Bio- Chemical and Molecular Mechanisms of Vitamin D

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Abstract

1,25-Dihydroxyvitamin D3 (1,25D) is a vitamin D hormone metabolite that activates the vitamin D receptor to have biological effects (VDR). In the presence of 1,25D, the retinoid X receptor (RXR) and vitamin D receptor (VDR) create a heterodimer that binds to vitamin D responsive elements in the area of genes directly regulated by 1,25D. To promote the classic vitamin D activities including intestinal calcium and phosphate absorption and bone and calcium homeostasis, ligand-activated VDR-RXR recruits' coactivator or corepressor complexes to control transcription of genes encoding the relevant protein genes. Thus, vitamin D action in a particular cell is dependent on the metabolic production or delivery of sufficient concentrations of the 1,25D ligand, expression of adequate VDR and RXR coreceptor proteins, and cell-specific programming of transcriptional responses to regulate select genes that encode proteins that function in mediating the effects of vitamin D. RANKL, SPP1 (osteopontin), and BGP control bone mineral remodeling; TRPV6, CaBP9k, and claudin 2 increase intestinal calcium absorption; TRPV5, klotho, and Npt2c regulate renal calcium and phosphate reabsorption. In keratinocytes, VDR seems to act without 1,25D ligand to regulate mammalian hair cycle by regulating genes such as CASP14, S100A8, SOSTDC1, and others that influence Wnt signaling. Other low-affinity non-vitamin D VDR ligands, such lithocholic acid, docosahexaenoic acid, and curcumin, have also been identified. Chronic diseases of aging including as osteoporosis, type 2 diabetes, cardiovascular disease, and cancer may be slowed down by a combination of alternate VDR ligands and 1,25D/VDR gene expression regulation.

Keywords: *Vitamin D, Gene, Expression, Biological, Signaling.*

1. INTRODUCTION

Vitamin D's role in mineral homeostasis and bone health is well-known. Oral vitamin D supplements may be used to treat or prevent rickets in children and osteomalacia in adults who are vitamin D deficient. Investigations on vitamin D's role in cancer, cardiovascular disease, type 2 diabetes obesity and autoimmune diseases are still inconclusive despite encouraging pre-clinical findings and vitamin D-deficiency-related studies. To further understand vitamin D's functions outside of the skeleton, randomized, controlled experiments are needed. The steroid hormones and hormone precursors that vitamin D and its metabolites produce are called steroid hormones. As much as 80% comes through the skin's photoconversion of 7-dehydrocholesterol to pre-vitamin D3, while the rest comes from the diet and from dietary supplements. It doesn't matter where pre-vitamins D2 and D3 come from; they remain inactive in the body until they are hydroxylated in the liver and kidneys into the active form of vitamin D, 1,25(OH)2D. Upon reaching their destination cells, vitamin D and its metabolites break from vitamin D binding protein (VDBP) and enter the cells.

2. LITERATURE REVIEW

Wanvisa Udomsinprasert et.al (2019) A key function for vitamin D in bone metabolism and calcium homeostasis has been shown. Intriguingly, new research reveals that vitamin D may protect against liver fibrosis, as previously thought. This activity, however, remains a mystery as to how it works. Vitamin D's significance in liver fibrosis pathophysiology and its therapeutic value in the treatment of liver fibrosis have been summarized and updated in this study. Specifically, vitamin D receptor-mediated signal transduction pathways, which in turn suppress the expression of pro-fibrogenic genes, have an anti-fibrotic impact on hepatic stellate cells, with respect to its influence on liver fibrosis. Low vitamin D levels are also linked to an increased risk of liver fibrosis in many studies. Patients with advanced liver fibrosis had a higher incidence of vitamin D insufficiency, indicating that vitamin D status might be used as a biochemical diagnostic for the advancement of hepatic fibrosis. As a result, it's logical to assume that supplementing with vitamin D, which is inexpensive and easy to administer, might help treat liver fibrosis. Vitamin D supplementation as a very low-cost therapy for

liver fibrosis in individuals with chronic liver disorders still need more investigation to better understand its regulatory function in preventing fibrogenesis and its safety and efficacy.

Sang-Min Jeon et.al (2018) Vitamin D, which has long been considered an important vitamin, is really a precursor to a powerful steroid hormone that affects a wide range of bodily functions. It was discovered that vitamin D may have an important role in the treatment of numerous extra-skeletal disorders, such as cancer, as well as its more traditional involvement in bone metabolism in epidemiological, preclinical, and cell studies throughout recent decades. Calectriol, the physiologically active form of vitamin D, undergoes a two-step metabolic process in the liver and kidneys to bind to the vitamin D receptor (VDR) and regulate gene expression. In addition, a non-canonical metabolic route mediated by CYP11A1 may also metabolize and activate vitamin D, according to recent investigations. It has been hypothesized that vitamin D may have a variety of anticancer qualities, with varying impacts on cancer progression. Evidence is mounting suggesting vitamin D metabolism and functions are dysregulated in many forms of cancer, making it resistant to vitamin D's antitumorigenic actions and contributing to cancer's emergence and spread. Since vitamin D metabolism and function are dysregulated in cancer, new ways for effective vitamin D-based cancer treatment will need to be developed.

KEVIN N KEANE ET.AL (2017) Due to widespread vitamin D insufficiency and its ability to influence molecular pathways linked to chronic and inflammatory illnesses, vitamin D (VitD) has received a great deal of interest as a crucial secosteroid. Many tissues, including those of the reproductive system, are influenced by VitD metabolites and the VitD receptor. VDR expression has been found in spermatozoa and germ cells in the male reproductive tract as well as in the ovaries, placenta, and endometrium in the female reproductive system. This has to be shown, however, in terms of how VitD signaling and metabolism affect reproductive health at the molecular level. Thus, the purpose of this study is to examine the present metabolic and molecular components of the VitD–VDR axis in reproductive medicine and to suggest future research directions in this field. Particularly VitD's effect on sperm motility, calcium handling and capacitation, acrosin response and lipid metabolism are being investigated in this study." As a result, we'll also address the influence of Vitamin D on primary granulosa cells, as well as trophoblast cells, on sex hormone release and receptor expression. An overview of current advancements in VitD–VDR signaling, especially linked to changed cellular bioenergetics, is included in the review's conclusion.

3. VITAMIN D AND ITS BIOACTIVATION

A. Vitamin D and 25(OH)D3

The skin produces vitamin D3 (cholecalciferol) from 7-dehydrocholesterol, the natural form of vitamin D. Vitamin D3 is created via the temperature-sensitive rearrangement of three double bonds in 7-dehydrocholesterol, which is then converted into vitamin D3 by irradiation. As the most significant source of vitamin D, the skin's ability to synthesize it relies on exposure to sunlight, which varies according to season and latitude. From November to February, the skin in Boston (42.2°N) generates no vitamin D, but in San Juan (18°N), the skin produces vitamin D all year round. Sunscreen and melanin both reduce vitamin D synthesis. In addition to supplementation, food sources of vitamin D exist. Vitamin D, on the other hand, can be found in just a few foods (which include fortified dairy products and fish oils). Vitamin D3 is not physiologically active in and of itself. The vitamin D binding protein (DBP; which binds vitamin D and its metabolites in serum) transports vitamin D to the liver in the bloodstream. To form 25(OH)D3, vitamin D is hydroxylated at C-25 in the liver. Vitamin D in the form of 25(OH)D3 is the most often circulating type. One of the most dependable indicators of vitamin D status has been its blood concentration. There is no evidence that 25(OH)D3 synthesis is tightly controlled. A number of cytochrome P-450 enzymes (CYPs), including CYP2R1, CYP27A1, and CYP2D25, have been examined as potential candidates for the enzyme responsible for the conversion of vitamin D to 25(OH)D3. Some researchers believe that the microsomal vitamin D 25-hydroxylase, initially discovered by Cheng et al. as CYP2R1, is the primary enzyme in the biosynthesis of 25(OH)D3 and is responsible for patients' 25(OH)D3 shortage and the development of vitamin D-dependent rickets. It has been reported that the crystal structure of CYP2R1 in association with vitamin D3 shows that the 17-aliphatic side chain of vitamin D is situated above the heme plate that is suited for 25-hydroxylation. Recent research utilizing Cyp2r1 null mutant mice have shown that CYP2R1 is the primary enzyme responsible for the 25-hydroxylation of vitamin D, adding weight to the notion that CYP2R1 has a physiological function. 25(OH)D3 production is not completely inhibited in the Cyp2r1-null animals, indicating the existence of additional 25(OH)D3 25-hydroxylases that have yet to be discovered.

DBP transports 25(OH)D3 to the kidney, where the glomerulus filters it. DBP is taken up by the tubular epithelial cells through endocytic internalization by megalin, a 600-kDa transmembrane protein and a member of the low-density lipoprotein receptor superfamily. According to research with mice lacking the megalin gene, the renal absorption and metabolism of 25(OH)D3 are critical functions of megalin. DBP and 25(OH)D3 are lost in the urine of Megalin-deficient mice and bone metabolism is impaired, mimicking vitamin D deficiency rickets. DBP and 25(OH)D3 are internalized by cubulin, a second surface receptor in the proximal tubule for cubulin and megalin. Dab 2 (Dab) 2, an adapter protein, also functions in combination with megalin for the cellular absorption of DBP/25(OH)D3 by attaching to the cytoplasmic tail of megalin and therefore facilitating the correct routing of the receptor.

4. THE VITAMIN D RECEPTOR AND GENOMIC MECHANISM OF 1,25(OH)2D3 ACTION

A. Vitamin D Receptor

1. Structure and function of VDR have been studied in detail. The vitamin D receptor (VDR) is responsible for 1,25(OH)2D3's biological effects. Retinoic acid, thyroid hormones, sexual hormones, and adrenal steroids are all part of the steroid receptor family that includes VDR. The VDR gene is found in fish, birds, and mammals, all of which have a similar evolutionary history. There are two VDR genes in the human and mouse genomes, located on chromosome 12. The eight coding exons in both the human and mouse genomes are the same. At least six noncoding exons are detected in the human gene, two of which are in the mouse gene. At least two promoters can be found in the human gene. It has been recommended that tissue-specific promoters be used. Vitamin D target genes are activated by VDR protein, which has 423 or 427 amino acids, depending on the species (mouse VDR or human VDR). NH2-terminal DNA-binding domain (DBD) and COOH-terminal ligand binding domain (LBD) are the two key functional domains of the VDR (LBD). Cysteine is abundant in the zinc finger area of the DBD. It has four invariant cysteine residues, which form two zinc fingers, each of which holds one zinc atom in a tetrahedral structure. H1-H12, which corresponds to H12 in the ligand-dependent activation function (AF2), and 3 sheets make up the LBD (S1-3). As a result of the binding of 1,25(OH)2D3, RXR and coregulatory complexes are able to interact more easily with target genes. Coactivator protein recruitment has been shown to be dependent on H12 relocation following 1,25(OH)2D3 binding, despite the discovery of alternative coactivator interfaces in the LBD region of VDR. The DBD and LBD are linked by a hinge point. Even though the X-ray crystallographic data of the complex of VDR and RXR has been published, it is now unavailable. DNA liganded to VDR/RXR was recently studied using cryoelectron microscopy to determine its structure (Figure 1). Results from this research reveal that LBD and DBD work together and allosterically to regulate gene expression through VDR. To make recruitment of coregulators easier, LBD may be positioned closer to the hinge region of H12, which may help stabilize the whole complex. Small angle X-ray scattering and hydrogen-deuterium exchange technologies have also been used to characterize the DNA complex between the VDR and RXR ligands, indicating that ligands may operate along with DNA to fine-tune gene expression, as has been shown in previous research. A better knowledge of the structural basis for VDR and coactivator activation will be gained as a consequence of these recent technological developments.

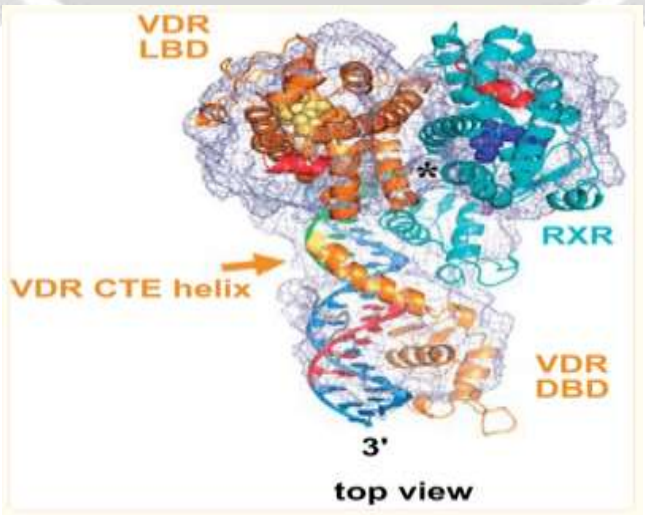

Figure 1: Structure of the full human RXR/VDR nuclear receptor heterodimeric complex with its target DNA.

2. Hereditary vitamin D-resistant rickets

In hereditary vitamin D-resistant rickets (HVDRR), which is an uncommon autosomal recessive condition, the symptoms include hypocalcemia, hyperparathyroidism and early-onset rickets. The VDR mutations that induce resistance to 1,25(OH)2D3 are diverse in nature. In youngsters, alopecia may also be a symptom. VDR's structure and heterodimeric complex, as well as biochemical and genetic research of HVDRR patients, have yielded vital insights into VDR's functional domains and signaling processes. When HVDRR was discovered, zinc finger DBD point mutations in the steroid receptor gene superfamily were the first disease-causing mutations discovered. HVDRR has already been linked to over 100 instances and 45 different VDR mutations since that first publication. As well as mutations that disrupt ligand binding (such as R274H, which interacts with the 1-hydroxyl group) and VDR/RXR interaction (such as R391S, which is located in helix 10) or prevent coactivator recruitment (such as E420 K, which is located in helix 12), variations in the LBD have been discovered as well. The patient with the E420K mutation exhibited rickets but no alopecia, demonstrating that VDR recruitment of coactivator cofactors is essential to prevent rickets but not for hair growth. HVDRR individuals have had their mineral and skeletal phenotypes reversed by intravenous or oral calcium treatment, showing that the activity of VDR/1,25(OH)2D3 on intestinal calcium absorption is crucial. HVDDR without alopecia in a humanized mouse model has recently been discovered It has been shown that transgenic mice with the 1,25(OH)2D3-binding deficiency hVDR-L233S mutant have no hair loss, but they nonetheless exhibit all of the rickets-like symptoms of a Vdr null mouse. Another research presents VDRgem mice, which express a VDR mutant that does not bind 1,25(OH)2D3 at supraphysiological concentrations, but may be selectively triggered by the binding of the gemini vitamin D analog. They have a greater deficit in calcium and bone homeostasis than normal Vdr mice. VDR signaling pathways will be further understood in future studies using both fascinating models.

B. Genomic Mechanism of 1,25(OH)2D3 Action

1. Coenzyme diversity As a consequence of this direct binding of 1,25(OH)2D3-activated VDR/RXR to particular DNA sequences, the target genes' transcription is either activated or repressed, depending on the state of 1,25(OH)2D3 activity. VDREs with strong affinity for VDR are composed of two direct imperfect repetitions of hexanucleotides with a spacer of three nucleotides, despite reports of substantial sequence diversity. 1,25(OH)2D3-VDR forms high-affinity heterodimers with RXR in order to bind VDREs. Gene expression modifications are caused by recruiting transcriptional coactivators once the liganded receptor binds to the VDRE and binds to the coactivators. The principal coactivators that bind to the AF2 domain of liganded VDR are the p160 coactivators steroid receptor activator 1, 2, and 3 (SRC-1, SRC-2, and SRC-3), which contain histone acetylase (HAT) activity. LxxL (x = any amino acid) patterns in the SRCs allow them to bind to VDR and other nuclear receptors. Secondary coactivators, such as CBP/p300 (which also have HAT activity), are recruited by members of the p160 family, resulting in a multisubunit complex that alters chromatin and destabilizes histone/DNA interaction. Core histones are also methylated in addition to acetylation. Methyltransferases may possibly play an important role in VDR-mediated transcription, according to recent findings. A stable preinitiation complex is formed when liganded VDR interacts directly or indirectly with basal transcription factors [TFIIB and numerous TAT binding protein associated factors (TAFs)]. Mediator, a multi-protein complex (the 205 subunit interacts to VDR) that operates by recruiting RNA polymerase II and promoting the development of the preinitiation complex, facilitates VDR-mediated transcription.

VDR's transcriptional activity has been shown to be influenced by a slew of other transcription factors. As a result of Ras activation of the Ets transcription factor, Cyp24a1 has been shown to be induced. Rat osteocalcin (Bglap) transcription is suppressed by YY1, an all-purpose transcription factor, after being activated by VDR. Some data suggests that the 1,25(OH)2D3 effect is mediated by members of the CAAT enhance binding protein (C/EBP) family. When 1,25(OH)2D3 and VDR work together to enhance Cyp24a1 and Bglap transcription, C/EBP is activated in kidney and osteoblastic cells. C/EBP and VDR, as well as Runx2 and C/EBP, have been shown to work together in the control of Bglap transcription. Transcriptional control of the VDR by C/EBPs and Runx2 has also been described. In lung epithelial cells, C/EBP and VDR work together to regulate the transcription of the human antimicrobial peptide cathelicidin, whereas Runx2 and VDR work together to control the transcription of mouse osteopontin in osteoblastic cells. Matrix metalloproteinase 13 (Mmp13) gene transcription is regulated by C/EBP, Runx2, and VDR.

Vitamin D Bioactivation and Its Endocrine/Mineral Feedback Control

A nonenzymatic, UV light-dependent reaction in the skin produces vitamin D3 from 7-dehydrocholesterol, a precursor to vitamin D. (Fig. 1). There, it undergoes hydroxylation at C-25 of the side chain to generate 25-hydroxyvitamin D3 (25D), which is the primary circulating form of vitamin D3 in the human body. 1a-hydroxylation, a key step in the synthesis of hormones, occurs mostly but not solely in the kidneys (Fig. 1). The microsomal CYP2R1 and mitochondrial CYP27B1 enzymes are responsible for 25- and 1a-hydroxylations, respectively. As shown in Fig. 1, 1,25-dihydroxyvitamin D3 (1,25D) circulates in the bloodstream attached to plasma vitamin D binding protein (DBP), where it exerts its endocrine effects through the vitamin D receptor (VDR). In addition to regulating calcium and phosphate metabolism, 1,25D is well-known for its role in the skeletal remodeling cycle, which involves initiating the breakdown of bone as part of the process of bone mineralization.

Parathyroid hormone (PTH) production is suppressed by 1,25D liganded VDR in a direct impact on gene transcription. PTH's inhibition of CYP27B1 activation by low calcium (Fig. 1) creates a negative feedback loop that prevents hypercalcemia by limiting the bone-resorbing effects of PTH before 1,25D-mediated increases in intestinal calcium absorption and bone resorption occur. FGF23 deficiency has been linked to familial hypophosphatemia and hyperphosphatemia, which we now know to be produced by disordered bone-derived FGF23 levels in the blood. FGF23 has emerged as a novel phosphate regulator and a second phosphaturic hormone, after PTH, in recent years. In addition to 1,25D, large levels of circulating phosphate have been shown to trigger the release of FGF23 from osteoblastic osteocytes (Fig. 1), a mechanism that is independent of 1,25D-induced release from bone (Fig. 1). Because PTH is inhibited by 1,25D and calcium while FGF23 is stimulated by 1,25D and phosphate, mammals are protected against the ectopic formation of calcium deposits by either 1,25D or phosphate as an exquisite example. The small intestine, kidneys, bone, and parathyroid all play a role in maintaining bone mineral homeostasis. It seems that optimum 1,25D intracrine effects need larger levels of circulating 25D. (Fig. 1). An abundance of epidemiological evidence linking low 25D levels to chronic illness, as well as statistically significant protection against a wide range of diseases conferred by much greater levels of 25D in the blood, has led to this conclusion. Fig. 1 schematically depicts how 1,25D may protect the cardiovascular system, lower the risk of heart attack and strokes, control the adaptive immune system to reduce the incidence of autoimmune disease while enhancing innate immunity to fight infection, and exert anti-inflammatory and anticancer pressure on epithelial cells prone to fatal malignant tumors.

Catalytic activity of CYP24A1, an enzyme that begins 1,25D catabolism, is a key mechanism by which the 1,25D/VDR-mediated endocrine or intracellular signal is stopped in all target cells, as seen in Fig. 2. 1,25D and FGF23 both induce transcription of the CYP24A1 gene (Fig. 1). CYP27B1 is regulated by FGF23 and 1,25D, with the latter hormone operating through a brief negative feedback loop to reduce the synthesis of 1,25D. Therefore, the

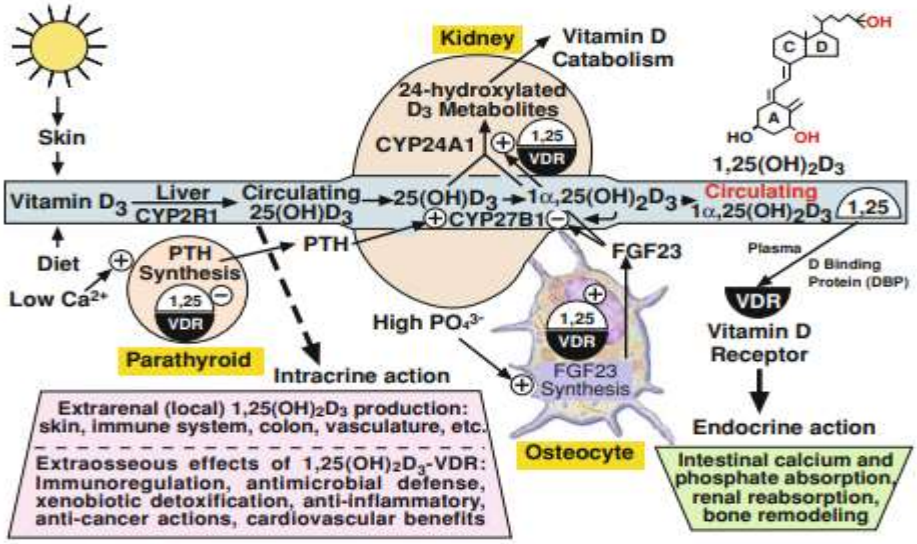

Fig. 2 Vitamin D acquisition, regulation of metabolic activation/catabolism, and receptor-mediated endocrine and intracrine actions of the 1,25D hormone in selected tissues

Feedback controls of vitamin D bioactivation read bone mineral ion status and avoid hypervitaminosis D diseases through feedforward induction of 1,25D catabolism in the vitamin endocrine system. However, the vitamin D intranasal system seems to rely heavily on the availability of sufficient 25D substrate to create local 1,25D to reduce the risk of epithelium (skin, colon, etc.) and immune and cardiovascular system chronic illnesses.

Effects of Vitamin D on Skin Biology

Vitamin D's involvement in skin physiological regulation is complicated. Keratinocytes in the basal and spinous layers of the epithelium express vitamin D and its receptor, and the 1,25(OH)2D/VDR signaling affects keratinocyte proliferation, differentiation, and death, as well as cutaneous immunological responses.

1,25(OH)2D promotes basal cell keratinocyte proliferation in the epithelium's basal cell layer, whereas in the spinous cell layer it increases production of differentiation agents that mediate keratin synthesis (K1, K10), involucrin, transglutaminase, loricrin, and filaggrin. 1,25(OH)2D reduces proliferation of keratinocytes in vitro through VDR-mediated genomic processes and promotes differentiation of keratinocytes by increasing intracellular calcium levels via nongenomic mechanisms.

It is the more developed keratinocytes in the epithelium's higher layers that create proteins and lipids, as well as glucosylceramides, which form an epidermal barrier to prevent infectious and poisonous chemicals from penetrating the tissue underneath it.

E-cadherin/catenin complexes that form adherent junctions in keratinocytes are regulated by cross-talk between 1,25(OH)2D/VDR signaling and Ca/Casr signaling pathways, which are plasma membrane-bound members of the G protein-coupled receptor family. When Ca and 1,25(OH)2D/VDR signaling work together, it prevents the translocation of Ca2+ of β-catenin from the plasma membrane E-cadherin/catenin complex, thus diminishing the nuclear transcriptional activity of β-catenin.

The differentiation and proliferation of keratinocyte stem cells are regulated physiologically by the hedgehog intracellular signaling system. In the etiology of cutaneous basal cell carcinoma, functional activation of this pathway is an early genetic event that grants afflicted basal keratinocytes increased proliferative potential, raising their basal cell carcinoma risk. The etiology of cutaneous basal cell carcinoma is also linked to a decreased functional activity of genes that repair DNA damage produced by UVB exposure. Considering that $1,25(OH)_2D$/VDR signaling inhibits the hedgehog signaling pathway and increases the activity of DNA nucleotide excision repair enzymes, $1,25(OH)_2D$ might lower basal cell carcinoma incidence. $1,25(OH)_2D$/VDR signaling suppresses cell growth in cutaneous squamous cell carcinoma by causing cell cycle arrest, inducing apoptosis, decreasing DNA synthesis, and encouraging repair of UVB radiation-induced DNA damage, as shown in in vitro studies.

5. CONCLUSION

Vitamin D's mode of action and its biological implications have been discussed in this review. With 1,25D-VDR activation of FGF23 (in bone and kidney) and klotho (in kidney) taking center stage as the mechanism whereby vitamin D regulates phosphate homeostasis and maybe delays chronic illnesses of ageing, the new knowledge of vitamin D bioactivation has been gained. A founding member of the nuclear receptor superfamily, VDR is a member of the VDR/PXR/CAR subfamily, which evolved as a means of signaling detoxification in response to environmental toxins. There is little doubt that new 1,25(OH)2D3 target genes will be discovered in a variety of diverse systems. Regulation mechanisms, novel transcription factors, and epigenetic alterations that mediate these many biological responses will be the subject of new research. Furthermore, by studying VDR in the presence of protein partners, we hope to get a better knowledge of how 1,25(OH)2D3 activity in various target tissues might be modulated. Chemoprevention and chemotherapy might now have new targets thanks to these results.

6. REFERENCE

1. Wanvisa Udomsinprasert et.al “Vitamin D and liver fibrosis: Molecular mechanisms and clinical studies” Volume 109, January 2019
2. Jeon, SM., Shin, EA. Exploring vitamin D metabolism and function in cancer. Exp Mol Med 50, 1–14 (2018). https://doi.org/10.1038/s12276-018-0038-9

3. Abbas S, Linseisen J, Slanger T, Kropp S, Mutschelknauss EJ, FleschJanys D, Chang-Claude J (2008). Serum 25-hydroxyvitamin D and risk of post-menopausal breast cancer--results of a large case-control study. Carcinogenesis, 29(1): 93-99. Epub 2007, Oct 31.
4. El-Hajj FG, Nabulsi M, Choucair M (2001). Hypovitaminosis D in healthy schoolchildren. Pediatrics, 107: E53.
5. Kahles H, G Morahan, JA Todd, Baden hoop K (2009). Association analysis of the vitamin D receptor gene in 1654 families with type 1 diabetes mellitus. Genes Immun., 10(Suppl 1): S60–S63.
6. Standing Committee on the Scientific Evaluation of Dietary Reference Intakes Food and Nutrition Board, Institute of Medicine (1999). Vitamin D. In: Dietary reference intakes for calcium, phosphorus, magnesium, vitamin D, and fluoride. Washington, DC: National Academy Press, pp. 250-287.
7. Zuberi LM, Habib A, Naeemulhaque, Abdul-Jabbar (2008). Vitamin D deficiency in ambulatory patients. J. Pak. Med. Assoc., 58(9): 482- 484.
8. Berridge MJ. Vitamin D, reactive oxygen species and calcium signalling in ageing and disease. Philos Trans R Soc Lond B Biol Sci. 2016; 371 (1700): doi: 10.1098/rstb.2015.0434. [DOI:10.1098/rstb.2015.0434]
9. Hill TR, Granic A, Aspray TJ. Vitamin D and ageing. Sub-Cellular Biochemistry. 2018; 90: 191-220. [DOI:10.1007/978-981-13-2835-0_8]
10. Christakos S, Dhawan P, Verstuyf A, Verlinden L, Carmeliet G. Vitamin D: metabolism, molecular mechanism of action, and pleiotropic effects. Physiol Rev. 2016; 96(1): 365-408. [DOI:10.1152/physrev.00014.2015]
11. Wang Y, Zhu J, DeLuca HF. Where is the vitamin D receptor? Arch Biochem Biophys. 2012; 523(1): 123-33. [DOI: 10.1016/j.abb.2012.04.001]
12. Baeke F, Takiishi T, Korf H, Gysemans C, Mathieu C. Vitamin D: modulator of the immune system. Curr Opin Pharmacol. 2010; 10(4): 482-96. [DOI: 10.1016/j.coph.2010.04.001]