risk of colorectal cancer in the majority of observational studies [22]. High-dose vitamin D supplementation appears to protect postmenopausal women with colorectal cancer, according to a small trial [14]. However, a trial comprising 36,282 patients who were administered vitamin D (400 IU of vitamin D_3 daily) found no reduction in the incidence of colorectal cancer.

The importance of maintaining appropriate $25(OH)D_3$ levels to reduce the risk of a variety of diseases is demonstrated by the association of calcitriol in limiting prostate and colon cancer growth.

5.4.1 Colon cancer

Calcitriol has been found to be critical for colon cancer cell growth and development. Scientists have discovered that highly differentiated human malignant colon cancer tissue and surrounding normal tissue had similar levels of 1(OH)ase. The expression of 1(OH)ase was 20 to 30 times higher in the less differentiated cancer colon tissue than in the normal neighboring tissue. Moreover, calcitriol has the potential to play a key role in the production of Id proteins or inhibitors of DNA binding and cell differentiation proteins, which interfere with basic helix-loop-helix transcription factors and hinder DNA binding and differentiation [8]. These proteins are important parts of the signaling pathways which are involved in development, cell cycle and tumorigenesis. Furthermore, the antiproliferative and pro-differentiating effect of calcitriol on human and mouse skin cells in vitro was well established, implying that the vitamin would have a similar effect on colon cells. As a result, scientists treated HT-29, which is a cell line from human colon cancer that expressed the VDR, with calcitriol [24]. The addition of the vitamin effectively suppressed cellular proliferation and caused the colon cancer cells to differentiate in a dose-dependent manner. Using a mouse colon cancer model, researchers looked at the protective effect of vitamin D deficiency on tumor growth in colon cancer. For a period of time, the animals were fed a vitamin D-sufficient or vitamin D-deficient diet. The mice were given subcutaneous implantation of 10,000 mouse colon cancer cells after it was determined that the vitamin D deficient group had blood vitamin levels of less than 5 ng/mL and the vitamin D-sufficient group had levels in the normal range with a mean of 26 ng/mL. The vitamin D-deficient mice had a 60 percent (mean < p0.05) higher tumor volume on the final day of the trial than the vitamin D-sufficient mice. The researchers came to the conclusion that getting enough vitamin D in the diet protects from colon cancer. Melanoma, leukemia, lung, breast, and prostate cancer cell lines have all been found to respond to the antiproliferative and prodifferentiating activities of calcitriol, the active form of vitamin D [8].

5.4.2 Prostate cancer

It has been suggested that the expression of prostatic 1(OH)ase diminishes with age in prostate cancer. When $25(OH)D_3$ was introduced to a culture of primary prostatic epithelial cells with 1(OH)ase activity, the $25(OH)D_3$ was converted to its active metabolite, calcitriol, and cellular proliferation was suppressed [25]. Reduced expression of 1(OH)ase in prostate cells has also been postulated as a possible explanation for the link between aging and the development of prostate cancer [25].When compared to normal prostate cells, benign prostatic hyperplasia cells had a 60 percent drop in 1(OH)ase activity, while prostate cancer cells from primary cultures had an average of 85 percent reduction in 1(OH)ase activity. Moreover, the LNCaP(Lymph Node Carcinoma of the Prostate) cell line, which is frequently employed as a model for human prostate cancer, was discovered to have undetectable levels of 1(OH)ase expression. There was no influence on cellular proliferation when these cells were treated with $25(OH)D_3$. When the 1(OH)ase cDNA was transfected into LNCaP cells, the cells produced the 1(OH)ase enzyme, which allowed them to convert $25(OH)D_3$ to $1,25(OH)_2D_3$or calcitriol. $25(OH)D_3$ then reduced the proliferation of these 1(OH)ase transfected cells [8].

5.4.3 Colorectal cancer

Many epidemiological studies have been developed to test this theory since 1980, when a preventive role for

vitamin D in colorectal cancer was first hypothesized. The observation that colorectal cancer mortality increased with geographical latitude was taken under consideration to explain cancer mortality according to the geographical pattern. The gradual decrease in vitamin D levels was considered as a consequence to weak UV-B irradiation at the higher geographical latitudes [26].

Vitamin D and its metabolites have long been known to suppress colorectal cancer progression through a variety of mechanisms, some of which have been explained in recent years. Vitamin D interacts with Wnt/β-catenin signaling (a highly conserved pathway through evolution, regulates key cellular functions including proliferation, differentiation, migration, genetic stability, apoptosis, and stem cell renewal), a known factor to colorectal cancer progression, and the innate immune response to influence both the initiation and advancement of colorectal cancer. As such, low vitamin D levels have been linked to an increased risk of death and aggressiveness in colorectal cancer, as well as a 30 percent reduction in the production of colorectal adenomas in people with greater versus lower $25(OH)D_3$levels. These findings, along with in vitro evidence that colorectal epithelial cells express the VDR, provide a foundation for hypothesizing a therapeutic impact of vitamin D in colorectal cancer and designing a number of clinical trials [22].

5.4.4 Breast cancer

There is an increasing interest in determining the effect of environmental risk factors, lifestyle, and food habits in case of breast cancer or tumor, as it is one of the most frequent malignancies in women. Laboratoristic, epidemiological, and genetic studies all indicate the link between vitamin D and breast cancer (22). Because both healthy and malignant cells display the 1(OH)ase enzyme activity, it is quite likely that breast cells can locally manufacture calcitriol. Its antiproliferative and prodifferentiating effects in the breast appear to be regulated by a balance between the activity of the 1(OH)ase enzymes, which are responsible for the synthesis and the 24(OH)ase enzymes, which are responsible for the breakdown of the active form of the hormone-like vitamin [27].

It has been stated that VDR is expressed in both healthy and cancerous breast cells, and gene ablation studies have also revealed that VDR plays a function in mammary gland development. In lieu to this, a study was carried out on mice having deficiency of the vitamin receptor genes. Mice deficient for the VDR gene produced a larger number of pre-malignant lesions than wild-type mice, after being stimulated with the carcinogen DMBA [27].

5.5 Recent Approaches for Cancer Treatment

The rapidly developing understanding of biological processes driving carcinogenesis has aided in the identification of novel cellular targets for anticancer medicines and the exploiting of biological derangements particular to cancer cells. It includes, Matrix metalloproteinase inhibitors to prevent invasion and metastasis, angiogenesis inhibitors to prevent new blood vessel formation, signal transduction inhibitors to interrupt crucial signaling pathways which are compulsory for cellular growth and proliferation, differentiation agents keeping neoplastic cells in a stage where they have little or no proliferative potential, and designer molecules to inhibit tumor growth [28].

6. RECOMMENDATIONS FOR VITAMIN D INTAKE AND TOXICITY OF OVERDOSE

Amount of vitamin D recommended for regular intake is about 400 International Units which can be regarded as equal to about 10 mg of Cholecalciferol [4]. People in countries having moderate temperatures throughout the year or where people are more exposed to sunlight, may require lesser amounts of the vitamins to intake. In some countries the amount required might even be half of the regular recommendation. According to a study, the current recommended acceptable intake (AI) of 200 IU for all children and adults 50 years old, 400 IU for adults 50-70 years old, and 600 IU for those 71 years and above is four to five times too low to achieve optimal levels of the vitamin. Even when these AIs are followed, just 4% of adults 51 years old consume the required AI. Due to the AI's insufficiency, many people continue to consume insufficient dietary vitamin D, putting them at risk for diseases that could be avoided.

However, it's important to remember that taking too much of this vitamin might be dangerous. Overdosing on vitamin D is regarded the most dangerous of all vitamins since it causes many negative consequences such as bone demineralization and increased calcium absorption from the intestine. Overdose on vitamin D also causes hypercalcemia, which is characterized by high amounts of calcium in the blood as calcium deposits in various soft tissues, including the kidneys and arteries, as a result of prolonged hypercalcemia. Overdosing can result in the production of kidney stones as well.

7. CONCLUSION

As it functions as a hormone, vitamin D is essential for survival. Hence its source and proper mechanism of action in the body must be well understood. The current review aims at reaching the proper knowledge of the vitamin's pathway of carrying out different functions in different systems of the body including both classical and non-classical functions of the vitamin. It has been mentioned that its biological activity and relationship with vitamin D receptors is not restricted to the typical organs linked with calcium homeostasis and PTH generation. It regulates a range of physiologic processes throughout the body. By understanding the vitamin's mechanism of action on various cells and tissues and its effects on increasing or decreasing specific cellular functions we can reach to the conclusion that vitamin D can also manipulate the proliferation of cancer cells. In various tissues, vitamin D functions as an antiproliferative agent, slowing the growth of cancerous cells. It has also proven to be antiangiogenic in vivo and in vitro, in addition to its antiproliferative and pro-differentiating properties. As it deprives cancer cells of nutrition and oxygen for growth and survival due to its angiogenesis inhibiting activity, it can also induce tumor growth retardation and tumor regression in addition to cancer prevention and cell death. Moreover, cancer cells are immortal, these never grow up, mature, or die, whereas vitamin D derivatives are known to stimulate normal cell growth and maturation (differentiation), all of which cause adverse effects towards the cancer cells. Thus it plays significant role against cancer. Owing to the anticancer properties of the vitamin, some experts believe vitamin D supplementation could help in the treatment and prevention of some cancers. Furthermore, epidemiological studies have revealed that ultraviolet-B exposure can reduce cancer risk and prevalence, implying that vitamin D could play a role in cancer prevention and recurrence. Because of the preventive potential of this biologically active agent, it has been suggested that countries where cancer is on the rise and vitamin D is readily available should implement awareness, education, and strategies to increase vitamin D supplementation in people of all ages as prevention to bring down the risk and prevalence of cancer. Understanding the physiology and consequences of vitamin D throughout the body is only getting a glimpse of scientific knowledge. In order to learn more about this important hormone, more research and development is now underway.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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