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The Anti-Cancer and Anti-Inflammatory Actions of 1,25(OH)₂D₃

Els Vanoirbeek,

Laboratory for Experimental Medicine and Endocrinology (LEGENDO), Katholieke Universiteit Leuven, Herestraat 49 bus 901 O&NI, Leuven, Belgium, tel. +32 16 347145, fax +32 16 345934

Dr. Aruna V Krishnan,

Dept. of Medicine, Division of Endocrinology, Stanford University School of Medicine 300 Pasteur Drive, Stanford, CA 94305-5103, USA, tel: 650-725-2910, fax: 650-725-7085

Dr. Ir Guy Eelen,

Laboratory for Experimental Medicine and Endocrinology (LEGENDO), Katholieke Universiteit Leuven, Herestraat 49 bus 901 O&NI, Leuven, Belgium, tel. +32 16 347145, fax +32 16 345934

Dr. Lieve Verlinden,

Laboratory for Experimental Medicine and Endocrinology (LEGENDO), Katholieke Universiteit Leuven, Herestraat 49 bus 901 O&NI, Leuven, Belgium, tel. +32 16 347145, fax +32 16 345934

Prof. Dr. Roger Bouillon,

Laboratory for Experimental Medicine and Endocrinology (LEGENDO), Katholieke Universiteit Leuven, Herestraat 49 bus 901 O&NI, Leuven, Belgium, tel. +32 16 345970, fax +32 16 345934

Prof. Dr. David Feldman, and

Dept. of Medicine, Division of Endocrinology, Stanford University School of Medicine 300 Pasteur Drive, Stanford, CA 94305-5103, USA, tel: 650-725-2910, fax: 650-725-7085

Prof. Dr. Annemieke Verstuyf

Laboratory for Experimental Medicine and Endocrinology (LEGENDO), Katholieke Universiteit Leuven, Herestraat 49 bus 901 O&NI, Leuven, Belgium, tel. +32 16 346209, fax +32 16 345934

Els Vanoirbeek: Els.Vanoirbeek@med.kuleuven.be; Aruna V Krishnan: akrish@stanford.edu; Ir Guy Eelen: Guy.Eelen@med.kuleuven.be; Lieve Verlinden: Lieve.Verlinden@med.kuleuven.be; Roger Bouillon: Roger.Bouillon@med.kuleuven.be; David Feldman: dfeldman@stanford.edu; Annemieke Verstuyf: Mieke.Verstuyf@med.kuleuven.be

Abstract

Various epidemiological studies have shown an aetiological link between vitamin D deficiency and cancer incidence. The active metabolite of vitamin D, 1,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃], has potent anti-cancer activities both *in vitro* and *in vivo*. These anti-cancer effects are attained by regulating the transcription of numerous genes that are involved in different pathways to reduce tumorigenesis and are dependent on the cancer cell type. Besides reducing cell growth and inducing apoptosis, 1,25(OH)₂D₃ also inhibits angiogenesis and metastasis. Moreover, its potency to inhibit inflammation also contributes to its anti-tumoral activity. Here, we report the different ways in which 1,25(OH)₂D₃ interferes with the malignant processes that are activated in cancer cells.

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Correspondence to: Annemieke Verstuyf, Mieke.Verstuyf@med.kuleuven.be.

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Keywords

Vitamin D; Cancer; Proliferation; Apoptosis; Metastasis; Angiogenesis; Inflammation

Introduction

Several epidemiological studies have revealed a relationship between an individual's vitamin D status and the risk of developing cancer [1]. The vitamin D receptor (VDR) is essential to initiate the genomic and non-genomic signaling pathways that are induced by $1,25(\text{OH})_2\text{D}_3$ and have been shown to play a critical role in its anti-cancer activity. This is evidenced by multiple studies which report that VDR expression is gradually reduced when the tumor disease stage advances [2] and by the existence of VDR polymorphisms that are associated with cancer risk [3]. Furthermore, knockout studies in mice show that optimal VDR signaling is important to suppress carcinogenesis [4, 5]. Importantly, locally circulating $25(\text{OH})\text{D}_3$ can be converted into $1,25(\text{OH})_2\text{D}_3$ by the enzyme 25-hydroxyvitamin D-1 α -hydroxylase (CYP27B1), which is not only expressed in kidney but in many other tissues such as colon, breast, prostate, placenta and different cells of the immune system. In some tumors, the anti-cancer activities of $1,25(\text{OH})_2\text{D}_3$ include the effects of its local synthesis, which induces autocrine/paracrine actions within the tumors in addition to the effects of circulating $1,25(\text{OH})_2\text{D}_3$ acting in an endocrine capacity.

The molecular mechanisms of $1,25(\text{OH})_2\text{D}_3$ actions on cancer cells have been extensively studied, and include the regulation of cell proliferation and apoptosis [6, 7]. Moreover, $1,25(\text{OH})_2\text{D}_3$ attenuates the ability of cancer cells to metastasize and inhibits angiogenesis as well as the inflammatory process associated with cancer formation. The insights into these anti-cancer activities of $1,25(\text{OH})_2\text{D}_3$ will be discussed in this chapter.

Anti-Cancer Actions of $1,25(\text{OH})_2\text{D}_3$

a. Anti-proliferative actions

In 1981, Colston *et al.* reported the anti-proliferative effect of $1,25(\text{OH})_2\text{D}_3$ in malignant melanoma cells [8]. In that same year, Abe *et al.* described the pro-differentiating effects of $1,25(\text{OH})_2\text{D}_3$ on myeloid leukemia cells [9]. Since then, the molecular mechanisms by which $1,25(\text{OH})_2\text{D}_3$ mediates anti-cancer effects have been shown to involve multiple pathways and in some cases to be cell type-specific. Nevertheless, in most cell types that express a functional VDR, exposure to $1,25(\text{OH})_2\text{D}_3$ results in the accumulation of cells in the G0/G1 phase of the cell cycle. The transition from G- to S-phase is controlled by the pocket proteins retinoblastoma (Rb), p107 and p130. The phosphorylation status of these proteins determines their association with members of the E2F family of transcriptional regulators that play a pivotal role in mediating gene expression during cell proliferation. In quiescent and early G1 cells E2F4 and E2F5 act as transcriptional repressors by associating with p107 and p130. In proliferating cells, pocket proteins are phosphorylated. Upon release by Rb, E2Fs 1–3 function as transcriptional activators and induce the transcription of target genes that regulate cell cycle progression (e.g. cyclin E). Dephosphorylation of Rb by $1,25(\text{OH})_2\text{D}_3$ results in the modulation of the expression and the activity of a number of genes. In general, activated cyclin/cyclin dependent kinase (CDK) complexes phosphorylate Rb, and a loss of their kinase activity generates hypophosphorylated Rb. Both the expression of cyclins (D1, E, A) and CDKs (2,4,6) can be decreased by $1,25(\text{OH})_2\text{D}_3$, albeit in a cell-type type specific way [10].

More upstream, CDK Inhibitors (CDKIs) regulate the activity of the cyclin/CDK complex. The expression of the CDKIs p19, p21 and p27 are increased after treatment with

1,25(OH)₂D₃, although their importance in the 1,25(OH)₂D₃-induced cell cycle arrest is not yet completely established. The effect appears to be dependent on the cell type. In different cancer cell lines, increased p27 protein levels are attained by secondary effects that expand the half-life of the protein. Treatment with 1,25(OH)₂D₃ reduces phosphorylation of Thr187 of p27, which is important for its recognition by the ubiquitin-ligase SKP2. A reduced phosphorylation thus renders the p27 protein less prone to degradation. Also the protein levels of SKP2 are directly decreased by 1,25(OH)₂D₃ [11]. Moreover, in HL60 cells, 1,25(OH)₂D₃ controls p27 levels by decreasing the expression of miR181s, a microRNA known to target p27 [12]. The upregulation of p21 mRNA and protein by 1,25(OH)₂D₃ on the other hand seems to be a direct effect in some cells. Three functional VDREs are present in the proximal promoter of the gene [13]. In addition, in RWPE-1 prostate epithelial cells, VDR also epigenetically regulates p21 expression by generating histone modifications in the promoter. Moreover, 1,25(OH)₂D₃ regulates miR-106b expression to control p21 expression levels [14].

Several other growth regulating pathways are affected by 1,25(OH)₂D₃. The EGFR signaling cascade is inhibited by 1,25(OH)₂D₃ because the hormone targets the ligand-activated EGFR to early endosomes [15]. Furthermore, a consensus VDRE is present in the EGFR promoter. The ligand-activated VDR, together with a yet unidentified nuclear partner, binds this site and represses EGFR expression through displacement of the transcriptional activator Sp1 from its binding site in the promoter [16].

The anti-proliferative action of 1,25(OH)₂D₃ also involve down-regulation of the mitogenic insulin-like growth factors (IGFs) and up-regulation of factors that control their activity, IGF binding proteins (IGFBPs) [17]. In addition, IGFBPs also have IGF-independent actions and can directly inhibit cancer growth and promote apoptosis [18]. A screening of the six IGFBPs showed that only IGFBP 1, 3 and 5 are direct targets of 1,25(OH)₂D₃ [19]. Moreover, IGFBP3 up-regulation by 1,25(OH)₂D₃ is important for the induction of p21 expression in some cancer cells where it contributes to growth inhibition [20]. Other tumor suppressing signaling cascades, such as TGFβ secretion and TGFβ-receptor II expression, are induced by 1,25(OH)₂D₃ and can play an important role in the growth reducing action of 1,25(OH)₂D₃ [21].

b. Apoptosis

The ability of 1,25(OH)₂D₃ to induce apoptosis has been demonstrated in breast (BCa), colon and prostate (PCa) cancer cells. Although the mechanisms of the apoptotic effect are not fully unraveled, 1,25(OH)₂D₃ can trigger the intrinsic, mitochondria-dependent pathway that induces cell death. Depending on the cell type, 1,25(OH)₂D₃ decreases the expression of the anti-apoptotic factors (Bcl-2, Bcl-X_L) and/or increases the pro-apoptotic equivalents (Bax, Bak), thus directing the cells more towards apoptosis than to survival [22].

In LNCaP human PCa cells, 1,25(OH)₂D₃-induced apoptosis is accompanied by a down-regulation of anti-apoptotic proteins Bcl-2 and Bcl-X_L, while other proteins important in apoptotic control, Bax, Mcl-1, and Bcl-X_S, are unaffected. Overexpression of Bcl-2 blocks 1,25(OH)₂D₃-stimulated apoptosis and reduces, but does not eliminate, 1,25(OH)₂D₃-induced growth inhibition [23].

Additionally, 1,25(OH)₂D₃ sensitizes cancer cells to different cytotoxic substances and radiation. For example, BCa cells are more susceptible to apoptosis, triggered by reactive oxygen species (ROS), when they are pretreated with 1,25(OH)₂D₃. Both caspase-dependent and -independent pathways are enhanced which results in increased mitochondrial membrane damage and cytochrome c release [24]. Likewise, 1,25(OH)₂D₃ increases TNFα-induced cytotoxicity [25]. Further, 1,25(OH)₂D₃ increases the intracellular calcium levels by

depleting calcium stores in the endoplasmic reticulum and by activating calcium entry from the extracellular space. This increase is sustained in MCF-7 BCa cells since they do not express calbindin-D(28k), which buffers intracellular calcium levels in normal cells. As a result, mu-calpain and caspase-12 proteases are activated and generate an apoptotic response [26].

Furthermore, activation of mitogen activated protein kinase (MAPK) pathways p38 and JNK by $1,25(\text{OH})_2\text{D}_3$ is implicated in $1,25(\text{OH})_2\text{D}_3$ -induced apoptosis in BCa cells, while ERK1/2 signaling is not activated [27]. Moreover, attenuation of the PI3 kinase pathway is accomplished in gastric cancer cells by inducing the expression of PTEN, a negative regulator of the kinase and is associated with an increase in apoptosis. The PTEN promoter contains a functional VDRE and its upregulation is thus realized through the VDR [28].

$1,25(\text{OH})_2\text{D}_3$ down-regulation of mitogenic pathways, such as those induced by IGFs, is another mechanism to induce apoptosis. This is achieved by a decrease in the IGF receptors, by stimulation of the IGF binding proteins (IGFBPs) that sequester IGF and by independent IGFBP-3 hormonal actions [17, 29]. Additionally, down-regulation of Iroquois homeobox gene 5 (*Irx5*) by $1,25(\text{OH})_2\text{D}_3$ has been shown in LNCaP and MCF-7 cells and results in an induction of apoptosis, which is partially mediated by increasing p53 expression [30].

The mechanism by which $1,25(\text{OH})_2\text{D}_3$ induces apoptosis varies, depending on the cancer type. Also, within different normal and malignant breast epithelial cell lines $1,25(\text{OH})_2\text{D}_3$ provokes cell death through different mechanisms. In non-malignant MCF-12A cells, $1,25(\text{OH})_2\text{D}_3$ activates caspase 7 and 8, while in malignant MCF-7 cells, there is no caspase activation that has been identified. Nevertheless, in both cell lines, cleavage of downstream target PARP-1 is present, albeit more rapid in MCF-12A cells. This suggests differential mechanisms in both cell lines to induce PARP-1 cleavage and to activate apoptosis [27].

Conceivably, cancer cells evolve different mechanisms to escape cell death and some of these might help some cancer cells to more efficiently evade the proapoptotic effects of $1,25(\text{OH})_2\text{D}_3$. This may explain why different cell lines have different apoptotic responses to $1,25(\text{OH})_2\text{D}_3$. One such mechanism could be the expression of a mutant p53 protein (mutp53). In contrast with wtp53, mutp53 is characterized by gain-of-function capacities and can bind the VDR, resulting in altered VDR-induced gene expression. In general, the expression of survival genes is enhanced, while the expression of proapoptotic genes is reduced. This way, mutp53 converts the VDR to an anti-apoptotic agent [31]. In support of this hypothesis, cell lines such as MDA-MB-231 contain a mutated p53 gene and are resistant to $1,25(\text{OH})_2\text{D}_3$ -induced cell death [27]. Although $1,25(\text{OH})_2\text{D}_3$ induces apoptosis in glioma, melanoma, and mammary cancer, normal astrocytes, melanocytes, and mammary cells are not sensitive to its apoptotic activity. In addition, keratinocytes treated with UV or chemotherapy with the aim of inducing cell death are even less responsive to these assaults after treatment with $1,25(\text{OH})_2\text{D}_3$. Likewise, primary melanocytes treated with TNF α or UV are protected from cell death by $1,25(\text{OH})_2\text{D}_3$ [32].

Induction of apoptosis is often not a component of the growth arresting effects of $1,25(\text{OH})_2\text{D}_3$. Most likely, the cancer cells that are not induced to undergo apoptosis are instead directed to a more advanced differentiation status by $1,25(\text{OH})_2\text{D}_3$. The decision of whether to induce apoptosis or differentiation most likely depends on the presence of other factors. For instance, calbindin-D(28k), a $1,25(\text{OH})_2\text{D}_3$ -induced gene, is possibly important in determining the response to $1,25(\text{OH})_2\text{D}_3$ signaling. This calcium binding protein buffers the intracellular calcium concentrations that are raised by $1,25(\text{OH})_2\text{D}_3$, and can therefore inhibit apoptosis. But independent of this buffering function, calbindin-D(28k) is also able to directly bind and inhibit caspase 3, resulting in reduced levels of PARP-1 cleavage and it

can, moreover, inhibit the calcium-mediated activation of calpain, leading to reduced processing of Bax. Thus Calbindin-D(28k) protects cells from caspase-dependent and -independent apoptosis signaling [33]. Additionally, over-expression of calbindin-D(28-k) in human endometrial cells prevents the activation of proapoptotic signaling by Bax and p53, that is generated after treatment with H₂O₂ [34].

Finally, recent studies suggest that 1,25(OH)₂D₃ promotes autophagic cell death [35]. Initiation of autophagy by 1,25(OH)₂D₃ relies on the increase of intracellular calcium that activates Ca²⁺/calmodulin-dependent kinase kinase-β (CaMKK-β), followed by the activation of AMP-activated kinase (AMPK). AMPK further inhibits mammalian target of rapamycin complex 1 (mTORC1), important for the initiation of the autophagic process. Downstream of mTORC1, several Atg proteins are activated. Other compounds can trigger autophagy in cancer cells by stimulating purinergic receptors in an ATP-mediated way, leading to the activation of a calcium dependent signaling cascade that initiates autophagy [36].

c. Angiogenesis and metastasis

In vitro and *in vivo* experiments showed that 1,25(OH)₂D₃ can also modulate angiogenesis, a key step in continued tumor growth and progression. The formation of new blood vessels is an important ability of malignant cells to guarantee their oxygen supply, and tumor suppressors often interfere with this process. Vascular endothelial growth factor (VEGF) is a key mediator that positively regulates the formation of new vessels. The anti-angiogenic effect of 1,25(OH)₂D₃ has been shown *in vitro* using different assays. 1,25(OH)₂D₃ inhibits the proliferation of endothelial cells [37] and reduces VEGF-induced endothelial cell sprouting and elongation. Moreover, the formation of networks of elongated endothelial cells within 3D collagen gels is hampered. Additionally, the anti-angiogenic activity of 1,25(OH)₂D₃ was demonstrated *in vivo* in a tumor transplantation model system in which animals treated with 1,25(OH)₂D₃ produced tumors that were less vascularized than the control treated mice [38]. TRAMP (Transgenic Adenocarcinoma of the Mouse Prostate)-2 tumors established in VDR knockout mice had enlarged vessels and increased vessel volume. Moreover, the expression of pro-angiogenic factors was increased compared to the tumors in wild-type mice, suggesting an inhibitory role for VDR and 1,25(OH)₂D₃ in tumor angiogenesis [39]. At the molecular level, the effects of 1,25(OH)₂D₃ are mediated by decreasing the expression of hypoxia-inducible factor-1 (HIF-1), a transcription factor that directly induces VEGF expression [40].

Elimination of blood vessels is further important to reduce the possibility of cancer cells from invading the circulatory system and metastasizing to secondary sites. Moreover, 1,25(OH)₂D₃ also directly reduces the migration and invasion capacity of cancer cells. This was shown with *in vitro* assays using Boyden chambers [41] and involves modulation of adhesion to the extracellular matrix. Both laminin, a component of the extracellular matrix, and its receptors integrins α6 and β4 are down-regulated after 1,25(OH)₂D₃ administration. 1,25(OH)₂D₃-induced disruption of the interaction between these molecules results in reduced migration and invasion [41]. Moreover, the adhesion of circulatory cancer cells to endothelial cells in blood vessels is associated with their capacity to metastasize. This heterotypic type of adhesion is prevented by 1,25(OH)₂D₃ in LNCaP cells through induction of E-cadherin, which increases homotypic adhesion [42]. In SW480-ADH colon cancer cells, the induction of E-cadherin by 1,25(OH)₂D₃ is moreover associated with sequestration of nuclear β-catenin at the plasma membrane [43]. As a consequence, β-catenin is exported out of the nucleus, and its transcriptional activity is reduced. Additionally, 1,25(OH)₂D₃ increases the binding of the VDR to β-catenin, resulting in a decreased interaction with Tcf-4 and thus decreased transcription of target genes. All together, these changes induce differentiation of colon cancer cells. Nevertheless, advanced colon cancer cells are often

resistant to $1,25(\text{OH})_2\text{D}_3$ treatment, possibly due to overexpression of snail transcription factors, which downregulate VDR expression [44]. $1,25(\text{OH})_2\text{D}_3$ reduces also the expression of the adhesion molecule carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1), which is highly expressed on endothelial cells, resulting in decreased metastasis [45].

Proteases degrade the extracellular matrix and consequently allow the cancer cells to invade through the matrix. The proteases that are involved in metastasis can be subdivided into three different classes: matrix metalloproteinases (MMPs), plasminogen activators (PAs) and cathepsins (CPs). Specific inhibitors are secreted to keep their activity in balance in normal tissues. Tissue inhibitors of metalloproteinases (TIMPs) are the natural counterparts of the MMPs [46], PAs are negatively regulated by plasminogen activator inhibitors (PAIs) [47], and cathepsins by cathepsin inhibitors (CIs) [48]. Treatment of cancer cells with $1,25(\text{OH})_2\text{D}_3$ reduces the activity of MMP-9 and CPs, but does not affect PAs, at least in some studies. Moreover the activity of TIMP-1 and CIs is increased [49]. The combined effect of $1,25(\text{OH})_2\text{D}_3$ on the protease actions results in inhibition of metastasis.

Furthermore, transcription factor Stat3 is constitutively activated in PCa and is related to a more advanced stage of the disease. Overexpression of Stat3 in PCa cells stimulates the formation of lamellipodia and decreases cell clustering. These morphological changes resemble epithelial-to-mesenchymal transition and are consistent with the increased motility of the cells and the higher potential to metastasize *in vivo* [50]. Multiple studies showed that $1,25(\text{OH})_2\text{D}_3$ interferes with Stat3 phosphorylation and activation and it was suggested that this activity may be an important factor in reducing cancer cell metastasis [51]. Additionally, rapid non-genomic activation of the JNK/MAPK pathway was associated with decreased invasiveness of PCa cells [52].

Finally, the bone microenvironment is especially hospitable to metastasis from many cancers, including BCa and PCa [53]. It has been shown that bone resorption is crucial for cancer cell metastasis and survival and this resorption can be modulated by $1,25(\text{OH})_2\text{D}_3$ and calcium [54]. Bone metabolism can influence the metastasis of primary tumors at different levels. For example, cancer cells can be actively recruited through promoting adhesion to endothelial cells within the blood vessels of bone. By increasing the blood flow, this process can be modulated by the bone metabolic rate. It is however more likely that cancer cells are passively entrapped within the bone, a tissue that is able to support survival and proliferation of BCa and PCa cells, apparently because it provides the necessary growth factors. These are released from the bone mineral matrix during the osteoclastic bone resorption [55]. As a consequence, blocking bone resorption inhibits tumor growth. The use of anti-resorption drugs, which target the osteoclast, for the treatment of BCa and PCa is currently being clinically investigated in patients [56]. In contrast, enhanced growth of BCa cells implanted in the tibia of $1,25(\text{OH})_2\text{D}_3$ -deficient mice has been observed. This is partially due to an increase in bone resorption [57].

d. Non-genomic actions of $1,25(\text{OH})_2\text{D}_3$

In addition to the activation of genomic pathways, $1,25(\text{OH})_2\text{D}_3$ also triggers rapid, non-genomic responses that activate a transmembrane signaling cascade [58]. Some studies, performed in VDR knockout mice, suggest that VDR expression is needed to induce rapid responses [59], while other studies contradict this finding [60]. Furthermore, phosphorylation of the VDR has been suggested to modify the actions of the receptor. Treatment with $1,25(\text{OH})_2\text{D}_3$ results in activation of MAPKs, which in turn leads to phosphorylation of transcription factors involved in either cell proliferation or apoptosis [27]. Moreover, activation of the p38 and JNK pathway stimulates VDR expression and increases $1,25(\text{OH})_2\text{D}_3$ -induced growth inhibition in BCa cells [61]. Photoprotection from

ultraviolet rays is another pathway considered to be mediated by non-genomic actions that may have importance for decreasing the DNA damage induced by sun exposure that leads to skin cancer [62].

Anti-inflammatory effects

Inflammation contributes to the development and progression of many cancers [63]. Recent research indicates that $1,25(\text{OH})_2\text{D}_3$ has anti-inflammatory actions that likely contribute to its beneficial effects in multiple cancers. Some of the molecular mechanisms underlying the anti-inflammatory actions of $1,25(\text{OH})_2\text{D}_3$ are depicted in Figure 1 and are discussed below.

a. Regulation of prostaglandin metabolism and signaling

Prostaglandins (PGs) promote carcinogenesis and cancer progression by stimulating cellular proliferation, inhibiting apoptosis, promoting angiogenesis and by activating carcinogens [64]. Cyclooxygenase-2 (COX-2), the enzyme responsible for PG synthesis is regarded as an oncogene and is an important molecular target in cancer therapy [65–67]. Increased COX-2 expression is one of the key steps in carcinogenesis [68]. Long-term use of non-steroidal anti-inflammatory drugs (NSAIDs), which inhibit COX enzyme activity, is associated with a decrease in death rate from several cancers [69]. COX-2 expression in PCa surgical specimens is inversely correlated with disease-free survival [70] and is an independent predictor of disease recurrence [71]. COX-2 protein levels are elevated in ~40% of invasive BCa [65]. COX-2 is significantly over-expressed in colorectal cancer (CRC) [67]. 15-hydroxyprostaglandin dehydrogenase (15-PGDH) is the enzyme that catalyzes the conversion of PGs to their corresponding 15-keto derivatives with reduced biological activity. 15-PGDH has been shown to act as a tumor suppressor in colon cancer [72] and BCa [73].

$1,25(\text{OH})_2\text{D}_3$ regulates the expression of several PG pathway genes in PCa cell lines and primary prostatic epithelial cells [74] as well as in BCa cells [75]. In these cells $1,25(\text{OH})_2\text{D}_3$ decreases the expression of COX-2 and increases that of 15-PGDH [74, 75] and consequently decreases the levels of biologically active PGs and limits growth stimulation due to PGs. In PCa cells $1,25(\text{OH})_2\text{D}_3$ also decreases the expression of EP and FP PG receptors and thereby inhibits PG signaling [74]. Cumulatively these actions contribute to the suppression of the proliferative and angiogenic stimuli provided by PGs in malignant cells by $1,25(\text{OH})_2\text{D}_3$. Importantly, combinations of $1,25(\text{OH})_2\text{D}_3$ with NSAIDs exhibit synergistic enhancement of the inhibition of PCa cell growth [74]. A single arm, open label phase II clinical study investigated the effects of a combination of high dose $1,25(\text{OH})_2\text{D}_3$ and the non-selective NSAID naproxen in patients with early recurrent PCa and demonstrated a beneficial effect of the prolongation of serum prostate specific antigen (PSA) doubling time in 75% of the patients [76]. COX-2 generated PGE_2 also exerts pro-angiogenic effects due to its action to increase HIF-1 α protein synthesis in cancer cells [77]. Suppression of COX-2 expression is therefore an important additional mechanism by which $1,25(\text{OH})_2\text{D}_3$ inhibits angiogenesis.

b. Inhibition of the PG pathway by $1,25(\text{OH})_2\text{D}_3$ and suppression of estrogen synthesis and signaling in BCa

The $1,25(\text{OH})_2\text{D}_3$ -mediated decrease in COX-2 expression is especially important in the BCa setting because, as discussed above, PGs are major stimulators of aromatase expression as well as stimulating proliferation, angiogenesis and invasion. In tumor samples from BCa patients there is a tight coupling between the expression levels of COX-2 and aromatase, the enzyme that catalyzes the synthesis of estrogens from androgenic precursors [78].

Aromatase expression in the breast is critical for the progression of estrogen receptor (ER)-

positive BCa in postmenopausal women. While BCa cells express aromatase and have the capacity to synthesize estrogens, in the normal breast, aromatase is primarily expressed in the stromal mesenchymal cells of the breast adipose tissue (adipose stromal cells, ASCs). In normal breast adipose tissue aromatase transcription is primarily driven by the tissue specific promoter I.4 [79]. However, in the presence of BCa, the transcription switches predominantly from promoter I.4 to promoters I.3 and II, both in the cancerous epithelial cells and the surrounding ASCs [79]. $1,25(\text{OH})_2\text{D}_3$ regulates the expression of aromatase in a tissue-selective manner [75]. $1,25(\text{OH})_2\text{D}_3$ significantly decreases aromatase expression by a direct transcriptional repression of promoter II/I.3 in human BCa cells and in preadipocytes and increases it in osteoblastic cells [75]. $1,25(\text{OH})_2\text{D}_3$ also decreases aromatase expression in xenografts of human BCa cells established in immunocompromised mice and in the mammary adipose tissue surrounding the xenograft tumors [75]. Both promoters I.3 and II are responsive to cAMP and are significantly stimulated by PGE_2 [80, 81]. Therefore $1,25(\text{OH})_2\text{D}_3$ -mediated reduction in PG levels in BCa cells provides an important second, indirect mechanism for its suppressive effect on aromatase expression. The growth stimulating actions of estrogen require the presence of $\text{ER}\alpha$, which mediates BCa proliferation in response to estrogens. $1,25(\text{OH})_2\text{D}_3$ down-regulates $\text{ER}\alpha$ expression in BCa cells [82–85] by a direct transcriptional repression of the $\text{ER}\alpha$ gene [84, 85]. Thus by reducing both estrogen synthesis and down-regulating $\text{ER}\alpha$ levels, $1,25(\text{OH})_2\text{D}_3$ attenuates the mitogenic stimulus provided by estrogens in BCa.

Aromatase inhibitors (AIs) have become the major therapeutic agents to treat ER-positive BCa in postmenopausal women after primary therapy [86]. Combinations of $1,25(\text{OH})_2\text{D}_3$ with AIs exhibited enhanced growth inhibitory effects in BCa cell cultures [75]. AIs inhibit estrogen synthesis globally and therefore have a detrimental effect at sites such as bone where normal estrogen function is required for the maintenance of bone homeostasis. The development of selective aromatase modulators (SAMs) that inhibit aromatase expression in breast, but allow unimpaired estrogen synthesis at other desirable sites such as bone, would have great utility in BCa therapy [79]. $1,25(\text{OH})_2\text{D}_3$ acts as a SAM, decreasing aromatase expression in BCa cells and breast ASCs [75], while increasing it in bone cells [75, 87]. Thus it has the potential to ameliorate the AI-induced side effect of osteoporosis when used in combination with an AI in BCa patients.

c. Induction of MAP kinase phosphatase 5 (MKP5) and inhibition of stress-activated kinase signaling

In normal human prostate epithelial cells $1,25(\text{OH})_2\text{D}_3$ increases the expression of MKP5, a member of the dual specificity MKP family of enzymes that dephosphorylate, and thereby inactivate, MAPKs [88]. This action leads to downstream anti-inflammatory responses by causing the dephosphorylation and inactivation of the p38 stress-induced kinase, resulting in a decrease in the production of pro-inflammatory cytokines such as interleukin-6 (IL-6) [88]. Interestingly, $1,25(\text{OH})_2\text{D}_3$ up-regulation of MKP5 was seen only in primary cells derived from normal prostatic epithelium and primary localized adenocarcinoma but not in the established PCa cell lines derived from PCa metastasis, suggesting that a loss of MKP5 might occur during PCa progression, as a result of a selective pressure to eliminate the tumor suppressor activity of MKP5 and/or $1,25(\text{OH})_2\text{D}_3$.

d. Inhibition of NF κ B activation and signaling

NF κ B comprises a family of inducible transcription factors ubiquitously present in all cells that are important regulators of innate immune responses and inflammation [89]. In contrast to normal cells many malignant cells have elevated levels of active NF κ B [90]. $1,25(\text{OH})_2\text{D}_3$ directly modulates basal and cytokine-induced NF κ B activity in many cells [91, 92]. Mice lacking the VDR have reduced levels of the NF κ B inhibitory protein I κ B α

[93]. The addition of a VDR antagonist to colon cancer cells up-regulates NF κ B activity by decreasing the levels of I κ B α [94]. 1,25(OH) $_2$ D $_3$ inhibits NF κ B signaling in prostate cells and thereby decreases the levels of the angiogenic and pro-inflammatory cytokine IL-8 [49]. In addition 1,25(OH) $_2$ D $_3$ also indirectly inhibits NF κ B signaling by up-regulating the expression of IGFBP-3, which interferes with NF κ B signaling in PCa cells [29]. 1,25(OH) $_2$ D $_3$ also significantly enhances the sensitivity of PCa cells to ionizing radiation by selectively suppressing radiation-mediated RelB activation [95]. Thus 1,25(OH) $_2$ D $_3$ may serve as an effective agent for sensitizing PCa cells to radiation therapy via suppression of the NF κ B pathway.

e. The role of anti-inflammatory effects of 1,25(OH) $_2$ D $_3$ in cancer chemoprevention

As discussed above, current perspectives suggest that inflammation plays a role in the development of cancer. De Marzo et al [96] have proposed that areas of proliferative inflammatory atrophy (PIA) in the prostate, which are associated with acute or chronic inflammation, are precursors of prostate intraepithelial neoplasia (PIN) and PCa. The epithelial cells in PIA lesions exhibit many molecular signs of stress including elevated expression of COX-2 [97]. Inflammatory bowel disease (IBD) is associated with the development of CRC [98]. Since 1,25(OH) $_2$ D $_3$ exhibits significant anti-inflammatory effects, it has the potential to be useful as a chemopreventive agent in several cancers. In Nkx3.1; Pten mutant mice, which recapitulate stages of prostate carcinogenesis from PIN lesions to adenocarcinoma, 1,25(OH) $_2$ D $_3$ significantly reduces the progression of low grade PIN to a high grade PIN thereby inhibiting the development of invasive cancer [99]. 1,25(OH) $_2$ D $_3$ is more effective when administered before, rather than subsequent to the initial occurrence of PIN.

1,25(OH) $_2$ D $_3$ also plays an important role in the normal development of mammary glands, where it functions to oppose estrogen driven proliferation and to maintain differentiation [100]. Several studies using various rodent models of chemical carcinogen-induced BCa [reviewed in [100]] have concluded that 1,25(OH) $_2$ D $_3$ exerts a beneficial effect in preventing the growth of tumors induced by the carcinogens suggesting that it might be useful in BCa chemoprevention [100]. In the case of CRC, a study in the APC $_{(min)}$ mouse model has demonstrated that both vitamin D and calcium individually exert inhibitory effects on the development of polyps and exhibit a synergistic effect when used together [101].

Summary

1,25(OH) $_2$ D $_3$ is a hormone with powerful anti-cancer and anti-inflammatory activities. It interferes with carcinogenesis via different mechanisms in a cell type specific way. Besides inducing a cell cycle block to reduce growth, cell death is provoked by mediating the expression of pro- and anti-apoptotic genes. This leads to apoptosis through caspase dependent and independent pathways. The effects are realised either by regulation of gene transcription or by activation of rapid non-genomic signaling cascades. Furthermore, angiogenesis and metastasis are reduced via different mechanisms. Additionally, anti-inflammatory effects including inhibition of PG synthesis and NF κ B and MAPK activity, further contribute to the anti-cancer effects of 1,25(OH) $_2$ D $_3$. Collectively these data demonstrate potent anti-cancer activity in a variety of cell and animal models. The compelling benefits seen in these pre-clinical studies suggest that clinical trials in cancer patients are warranted. Recognition of vitamin D deficiency and supplementation to optimum levels may well have chemoprevention activity and the ability to reduce cancer risk. Treatment with 1,25(OH) $_2$ D $_3$ or its analogs, either alone or in combination with active anti-cancer drugs, may be able to prevent cancer initiation and/or delay cancer progression.

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Practice points

- Specific guidelines for vitamin D supplementation exist for maintenance of bone homeostasis and fall prevention, however it is not known if these doses are sufficient for the prevention of cancer development.
- Preclinical studies suggest that $1,25(\text{OH})_2\text{D}_3$ is a potent anti-cancer agent but clinical trials in cancer patients evaluating $1,25(\text{OH})_2\text{D}_3$ and its analogs as agents that prevent and/or delay cancer progression, are needed.

Research agenda

- Prospective, randomized trials of cancer prevention in people by vitamin D supplementation are warranted.
- Further controlled trials are needed to investigate the efficacy of $1,25(\text{OH})_2\text{D}_3$ treatment of cancer patients used alone and in combination with other anti-cancer drugs.
- The development and the screening of non-calcemic analogs in patients would be desirable.

Figure 1. Overview of the molecular mechanisms underlying the anti-inflammatory actions of $1,25(\text{OH})_2\text{D}_3$. Figure adapted from Krishnan AV, Feldman D; Ann. Rev. Pharmacol. Toxicol. 51: 311–336, 2011 with permission.