Send Orders of Reprints at bspsaif@emirates.ae

126 *Anti-Cancer Agents in Medicinal Chemistry,* **2013,** *13,* **126-139**

The Anti-cancer Actions of Vitamin D

Kun-Chun Chiang[1,2] and Tai C. Chen[3,*]

[1]*Department of General surgery, Chang Gung Memorial Hospital, 222, Mai-Chin Road, Keelung, Taiwan, 204, R.O.C*; [2]*Graduate Institute of Clinical Medical Sciences, College of Medicine, Chang Gung University, 259 Wen-Hwa 1st Road, Kwei-Shan Tao-Yuan, Taiwan, 333, R.O.C;* [3]*Boston University School of Medicine, M-1022, 85 E. Newton Street, Boston, MA 02118 USA*

Abstract: Vitamin D_3 is biologically inert. To become active, it requires two successive hydroxylation steps catalyzed by two cytochrome P450 enzymes, first to synthesize the pro-hormone 25-hydroxyvitamin D_3 [$25(OH)D_3$] and then the active hormone 1α,25-dihydroxyvitamin D_3 [$1\alpha,25(OH)_2D_3$]. $1\alpha,25(OH)_2D_3$ has high affinity for the vitamin D receptor (VDR), a transcription factor and a member of the steroid receptor superfamily. Through VDR, $1\alpha,25(OH)_2D_3$ regulates more than 200 genes in mammals, including those involved in the calcium and phosphorus homeostasis, immune function, reproduction, cardiovascular, central nerve system, inflammation, angiogenesis, and cellular proliferation, differentiation and apoptosis. Due to its versatile roles in maintaining and regulating normal cellular phenotypes and functions, $1\alpha,25(OH)_2D_3$ has been implicated as an anti-cancer agent. In fact, ecological and epidemiologic data have linked vitamin D deficiency with the incidence and mortality of many types of cancer. More importantly, *in vitro* and *in vivo* animal model studies have clearly demonstrated the anti-tumor effects of vitamin D. In this review, we describe the anti-cancer actions of vitamin D, with special emphasis on different pathways underlying the VDR-mediated genomic as well as less-defined non-genomic actions of vitamin D.

Keywords: Cancer, Vitamin D, Cell cycle, Hedgehog, Growth factors, Apoptosis, Angiogenesis, Differentiation, Kinases, Transcription factors, Metastasis.

1. INTRODUCTION

Vitamin D was discovered because of its ability to cure the childhood bone disease, rickets. Up until 1960's, vitamin D, either vitamin D_2 or vitamin D_3, was believed to be the active principle responsible for all the known vitamin D actions known during that period. Professor Hector F. DeLuca and his associates first challenged this concept and demonstrated that radioactive vitamin D_3 injected into vitamin D deficient rats was metabolized to more polar compounds, and one of them was shown to be more potent than the parent compound vitamin D_3 in stimulating intestinal calcium transport and mobilization of calcium from bone, implicating that vitamin D_3 might be further metabolized before becoming active [1-2]. Subsequently, the unknown compound was purified and identified as 25-hydroxyvitamin D_3 [$25(OH)D_3$] in 1968 [3]. Later, when radioactive $25(OH)D_3$ was synthesized and injected into rats, several more polar metabolites were found and isolated. One of them was shown to act even faster and to a greater extent than $25(OH)D_3$. The compound was then identified in 1971 as 1α,25-dihydroxyvitamin D_3 [$1\alpha,25(OH)_2D_3$] [4-6].

Vitamin D is rare in food. The primary source of vitamin D for most humans is the exposure to sunlight, accounting for about 90% of vitamin D requirement [7]. However, due to the concern of UV-induced skin cancers, we increasingly depend on vitamin D supplementation to meet our vitamin D requirement, including vitamin D pills and vitamin D-fortified food and drink. In spite of many forms of vitamin D in nature with the same secosteroid ring structure but with different side chains, vitamin D_2 and vitamin D_3 are the two major types. Vitamin D_2 is synthesized from ergosterol found in plants and phytoplankton, whereas vitamin D_3 is produced from 7-dehydrocholesterol (7-DHC) in humans and some zooplankton species. When human skin is exposed to UV irradiation (wavelength 290-315 nm), 7-DHC, stored in the basal and suprabasal layers of skin, is photolyzed to form previtamin D_3, which is then thermoisomerized to vitamin D_3 [7]. Either endogenously produced vitamin D_3 or ingested vitamin D_2 or vitamin D_3 is then entering the blood circulation and bound to vitamin D binding protein (DBP). After vitamin D-DBP complex reaches liver, vitamin D is hydroxylated at carbon-25 mainly by CYP2R1 to form 25(OH)D [8-9]. Serum 25(OH)D, an index of vitamin D status in humans, has the highest affinity for DBP among all vitamin D related compounds [10-11]. 25(OH)D-DBP complex is then delivered to the kidneys to be hydroxylated at carbon-1 by CYP27B1 to form $1\alpha,25(OH)_2D$, the biologically active form of vitamin D. Both 25(OH)D and $1\alpha,25(OH)_2D$ can be hydroxylated by CYP24A1 at caron-24 in the kidneys to form their corresponding 24-hydroxylated metabolites. Hydroxylation at carbon-24 of the $1\alpha,25(OH)_2D$ molecule by CYP24A1 is the first step of catabolic process of the active hormone [12] and is responsible for terminating its cellular actions. In addition, when there is an excess of 25(OH)D, the renal CYP24A1 can convert it to $24,25(OH)_2D$ to prevent the over-production of $1\alpha,25(OH)_2D$ [12]. However, it is now established that CYP27B1 and CYP24A1 are expressed in many other tissues and cell types in addition to the kidneys [12-13], that clearly suggests that the production and degradation of $1\alpha,25(OH)_2D$ can take place in an autocrine/paracrine fashion. Given that no detectable ^3H-$1\alpha,25(OH)_2D_3$ was found in the circulation of anephric animals after the injection of ^3H-$25(OH)D_3$ [14-15], and very low concentrations of circulating $1\alpha,25(OH)_2D_3$ were detected in some but not all anephric patients [16-18], it is believed that any $1\alpha,25(OH)_2D_3$ generated in the extra-renal tissues acts and is degraded locally with very little (or under the detection limit) leaking into the circulation. Recently, Wagner *et al.* [19] determined $1\alpha,25(OH)_2D$ concentration in 30 human colon tissues and concluded that the $1\alpha,25(OH)_2D$ detected was mainly synthesized within the tissue. Although it is widely believed that higher serum concentrations of 25(OH)D would lead to higher tissue concentrations of 25(OH)D and would produce more $1\alpha,25(OH)_2D$ by CYP27B1, Wagner *et al*. however did not find a significant correlation between the colon $1\alpha,25(OH)_2D$ levels and 25(OH)D concentrations from the matched serum samples. The results support the previous observation that this autocrine/paracrine pathway is likely regulated in a tissue-specific manner, depending on the relative expression of CYP24A1 to CYP27B1 [12].

*Address correspondence to this author at the Boston University School of Medicine, M-1022, 85 E. Newton Street, Boston, MA 02118 USA;
Tel: 617-638-4543; Fax: 617-638-8882; E-mail: taichen@bu.edu

1875-5992/13 $58.00+.00

The first evidence indicating that $1\alpha,25(OH)_2D_3$ might have actions other than regulating calcium and phosphate homeostasis was obtained by an autoradiographic study [20]. In this study, ^{3}H-lebeled $1\alpha,25(OH)_2D_3$ was injected into vitamin D-deficient rats and was found localized in the nuclei of the cells in epidermis, stomach, pituitary and parathyroid in addition to the small intestine, renal tubules and glomeruli. The data therefore suggest that the target cells for $1\alpha,25(OH)_2D_3$ could include those not known to be involved in calcium and bone metabolism [21].

2. VITAMIN D RECEPTOR (VDR)

2.1. Discovery of VDR

Following the identification of $1\alpha,25(OH)_2D_3$ as the active metabolite of vitamin D_3 in 1971 [4-6], ^{3}H-labeled $1\alpha,25(OH)_2D_3$ became available and was used to study the subcellular localization of this active hormone in rat [22] and chick small intestine [23]. Both studies found that the radioactive $1\alpha,25(OH)_2D_3$ was mostly localized in or associated with the nuclear fraction. Later, the specific binding of ^{3}H-$1\alpha,25(OH)_2D_3$ to nuclear components was demonstrated in chick intestine [24] and in chick parathyroid gland [25]. Using two VDR-specific monoclonal antibodies, McDonnell *et al*. were able to recover the cDNA for VDR from a chicken intestinal lambda gt11 cDNA expression library [26]. cDNA for rat VDR was soon cloned from a rat kidney lambda gt11 expression library [27], followed by the cloning of human VDR cDNA from the human intestine and T47D cell cDNA library by Baker *et al*. [28], who also demonstrated that transfection of the cloned cDNA into COS-1 cells resulted in the synthesis of a single peptide species indistinguishable from the native VDR. Further structural analysis revealed that the human cDNA has 4,605 nucleotides including a noncoding leader sequence of 115 bases, a 1,281-bases open reading frame and 3,209 bases of 3' noncoding sequence. The cDNA codes for a single mRNA species of about 4,600 nucleotides and a peptide of 427 amino acids [29]. When the VDR cDNA sequence and the translated peptide were compared with those from thyroid hormone receptor, estrogen receptor and androgen receptor, similar characteristic features were found among all four receptors, and therefore they are members of the steroid hormone receptor supergene family. These receptors are ligand-dependent transcription factors. The VDR gene is located on chromosome 12q13 with a promoter sequence proceeding the coding region [29].

2.2. The Structure and Actions of VDR

Human VDR consists of 427 amino acids with a molecular weight of 52 kDa. The molecule has four major functional domains, including the highly variable N-terminal A/B domain, highly conserved zinc finger-containing DNA-binding domain (DBD) or C domain, the hinge domain (D domain), and the ligand-binding domain (LBD) or E domain. The A/B domain is known to be involved in VDR transactivation. The LBD has a characteristic secondary domain structure which is common for all nuclear receptors. The crystal structure of human VDRΔ LBD (Δ means lacking D hindge domain) shows that it has canonical shape with 13 α-helices sandwiched in three layers and a three-stranded β sheet [30]. The ligand-binding pocket is rather large (697 $Å^3$) with $1\alpha,25(OH)_2D_3$ occupying only 56% of this volume [30]. The LBD is multifunctional and facilitate ligand binding, nuclear localization, dimerization and interaction with coactivator and co-repressor proteins. Upon binding to its ligand, such as $1\alpha,25(OH)_2D_3$, VDR is stabilized as a result of phosphorylation at serine 51 and serine 208 [31-34].

2.3. Genomic Actions of Vitamin D

All the genomic actions of $1\alpha,25(OH)_2D$ are mediated through its binding to VDR [33]. Although nuclear receptors can function as monomers, homodimers or heterodimers in complex with RXR, VDR functions as an obligate heterodimer with RXR and recognizes specific DNA elements known as vitamin D response elements (VDREs) [34] located in the promoter region of vitamin D responsive genes to exert its genomic actions. Furthermore, the VDR-mediated gene expression is modulated by a multiple of co-activators and co-repressors [29]. As $1\alpha,25(OH)_2D$ binds to VDR, phosphorylation occurs, carried out by protein kinase C and casein kinase II over serine 51 located in the DNA-binding domain and serine 208 located in the hinge region [31-32], and leads to subsequent conformational change of VDR, which, in turn, results in the release of co-repressors, including NCOR1 and NCoR2/SMRT and recruitment of co-activators, such as HAT and steroid receptor-coactivator 1 (SRC1) [35-37], to remodel chromatin and initiate gene transcription in a ligand-dependent manner [38].

A spectrum of naturally occurring VDRE sequences has been identified [39]. The most commonly found VDREs are direct repeat of two hexanucleotide half sites with a three nucleotides (DR3) spacer in between. Less common are everted repeats of two half-sites with a spacer of six nucleotides (ER6) motif. In the direct DR3 VDREs, VDR has been found to occupy the 3' half-site, whereas RXR resides on the 5' half-site [40]. The highest affinity 3' half-site for VDR is PGTTCA, where P is a purine base, and the highest affinity 5' half-site for RXR is PGGTCA. The multiple sequences found in natural VDREs may offer a tissue-specific preferential binding to exert differential actions in different tissues. Although most VDR-regulated genes have a single copy of VDRE in their proximal promoter region, some genes do have more than one copy. For example, CYP24A1 promoter has at least two copies. Genes with multiple VDREs require all VDR/RXR docking sites for maximal induction by $1\alpha,25(OH)_2D_3$ and individual VDREs appear to function synergistically in recruiting coactivators and basal regulators for transactivation [40]. A model proposing that VDREs may juxtapositioned *via* DNA looping in chromatin to create a single platform and support the transcription machinery has been advanced to explain the synergistic effect in the presence of multiple VDREs [41].

Recently, a hydrogen-deuterium exchange (HDX) technique has been employed to study the dynamics of the human RXRα/VDR heterodimerization and the influence of the VDR interaction with DNA (VDRE), and the coactivators, such as receptor-interacting domain of human SRC1 in an effort to understand the molecular mechanism of the VDR/RXR complex-induced transactivation [42]. In the absence of the crystal structure of the RXRα/VDR heterodimer, the authors created a model for it by docking the VDR LBD into the known PPARγ/RXRα crystal structure. The docking was accomplished by using superimposition with Coot [43] and minimization with Chimera [44] and then remodel the DBD structure on the VDRE using the structure of the RXR-VDR DBD on the VDRE direct repeat with three intervening nucleotides DR3 [45]. Furthermore, the potential effect of different VDRE nucleotide sequences on the dynamics of RXRα/VDR heterodimer was studied. Briefly, the authors were able to demonstrate that (a) in the absence of either ligand for these two receptors, heterodimerization of VDR with RXRα increased the stability of the region responsible for a high affinity interaction between the two subunits of the heterodimer. (b) Upon binding of $1\alpha,25(OH)_2D_3$ to the heterodimer, a $1\alpha,25(OH)_2D_3$-dependent allosteric communication between co-receptors was observed, which favors co-receptor interaction to stabilize the heterodimer. (c) Similar enhanced allosteric modulation of the co-receptor interaction occurred when RXRα was bound to its ligand 9-cis-retinoic acid. However, the binding of 9-cis-retinoic acid to its receptor induced a subtle but statistically significant increase in HDX within the VDR DBD, suggesting a destabilization of the DBD of VDR. (d) Binding of RXRα/VDR to DNA response elements differentially altered the conformational dynamics and stability of AF-2 of both co-receptors, depending on the specific

sequence of the response elements. This suggests that DNA binding could directly influence coactivator recognition and binding. (e) The RXRα/VDR heterodimer was activated in a synergistic manner in the presence of both 9-cis-retinoic acid and 1α,25(OH)$_2$D$_3$ by a concerted interaction between both co-receptors and one molecule of SRC1. In summary, the HDX data indicate that there is extensive allosteric communication throughout the heterodimer, leading to dynamic changes in the stability of the DBD of VDR. The results suggest that the ligand itself may alter the DNA-binding properties of this nuclear receptor heterodimer, and further imply that different classes of ligands may differentially affect the DBD stability and thus provide unique pharmacological profiles in terms of target gene activities. This study also renders direct structural evidence for DNA (VDRE)-dependent allosteric communication between the DBD and LBD of an intact nuclear receptor (e.g., VDR), as well as between two heterodimer partners.

It is now well-established that VDR regulates at least 229 genes through binding to at least 2,776 genomic DNA binding sites [46]. The genes include those involved in anti-proliferation, pro-differentiation, anti-inflammation, pro-apoptosis, immune regulation and many other functions in a tissue- and cell-specific manner [47-53].

3. VDR-DEPENDENT ANTI-CANCER ACTIONS

Several approaches have been used to demonstrate that VDR is required for the antiproliferative effect of 1α,25(OH)$_2$D$_3$ in cancer cells. Using JCA-1 prostate carcinoma cells stably transfected with cDNA that encodes VDR to increase its concentration, Miller and colleagues [54] were able to demonstrate that the degrees of 1α,25(OH)$_2$D$_3$-induced antiproliferative action and CYP24A1 up-regulation were proportional to VDR concentrations within the transfected cells. Conversely, stable transfection of antisense VDR cDNA to ALVA-31 prostate cancer cells to knockdown VDR attenuated the ability of 1α,25(OH)$_2$D to inhibit cell growth and induce CYP24A1 expression [54]. Using a different approach, Zinser *et al*. [55] studied cancer cells derived from VDR knock-out animals and showed that the cells were completely resistant to 1α,25(OH)$_2$D$_3$-mediated growth arrest and apoptosis over the range of 0.01-100 nM 1α,25(OH)$_2$D$_3$. Overall, these studies demonstrate that the 1α,25(OH)$_2$D-dependent induction of cell cycle arrest, CYP24A1 up-regulation, and apoptosis in cancer cells are dependent on VDR.

3.1. Antiproliferative Effects of Vitamin D

A central part of the antiproliferative effects of vitamin D lies in its ability to interrupt cell cycle progression (Fig. **1**). Cell cycle progression through four phases (G_1, S, G_2 and M) is regulated by multiple molecular pathways and checkpoints, including the expression of one family of evolutionarily conserved proteins, called cyclin-dependent kinases (CDKs), and their inhibitors (CKIs) in a coordinated fashion [56]. CDKs are serine/threonine-specific kinases that consist of a catalytic subunit (CDK) and a regulatory subunit (the cyclin). There are five CDKs-CDK1, CDK2, CDK3, CDK4 and CDK6, and several cyclins, which are directly involved in driving the cells through G_1 into S phase cycle [57]. The primary substrates of CDKs in G_1 progression are the members of the retinoblastoma protein family (pRb). These proteins function as "docking" sites for a series of proteins that are tightly regulated through the cell cycle progression [58]. For example, pRb proteins bind to the E2F family of transcription factors to ensure that they remain inactive during M and G_0 phases. The activity of the pRb proteins is modulated by sequential phosphorylation by CDK4/6-cyclin-D and CDK2-cyclin-E complexes that allow the release of molecules (e.g., E2Fs) which are bound to hypophosphorylated pRB isoforms.

Once they are released, these molecules will be able to carry out their specific tasks (e.g. as transcription factors) in cell cycle progression. Most adult mammalian cells are quiescent remaining in G_0 phase and do not express many cell cycle genes. Therefore, the first step to re-entry into the cell cycle is to activate the transcriptional and translational machinery essential for the specific expression of cell cycle genes following a mitogenic signal. This

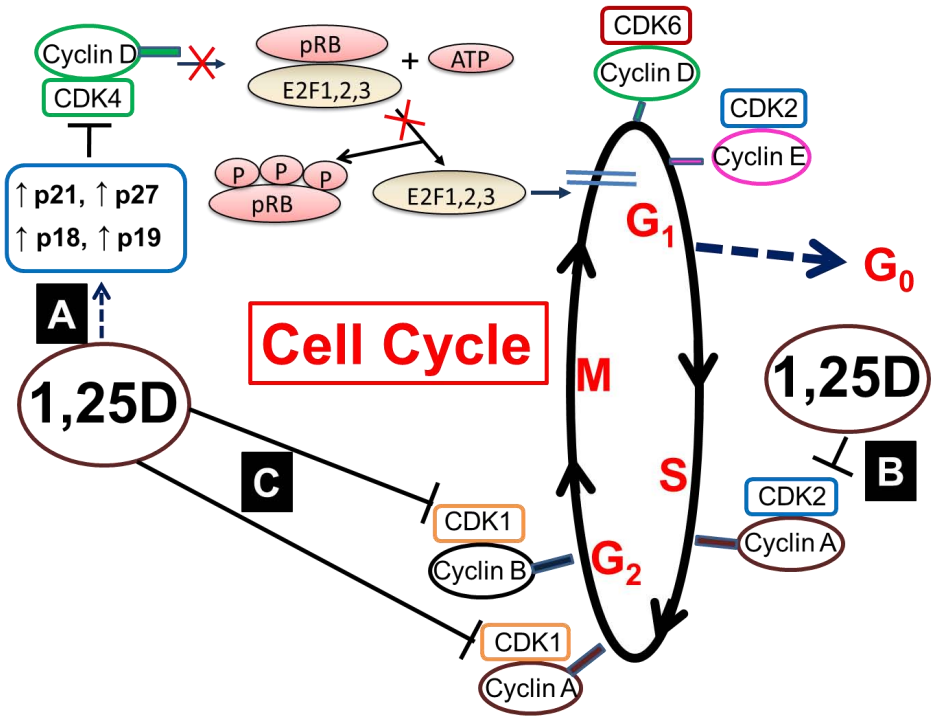

Fig. (1). Effects of 1α, 25(OH)$_2$D$_3$ (1,25D) on cell cycle progression. 1α,25(OH)$_2$D$_3$ has been shown to regulate the cell cycle in eukaryotic cells at 3 different check points: (**A**) inducing the expression of CDKIs, including p21, p27, p18 and p19, which in turn block Cdk4 activation and arrest cells in G1 phase, (**B**) inhibiting CdK2 activity and leading to arrest in the S phase, and (**C**) down-regulating CDK1 leading to the arrest in the G2 phase.

machinery is active during early cell divisions in the embryo in order to generate new cells, but a repression mechanism, which is generally represented by the pRb, becomes crucial for those cells that are terminally differentiated during organogenesis. One of those genes which are turned on is D-type cyclins. The activation of cyclin D expression leads to direct phosphorylation of pRb which binds E2F transcription factors. In mid to late G_1 phase, CDKs phosphorylate pRb, and the hyperphosphorylated pRb displaces the E2F transcriptional factors which in turn induces the expression of S phase genes required for DNA replication [56]. The activity of CDKs required for G_1/S transition is regulated by endogenous CDK inhibitors, including tumor repressors such as p21 and p27. In other words, p21 and p27 could interfere with cell cycle progression by triggering G_1 cell cycle arrest and withdrawal from the cell cycle. It is known that many of the genes involved in cell cycle progression are frequently mutated in human cancers leading to aberrant CDK activity and thereby uncontrolled cell division and tumor growth.

3.1.1. Cell Cycle Arrest

The cyclin-dependent kinase inhibitor $p21^{waf1/cip1}$ was first suggested as a VDR target gene in the human promyelocytic HL-60 leukemia cells by Jiang *et al*. [59]. Later, multiple VDREs were identified within the $p21^{waf1/cip1}$ promoter at -770 in relation to the transcriptional start site in the human myelomonocytic cell line U937 [60] and in MCF7 human breast cancer cells [61]. Induction of $p21^{waf1/cip1}$ mRNA occurred within 2 h of $1\alpha,25(OH)_2D_3$ addition and is a direct effect of liganded VDR. The expression of other CDK inhibitors, such as $p27^{kip1}$ and the Ink4 family member p15, p16, and p18, were also found to be induced by the ligand [60]. In pancreatic cancer cells, $1\alpha,25(OH)_2D_3$ inhibited their proliferation through cell cycle arrest at the G_0/G_1 phase [62], which in turn is mediated through the up-regulation of $p21^{waf1/cip1}$ and $p27^{kip1}$, followed by the down-regulation of cyclins, CDKs and CDKI [63]. Interestingly, data from studies using LNCaP prostate cancer cells and HepG2 liver cancer cells indicate that the up-regulation of $p27^{kip1}$ proteins induced by $1\alpha,25(OH)_2D_3$ or its analogs may not involve new $p27^{kip1}$ mRNA synthesis [64-66]. Along this line, Flores *et al*. [67] infected LNCaP cells with a retrovirus containing a sh-p27 or sh-Luc (served as control) and found that while $p27^{kip1}$ was depleted by 74% in sh-$p27^{kip1}$ infected cells, compared to the cells infected with sh-Luc, the knockdown of $p27^{kip1}$had no effect on $1\alpha,25(OH)_2D_3$-induced cell growth inhibition. Thus, it was concluded that $p27^{kip1}$ up-regulation in LNCaP prostate cancer cells was not essential for $1\alpha,25(OH)_2D_3$-mediated growth inhibition, and the up-regulation of $p27^{kip1}$ protein expression is likely the consequence rather than the cause of $1\alpha,25(OH)_2D_3$-induced growth inhibition. Thus, further investigation into the exact role $p27^{kip1}$ plays in $1\alpha,25(OH)_2D_3$-meidated cell cycle arrest in other cell lines is warranted.

In addition to the direct effects of $1\alpha,25(OH)_2D_3$ on $p21^{waf1/cip1}$ and other cell cycle-related genes [68], the hormone may act indirectly through other mechanisms on these genes. For example, $1\alpha,25(OH)_2D_3$ has been shown to inhibit the Hedgehog (Hh)-signaling pathway [69-71], up-regulate the insulin-like growth factor binding protein-3 (IGFBP3) and transforming growth factor-β (TGF-β) signaling pathways and down-regulate the EGFR signaling cascade [72-74]. In prostate cells, it has been proposed that the modulation of prostaglandin concentration by $1\alpha,25(OH)_2D_3$ may be one mechanism responsible for the growth inhibitory action of $1\alpha,25(OH)_2D_3$ [75].

3.1.2. Hedgehog Signaling Pathway

The hedgehog (Hh) gene was initially identified in fruit fly Drosophila [76] and subsequently three homologs, Sonic Hedgehog (Shh), Indian Hedgehog (Ihh), and Desert Hedgehog (Dhh), were identified in vertebrates [77-81]. In humans, the genes were found to be an essential developmental signaling pathway in maintaining tissue polarity and stem cell population [82]. Inactivation or hyperactivation of this pathway can cause serious health problems, including developmental defects such as holoprosencephaly [83], and different forms of cancer including basal cell carcinomas (BCCs), medulloblastomas, leukemia, gastrointestinal, prostate, ovarian, breast and lung cancers [82, 84]. The receptor for Hhs is a transmembrane protein called Patched (PTC). In the absence of Hh ligands, PTC is bound to another transmembrane protein, smoothened (SMO), and functions as an inhibitor of SMO (Fig. **2**). The binding of Hh ligands to PTC releases SMO from the inhibitory effect of PTC and allows SMO to transduce signals leading to the activation of transcription factor, called glioma associated (Gli), and the expression of genes involved in regulating embryonic and postnatal development, and the transformation of cancer- and metastasis-initiating cells [85]. Recent studies indicate a cross-talk between vitamin D_3 and Hh signaling mediated by at least two mechanisms. First, PTC has been shown to stimulate the secretion of a vitamin D_3-related compound, which is likely responsible for the inhibitory action of PTC on SMO [86]. Second, $1\alpha,25(OH)_2D_3$ can down regulate the expression of some members of the Hh pathway genes, including PTC, SMO and Gli in an epidermal explants culture system, suggesting a direct regulation by $1\alpha,25(OH)_2D_3$ [70]. These results are in agreement with the increased expression of Shh in the keratinocytes of the VDR-null animal and hyperactivation of the Hh pathway, predisposing the skin to the development of both malignant and benign epidermal neoplasms [70]. More interestingly, Uhmann *et al*. [69] demonstrated that $1\alpha,25(OH)_2D_3$ was capable of inhibiting Hh signaling at the level of SMO in the absence of VDR. Similar conclusion was obtained by Tang *et al*. [71] who studied murine basal cell carcinomas (BCC) *in vitro* and *in vivo*, and found that the effect of $1\alpha,25(OH)_2D_3$ on Gli expression is likely independent of VDR. The results provide strong evidence of the non-genomic action of $1\alpha,25(OH)_2D_3$ on cell growth and differentiation mediated by Hh/Gli signaling pathway.

3.1.3. Insulin-Like Growth Factor-Binding Protein 3 (IGFBP-3) Pathway

Several types of IGFBP have been identified as modulators of insulin-like growth factor (IGF) actions as well as other cellular functions unrelated to their ability to bind IGFs. Among the binding proteins, IGFBP-3 has generated more attention. The association between IGFBP-3 and vitamin D was first reported in 1992 in a study describing the $1\alpha,25(OH)_2D_3$-dependent IGFBP-3 secretion in cultured human osteosarcoma cells [87]. Later, it was shown that the effect was a consequence of increased IGFBP-3 mRNA expression [88] and was accompanied by growth arrest and the induction of differentiation in human osteosarcoma cell line [88], MCF-7 and Hs578T human breast cancer cell lines [89], PC-3 prostate cancer cell line [73], ventral prostate tissue [90], non-tumorigenic cervical ECE16-1 cell line [91], LNCaP prostate cancer cell line [92], primary cultures of benign human prostate epithelial cells and non-tumorigenic prostate epithelial P153 cell line [93]. Thus, the results indicate a common role for IGFBP-3 in the growth inhibitory effects of vitamin D and its analogs in a variety of cell types. The presence of a functional VDRE in the distal region of the cloned human IGFBP-3 promoter [94] points to a strong likelihood that the induction of IGFBP-3 by $1\alpha,25(OH)_2D_3$ may be directly mediated *via* the genomic action of VDR. Based on the finding that IGFBP-3 can up-regulate the cyclin-dependent kinase inhibitor protein $p21^{WAF/CIP1}$ and the addition of antibody against IGFBP-3 completely prevented the up-regulation of $p21^{WAF/CIP1}$ by $1\alpha,25(OH)_2D_3$ in LNCaP cells, Boyle *et al*. [92] concluded that $1\alpha,25(OH)_2D_3$- induced growth inhibition in LNCaP prostate cancer cell line was mediated through its up-regulation of IGFBP-3 which in turn increased the expression of $p21^{WAF/CIP1}$. On the contrary, Murthy and Weigel [95] studied $1\alpha,25(OH)_2D_3$-induced growth inhibition of PC-3 prostate cancer cells and found that the blocking of TGF-β pathway substantially reduced the

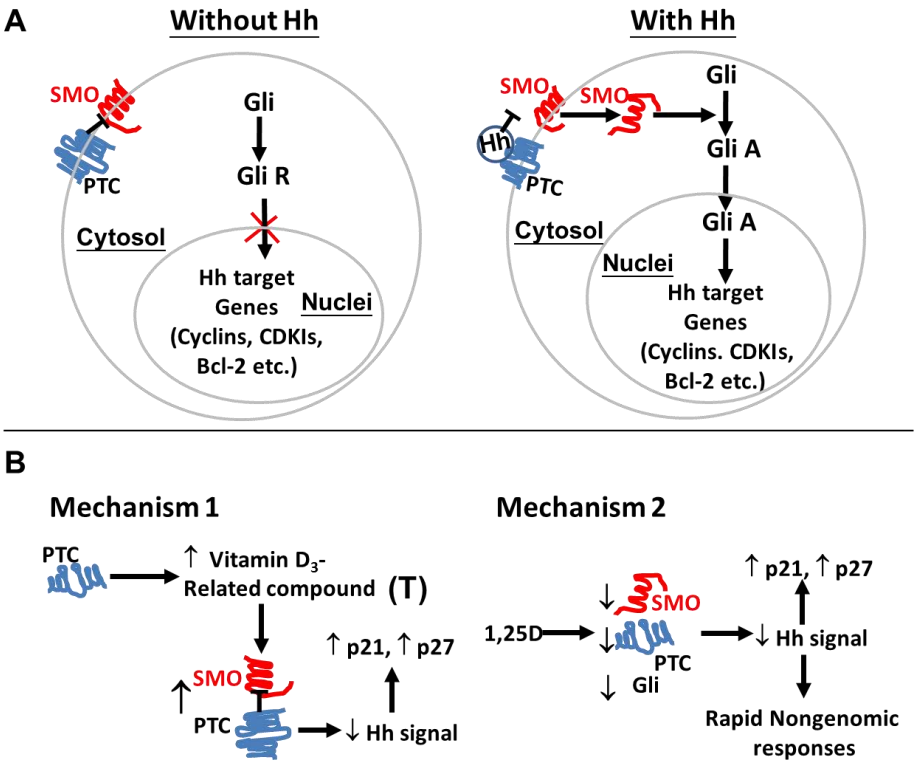

Fig. (2). Simplified Hedgehog (Hh) pathway and its potential effects on the anti-proliferative action of vitamin D. (**A**) A simplified model for Hh signaling in mammalian cells. Left panel: In the absence of the Hh ligands, Hh receptor (PTC) binds SMO, the key Hh signal transducer, *via* a vitamin D_3-related compound (shown as T). Under this condition, Gli molecules are processed into repressor forms (Gli R), which turn off the expression of Hh-inducible genes. Right panel: In the presence of Hh, Hh binds to PTC. The binding causes the release of SMO from T. After SMO is freed from the PTC inhibition, it undergoes conformational changes to activate Gli to form active Gli (Gli A) in the cytosol. Cytosolic Gli A is then transported into the nuclei to act as a transcription factor to induce gene expression. (**B**) Vitamin D may affect Hh signaling pathway *via* two potential mechanisms. Left panel shows that PTC can stimulate the secretion of a vitamin D_3-related compound (T), which is responsible for the inhibitory action of PTC on SMO in the absence of Hh. This results in a down-regulation of Hh signaling and increased expression of p21 and p27. Right panel depicts that the active form of vitamin D_3, $1\alpha,25(OH)_2D_3$, can down-regulate the expression of PTC, SMO and Gli proteins, that in turn decreases Hh signaling, leading to enhanced p21 and p27 expression and other actions, including rapid nongenomic responses.

$1\alpha,25(OH)_2D_3$- mediated growth without affecting IGFBP-3 expression in PC-3 prostate cells [86], suggesting an active TGF-β signaling pathway is required for the growth inhibition induced by $1\alpha,25(OH)_2D_3$ in PC-3 cells. Stewart and Weigel [96] further demonstrated that $1\alpha,25(OH)_2D_3$-induced up-regulation of IGFBP-3 was not required for the growth inhibitory effects of $1\alpha,25(OH)_2D_3$ either in LNCaP or PC-3 cell line if serum was present in cultures. The apparent discrepancy between different cell lines and the effect of serum on $1\alpha,25(OH)_2D_3$ and IGFBP-3 interactions suggest a complicated cross-talk among these cell growth regulators in a cell specific manner. The complexity can be partially explained by the finding that ten functional VDREs, which are distributed within the promoter region of the IGFBP-3 gene, may be under the regulatory control of not only $1\alpha,25(OH)_2D_3$ [97-98] but also a putative androgen response element [99], as well as unknown factors present in the serum. In summary, $1\alpha,25(OH)_2D_3$ may up-regulate the expression of IGFBP-3 in certain cancer cell types, and the up-regulation may play a critical role in inhibiting the cancer cells by increasing the expression of cyclin-dependent kinase inhibitor $p21^{WAF/CIP1}$.

3.1.4. Transforming Growth Factor-beta (TGF-β) Pathway

TGF-β is a 25 kDa peptide. Similar to vitamin D, TGF-β regulates diverse biological processes through modulation of the expression of target genes in a variety of cell types. For example, TGF-β is a potent growth inhibitor of many epithelial cell types *in vitro*. TGF-β transduces signals by simultaneously contacting two transmembrane receptors, type I and type II. Upon binding to its type II receptor, the liganded type II receptor is recognized by type I receptor to form type I/type II heterotetramer, causing the phosphorylation of the type I receptor catalyzed by type II receptor serine/threonine kinase domain and the activation of certain members of the Smads protein family. Several studies have examined the molecular mechanism underlying the interplay between TGF-β and vitamin D, and suggested that the interplay between the two signaling pathways mainly is mediated by Smads, downstream components of the TGF-β signaling pathway [72,100]. The activation of Smad3 stimulates the ligand-induced transactivation of VDR [71], whereas Smad-7 inhibits formation of the VDR-Smad3 complex, and negatively regulates VDR transactivation function [100]. On the other hand, Wu *et al*. [101] reported two direct repeats of VDRE in the human TGF-β2 gene promoter, which can bind to VDR/RXR heterodimers. Furthermore, they demonstrated that $1\alpha,25(OH)_2D_3$ up-regulated the expression of TGF-β type II receptor mRNA and protein in human breast cancer MCF-7 cells [102]. The later finding is in agreement with a previous report showing that $1\alpha,25(OH)_2D_3$ up-regulated the expression of TGF-β1 and its latent binding protein in cultured breast carcinoma BT-20 cells [103]. TGF-β1-dependent pathway through up-regulation of Smad4 and phosphorylated-Smad3 levels was also shown to mediate the synergistic growth inhibition of MCF-7 breast cancer cells in the presence of melatonin and $1\alpha,25(OH)_2D_3$ [104] and the up-regulation of VDR expression following butyrate treatment in CaCo-2 colon cancer cells [105].

Other potential interactions between TGF-β and vitamin D pathway may include the blockade by $1\alpha,25(OH)_2D_3$ of the TGFβ-1-induced up-regulation of mesenchymal cell markers and abnormal expression of epithelial cell markers, and therefore inhibits the pro-fibrotic phenotype of lung fibroblasts and epithelial cells [106]. The anti-fibrotic role of vitamin D was also reported in a study using human uterine leiomyoma cells [107]. It was shown that an attenuation of TGF-β induced phosphorylation of Smad2 as well as nuclear translocation of Smad2 and Smad3 by $1\alpha,25(OH)_2D_3$ in human uterine leiomyoma cells led to the inhibition of uterine fibrosis caused by TGF-β3-induced up-regulation of extracellular matrix proteins. Taken together, interactions between vitamin D and TGF-β signaling pathway may involve different Smad proteins and TGF-β receptors in different cell types and systems studied.

3.2. Apoptosis

Apoptosis or programmed cell-death is a regulated process, which is fundamental to the advantage of the organism. In tumor cells, activation of apoptosis pathways is a key mechanism by which chemotherapeutic or cytotoxic drugs eliminate those tumor cells [108]. Conversely, lack of apoptosis may contribute to the initiation and progression of cancer. During the past two decades, extensive research aimed at understanding the mechanisms involved in the complex signaling pathways that control apoptosis in normal as well as cancer cells has revealed that Bcl-2 proteins are the "master regulator" of the intrinsic apoptosis pathway that is crucial to the apoptotic response from DNA-damage and other carcinogenic challenges [109]. The Bcl-2 family of proteins has two subclasses that either promote (e.g., Bcl-X_s, Bax) or suppress (e.g., Bcl-2, Bcl-X_L, Mcl-1) apoptosis [110-111]. These proteins can form heterodimers and homodimers. The ratio of apoptotic promoters and suppressors is one determinant of cellular response. Increased Bcl-2 expression has been reported in many types of cancer, including breast, prostate and lung, and is often found in the advanced stages of cancers [112]. $1\alpha,25(OH)_2D_3$ induces apoptosis in a variety of cancer cells to exert anti-tumor effects by repressing the expression of the anti-apoptotic proteins Bcl-2 and Bcl-X_L, or inducing the expression of pro-apoptotic proteins, such as BAX, BAK and BAD [47,51,113,114]. Studying MCF-7 breast cancer cells, James *et al*. [115] and Simboki-Campbell *et al*. [116] reported $1\alpha,25(OH)_2D_3$-induced apoptosis by demonstrating reduced Bcl-2 protein, induction of TRPM-2 (clusterin) mRNA expression and increased DNA fragmentation after $1\alpha,25(OH)_2D_3$ treatment. In a study using LNCaP prostate cancer cells, Blutt *et al*. [112] found a down-regulation of Bcl-2 and Bcl-X_L proteins without affecting other proteins important in apoptotic control after $1\alpha,25(OH)_2D_3$ treatment. In addition, $1\alpha,25(OH)_2D_3$ may directly activate caspase effector molecules to induce apoptosis. Activation of the caspase cascades, particularly caspase-3, -6, -7 and -9, is mainly induced by the release of cytochrome c from the mitochondrial membrane, into the cytosol. Among them, caspase-3 is the prime inducer of apoptosis [117-119]. In our studies with HepG2 cells, no expression of active caspase 3 protein was found either in the control or the group treated with $1\alpha,25(OH)_2D_3$ as measured by western blot analysis [120]. Flow cytometry study with Annexin V-FITC and PI staining to analyze apoptotic and necrotic cell populations of HepG2 cells after $1\alpha,25(OH)_2D_3$ treatment also showed similar apoptotic and necrotic cell populations between the control and the treated groups, suggesting that the decrease in cell number after $1\alpha,25(OH)_2D_3$ treatment in HepG2 cells is apoptosis-independent. The $1\alpha,25(OH)_2D_3$-induced apoptotic response may also be mediated by the destabilization of telomerase reverse transcriptase mRNA, that will lead to the down-regulation of telomerase activity [121]. A recent report by Brosseau *et al*. [122] demonstrates that, by combining with immunomodulatory drug lenalidomide, $1\alpha,25(OH)_2D_3$ was able to cause apoptosis of MDA-MB-231 cells, a triple negative and vitamin D-resistant cell line, through the Bcl-2 inhibition mechanism, whereas the combination did not affect Bcl-2 in two other vitamin D resistant breast cancer cell lines MCF7VDR and HBL-100. Therefore, $1\alpha,25(OH)_2D_3$ induces apoptosis in a cell-specific manner [123,124] and may involve different mechanisms in different cells.

3.3. Anti-angiogenesis

Angiogenesis is generally applied to the formation of thin-walled endothelium-lined new blood microvessels from pre-existing vessels, a process that is regulated by a range of endogeneous angiogenic factors and inhibitors [125]. It plays an important role in reproduction, development and wound healing. It is usually focal and self-limited in time. On the contrary, pathological angiogenesis can last for years, and is necessary for tumors and their metastases to grow beyond a microscopic size [126]. It can also cause bleeding, vascular leakage and tissue destruction. There are several hypotheses regarding the onset of tumor-induced angiogenesis. The most important one is hypoxia-induced angiogenesis. Inefficient vascular supply and the resultant reduction in tissue oxygen tension often lead to neovascularization in order to satisfy the needs of the tissue [127-128], which in turn stimulates the expression of hypoxia-inducible factors (HIFs) [129]. Tumor cells induce angiogenesis through a multistep process, called the "angiogenic switch", which ultimately tips the balance toward pro-angiogenic factors [130]. HIFs can directly activate the expression of a number of pro-angiogenic factors, including vascular endothelial growth factor (VEGF), VEGF receptors FLT-1 and FLK-1, plasminogen activator inhibitor-1 (PAI-1), angiopoietins, platelet-derived growth factor β, and matrix metalloproteinases MMP-2 and -9 [131]. There is considerable evidence that VEGF is a major tumor angiogenesis factor [132]. Many tumor cell lines secrete VEGF *in vitro*. Several *in situ* hybridization studies have demonstrated that the VEGF mRNA is expressed in a vast majority of human tumors so far examined [133]. Anti-VEGF monoclonal antibodies exert a potent inhibitory effect on the growth of many tumor cell lines in nude mice. Several inhibitors of the VEGF pathway have been approved by FDA for cancer treatment, which is a significant advance in the therapy of cancer [134]. In addition, platelet derived growth factor (PDGF), fibroblast growth factors (FGFs) and Notch Delta-like ligand 4 (DLL4) have been reported to promote angiogenesis independent of VEGF [135]. Other than the hypoxia-induced pathological angiogenesis, it has been proposed that deficiency in MMP-19 may contribute to an earlier onset of tumoral angiogenesis, in contrast to most MMPs that promote tumor progression [136].

The evidence that vitamin D might exert anti-angiogenic action was first provided by Merke *et al*. [137], who demonstrated VDR expression in venular and capillary endothelial cells of human skin biopsies. They also demonstrated the conversion of $25(OH)D_3$ to $1\alpha,25(OH)_2D_3$, and the growth inhibitory effect of $25(OH)D_3$ and $1\alpha,25(OH)_2D_3$ in the bovine aortic endothelial cells, suggesting the existence of a vitamin D autocrine loop in endothelium that may play a role in the development and/or functions of blood vessels. Subsequently, numerous studies showed $1\alpha,25(OH)_2D_3$ inhibited the proliferation of cultured endothelial cells and anti-angiogenesis in animal models [138-140]. Furthermore, the expression of VEGF-related protein has been shown to be down-regulated by EB1089, a less calcemic analog of $1\alpha,25(OH)_2D_3$ [141], and $1\alpha,25(OH)_2D_3$ inhibited HIF-1 transcriptional activity as well as its target genes, including VEGF, ET-1, and Glut-1 in wild type human cancer cells, but failed to suppress VEGF expression in HIF-1α knockout human colon cancer cells [142]. Taken together, the anti-angiogenesis effect of $1\alpha,25(OH)_2D_3$ in cancer cells is likely mediated by HIF-1/VEGF pathway [142]. $1\alpha,25(OH)_2D_3$ can also up-regulate mRNA levels of the potent anti-angiogenic factor thrombospondin 1 in human colon tumor cells [143]. The hormone can also interrupt the

angiogenic factor interleukin 8 signaling, leading to the inhibition of endothelial cell migration and tube formation [144]. It has been proposed that the inhibitory effect of $1\alpha,25(OH)_2D_3$ on metastasis observed in the prostate and lung murine models may partially depend on its anti-angiogenic property [145-146]. It should be noted that in vascular smooth muscle cells, $1\alpha,25(OH)_2D_3$ was shown to upregulate VEGF mRNA [147].

3.4. Pro-differentiation

The seminal observation by Abe *et al*. [148] that $1\alpha,25(OH)_2D_3$ was capable of inducing mouse myeloid leukemia cells to differentiate into multinucleated macrophages provides the first evidence that $1\alpha,25(OH)_2D_3$ has functions other than regulating calcium and phosphate homeostasis and paved the way for a new era of vitamin D research. Subsequently, it was shown that $1\alpha,25(OH)_2D_3$ was able to inhibit proliferation and increase the expression of a variety of differentiation markers, including involucrin, transglutaminase, loricrin and filaggrin, and enhance cornified envelope formation in cultured keratinocytes [49]. By maintaining the ordered cellular proliferation and differentiated epithelium, $1\alpha,25(OH)_2D_3$ is able to contribute to skin cancer prevention [49]. Now, we know that in cultured cells, administration of $1\alpha,25(OH)_2D_3$ or its analogs can regulate the expression of numerous genes that are associated with the differentiated cell of origin [149-151], and thus inhibit the processes critical for tumor growth and metastases [143]. In VDR-expressing SW 480-ADH human colon carcinoma cell line, $1\alpha,25(OH)_2D_3$ induces differentiation by promoting the expression of proteins implicated in adherent junction formation, including differentiation marker E-cadherin, and other adhesion proteins, such as occludin and vinculin [152]. This process is mediated by the up-regulation of Id1gene and down-regulation of Id2 gene in response to $1\alpha,25(OH)_2D_3$ [141]. In breast cancer cell lines, the induction of differentiation markers, such as E-cadherin, casein, lipid droplets, was also observed following $1\alpha,25(OH)_2D_3$-induced growth arrest [153-154]. E-cadherin is a member of the cadherin family of cell membrane adhesion glycoproteins that play an important role during cell migration. E-cadherin is expressed on the epithelial cells and forms the cell-cell tight junction. Decreased expression of this protein will cause a decrease of homotypic cell adhesion and increased cell migration and invasion, suggesting that E-cadherin may act as a tumor suppressor. In MCF-7 and HEC-1B endometrial cancer cells, Icb-1, a human gene product involved in differentiation processes of cancer cells, has been shown to be a mediator of the $1\alpha,25(OH)_2D_3$-induced up-regulation of E-cadherin expression [155]. Hsu *et al*. [156] demonstrated that $1\alpha,25(OH)_2D_3$ promoted prostate cancer cell aggregation by up-regulating E-cadherin expression, and therefore interfering their adhesion to microvascular endothelial cells and reducing their metastatic potential. Thus, $1\alpha,25(OH)_2D_3$-induced differentiation may be one mechanism responsible for inhibiting tumor growth and metastases.

Recently, a time- and dose-dependent induction of E-cadherin by $1\alpha,25(OH)_2D_3$ was observed in the triple-negative breast cancer cell line MDA-MB-231 [157], suggesting that metaplastic breast carcinomas may respond to $1\alpha,25(OH)_2D_3$ treatment. Other differentiation markers affected by $1\alpha,25(OH)_2D_3$ in breast cancer cells include claudin-7, occludin, actin filaments and microtubules, filopodia and lamellipodia. Therefore, the addition of $1\alpha,25(OH)_2D_3$ to breast cancer cells will result in increased cell and nuclear size and induce a change of nuclear shape and cell morphology from a rounded to a flattened shape [154]. In addition, $1\alpha,25(OH)_2D_3$ down-regulates SPROUTY-2 (SPRY2) expression in colon cancer cells through E-cadherin-dependent and -independent mechanisms [158]. In turn, SPRY2 suppresses both basal and $1\alpha,25(OH)_2D_3$-induced E-cadherin expression, and induces ZEB1 expression. For this reason, SPRY2 and E-cadherin protein levels were found inversely correlated in colon cancer cell lines, xenograft tumors and human colon tumor tissues.

Wnt/β-catenin is an evolutionarily conserved signaling pathway that plays an essential role in a diverse array of biologic processes, including organogenesis, tissue homeostasis and, in some instances, pathogenesis of diseases, including cancers [159]. Earlier studies indicate that $1\alpha,25(OH)_2D_3$ and its analogs are able to promote the differentiation of colon cancer cells by inhibiting Wnt/β-catenin signaling pathway mediated by VDR competing with transcription factor TCF-4 for β-catenin binding [152,160-161]. The molecular mechanism of the induction by $1\alpha,25(OH)_2D_3$ was further studied in LS180 colon cancer cells using chromatin immunoprecipitation-seq and gene expression analyses [162]. It was found that VDR and RXR co-localized to VDRE sites in a ligand-dependent manner near a set of genes that included c-FOS and c-MYC. The expression of both c-FOS and c-MYC was modulated by $1\alpha,25(OH)_2D_3$. At the c-FOS gene, both VDR/RXR and TCF4/β-catenin bound to a single distal enhancer located 24kb upstream of the transcriptional start site. At the c-MYC locus, binding was at a cluster of sites between -139 and -165 kb and at a site located -335 kb upstream, where both VDR and β-catenin activation was interlinked to basal and $1\alpha,25(OH)_2D_3$-inducible activities. In addition, $1\alpha,25(OH)_2D_3$ is known to regulate two genes encoding two extracellular Wnt inhibitors, DICKKOPF-1(DKK-1) and DICKKOPF-4 (DKK-4), in opposite directions; $1\alpha,25(OH)_2D_3$ up-regulates DKK-1 which acts as a tumor suppressor in human colon cancer cells, whereas $1\alpha,25(OH)_2D_3$ down-regulates DKK-4, an oncogenic protein and a target of the Wnt/β-catenin pathway [159-160]. Taken together, these data reveal complex modes of action in the regulation of target genes by$1\alpha,25(OH)_2D_3$.

3.5. Anti-inflammation

Animal studies have linked the anti-cancer effects of $1\alpha,25(OH)_2D_3$ to its ability to regulate inflammation [163]. In colon cancer cells, $1\alpha,25(OH)_2D_3$ can interrupt the wnt-mediated crosstalk between tumor epithelial cells and macrophages in the tumor microenvironment by blocking the production of IL-1β, an inflammatory cytokine produced by tumor-associated macrophages [164]. Conversely, any disruption of the $1\alpha,25(OH)_2D_3$/VDR signaling pathway, such as up-regulation of the transcriptional repressor SNAIL, may enhance epithelial cell inflammation and exacerbate colon cancer progression. Feldman and colleagues performed cDNA-microarray analyses of normal and cancer-derived primary prostate epithelial cells [165] and LNCaP cells [166] and revealed that $1\alpha,25(OH)_2D_3$ regulated a wide array of genes, including those involved in the synthesis and catabolism of prostaglandins, which are well-established inflammatory mediators. They showed that $1\alpha,25(OH)_2D_3$ up-regulated the expression of NAD^+-dependent 15-hydroxy-prostaglandin dehydrogenase (15-PGDH) gene and down-regulated cyclooxygenase-2 (COX-2) expression. Since prostaglandins are known to play a role in the development and progression of many cancers [167], the ability of $1\alpha,25(OH)_2D_3$ to decrease prostaglandin concentration strongly suggests that one mechanism of anti-cancer effect of vitamin D may be mediated through its anti-inflammatory action.

3.6. DNA Repair and the Prevention of Tumor Initiation and Progression

Gene array profiling studies have demonstrated that $1\alpha,25(OH)_2D_3$ up-regulates the expression of DNA repair genes [166,168], suggesting that $1\alpha,25(OH)_2D_3$ may be involved in DNA repair pathways. To investigate the mechanism of $1\alpha,25(OH)_2D_3$-induced DNA repair, Ting *et al*. [169] utilized a well-established *in vitro* model of chemical carcinogenesis. In this model, they found that $1\alpha,25(OH)_2D_3$ promoted the expression of the DNA repair genes RAD50 and ataxia telangiectasia mutated (ATM), both of them are known to be critical for mediating the signaling responses to DNA damage. They also found that $1\alpha,25(OH)_2D_3$ protected cells from genotoxic stress and growth inhibition by promoting double-strand break DNA repair. They noted that depletion of VDR

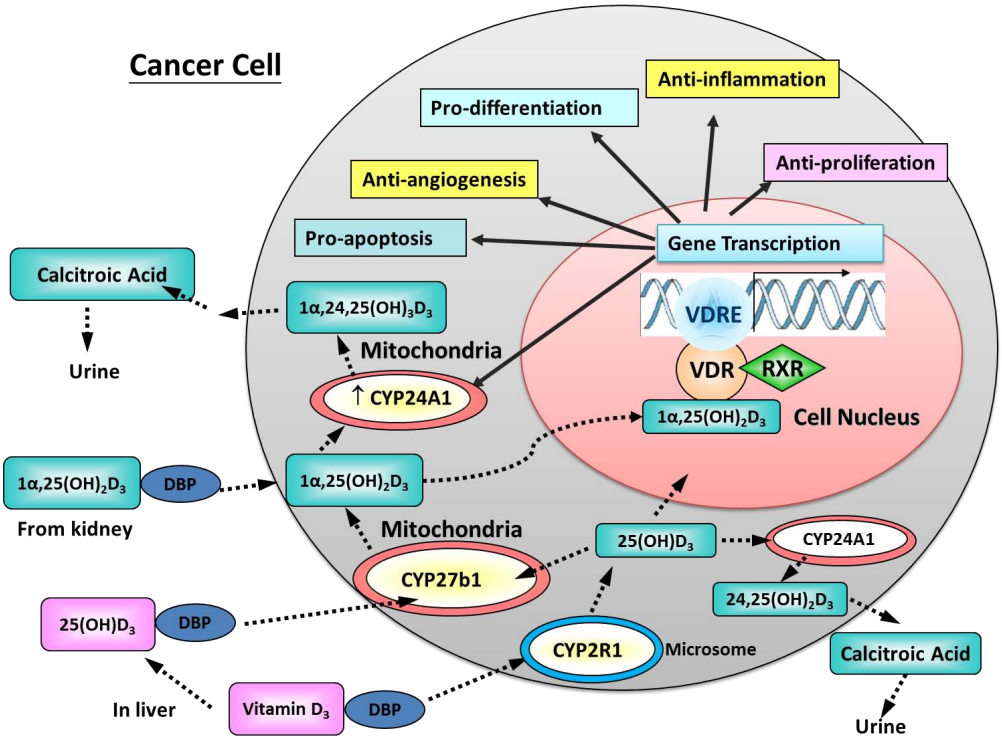

Fig. (3). The genomic anti-cancer actions of 1α,25(OH)$_2$D$_3$. The genomic actions of 1α,25(OH)$_2$D are mediated through its binding to vitamin D receptor (VDR), a member of the nuclear receptor superfamily, to modulate the expression of various genes. The liganded VDR forms a heterodimer with retinoid X receptor (RXR) and binds to vitamin D response element (VDRE) to modulate the gene expression. Since VDR is expressed in almost all tissues, 1α,25(OH)$_2$D$_3$ has been found to exert various anti-cancer actions, including anti-proliferation, anti-inflammation, pro-differentiation, pro-apoptosis and anti-angiogenesis in a tissue- and cell-specific manner. Recent evidence indicates that 25(OH)D$_3$ is a natural ligand for VDR, and is capable for causing biological effects without converting to 1α,25(OH)$_2$D$_3$.

reduced the observed genoprotective effects and caused malignant transformation that could not be prevented by 1α,25(OH)$_2$D$_3$ in a xenograft mouse model, indicating an essential role for VDR in mediating the anticancer effects of 1α,25(OH)$_2$D$_3$. Since genotoxic stress can activate ATM and VDR through phosphorylation of VDR, mutations in VDR at putative ATM phosphorylation sites impaired the ability of ATM to enhance VDR transactivation activity, leading to decreased induction of ATM and RAD50 expression by 1α,25(OH)$_2$D$_3$. Taken together, the authors propose a positive feedback signaling loop between ATM and VDR to enhance the up-regulation of DNA repair proteins and thereby prevent tumor initiation and progression resulting from DNA damages.

4. NON-GENOMIC ANTI-CANCER ACTIONS OF VITAMIN D

1α,25(OH)$_2$D$_3$ and many of its analogs are structurally flexible, and are capable of facile rotation about its 6,7 single carbon bond to permit generation of a continuum of potential ligand shapes extending from the 6-s-cis (6C) to the 6-s-trans (6T) [170]. This structural flexibility has been proposed as a base of rapid and non-genomic biological responses induced by 1α,25(OH)$_2$D$_3$ and some of its analogs. The non-genomic rapid response has been shown in several biological systems [39], that may be mediated through a functional VDR in some systems [171] or may be independent of VDR in other systems [172]. To support the involvement of VDR in the rapid non-genomic actions of vitamin D, it has been reported that VDR is present in caveolae-enriched plasma membranes and binds 1α,25(OH)$_2$D$_3$ with high affinity *in vivo* and *in vitro* [173]. Furthermore, an alternative ligand-binding pocket in the VDR has been identified by molecular docking using a receptor conformational ensemble model generated from x-ray crystal structure of the VDR ligand binding domain [174]. The existence of non-classical membrane VDR has been found to be related to the rapid actions, including activation of protein kinase C and protein phosphatase PPIc [114]. The actions have been shown to result in subsequent ion channel activity modulation [171]. Furthermore, it has been known for some time that 1α,25(OH)$_2$D$_3$ reduces UV-induced DNA damage [175] in the form of cyclobutane pyrimidine dimers (CPD) in human keratinocytes in culture, and in mouse and human skin [176-177]. The photoprotection by 1α,25(OH)$_2$D$_3$ against oxidative insults [175,177-178] is thought to be mediated by a non-genomic signaling mechanism, because 1α,25(OH)$_2$ lumisterol$_3$, which has almost no transactivating activity, reduces UV-induced DNA damage, apoptosis and immunosuppression with similar potency as 1α,25(OH)$_2$D$_3$ [179].

In the earlier section under Hedgehog Hh/Gli signaling pathway, we have mentioned the work by Uhmann *et al*. [69] who demonstrated that 1α,25(OH)$_2$D$_3$ was capable of inhibiting Hh signaling at the level of SMO in the absence of VDR, and by Tang *et al*. [71] who also found that 1α,25(OH)$_2$D$_3$-induced Gli expression in murine BCC cells was independent of VDR. Thus, in addition to the report by Wali *et al* [172], these two studies provide strong evidence of the non-genomic and rapid non-VDR action of 1α,25(OH)$_2$D$_3$ on cell growth and differentiation.

5. CONCLUSIONS AND FUTURE DIRECTIONS

Vitamin D is a pleiotropic hormone. 1α,25(OH)$_2$D$_3$, the most active metabolite of vitamin D$_3$, is a natural ligand for the vitamin D receptor VDR. Upon binding of the hormone to VDR, the liganded receptor heterodimerizes with RXR and binds to VDRE to either induce or repress the expression of affected genes. The spectrum of genes regulated by vitamin D is vast, essentially including every aspect of human physiology.

This brief overview clearly indicates that $1\alpha,25(OH)_2D_3$ is a potent anti-cancer agent, affecting cancer cells in cultures and tumor progression in animal models by a variety of mechanisms, including but not limited to anti-proliferation, anti-angiogenesis, pro-apoptosis, pro-differentiation and anti-inflammation (Fig. **3**). The underlying mechanisms for its anti-proliferative action, mediated through the VDR-dependent transactivation of genomes, may include cell cycle arrest, and pathways related to hedgehog, IGFBP-3, TGF-β-signaling and etc.

Emerging evidence indicates that $1\alpha,25(OH)_2D_3$ can have rapid actions with or without involving VDR. This will be an area of focus in the coming years. Certainly, we need to continue exploring the VDR-mediated actions in new areas, such as central nervous system, stem cell biology and new ways of diagnosing diseases, including cancers.

Despite the progress in basic laboratory research, the clinical application of vitamin D compounds for cancer treatment is still lacking. The major hurdle is the hypercalcemic side effect induced by administering a high dose of $1\alpha,25(OH)_2D_3$, that is necessary to exert the anti-tumor effects of vitamin D in humans. The potential of using less calcemic analogs of vitamin D with much higher potency than $1\alpha,25(OH)_2D_3$ for treating cancers still exists, especially in combination with other anti-cancer agents or immunomodulatory drugs. The association between decreased sun exposure/vitamin D deficiency and the risk of chronic diseases, including many types of cancer, indicates that maintaining adequate vitamin D nutrition should be a paramount priority for men and women of all ages.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

Declared none.

ABBREVIATIONS

$25(OH)D_3$	=	25-hydroxyvitamin D_3
$1\alpha,25(OH)_2D$	=	1α,25-dihydroxyvitamin D
7-DHC	=	7-dehydrocholesterol
DBP	=	vitamin D binding protein
DBD	=	DNA-binding domain
LBD	=	ligand-binding domain
VDRE	=	vitamin D response element
VDR	=	vitamin D receptor
RXR	=	retinoid X receptor
HDX	=	hydrogen-deuterium exchange
CDKs	=	cyclin-dependent kinases
CDKIs	=	cyclin-dependent kinase inhibtors
pRB	=	retinoblastoma protein
IGFBP3	=	insulin-like growth factor binding protein-3
TGF-β	=	transforming growth factor-beta
EGFR	=	epidermal growth factor receptor
E2F1	=	transcription factor 1
Hh	=	Hedgehog
Shh	=	Sonic Hedgehog
Ihh	=	Indian Hedgehog
Dhh	=	Desert Hedgehog
Gli	=	glioma associated
PTC	=	Patched or Hh receptor
SMO	=	smoothened
SPTHIFs	=	hypoxia-inducible factors
VEGF	=	vascular endothelial growth factor
MMPs	=	matrix metalloproteinases
PDGF	=	platelet derived growth factor
FGFs	=	fibroblast growth factors
DLL4	=	Notch Delta-like ligand 4
15-PGDH	=	15-hydroxy-prostaglandin dehydrogenase
COX-2	=	cyclooxygenase-2
ATM	=	ataxia telangiectasia mutated
CPD	=	cyclobutane pyrimidine dimmers

REFERENCES

[1] Norman, A.W.; Lund, J.; Deluca, H.F. Biologically Active Forms of Vitamin D_3 in Kidney and Intestine. *Arch. Biochem. Biophys.*, **1964**, *108*, 12-21.

[2] Morii, H.L., J.; Neville, P.F.; DeLuca, H.F. Biological activity of a vitamin D metabolite. *Arch. Biochem. Biophys.*, **1967**, 120, 508-512.

[3] Blunt, J.W.; Tanaka, Y.; DeLuca, H.F. The biological activity of 25-hydroxycholecalciferol, a metabolite of vitamin D3. *Proc. Natl. Acad. Sci. U. S. A.*, **1968**, *61*(2), 717-718.

[4] Lawson, D.E.; Fraser, D.R.; Kodicek, E.; Morris, H.R.; Williams, D.H. Identification of 1,25-dihydroxycholecalciferol, a new kidney hormone controlling calcium metabolism. *Nature*, **1971**, *230*(5291), 228-230.

[5] Norman, A.W.; Myrtle, J.F.; Midgett, R.J.; Nowicki, H.G.; Williams, V.; Popjak, G. 1,25-dihydroxycholecalciferol: identification of the proposed active form of vitamin D_3 in the intestine. *Science*, **1971**, *173*(991), 51-54.

[6] Holick, M.F.; Schnoes, H.K.; DeLuca, H.F. Identification of 1,25-dihydroxycholecalciferol, a form of vitamin D_3 metabolically active in the intestine. *Proc. Natl. Acad. Sci. U. S. A.*, **1971**, *68*(4), 803-804.

[7] Holick, M.F.; MacLaughlin, J.A.; Clark, M.B.; Holick, S.A.; Potts, J.T., Jr.; Anderson, R.R.; Blank, I.H.; Parrish, J.A.; Elias, P. Photosynthesis of previtamin D_3 in human skin and the physiologic consequences. *Science*, **1980**, *210*(4466), 203-205.

[8] Ponchon, G.; DeLuca, H.F. The role of the liver in the metabolism of vitamin D. *J. Clin. Invest.*, **1969**, *48*(7), 1273-1279.

[9] Cheng, J.B.; Levine, M.A.; Bell, N.H.; Mangelsdorf, D.J.; Russell, D.W. Genetic evidence that the human CYP2R1 enzyme is a key vitamin D 25-hydroxylase. *Proc. Natl. Acad. Sci. U. S. A.*, **2004**, *101*(20), 7711-7715.

[10] Haddad, J.G., Jr.; Walgate, J. 25-Hydroxyvitamin D transport in human plasma. Isolation and partial characterization of calcifidiol-binding protein. *J. Biol. Chem.*, **1976**, *251*(16), 4803-4809.

[11] Bouillon, R.; Van Baelen, H.; Tan, B.K.; De Moor, P. The isolation and characterization of the 25-hydroxyvitamin D-binding protein from chick serum. *J. Biol. Chem.*, **1980**, *255*(22), 10925-10930.

[12] Schuster, I. Cytochromes P450 are essential players in the vitamin D signaling system. *Biochim. Biophys. Acta*, **2011**, *1814*(1), 186-199.

[13] Zehnder, D.; Bland, R.; Williams, M.C.; McNinch, R.W.; Howie, A.J.; Stewart, P.M.; Hewison, M. Extrarenal expression of 25-hydroxyvitamin d(3)-1 alpha-hydroxylase. *J. Clin. Endocrinol. Metab.*, **2001**, *86*(2), 888-894.

[14] Shultz, T.D.; Fox, J.; Heath, H., 3rd; Kumar, R. Do tissues other than the kidney produce 1,25-dihydroxyvitamin D_3 *in vivo*? A reexamination. *Proc. Natl. Acad. Sci. U. S. A.*, **1983**, *80*(6), 1746-1750.

[15] Reeve, L.; Tanaka, Y.; DeLuca, H.F. Studies on the site of 1,25-dihydroxyvitamin D_3 synthesis *in vivo*. *J. Biol. Chem.*, **1983**, *258*(6), 3615-3617.

[16] Mawer, E.B.; Backhouse, J.; Lumb, G.A.; Stanbury, S.W. Evidence for formation of 1,25-dihydroxycholecalciferol during metabolism of vitamin D in man. *Nat. New. Bi.l.*, **1971**, *232*(2), 188-189.

[17] Gray, R.W.; Weber, H.P.; Dominguez, J.H.; Lemann, J., Jr. The metabolism of vitamin D3 and 25-hydroxyvitamin D_3 in normal and anephric humans. *J. Clin. Endocrinol. Metab.*, **1974**, *39*(6), 1045-1056.

[18] Catto, G.R.; Muirhead, N. Metabolic consequences of bilateral nephrectomy. *Br. Med. J. (Clin Res Ed)*, **1984**, *289*(6438), 146-147.
[19] Wagner, D.; Dias, A.G.; Schnabl, K.; Van der Kwast, T.; Vieth, R. Determination of 1,25-dihydroxyvitamin D concentrations in human colon tissues and matched serum samples.*Anticancer Res.*, **2012**, *32*(1), 259-263.
[20] Stumpf, W.E.; Sar, M.; Reid, F.A.; Tanaka, Y.; DeLuca, H.F. Target cells for 1,25-dihydroxyvitamin D_3 in intestinal tract, stomach, kidney, skin, pituitary, and parathyroid. *Science*, **1979**, *206*(4423), 1188-1190.
[21] Colston, K.; Hirt, M.; Feldman, D. Organ distribution of the cytoplasmic 1,25-dihydroxycholecalciferol receptor in various mouse tissues. *Endocrinology*, **1980**, *107*(6), 1916-1922.
[22] Chen, T.C.; DeLuca, H.F. Receptors of 1,25-dihydroxy-cholecalciferol in rat intestine. *J. Biol. Chem.*, **1973**, *248*(14), 4890-4895.
[23] Tsai, H.C.; Wong, R.G.; Norman, A.W. Studies on calciferol metabolism. IV. Subcellular localization of 1,25-dihydroxy-vitamin D_3 in intestinal mucosa and correlation with increased calcium transport. *J. Biol. Chem.*, **1972**, *247*(17), 5511-5519.
[24] Brumbaugh, P.F.; Haussler, M.R. Specific binding of 1alpha,25-dihydroxycholecalciferol to nuclear components of chick intestine. *J .Biol. Chem.*, **1975**, *250*(4), 1588-1594.
[25] Brumbaugh, P.F.; Hughes, M.R.; Haussler, M.R. Cytoplasmic and nuclear binding components for 1alpha25-dihydroxyvitamin D_3 in chick parathyroid glands. *Proc. Natl. Acad. Sci. U S A*, **1975**, *72*(12), 4871-4875.
[26] McDonnell, D.P.; Mangelsdorf, D.J.; Pike, J.W.; Haussler, M.R.; O'Malley, B.W. Molecular cloning of complementary DNA encoding the avian receptor for vitamin D. *Science*, **1987**, *235*(4793), 1214-1217.
[27] Burmester, J.K.; Maeda, N.; DeLuca, H.F. Isolation and expression of rat 1,25-dihydroxyvitamin D_3 receptor cDNA. *Proc. Natl. Acad. Sci. U S A*, **1988**, *85*(4), 1005-1009.
[28] Baker, A.R.; McDonnell, D.P.; Hughes, M.; Crisp, T.M.; Mangelsdorf, D.J.; Haussler, M.R.; Pike, J.W.; Shine, J.; O'Malley, B.W. Cloning and expression of full-length cDNA encoding human vitamin D receptor. *Proc. Natl. Acad. Sci. U S A*, **1988**, *85*(10), 3294-3298.
[29] Haussler, M.R.; Whitfield, G.K.; Haussler, C.A.; Hsieh, J.C.; Thompson, P.D.; Selznick, S.H.; Dominguez, C.E.; Jurutka, P.W. The nuclear vitamin D receptor: biological and molecular regulatory properties revealed. *J. Bone. Miner. Res.*, **1998**, *13*(3), 325-349.
[30] Rochel, N.; Wurtz, J.M.; Mitschler, A.; Klaholz, B.; Moras, D. The crystal structure of the nuclear receptor for vitamin D bound to its natural ligand. *Mol. Cell.*, **2000**, *5*(1), 173-179.
[31] Hsieh, J.C.; Jurutka, P.W.; Galligan, M.A.; Terpening, C.M.; Haussler, C.A.; Samuels, D.S.; Shimizu, Y.; Shimizu, N.; Haussler, M.R. Human vitamin D receptor is selectively phosphorylated by protein kinase C on serine 51, a residue crucial to its trans-activation function. *Proc. Natl. Acad. Sci. U S A*, **1991**, *88*(20), 9315-9319.
[32] Jurutka, P.W.; Hsieh, J.C.; MacDonald, P.N.; Terpening, C.M.; Haussler, C.A.; Haussler, M.R.; Whitfield, G.K. Phosphorylation of serine 208 in the human vitamin D receptor. The predominant amino acid phosphorylated by casein kinase II, *in vitro*, and identification as a significant phosphorylation site in intact cells. *J. Biol. Chem.*, **1993**, *268*(9), 6791-6799.
[33] Haussler, M.R.; Haussler, C.A.; Jurutka, P.W.; Thompson, P.D.; Hsieh, J.C.; Remus, L.S.; Selznick, S.H.; Whitfield, G.K. The vitamin D hormone and its nuclear receptor: molecular actions and disease states. *J Endocrinol*, **1997**, *154* Suppl, S57-73.
[34] Tsai, M.J.; O'Malley, B.W. Molecular mechanisms of action of steroid/thyroid receptor superfamily members. *Annu. Rev. Biochem.*, **1994**, *63*, 451-486.
[35] Freedman, L.P. Increasing the complexity of coactivation in nuclear receptor signaling. *Cell*, **1999**, *97*(1), 5-8.
[36] Chen, H.; Lin, R.J.; Xie, W.; Wilpitz, D.; Evans, R.M. Regulation of hormone-induced histone hyperacetylation and gene activation *via* acetylation of an acetylase. *Cell*, **1999**, *98*(5), 675-686.
[37] Battaglia, S.; Maguire, O.; Campbell, M.J. Transcription factor co-repressors in cancer biology: roles and targeting. *Int. J. Cancer.*, **2010**, *126*(11), 2511-2519.
[38] Dilworth, F.J.; Chambon, P. Nuclear receptors coordinate the activities of chromatin remodeling complexes and coactivators to facilitate initiation of transcription. *Oncogene*, **2001**, *20*(24), 3047-3054.
[39] Haussler, M.R.; Jurutka, P.W.; Mizwicki, M.; Norman, A.W. Vitamin D receptor (VDR)-mediated actions of 1alpha,25(OH) vitamin D: genomic and non-genomic mechanisms. *Best Pract. Res. Clin. Endocrinol. Metab.*, **2011**, *25*(4), 543-559.
[40] Jin, C.H.; Kerner, S.A.; Hong, M.H.; Pike, J.W. Transcriptional activation and dimerization functions in the human vitamin D receptor. *Mol. Endocrinol.*, **1996**, *10*(8), 945-957.
[41] Pike, J.W.; Meyer, M.B.; Watanuki, M.; Kim, S.; Zella, L.A.; Fretz, J.A.; Yamazaki, M.; Shevde, N.K. Perspectives on mechanisms of gene regulation by 1,25-dihydroxyvitamin D_3 and its receptor. *J. Steroid Biochem. Mol. Biol.*, **2007**, *103*(3-5), 389-395.
[42] Zhang, J.; Chalmers, M.J.; Stayrook, K.R.; Burris, L.L.; Wang, Y.; Busby, S.A.; Pascal, B.D.; Garcia-Ordonez, R.D.; Bruning, J.B.; Istrate, M.A.; Kojetin, D.J.; Dodge, J.A.; Burris, T.P.; Griffin, P.R. DNA binding alters coactivator interaction surfaces of the intact VDR-RXR complex. *Nat. Struct. Mol. Biol.*, **2011**, *18*(5), 556-563.
[43] Chandra, V.; Huang, P.; Hamuro, Y.; Raghuram, S.; Wang, Y.; Burris, T.P.; Rastinejad, F. Structure of the intact PPAR-gamma-RXR- nuclear receptor complex on DNA. *Nature*, **2008**, *456*(7220), 350-356.
[44] Emsley, P.; Cowtan, K. Coot: model-building tools for molecular graphics. *Acta. Crystallogr. D. Biol. Crystallogr.*, **2004**, *60*(Pt 12 Pt 1), 2126-2132.
[45] Rastinejad, F.; Perlmann, T.; Evans, R.M.; Sigler, P.B. Structural determinants of nuclear receptor assembly on DNA direct repeats. *Nature*, **1995**, *375*(6528), 203-211.
[46] Ramagopalan, S.V.; Heger, A.; Berlanga, A.J.; Maugeri, N.J.; Lincoln, M.R.; Burrell, A.; Handunnetthi, L.; Handel, A.E.; Disanto, G.; Orton, S.M.; Watson, C.T.; Morahan, J.M.; Giovannoni, G.; Ponting, C.P.; Ebers, G.C.; Knight, J.C. A ChIP-seq defined genome-wide map of vitamin D receptor binding: associations with disease and evolution. *Genome Res.*, **2010**, *20*(10), 1352-1360.
[47] Chen, T.C.; Holick, M.F. Vitamin D and prostate cancer prevention and treatment. *Trends Endocrinol. Metab.*, **2003**, 14(9), 423-430.
[48] Stewart, L.V.; Weigel, N.L. Vitamin D and prostate cancer. *Exp. Biol. Med. (Maywood)*, **2004**, *229*(4), 277-284.
[49] Bikle, D. Nonclassic actions of vitamin D. *J. Clin. Endocrinol. Metab.*, **2009**, *94*(1), 26-34.
[50] Adams, J.S.; Hewison, M. Update in vitamin D. *J. Clin. Endocrinol. Metab.*, **2010**, *95*(2), 471-478.
[51] Chiang, K.C.; Yeh, C.N.; Chen, M.F.; Chen, T.C. Hepatocellular carcinoma and vitamin D: a review. *J. Gastroenterol. Hepatol.*, **2011**, *26*(11), 1597-1603.
[52] Krishnan, A.V.; Feldman, D. Mechanisms of the anti-cancer and anti-inflammatory actions of vitamin D. *Annu. Rev. Pharmacol. Toxicol.*, **2011**, *51*, 311-336.
[53] Fleet, J.C.; DeSmet, M.; Johnson, R.; Li, Y. Vitamin D and cancer: a review of molecular mechanisms. *Biochem. J.*, **2012**, *441*(1), 61-76.
[54] Miller, G.J. Vitamin D and prostate cancer: biologic interactions and clinical potentials. *Cancer Metastasis Rev.*, **1998**, *17*(4), 353-360.
[55] Zinser, G.M.; McEleney, K.; Welsh, J. Characterization of mammary tumor cell lines from wild type and vitamin D3 receptor knockout mice. *Mol. Cell. Endocrinol.*, **2003**, *200*(1-2), 67-80.
[56] Malumbres, M. Physiological relevance of cell cycle kinases. *Physiol. Rev.*, **2011**, *91*(3), 973-1007.
[57] Canavese, M.; Santo, L.; Raje, N. Cyclin dependent kinases in cancer: Potential for therapeutic intervention. *Cancer Biol. Ther.*, **2012**, *13*(7),
[58] Malumbres, M.; Barbacid, M. To cycle or not to cycle: a critical decision in cancer. *Nat. Rev. Cancer*, **2001**, *1*(3), 222-231.
[59] Jiang, H.; Lin, J.; Su, Z.Z.; Collart, F.R.; Huberman, E.; Fisher, P.B. Induction of differentiation in human promyelocytic HL-60 leukemia cells activates p21, WAF1/CIP1, expression in the absence of p53. *Oncogene*, **1994**, *9*(11), 3397-3406.
[60] Liu, M.; Lee, M.H.; Cohen, M.; Bommakanti, M.; Freedman, L.P. Transcriptional activation of the Cdk inhibitor p21 by vitamin D3 leads to the induced differentiation of the myelomonocytic cell line U937. *Genes Dev.*, **1996**, *10*(2), 142-153.
[61] Saramaki, A.; Banwell, C.M.; Campbell, M.J.; Carlberg, C. Regulation of the human p21(waf1/cip1) gene promoter *via*

multiple binding sites for p53 and the vitamin D3 receptor. *Nucleic Acids Res*., **2006**, *34*(2), 543-554.

[62] Kawa, S.; Yoshizawa, K.; Tokoo, M.; Imai, H.; Oguchi, H.; Kiyosawa, K.; Homma, T.; Nikaido, T.; Furihata, K. Inhibitory effect of 220-oxa-1,25-dihydroxyvitamin D_3 on the proliferation of pancreatic cancer cell lines. *Gastroenterology*, **1996**, *110*(5), 1605-1613.

[63] Kawa, S.; Nikaido, T.; Aoki, Y.; Zhai, Y.; Kumagai, T.; Furihata, K.; Fujii, S.; Kiyosawa, K. Vitamin D analogues up-regulate p21 and p27 during growth inhibition of pancreatic cancer cell lines. *Br. J. Cancer*, **1997**, *76*(7), 884-889.

[64] Yang, E.S.; Maiorino, C.A.; Roos, B.A.; Knight, S.R.; Burnstein, K.L. Vitamin D-mediated growth inhibition of an androgen-ablated LNCaP cell line model of human prostate cancer. *Mol. Cell. Endocrinol.*, **2002**, *186*(1), 69-79.

[65] Yang, E.S.; Burnstein, K.L. Vitamin D inhibits G1 to S progression in LNCaP prostate cancer cells through p27Kip1 stabilization and Cdk2 mislocalization to the cytoplasm. *J. Biol. Chem.*, **2003**, *278*(47), 46862-46868.

[66] Luo, W.; Chen, Y.; Liu, M.; Du, K.; Zheng, G.; Cai, T.; Zhang, W.; Zhao, F.; Yao, T.; Yang, R.; Chen, J. EB1089 induces Skp2-dependent p27 accumulation, leading to cell growth inhibition and cell cycle G1 phase arrest in human hepatoma cells. *Cancer Invest.*, **2009**, *27*(1), 29-37.

[67] Flores, O.; Wang, Z.; Knudsen, K.E.; Burnstein, K.L. Nuclear targeting of cyclin-dependent kinase 2 reveals essential roles of cyclin-dependent kinase 2 localization and cyclin E in vitamin D-mediated growth inhibition. *Endocrinology*, **2010**, *151*(3), 896-908.

[68] Jensen, S.S.; Madsen, M.W.; Lukas, J.; Binderup, L.; Bartek, J. Inhibitory effects of 1alpha,25-dihydroxyvitamin D(3) on the G(1)-S phase-controlling machinery. *Mol. Endocrinol.*, **2001**, *15*(8), 1370-1380.

[69] Uhmann, A.; Niemann, H.; Lammering, B.; Henkel, C.; Hess, I.; Nitzki, F.; Fritsch, A.; Prufer, N.; Rosenberger, A.; Dullin, C.; Schraepler, A.; Reifenberger, J.; Schweyer, S.; Pietsch, T.; Strutz, F.; Schulz-Schaeffer, W.; Hahn, H. Antitumoral effects of calcitriol in basal cell carcinomas involve inhibition of hedgehog signaling and induction of vitamin D receptor signaling and differentiation. *Mol. Cancer Ther.*, **2011**, *10*(11), 2179-2188.

[70] Teichert, A.E.; Elalieh, H.; Elias, P.M.; Welsh, J.; Bikle, D.D. Overexpression of hedgehog signaling is associated with epidermal tumor formation in vitamin D receptor-null mice. *J. Invest. Dermatol.*, **2011**, *131*(11), 2289-2297.

[71] Tang, J.Y.; Xiao, T.Z.; Oda, Y.; Chang, K.S.; Shpall, E.; Wu, A.; So, P.L.; Hebert, J.; Bikle, D.; Epstein, E.H., Jr. Vitamin D3 inhibits hedgehog signaling and proliferation in murine Basal cell carcinomas. *Cancer Prev. Res. (Phila)*, **2011**, *4*(5), 744-751.

[72] Yanagisawa, J.; Yanagi, Y.; Masuhiro, Y.; Suzawa, M.; Watanabe, M.; Kashiwagi, K.; Toriyabe, T.; Kawabata, M.; Miyazono, K.; Kato, S. Convergence of transforming growth factor-beta and vitamin D signaling pathways on SMAD transcriptional coactivators. *Science*, **1999**, *283*(5406), 1317-1321.

[73] Huynh, H.; Pollak, M.; Zhang, J.C. Regulation of insulin-like growth factor (IGF) II and IGF binding protein 3 autocrine loop in human PC-3 prostate cancer cells by vitamin D metabolite $1,25(OH)_2D_3$ and its analog EB1089. *Int. J. Oncol.*, **1998**, *13*(1), 137-143.

[74] Tong, W.M.; Hofer, H.; Ellinger, A.; Peterlik, M.; Cross, H.S. Mechanism of antimitogenic action of vitamin D in human colon carcinoma cells: relevance for suppression of epidermal growth factor-stimulated cell growth. *Oncol. Res.*, **1999**, *11*(2), 77-84.

[75] Moreno, J.; Krishnan, A.V.; Feldman, D. Molecular mechanisms mediating the anti-proliferative effects of Vitamin D in prostate cancer. *J. Steroid Biochem. Mol. Bil.*, **2005**, 97(1-2), 31-36.

[76] Nusslein-Volhard, C.; Wieschaus, E. Mutations affecting segment number and polarity in Drosophila. *Nature*, **1980**, 287(5785), 795-801.

[77] Echelard, Y.; Epstein, D.J.; St-Jacques, B.; Shen, L.; Mohler, J.; McMahon, J.A.; McMahon, A.P. Sonic hedgehog, a member of a family of putative signaling molecules, is implicated in the regulation of CNS polarity. *Cell*, **1993**, *75*(7), 1417-1430.

[78] Krauss, S.; Concordet, J.P.; Ingham, P.W. A functionally conserved homolog of the Drosophila segment polarity gene hh is expressed in tissues with polarizing activity in zebrafish embryos. *Cell*, **1993**, *75*(7), 1431-1444.

[79] Riddle, R.D.; Johnson, R.L.; Laufer, E.; Tabin, C. Sonic hedgehog mediates the polarizing activity of the ZPA. *Cell*, **1993**, *75*(7), 1401-1416.

[80] Chang, D.T.; Lopez, A.; von Kessler, D.P.; Chiang, C.; Simandl, B.K.; Zhao, R.; Seldin, M.F.; Fallon, J.F.; Beachy, P.A. Products, genetic linkage and limb patterning activity of a murine hedgehog gene. *Development*, **1994**, *120*(11), 3339-3353.

[81] Roelink, H.; Augsburger, A.; Heemskerk, J.; Korzh, V.; Norlin, S.; Ruiz i Altaba, A.; Tanabe, Y.; Placzek, M.; Edlund, T.; Jessell, T.M.; Dodd, J . Floor plate and motor neuron induction by vhh-1, a vertebrate homolog of hedgehog expressed by the notochord. *Cell*, **1994**, *76*(4), 761-775.

[82] Yang, L.; Xie, G.; Fan, Q.; Xie, J. Activation of the hedgehog-signaling pathway in human cancer and the clinical implications. *Oncogene*, **2010**, *29*(4), 469-481.

[83] Bale, A.E. Hedgehog signaling and human disease. *Annu. Rev. Genomics Hum. Genet.*, **2002**, 3, 47-65.

[84] Epstein, E.H. Basal cell carcinomas: attack of the hedgehog. *Nat .Rev. Cancer.*, **2008**, *8*(10), 743-754.

[85] Mimeault, M.; Batra, S.K. Frequent deregulations in the hedgehog signaling network and cross-talks with the epidermal growth factor receptor pathway involved in cancer progression and targeted therapies. *Pharmacol. Rev.*, **2010**, *62*(3), 497-524.

[86] Bijlsma, M.F.; Spek, C.A.; Zivkovic, D.; van de Water, S.; Rezaee, F.; Peppelenbosch, M.P. Repression of smoothened by patched-dependent (pro-)vitamin D3 secretion. *PLoS. Biol.*, **2006**, *4*(8), e232.

[87] Moriwake, T.; Tanaka, H.; Kanzaki, S.; Higuchi, J.; Seino, Y. 1,25-Dihydroxyvitamin D_3 stimulates the secretion of insulin-like growth factor binding protein 3 (IGFBP-3) by cultured human osteosarcoma cells. *Endocrinology*, **1992**, *130*(2), 1071-1073.

[88] Velez-Yanguas, M.C.; Kalebic, T.; Maggi, M.; Kappel, C.C.; Letterio, J.; Uskokovic, M.; Helman, L.J. 1 alpha, 25-dihydroxy-16-ene-23-yne-26,27-hexafluorocholecalciferol (Ro24-5531) modulation of insulin-like growth factor-binding protein-3 and induction of differentiation and growth arrest in a human osteosarcoma cell line. *J. Clin. Endocrinol. Metab.*, **1996**, *81*(1), 93-99.

[89] Colston, K.W.; Perks, C.M.; Xie, S.P.; Holly, J.M. Growth inhibition of both MCF-7 and Hs578T human breast cancer cell lines by vitamin D analogues is associated with increased expression of insulin-like growth factor binding protein-3. *J. Mol. Endocrinol.*, **1998**, *20*(1), 157-162.

[90] Nickerson, T.; Huynh, H. Vitamin D analogue EB1089-induced prostate regression is associated with increased gene expression of insulin-like growth factor binding proteins. *J. Endocrinol.*, **1999**, *160*(2), 223-229.

[91] Agarwal, C.; Lambert, A.; Chandraratna, R.A.; Rorke, E.A.; Eckert, R.L. Vitamin D regulates human ectocervical epithelial cell proliferation and insulin-like growth factor-binding protein-3 level. *Biol. Reprod.*, **1999**, *60*(3), 567-572.

[92] Boyle, B.J.; Zhao, X.Y.; Cohen, P.; Feldman, D. Insulin-like growth factor binding protein-3 mediates 1 alpha,25-dihydroxyvitamin d(3) growth inhibition in the LNCaP prostate cancer cell line through p21/WAF1. *J. Urol.*, **2001**, *165*(4), 1319-1324.

[93] Sprenger, C.C.; Peterson, A.; Lance, R.; Ware, J.L.; Drivdahl, R.H.; Plymate, S.R. Regulation of proliferation of prostate epithelial cells by 1,25-dihydroxyvitamin D_3 is accompanied by an increase in insulin-like growth factor binding protein-3. *J. Endocrinol.*, **2001**, *170*(3), 609-618.

[94] Peng, L.; Malloy, P.J.; Feldman, D. Identification of a functional vitamin D response element in the human insulin-like growth factor binding protein-3 promoter. *Mol. Endocrinol.*, **2004**, *18*(5), 1109-1119.

[95] Murthy, S.; Weigel, N.L. 1alpha,25-dihydroxyvitamin D_3 induced growth inhibition of PC-3 prostate cancer cells requires an active transforming growth factor beta signaling pathway. *Prostate*, **2004**, *59*(3), 282-291.

[96] Stewart, L.V.; Weigel, N.L. Role of insulin-like growth factor binding proteins in 1alpha,25-dihydroxyvitamin D(3)-induced growth inhibition of human prostate cancer cells. *Prostate*, **2005**, *64*(1), 9-19.

[97] Matilainen, M.; Malinen, M.; Saavalainen, K.; Carlberg, C. Regulation of multiple insulin-like growth factor binding protein

genes by 1alpha,25-dihydroxyvitamin D_3. *Nucleic Acids Res.*, **2005**, *33*(17), 5521-5532.

[98] Malinen, M.; Ryynanen, J.; Heinaniemi, M.; Vaisanen, S.; Carlberg, C. Cyclical regulation of the insulin-like growth factor binding protein 3 gene in response to 1alpha,25-dihydroxyvitamin D_3. *Nucleic Acids Res.*, **2011**, *39*(2), 502-512.

[99] Peng, L.; Malloy, P.J.; Wang, J.; Feldman, D. Growth inhibitory concentrations of androgens up-regulate insulin-like growth factor binding protein-3 expression *via* an androgen response element in LNCaP human prostate cancer cells. *Endocrinology*, **2006**, *147*(10), 4599-4607.

[100] Yanagi, Y.; Suzawa, M.; Kawabata, M.; Miyazono, K.; Yanagisawa, J.; Kato, S. Positive and negative modulation of vitamin D receptor function by transforming growth factor-beta signaling through smad proteins. *J. Biol. Chem.*, **1999**, *274*(19), 12971-12974.

[101] Wu, Y.; Craig, T.A.; Lutz, W.H.; Kumar, R. Identification of 1 alpha,25-dihydroxyvitamin D_3 response elements in the human transforming growth factor beta 2 gene. *Biochemistry*, **1999**, *38*(9), 2654-2660.

[102] Wu, G.; Fan, R.S.; Li, W.; Srinivas, V.; Brattain, M.G. Regulation of transforming growth factor-beta type II receptor expression in human breast cancer MCF-7 cells by vitamin D3 and its analogues. *J.Biol. Chem.*, **1998**, *273*(13), 7749-7756.

[103] Koli, K.; Keski-Oja, J. 1,25-Dihydroxyvitamin D_3 enhances the expression of transforming growth factor beta 1 and its latent form binding protein in cultured breast carcinoma cells. *Cancer Res.*, **1995**, *55*(7), 1540-1546.

[104] Proietti, S.; Cucina, A.; D'Anselmi, F.; Dinicola, S.; Pasqualato, A.; Lisi, E.; Bizzarri, M. Melatonin and vitamin D_3 synergistically down-regulate Akt and MDM2 leading to TGFbeta-1-dependent growth inhibition of breast cancer cells. *J. Pineal Res.*, **2011**, *50*(2), 150-158.

[105] Daniel, C.; Schroder, O.; Zahn, N.; Gaschott, T.; Steinhilber, D.; Stein, J.M. The TGFbeta/Smad 3-signaling pathway is involved in butyrate-mediated vitamin D receptor (VDR)-expression. *J. Cell. Biochem.*, **2007**, *102*(6), 1420-1431.

[106] Ramirez, A.M.; Wongtrakool, C.; Welch, T.; Steinmeyer, A.; Zugel, U.; Roman, J. Vitamin D inhibition of pro-fibrotic effects of transforming growth factor beta1 in lung fibroblasts and epithelial cells. *J. Steroid Biochem. Mol. Biol.*, **2010**, *118*(3), 142-150.

[107] Halder, S.K.; Goodwin, J.S.; Al-Hendy, A. 1,25-Dihydroxyvitamin D_3 reduces TGF-beta3-induced fibrosis-related gene expression in human uterine leiomyoma cells. *J. Clin. Endocrinol. Metab.*, **2011**, *96*(4), E754-762.

[108] Debatin, K.M. Apoptosis pathways in cancer and cancer therapy. *Cancer Immunol. Immunother.*, **2004**, *53*(3), 153-159.

[109] Jendrossek, V. The intrinsic apoptosis pathways as a target in anticancer therapy. *Curr. Pharm. Biotechnol.*, **2012**,

[110] McDonnell, T.J.; Beham, A.; Sarkiss, M.; Andersen, M.M.; Lo, P. Importance of the Bcl-2 family in cell death regulation. *Experientia*, **1996**, *52*(10-11), 1008-1017.

[111] Herrmann, J.L.; Bruckheimer, E.; McDonnell, T.J. Cell death signal transduction and Bcl-2 function. *Biochem. Soc. Trans.*, **1996**, *24*(4), 1059-1065.

[112] Blutt, S.E.; McDonnell, T.J.; Polek, T.C.; Weigel, N.L. Calcitriol-induced apoptosis in LNCaP cells is blocked by overexpression of Bcl-2. *Endocrinology*, **2000**, *141*(1), 10-17.

[113] Polek, T.C.; Stewart, L.V.; Ryu, E.J.; Cohen, M.B.; Allegretto, E.A.; Weigel, N.L. p53 Is required for 1,25-dihydroxyvitamin D3-induced G0 arrest but is not required for G1 accumulation or apoptosis of LNCaP prostate cancer cells. *Endocrinology*, **2003**, *144*(1), 50-60.

[114] Deeb, K.K.; Trump, D.L.; Johnson, C.S. Vitamin D signalling pathways in cancer: potential for anticancer therapeutics. *Nat. Rev. Cancer.*, **2007**, *7*(9), 684-700.

[115] James, S.Y.; Mackay, A.G.; Colston, K.W. Effects of 1,25 dihydroxyvitamin D_3 and its analogues on induction of apoptosis in breast cancer cells. *J. Steroid Biochem. Mol. Biol.*, **1996**, *58*(4), 395-401.

[116] Simboli-Campbell, M.; Narvaez, C.J.; Tenniswood, M.; Welsh, J. 1,25-Dihydroxyvitamin D_3 induces morphological and biochemical markers of apoptosis in MCF-7 breast cancer cells. *J. Steroid. Biochem. Mol. Biol.*, **1996**, *58*(4), 367-376.

[117] Gown, A.M.; Willingham, M.C. Improved detection of apoptotic cells in archival paraffin sections: immunohistochemistry using antibodies to cleaved caspase 3. *J. Histochem. Cytochem.*, **2002**, *50*(4), 449-454.

[118] Duan, W.R.; Garner, D.S.; Williams, S.D.; Funckes-Shippy, C.L.; Spath, I.S.; Blomme, E.A. Comparison of immunohistochemistry for activated caspase-3 and cleaved cytokeratin 18 with the TUNEL method for quantification of apoptosis in histological sections of PC-3 subcutaneous xenografts. *J. Pathol.*, **2003**, *199*(2), 221-228.

[119] Resendes, A.R.; Majo, N.; Segales, J.; Mateu, E.; Calsamiglia, M.; Domingo, M. Apoptosis in lymphoid organs of pigs naturally infected by porcine circovirus type 2. *J. Gen. Virol.*, **2004**, *85* (Pt 10), 2837-2844.

[120] Chiang, K.C.; Yeh, C.N.; Chen, H.Y.; Lee, J.M.; Juang, H.H.; Chen, M.F.; Takano, M.; Kittaka, A.; Chen, T.C. 19-Nor-2alpha-(3-hydroxypropyl)-1alpha,25-dihydroxyvitamin D_3 (MART-10) is a potent cell growth regulator with enhanced chemotherapeutic potency in liver cancer cells. *Steroids*, **2011**, *76*(13), 1513-1519.

[121] Jiang, F.; Bao, J.; Li, P.; Nicosia, S.V.; Bai, W. Induction of ovarian cancer cell apoptosis by 1,25-dihydroxyvitamin D_3 through the down-regulation of telomerase. *J. Biol. Chem.*, **2004**, *279*(51), 53213-53221.

[122] Brosseau, C.; Colston, K.; Dalgleish, A.G.; Galustian, C. The immunomodulatory drug lenalidomide restores a vitamin D sensitive phenotype to the vitamin D resistant breast cancer cell line MDA-MB-231 through inhibition of BCL-2: potential for breast cancer therapeutics. *Apoptosis*, **2012**, *17*(2), 164-173.

[123] Ylikomi, T.; Laaksi, I.; Lou, Y.R.; Martikainen, P.; Miettinen, S.; Pennanen, P.; Purmonen, S.; Syvala, H.; Vienonen, A.; Tuohimaa, P. Antiproliferative action of vitamin D. *Vitam. Horm.*, **2002**, *64*, 357-406.

[124] Chaudhry, M.; Sundaram, S.; Gennings, C.; Carter, H.; Gewirtz, D.A. The vitamin D3 analog, ILX-23-7553, enhances the response to adriamycin and irradiation in MCF-7 breast tumor cells. *Cancer Chemother. Pharmacol.*, **2001**, *47*(5), 429-436.

[125] Folkman, J. Angiogenesis: an organizing principle for drug discovery? *Nat. Rev. Drug. Discov.*, **2007**, *6*(4), 273-286.

[126] Folkman, J. Angiogenesis in cancer, vascular, rheumatoid and other disease. *Nat. Med.*, **1995**, *1*(1), 27-31.

[127] Adair, T.H.; Gay, W.J.; Montani, J.P. Growth regulation of the vascular system: evidence for a metabolic hypothesis. *Am. J. Physiol.*, **1990**, *259*(3 Pt 2), R393-404.

[128] Shweiki, D.; Itin, A.; Soffer, D.; Keshet, E. Vascular endothelial growth factor induced by hypoxia may mediate hypoxia-initiated angiogenesis. *Nature*, **1992**, *359*(6398), 843-845.

[129] Giaccia, A.; Siim, B.G.; Johnson, R.S. HIF-1 as a target for drug development. *Nat. Rev. Drug. Discov.*, **2003**, 2(10), 803-811.

[130] Hanahan, D.; Weinberg, R.A. The hallmarks of cancer. *Cell*, **2000**, *100*(1), 57-70.

[131] Rankin, E.B.; Giaccia, A.J. The role of hypoxia-inducible factors in tumorigenesis. *Cell Death Differ.*, **2008**, *15*(4), 678-685.

[132] Ferrara, N. Role of vascular endothelial growth factor in regulation of physiological angiogenesis. *Am. J. Physiol. Cell. Physiol.*, **2001**, *280*(6), C1358-1366.

[133] Ferrara, N. Vascular endothelial growth factor: basic science and clinical progress. *Endocr. Rev.*, **2004**, *25*(4), 581-611.

[134] Ferrara, N. Pathways mediating VEGF-independent tumor angiogenesis. Cytokine Growth Factor Rev, **2010**, 21(1), 21-26.

[135] Cao, Y.; Cao, R.; Hedlund, E.M. R Regulation of tumor angiogenesis and metastasis by FGF and PDGF signaling pathways. *J .Mol. Med. (Berl)*, **2008**, *86*(7), 785-789.

[136] Jost, M.; Folgueras, A.R.; Frerart, F.; Pendas, A.M.; Blacher, S.; Houard, X.; Berndt, S.; Munaut, C.; Cataldo, D.; Alvarez, J.; Melen-Lamalle, L.; Foidart, J.M.; Lopez-Otin, C.; Noel, A. Earlier onset of tumoral angiogenesis in matrix metalloproteinase-19-deficient mice. *Cancer Res.*, **2006**, *66*(10), 5234-5241.

[137] Merke, J.; Milde, P.; Lewicka, S.; Hugel, U.; Klaus, G.; Mangelsdorf, D.J.; Haussler, M.R.; Rauterberg, E.W.; Ritz, E. Identification and regulation of 1,25-dihydroxyvitamin D_3 receptor activity and biosynthesis of 1,25-dihydroxyvitamin D_3. Studies in cultured bovine aortic endothelial cells and human dermal capillaries. *J. Clin. Invest.*, **1989**, *83*(6), 1903-1915.

[138] Iseki, K.; Tatsuta, M.; Uehara, H.; Iishi, H.; Yano, H.; Sakai, N.; Ishiguro, S. Inhibition of angiogenesis as a mechanism for inhibition by 1alpha-hydroxyvitamin D3 and 1,25-dihydroxyvitamin D_3 of colon carcinogenesis induced by azoxymethane in Wistar rats. *Int. J. Cancer*, **1999**, *81*(5), 730-733.

[139] Mantell, D.J.; Owens, P.E.; Bundred, N.J.; Mawer, E.B.; Canfield, A.E. 1 alpha,25-dihydroxyvitamin D(3) inhibits angiogenesis *in vitro* and *in vivo*. *Circ. Res.*, **2000**, *87*(3), 214-220.

[140] Chung, I.; Wong, M.K.; Flynn, G.; Yu, W.D.; Johnson, C.S.; Trump, D.L. Differential antiproliferative effects of calcitriol on tumor-derived and matrigel-derived endothelial cells. *Cancer Res.*, **2006**, *66*(17), 8565-8573.

[141] Lin, R.; Nagai, Y.; Sladek, R.; Bastien, Y.; Ho, J.; Petrecca, K.; Sotiropoulou, G.; Diamandis, E.P.; Hudson, T.J.; White, J.H. Expression profiling in squamous carcinoma cells reveals pleiotropic effects of vitamin D_3 analog EB1089 signaling on cell proliferation, differentiation, and immune system regulation. *Mol. Endocrinol.*, **2002**, *16*(6), 1243-1256.

[142] Ben-Shoshan, M.; Amir, S.; Dang, D.T.; Dang, L.H.; Weisman, Y.; Mabjeesh, N.J. 1alpha,25-dihydroxyvitamin D_3 (Calcitriol) inhibits hypoxia-inducible factor-1/vascular endothelial growth factor pathway in human cancer cells. *Mol. Cancer. Ther.*, **2007**, *6*(4), 1433-1439.

[143] Fernandez-Garcia, N.I.; Palmer, H.G.; Garcia, M.; Gonzalez-Martin, A.; del Rio, M.; Barettino, D.; Volpert, O.; Munoz, A.; Jimenez, B. 1alpha,25-Dihydroxyvitamin D_3 regulates the expression of Id1 and Id2 genes and the angiogenic phenotype of human colon carcinoma cells. *Oncogene*, **2005**, *24*(43), 6533-6544.

[144] Bao, B.Y.; Yao, J.; Lee, Y.F. 1alpha, 25-dihydroxyvitamin D_3 suppresses interleukin-8-mediated prostate cancer cell angiogenesis. *Carcinogenesis*, **2006**, *27*(9), 1883-1893.

[145] Getzenberg, R.H.; Light, B.W.; Lapco, P.E.; Konety, B.R.; Nangia, A.K.; Acierno, J.S.; Dhir, R.; Shurin, Z.; Day, R.S.; Trump, D.L.; Johnson, C.S. Vitamin D inhibition of prostate adenocarcinoma growth and metastasis in the Dunning rat prostate model system. *Urology*, **1997**, *50*(6), 999-1006.

[146] Nakagawa, K.; Sasaki, Y.; Kato, S.; Kubodera, N.; Okano, T. 22-Oxa-1alpha,25-dihydroxyvitamin D_3 inhibits metastasis and angiogenesis in lung cancer. *Carcinogenesis*, **2005**, *26*(6), 1044-1054.

[147] Cardus, A.; Parisi, E.; Gallego, C.; Aldea, M.; Fernandez, E.; Valdivielso, J.M. 1,25-Dihydroxyvitamin D_3 stimulates vascular smooth muscle cell proliferation through a VEGF-mediated pathway. *Kidney Int.*, **2006**, *69*(8), 1377-1384.

[148] Abe, E.; Miyaura, C.; Sakagami, H.; Takeda, M.; Konno, K.; Yamazaki, T.; Yoshiki, S.; Suda, T. Differentiation of mouse myeloid leukemia cells induced by 1 alpha,25-dihydroxyvitamin D_3. *Proc. Natl. Acad. Sci. U S A*, **1981**, *78*(8), 4990-4994.

[149] Palmer, H.G.; Sanchez-Carbayo, M.; Ordonez-Moran, P.; Larriba, M.J.; Cordon-Cardo, C.; Munoz, A. Genetic signatures of differentiation induced by 1alpha,25-dihydroxyvitamin D_3 in human colon cancer cells. *Cancer Res.*, **2003**, *63*(22), 7799-7806.

[150] Akutsu, N.; Lin, R.; Bastien, Y.; Bestawros, A.; Enepekides, D.J.; Black, M.J.; White, J.H. Regulation of gene Expression by 1alpha,25-dihydroxyvitamin D_3 and Its analog EB1089 under growth-inhibitory conditions in squamous carcinoma Cells. *Mol. Endocrinol.*, **2001**, *15*(7), 1127-1139.

[151] Guzey, M.; Luo, J.; Getzenberg, R.H. Vitamin D_3 modulated gene expression patterns in human primary normal and cancer prostate cells. *J .Cell. Biochem.*, **2004**, *93*(2), 271-285.

[152] Palmer, H.G.; Gonzalez-Sancho, J.M.; Espada, J.; Berciano, M.T.; Puig, I.; Baulida, J.; Quintanilla, M.; Cano, A.; de Herreros, A.G.; Lafarga, M.; Munoz, A. Vitamin D(3) promotes the differentiation of colon carcinoma cells by the induction of E-cadherin and the inhibition of beta-catenin signaling. *J. Cell. Biol.*, **2001**, *154*(2), 369-387.

[153] Lazzaro, G.; Agadir, A.; Qing, W.; Poria, M.; Mehta, R.R.; Moriarty, R.M.; Das Gupta, T.K.; Zhang, X.K.; Mehta, R.G. Induction of differentiation by 1alpha-hydroxyvitamin D(5) in T47D human breast cancer cells and its interaction with vitamin D receptors. *Eur. J. Cancer*, **2000**, *36*(6), 780-786.

[154] Pendas-Franco, N.; Gonzalez-Sancho, J.M.; Suarez, Y.; Aguilera, O.; Steinmeyer, A.; Gamallo, C.; Berciano, M.T.; Lafarga, M.; Munoz, A. Vitamin D regulates the phenotype of human breast cancer cells. *Differentiation*, **2007**, *75*(3), 193-207.

[155] Haselberger, M.; Springwald, A.; Konwisorz, A.; Lattrich, C.; Goerse, R.; Ortmann, O.; Treeck, O. Silencing of the icb-1 gene inhibits the induction of differentiation-associated genes by vitamin D_3 and all-trans retinoic acid in gynecological cancer cells. *Int. J. Mol. Med.*, **2011**, *28*(1), 121-127.

[156] Hsu, J.W.; Yasmin-Karim, S.; King, M.R.; Wojciechowski, J.C.; Mickelsen, D.; Blair, M.L.; Ting, H.J.; Ma, W.L.; Lee, Y.F. Suppression of prostate cancer cell rolling and adhesion to endothelium by 1alpha,25-dihydroxyvitamin D_3. *Am. J. Pathol.*, **2011**, *178*(2), 872-880.

[157] Lopes, N.; Carvalho, J.; Duraes, C.; Sousa, B.; Gomes, M.; Costa, J.L.; Oliveira, C.; Paredes, J.; Schmitt, F. 1Alpha,25-dihydroxyvitamin D_3 induces de novo E-cadherin expression in triple-negative breast cancer cells by CDH1-promoter demethylation. *Anticancer Res.*, **2012**, *32*(1), 249-257.

[158] Barbachano, A.; Ordonez-Moran, P.; Garcia, J.M.; Sanchez, A.; Pereira, F.; Larriba, M.J.; Martinez, N.; Hernandez, J.; Landolfi, S.; Bonilla, F.; Palmer, H.G.; Rojas, J.M.; Munoz, A. SPROUTY-2 and E-cadherin regulate reciprocally and dictate colon cancer cell tumourigenicity. *Oncogene*, **2010**, *29*(34), 4800-4813.

[159] Pendas-Franco, N.; Garcia, J.M.; Pena, C.; Valle, N.; Palmer, H.G.; Heinaniemi, M.; Carlberg, C.; Jimenez, B.; Bonilla, F.; Munoz, A.; Gonzalez-Sancho, J.M. DICKKOPF-4 is induced by TCF/beta-catenin and upregulated in human colon cancer, promotes tumour cell invasion and angiogenesis and is repressed by 1alpha,25-dihydroxyvitamin D_3. *Oncogene*, **2008**, *27*(32), 4467-4477.

[160] Pendas-Franco, N.; Aguilera, O.; Pereira, F.; Gonzalez-Sancho, J.M.; Munoz, A. Vitamin D and Wnt/beta-catenin pathway in colon cancer: role and regulation of DICKKOPF genes. *Anticancer Res.*, **2008**, *28*(5A), 2613-2623.

[161] Shah, S.; Islam, M.N.; Dakshanamurthy, S.; Rizvi, I.; Rao, M.; Herrell, R.; Zinser, G.; Valrance, M.; Aranda, A.; Moras, D.; Norman, A.; Welsh, J.; Byers, S.W. The molecular basis of vitamin D receptor and beta-catenin crossregulation. *Mol. Cell.*, **2006**, *21*(6), 799-809.

[162] Meyer, M.B.; Goetsch, P.D.; Pike, J.W. VDR/RXR and TCF4/beta-catenin cistromes in colonic cells of colorectal tumor origin: impact on c-FOS and c-MYC gene expression. *Mol. Endocrinol.*, **2012**, *26*(1), 37-51.

[163] Welsh, J. Cellular and molecular effects of vitamin D on carcinogenesis. *Arch. Biochem. Biophys.*, **2011**,

[164] Kaler, P.; Augenlicht, L.; Klampfer, L. Macrophage-derived IL-1beta stimulates Wnt signaling and growth of colon cancer cells: a crosstalk interrupted by vitamin D3. *Oncogene*, **2009**, *28*(44), 3892-3902.

[165] Peehl, D.M.; Shinghal, R.; Nonn, L.; Seto, E.; Krishnan, A.V.; Brooks, J.D.; Feldman, D. Molecular activity of 1,25-dihydroxyvitamin D_3 in primary cultures of human prostatic epithelial cells revealed by cDNA microarray analysis. *J. Steroid. Biochem. Mol. Biol.*, **2004**, *92*(3), 131-141.

[166] Krishnan, A.V.; Shinghal, R.; Raghavachari, N.; Brooks, J.D.; Peehl, D.M.; Feldman, D. Analysis of vitamin D-regulated gene expression in LNCaP human prostate cancer cells using cDNA microarrays. *Prostate*, **2004**, *59*(3), 243-251.

[167] Mantovani, A.; Allavena, P.; Sica, A.; Balkwill, F. Cancer-related inflammation. *Nature*, **2008**, 454(7203), 436-444.

[168] Wu-Wong, J.R.; Nakane, M.; Ma, J.; Ruan, X.; Kroeger, P.E. Effects of Vitamin D analogs on gene expression profiling in human coronary artery smooth muscle cells. *Atherosclerosis*, **2006**, *186*(1), 20-28.

[169] Ting, H.J.; Yasmin-Karim, S.; Yan, S.J.; Hsu, J.W.; Lin, T.H.; Zeng, W.; Messing, J.; Sheu, T.J.; Bao, B.Y.; Li, W.X.; Messing, E.; Lee, Y.F. A positive feedback signaling loop between ATM and the vitamin D receptor is critical for cancer chemoprevention by vitamin D. *Cancer Res.*, **2012**, *72*(4), 958-968.

[170] Norman, A.W.; Song, X.; Zanello, L.; Bula, C.; Okamura, W.H. Rapid and genomic biological responses are mediated by different shapes of the agonist steroid hormone, 1alpha,25$(OH)_2$vitamin D_3. *Steroids*, **1999**, *64*(1-2), 120-128.

[171] Zanello, L.P.; Norman, A.W. Rapid modulation of osteoblast ion channel responses by 1alpha,25$(OH)_2$-vitamin D_3 requires the presence of a functional vitamin D nuclear receptor. *Proc. Natl. Acad. Sci. U S A*, **2004**, *101*(6), 1589-1594.

[172] Wali, R.K.; Kong, J.; Sitrin, M.D.; Bissonnette, M.; Li, Y.C. Vitamin D receptor is not required for the rapid actions of 1,25-dihydroxyvitamin D_3 to increase intracellular calcium and activate protein kinase C in mouse osteoblasts. *J. Cell. Biochem.*, **2003**, *88*(4), 794-801.

[173] Huhtakangas, J.A.; Olivera, C.J.; Bishop, J.E.; Zanello, L.P.; Norman, A.W. The vitamin D receptor is present in caveolae-

enriched plasma membranes and binds 1 alpha,25$(OH)_2$-vitamin D_3 *in vivo* and *in vitro*. *Mol. Endocrinol.*, **2004**, *18*(11), 2660-2671.

[174] Mizwicki, M.T.; Keidel, D.; Bula, C.M.; Bishop, J.E.; Zanello, L.P.; Wurtz, J.M.; Moras, D.; Norman, A.W. Identification of an alternative ligand-binding pocket in the nuclear vitamin D receptor and its functional importance in 1alpha,25$(OH)_2$-vitamin D_3 signaling. *Proc. Natl. Acad. Sci. U S A*, **2004**, *101*(35), 12876-12881.

[175] Hanada, K.; Sawamura, D.; Nakano, H.; Hashimoto, I. Possible role of 1,25-dihydroxyvitamin D3-induced metallothionein in photoprotection against UVB injury in mouse skin and cultured rat keratinocytes. *J. Dermatol. Sci.*, **1995**, *9*(3), 203-208.

[176] Wong, G.; Gupta, R.; Dixon, K.M.; Deo, S.S.; Choong, S.M.; Halliday, G.M.; Bishop, J.E.; Ishizuka, S.; Norman, A.W.; Posner, G.H.; Mason, R.S. 1,25-Dihydroxyvitamin D and three low-calcemic analogs decrease UV-induced DNA damage *via* the rapid response pathway. *J. Steroid. Biochem. Mol. Biol.*, **2004**, *89-90*(1-5), 567-570.

[177] De Haes, P.; Garmyn, M.; Verstuyf, A.; De Clercq, P.; Vandewalle, M.; Degreef, H.; Vantieghem, K.; Bouillon, R.; Segaert, S. 1,25-Dihydroxyvitamin D_3 and analogues protect primary human keratinocytes against UVB-induced DNA damage. *J. Photochem. Photobiol. B.*, **2005**, *78*(2), 141-148.

[178] Banakar, M.C.; Paramasivan, S.K.; Chattopadhyay, M.B.; Datta, S.; Chakraborty, P.; Chatterjee, M.; Kannan, K.; Thygarajan, E. 1alpha, 25-dihydroxyvitamin D_3 prevents DNA damage and restores antioxidant enzymes in rat hepatocarcinogenesis induced by diethylnitrosamine and promoted by phenobarbital. *World J .Gastroenterol.*, **2004**, *10*(9), 1268-1275.

[179] Mason, R.S.; Sequeira, V.B.; Dixon, K.M.; Gordon-Thomson, C.; Pobre, K.; Dilley, A.; Mizwicki, M.T.; Norman, A.W.; Feldman, D.; Halliday, G.M.; Reeve, V.E. Photoprotection by 1alpha,25-dihydroxyvitamin D and analogs: further studies on mechanisms and implications for UV-damage. *J. Steroid. Biochem. Mol. Biol.*, **2010**, *121*(1-2), 164-168.

Received: April 16, 2012 **Revised: May 02, 2012** **Accepted: May 06, 2012**