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REVIEW ARTICLE

Vitamin D in cancer chemoprevention

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Abstract

Context: There is increasing evidence that Vitamin D (Vit D) and its metabolites, besides their well-known calcium-related functions, may also exert antiproliferative, pro-differentiating, and immune modulatory effects on tumor cells *in vitro* and may also delay tumor growth *in vivo*.
Objective: The aim of this review is to provide fresh insight into the most recent advances on the role of Vit D and its analogues as chemopreventive drugs in cancer therapy.
Methods: A systematic review of experimental and clinical studies on Vit D and cancer was undertaken by using the major electronic health database including ISI Web of Science, Medline, PubMed, Scopus and Google Scholar.
Results and conclusion: Experimental and clinical observations suggest that Vit D and its analogues may be effective in preventing the malignant transformation and/or the progression of various types of human tumors including breast cancer, prostate cancer, colorectal cancer, and some hematological malignances. These findings suggest the possibility of the clinical use of these molecules as novel potential chemopreventive and anticancer agents.

Keywords

Calcitriol, cancer prevention, Vitamin D analogues

History

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Introduction

The clinical use of cytotoxic drugs has had a significant impact on neoplastic diseases. However, their therapeutic effectiveness is limited due to their narrow therapeutic index and the onset of chemoresistance. Therefore, many efforts are currently being directed to finding new therapeutic options that may overcome these problems. In this scenario, chemoprevention, i.e., the use of natural, synthetic, or biological substances to reverse, suppress, or prevent the development and progression of malignant diseases, holds great promise (Davis & Wu, 2012). In this context, a consistent body of investigation provides evidence that Vitamin D (Vit D) and its metabolites, in addition to its well-known involvement in calcium homeostasis, also appears to be effective in preventing the malignant transformation and the progression of various types of human tumors (Krishnan & Feldman, 2010; Nagpal et al., 2005; Vuolo et al., 2012). On one hand, experimental evidence indicates that these effects appear to be likely due to the antiproliferative, proapoptotic, and immunomodulatory activities with which these molecules are endowed (Krishnan & Feldman, 2010; Vanoirbeek et al., 2011). On the other hand, numerous epidemiological observations based on geographic variation in the incidence of cancer and\or mortality in relation to 18 different types of human cancer clearly demonstrate that Vit D exerts chemopreventive effects on at least three types of solid tumors at high risk of mortality, namely breast cancer (Khan et al., 2010), prostate cancer (Swami et al., 2011), and colorectal cancer (Pereira et al., 2012) and on squamous cell carcinoma (SCC) and some hematological malignances (Kim et al., 2012; Reddy, 2013). The aim of this paper is to provide insight into the most recent advances on the role of Vit D as chemopreventive drug in cancer therapy.

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Vit D chemical structure, biological functions, and metabolism

The Vit D complex includes a group of fat-soluble prohormones that contribute to maintaining calcium and phosphate homeostasis and bone and muscle integrity (Bouillon et al., 2008). On one hand, Vit D is known to be essential for the absorption of calcium and phosphate ions in the small intestine, their mobilization from the bone tissue, and their resorption in the kidney (Bouillon et al., 2008). These effects suggest an indirect involvement of this molecule in the regulation of the normal function of different tissues such as muscle contraction, nerve conduction, bone metabolism, and blood clotting (Bouillon et al., 2008). Interestingly, emerging evidence suggests that Vit D also appears to be implicated in the regulation of other important biological processes such as cell proliferation and differentiation (Gocek & Studzinski, 2009), immune response (Hewison, 2011), and insulin secretion (Teegarden & Donkin, 2009). Vit D is available in two main distinct forms: i.e., Vitamin D_2 (Vit D_2)

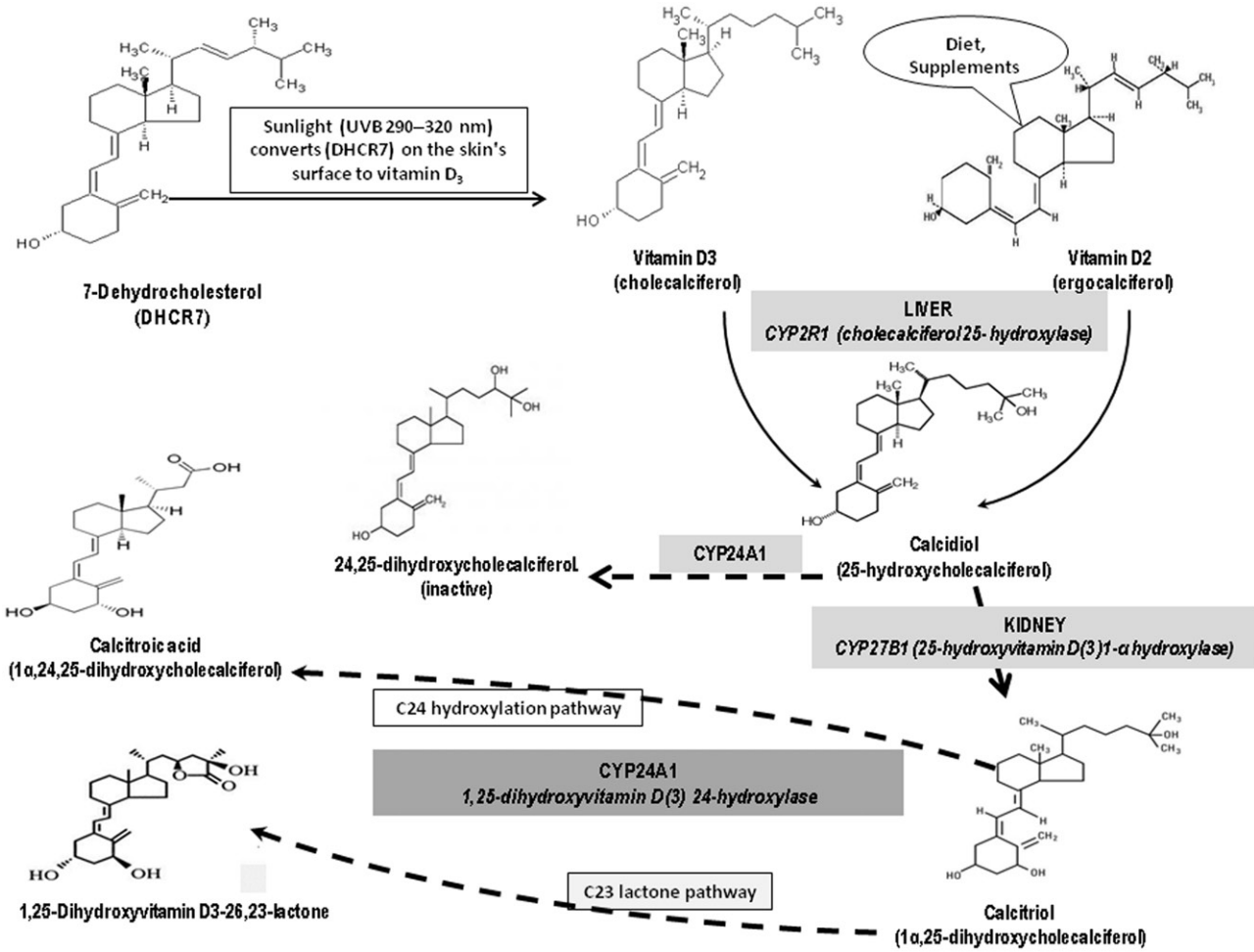

Figure 1. Synthesis and metabolism of secosteroids Vitamin D_3 and Vitamin D_2. In humans, cholecalciferol (Vitamin D_3) is synthesized from 7-dehydrocholesterol upon sunlight exposure. Vitamin D may also be obtained from dietary sources or supplements as ergocalciferol or Vitamin D_2. Vitamin D_3 binds to Vitamin D-binding protein (DBP) in the bloodstream and then is transported to the liver where it is first converted by the enzyme 25-hydroxylase (CYP2R1) to 25-hydroxyvitamin D [25(OH)D]. This molecule is converted by the renal enzyme 1-α hydroxylase (CYP27B1) to 1,25 dihydroxycholecalciferol (calcitriol), which is the active form of Vitamin D. The rate limiting step in catabolism is the degradation of $25(OH)D_3$ and $1,25(OH)_2D_3$ to $24,25(OH)D_3$ and $1,24,25(OH)_2D_3$, respectively, which occurs through 24-hydroxylation by mitochondrial 1,25-dihydroxyvitamin D_3 24-hydroxylase, (CYP24A1). $24,25(OH)D_3$ and $1,24,25(OH)_2D_3$ are excreted in this form.

(ergocalciferol) and Vitamin D_3 (Vit D_3) (cholecalciferol) (Figure 1). Vit D_2 is synthesized in plants, yeasts, and fungi whereas Vit D_3 is of animal origin (Bouillon et al., 2008; Jäpelt & Jakobsen, 2013) (Figure 1). Vit D_2 is derived from ergosterol, which is turned into viosterol by ultraviolet (UV) light and then converted into ergocalciferol. In this form, Vit D_2 can be ingested from the diet and from supplements (Figure 1). On the other hand, the exposure of skin to ultraviolet B radiation (UVB; 290–320 nm) converts 7-dehydrocholesterol (DHCR7) to pre-vitamin D_3 (1,25-dihydroxycholecalciferol or calcitriol) which isomerizes to Vit D_3 (Figure 1) (Bouillon et al., 2008; Jäpelt & Jakobsen, 2013). In this form, Vit D_3 binds to Vit D-binding protein (DBP) and is then transported into the liver. Vit D complex molecules are known as secosteroids, namely steroids in which one of the rings of its cyclopentanoperhydrophenanthrene structure has a broken carbon–carbon bond. Vit D_2 and Vit D_3 differ in their side chains in that the side chain of the D_2 form additionally contains a double bond between carbons 22 and 23 and a methyl group on carbon 24 (Figure 1). The biosynthetic pathway of Vit D_3 involves the hepatic hydroxylation of the carbon atom in position 25 by four cytochrome P450 isoenzymes, i.e., CYP2R1, CYP2J2, and CYP3A4 isoforms and the mitochondrial CYP27A1 isoform (Figure 1) (Jones et al., 2014; Schuster, 2011). This first hydroxylation generates the main circulating form of Vit D, namely 25-hydroxyvitamin D_3 [25(OH)D] or calcidiol (Figure 1). Normal serum values of 25(OH)D are comprised between 25 and 130 nmol/L depending on the geographic location (Ross et al., 2011). This form reflects dietary sources as well as Vit D production by UV light on the skin (Ross et al., 2011). A second hydroxylation in the 1-α position occurs in the kidney, at the tubule proximale levels, where it leads to the formation of $1\alpha,25(OH)_2D_3$ (calcitriol) which is the most biologically active form of Vit D (Figure 1) (Jones et al., 2014; Schuster, 2011). Once synthesized in the kidney, via CYP27B1 (25-hydroxyvitamin D_3 1-α-hydroxylase), this active form enters the bloodstream and it is then transported, by specific binding proteins (VDBP) to distant target tissues (Bouillon et al., 2008) (Figure 1). Vit D activity is tightly regulated by metabolic processes mediated by the CYP24A1 isoform (1,25-dihydroxyvitamin D_3 24-hydroxylase) that converts $1\alpha,25(OH)_2D_3$ into 1,24,25-trihydroxycholecalciferol [$1\alpha,24,25(OH)_3D_3$] which has a lower affinity for Vit

D receptors (VDR) (Schuster, 2011). This molecule, then, undergoes a further metabolization to generate calcitroic acid which is excreted in this form (Figure 1). In contrast, expression levels of renal 1-α hydroxylase (CYP27B1), namely the enzyme which converts 1,25-hydroxycholecalciferol into 1,25-dihydroxycholecalciferol, are positively regulated by high calcium and phosphate levels parathyroid hormone (PTH), calcitonin, growth hormone, and insulin-like growth factor-I (IGF-I) (Henry, 2011). Conversely, low calcium and phosphate levels, fibroblast growth factor 23 (FGF23), and $1,25(OH)_2D_3$ itself function as negative regulators of this enzyme (Fukumoto, 2014). However, PTH and $1\alpha,25(OH)_2D_3$ have no effect on the expression and/or activity of extrarenal 1α-hydroxylase. Other rate-limiting steps in Vit D metabolism involve the modulation of CYP2R1 (Vit D 25-hydroxylase) activity, which is induced by decreased level of 25(OH)D and that of CYP24A1 which is induced by 25(OH)D and $1,25(OH)_2D_3$ (Bouillon et al., 2008; Schuster, 2011).

Vit D receptor: structure and functions

The biological effects induced by the active form of Vit D and its semisynthetic analogues are mediated by the vitamin D receptor (VDR), also known as NR1I1 receptor (Wang et al., 2012). This receptor belongs to the superfamily of the nuclear receptor (NR) that includes receptors for steroid hormone, retinoids, and thyroid hormones. VDR is located in the nucleus of a variety of target cells including cells of the immune system (Wang et al., 2012). Similar to other nuclear receptors, VDR shows a domain structure which is homologous to that of these receptors (Bouillon et al., 2008; Haussler et al., 2011). This domain can be functionally divided into three regions with well-characterized functions, i.e., (a) an aminoterminal region factor that binds a short N-terminal activation-function 1 (AF-1) domain (A/B) and that plays an important role in the VDR-mediated transactivation; (b) a central region that contains a DNA-binding domain (DBD) which interacts directly with the cellular DNA at the level of Vit D-response elements (VDREs). This region contains two Zn^{2+}-finger (C) portions consisting of four cysteine residues that coordinate a zinc atom; (c) a carboxy-terminal region that encompasses a multifunctional domain named ligand-binding domain (LBD), which may interact with different ligands such as retinoid X receptor (RXR) and the transcriptional regulatory factor AF-2 (Bouillon et al., 2008).

Vit D and regulation of gene expression

The effects induced by Vit D may occur in three different ways through the modulation of the expression of specific genes responsive to Vit D (Haussler et al., 2011, 2013; Kriebitzsch et al., 2009). In particular, the transcription of genes that are involved in the regulation of bone-remodeling processes, such as receptor activator of NF-kB ligand (RANKL), carbonic anydrase II, osteocalcin, osteopontin, or other genes, such as phospholipase C (PLP C), 24-hydroxylase or CYP3A4, β3 integrin, tumor suppressor p21, insulin growth factor-binding protein-3 (IGFB-3), can be positively regulated by a direct interaction with vitamin D response elements (VDREs) present in their promoter regions (Cao et al., 1993; Haussler et al., 2011, 2013). Conversely, Vit D, through the interaction with negative VDREs, may negatively regulate the expression of gene encoding for several pro-inflammatory cytokines such as interleukin-2 (IL-2) and interleukin-12 (IL-12), tumor necrosis factor α (TNF-α), interferon γ (IFN-γ), and/or growth factors and receptors such as epidermal growth factor receptor (EGFR), *c-myc*, and hormones involved in calcium homeostasis including parathyroid hormone (PTH), parathyroid hormone-related peptide (PTHrP), and *rel-B* (Haussler et al., 2011, 2013). Gene transcription may also be inhibited by the expression of genes that antagonize the effects of specific transcription factors, such as (NF)-aT and NF-kB (Haussler et al., 2013).

Vit D signal transduction pathways

To date, two major signal transduction pathways activated by Vit D in target cells have been identified, namely the so-called ''genomic pathway,'' where the Vit D nuclear receptor plays a major role, and the ''non-genomic signal transduction pathway'' (Haussler et al., 2011). This latter pathway triggers those responses mediated by Vit D that are faster than those induced following changes in gene expression. In this case, Vit D is supposed to interact directly with a receptor present in the plasma membrane (mVDR). This interaction induces rapid changes in intracellular calcium concentrations, alterations in membrane phospholipid metabolism, and activation of several signaling transduction pathways (Haussler et al., 2011). In order to ensure the full biological activity of $1,25(OH)_2D_3$ both pathways needed to be activated. Following the binding of $1,25(OH)_2D_3$ to VDR, the receptor is phosphorylated. In this form, VDR may promote the recruitment of its preferred dimerization partner, namely the nuclear receptor for 9-*cis* retinoic acid (RXR), thus forming a heterodimer that, in turn, binds to VDR responsive elements (VDREs) (Haussler et al., 2011). In the absence of its ligand, most of the Vit D receptors are located in the cytoplasm. However, upon interaction with $1,25(OH)_2D_3$ and the subsequent heterodimerization the complex migrates from the cytoplasm into the nucleus. The $1,25(OH)_2D_3$-VDR–RXR complex then interacts with DNA at VDREs level that is located in the classic promoter regions of responsive genes, near the transcription start site of the gene (Haussler et al., 2011, 2013). Downstream targets of these genes are implicated in mineral metabolism and in the regulation of other metabolic pathways including those involved in the immune response and cancer.

Effects of vitamin D on tumor progression

The prophylactic and therapeutic activities of Vit D toward the most common types of cancer have been extensively investigated either *in vitro* or *in vivo* (Khan et al., 2010; Leyssens et al., 2013; Pereira et al., 2012; Swami et al., 2011). The most striking results have been obtained following studies on breast cancer, prostate cancer, and colorectal cancer (Krishnan & Feldman, 2010; Leyssens et al., 2013; McCulloug et al., 2009). Experimental observations suggest that the chemopreventive effects of Vit D appear to be mainly due to its modulating activity on important biological functions such as cell proliferation, cell differentiation,

growth factors gene expression, signal transduction, and apoptosis (Gocek & Studzinski, 2009; Haussler et al., 2013; Samuel & Sitrin, 2008) (Table 1). The inhibiting effects of Vit D on tumor cell growth were first described by Colston et al. (1981) who showed for the first time a dose-dependent decrease of cell proliferation in melanoma cells treated with $1,25(OH)_2D_3$. The growth inhibiting activity of this molecule was subsequently observed in other tumor cell lines including breast, prostate, and colon cancer cells (Welsh, 2012). These studies also highlighted the presence of specific receptors with high affinity for $1,25(OH)_2D_3$ that appeared to be essential for the growth inhibitory activity exerted by Vit D (Welsh, 2012). In line with these observations, other *in vitro* studied reported that antisense oligonucleotides, which decreased the intracellular levels of VDR, reduced the sensitivity of tumor cells to the antiproliferative effects of $1,25(OH)_2D_3$ (Hedlund et al., 1996; Welsh, 2012). On the contrary, VDR overexpression resulted in a potentiation of cell growth arrest (Hedlund et al., 1996; Welsh, 2012; Zhuang et al., 1997). Interestingly, recent studies have shown that $1,25(OH)_2D_3$ may also affect ovarian cancer cells proliferation by decreasing human telomerase reverse transcriptase (hTERT) mRNA through a small non-coding RNA (Ikeda et al., 2003; Kasiappan et al., 2012). Consistent with these observations a recent experimental investigation has shown that, in ovarian tumor and ovarian cancer cell lines, microRNA-498 (miR-498) induced by $1,25OH_2D_3$ decreased hTERT mRNA expression, fostered cell death, and suppressed tumor growth (Kasiappan et al., 2012). Conversely, the ability of $1,25OH_2D_3$ to decrease hTERT mRNA and to suppress ovarian cancer growth was compromised in the absence of miR-498, following its depletion in cell lines and in tumor-bearing mice (Kasiappan et al., 2012). Finally, Vit D has been reported to foster several types of malignant cells to undergo differentiation toward more mature phenotypes or to induce cell death by triggering apoptosis according to the cell type (Gocek & Studzinski, 2009).

Effects of Vit D on cyclin/cycline-dependent kinase system

Many investigations undertaken with the aim of assessing a direct effect of $1,25OH_2D_3$ on the expression levels of genes encoding for intracellular inhibitors of the cell cycle demonstrate that this molecule may increase the expression of cyclin-dependent protein kinase (CDK) inhibitors p21 and p27, while it decreases the expression of cycline regulatory proteins such as cyclin-dependent kinase 2 (Cdk2) (Colston & Hansen, 2002; Hager et al., 2001; Wang et al., 1996; Yang & Burnstein, 2003). These phenomena ultimately lead to a growth arrest of cells in the G0/G1 phase. In addition, Vit D has been shown to inhibit human breast cancer and prostate cancer cell-cycle progression by blocking cells in G1/S transition (Istfan et al., 2007; Jensen et al., 2001). This effect appears to be due to the conversion of the retinoblastoma gene (*Rb*), which is a direct target of cyclin-CDK complexes, in its active hypophosphorylated form (Jensen et al., 2001). Furthermore, other *in vitro* studies on SCC cells show that a 30 h exposure of these cells to $1,25(OH)_2D_3$ induces the overexpression of *p18* tumor suppressor gene but not that of *p27* or *p19* gene, which regulates G1 progression, by forming a stable complex with CDK4 or CDK6 and by preventing the activation of CDK kinases (Gedlicka et al., 2006). However, in LNCaP human prostate cancer cell line, $1,25(OH)_2D_3$ induces a marked increase of *p21* gene while, in RWPE-1 prostate epithelial cells, VDR may epigenetically regulate *p21* gene expression by generating histone modifications in the promoter (Flores et al., 2010). Moreover, $1,25(OH)_2D_3$ may also regulate *p21* expression levels by modulating miR-106b expression (Thorne et al., 2011). Conversely, in MCF-7 human breast cancer cells $1,25(OH)_2D_3$ show minimal effects on the expression levels of mRNA coding for *p21* (Verlinden et al., 1998). These findings suggest that, according to the cell types, the growth inhibitory effect of $1,25(OH)_2D_3$ does not appear to be related to an activation of VDR- mediated *p21* gene transcription. In contrast, Swami et al. (2003) highlighted the fact that, in MCF-7 MDA-MB-231 estrogen receptor α positive [$ER\alpha^{(+)}$] and estrogen receptor α negative [$ER\ \alpha^{(-)}$] human breast cancer cells, the treatment with $1,25(OH)_2D_3$ induces different profiles of gene expression with a few overlapping genes. These findings further support the hypothesis that different cellular pathways regulated by $1,25(OH)_2D_3$ may be involved in the growth inhibitory effects in different tumor cells.

Vit D-mediated regulation of the forkhead box O (FoxO) proteins

The forkhead box O (FoxO) proteins belong to a family of transcription factors that plays an important role in tumor suppression by upregulating target genes involved in cell-cycle arrest and apoptosis (An et al., 2010). In particular, FoxO1 (FKHR), FoxO3A (FKHRL1), FoxO4 (AFX), and FoxO6 regulate cell proliferation and differentiation. They are inhibited by phosphatidylinositol-3-kinase (PI3K), which stimulates their Akt-dependent phosphorylation and nuclear export. The biological functions of several members of the FoxO family are inhibited by phosphorylation induced by mitogen-activated protein kinases (MAPKs) such as ERK and p38. Interestingly, recent findings show that the interaction between $1,25(OH)_2D_3$ and VDR induces post-translational modifications and functional alterations of FoxO proteins (An et al., 2010). In fact, *in vitro* studies report that the treatment of human head and neck SCC (HNSCC) with $1,25(OH)_2D_3$ potentiates the binding of FoxO3A and FoxO4 proteins to FoxO promoter target genes and causes a block in the mitogen-induced FoxO protein nuclear export (An et al., 2010). Furthermore, *in vitro* investigations on human neuroblastoma cells show that a 4 h exposure of these cells to $1,25(OH)_2D_3$ induces the deacetylation and the dephosphorylation of FoxO. Consistent with these observations, the arrest of cell-cycle progression induced by Vit D is not observed in cells lacking FoxO3 and FoxO4 (An et al., 2010). These findings further indicate that FoxO proteins appear to play a key role as mediators of the anti-proliferative effects of Vit D in some human tumors.

Insulin growth factor modulation by Vit D

Experimental evidence shows that Vit D may also negatively affect cell proliferation by interfering with several growth

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Table 1. Proposed mechanisms underlying chemopreventive effects of Vitamin D.

Effect on	Mechanism	References
Cell proliferation	Increased expression of p18, p21, and p27 CDKs inhibitors	Wang et al. (1996), Hager et al. (2001), Colston et al. (2002), Yang and Burstein (2003), Gedlicka et al. (2006), Flores et al. (2010), and Thorne et al. (2011)
	Cell cycle blocking in G1/S transition	Jensen et al. (2001) and Istfan et al. (2007)
	Decreased human telomerase reverse transcriptase (hTERT)	Ikeda et al. (2003), Fedirko et al. (2009), and Kasiappan et al. (2012)
	Post-translational modifications and functional alterations of FoxO proteins	Kim et al. (2009) and An et al., (2010)
Growth factors gene expression	Inhibition of stimulating effects of IGF-I on cell growth and increased expression of IGFBP-3	Colston et al. (1998), Huynh et al. (1998), Nickerson and Huynh (1999), Boyle et al. (2001), Sprenger et al. (2001), Yang et al. (2001), Peng et al. (2004), Matilainen et al. (2005), and Teegarden and Donkin (2009)
	Increased expression levels of TGF-β or its secretion	Koli and Keski-Oja (1995), Wu et al. (1998), Yang et al. (2001), Chen et al. (2002), Lin et al. (2002), Bizzarri et al. (2003), Pálmer et al. (2003), Swami et al. (2003), Murthy and Weigel (2004), Peehl et al. (2004), Lambert et al. (20066), and Lee et al. (2006)
Signaling pathways	Blocking of the transcriptional regulation mediated by Wnt/β-catenin signaling pathway (decreasing formation of the transcriptional complex TCF4-β-catenin)	Larriba et al. (2013)
	Reduced expression of target genes for β-catenin	Xu et al. (2010)
	Prevention of β-catenin nuclear localization and transactivation by increasing E-cadherin expression	Pálmer et al. (2001), Shah et al. (2006), and Beildeck et al. (2009)
	Upregulation of the Wnt antagonist Dickkopf-1 (DKK-1) protein expression	Pendás-Franco et al. (2008b)
Cell differentiation	Increase of β-catenin and E-cadherin expression levels	Pendás-Franco et al. (2008a) and Lopes et al. (2012)
	Inhibitory effects on epidermal growth factor receptor (EGFR) expression	Gocek and Studzinski (2009)
	Accumulation and phosphorylation of integrins, paxillin, and focal adhesion kinase and down-regulation of mesechymal N-cadherin and myoepithelial P-cadherin	Pendás-Franco et al. (2007)
	Up-regulation of androgen receptor expression in LNCaP cells and increased secretion of prostate-specific antigen (PSA)	Gocek and Studzinski (2009)
	Increased expression of insulin-like growth factor binding protein-3 (IGFBP-3) → up regulating of p21/Cip1 → inhibition of cell proliferation	Boyle et al. (2001) and Krishnan et al. (2004)
	Up-regulation of CCAAT/enhancer-binding protein beta (C/EBP β), expression a transcriptional activator that regulates genes involved in immune and inflammatory response, and which cooperates with NF-κB in regulation of the secretion of IL-6 in neuroendocrine human prostate cancer cells	Xiao et al. (2004)
	Induction of human myeloid leukemia cells differentiation into functional monocytes via increased expression and/or activation of PKC isoforms, PI3K-AKT pathway, p42-ERK p38-ERK, and c-JNK families of MAPKs	Hughes et al. (2010)
Apoptosis	Down-regulation of the expression of anti-apoptotic and pro-survival proteins such as Bcl-2, Bcl-XL, or increase of the expression of pro-apoptotic proteins such as Bax, Bak, and Bad	Blutt et al. (2000) and Diaz et al. (2000)
	Up regulation of PTEN	Pan et al. (2010)
	Increase in the levels of the pro-apoptotic proteins death-associated protein (DAP-3), Fas-associated death domain (FADD), and caspase-3, -4, -6, and -8	Swami et al. (2003)
	Increased gene expression of pro-apoptotic protein G0–G1 switch 2 (G0S2)	Pálmer et al. (2003)
	Recruiting of Ca(2+)-dependent apoptotic effectors: Ca(2+)-dependent μ-calpain and Ca(2+)/calpain-dependent caspase-12	Sergeev (2012)

(*continued*)

Table 1. Continued

Effect on	Mechanism	References
Autophagy	Decreased inhibition of Bcl-2 on Beclin 1 to induce autophagy; decrease endoplasmic reticulum Bcl-2 to increase calcium to induce autophagy	Mathiasen et al. (1999) and Hoyer-Hansen et al. (2007)
	Decrease of mammalian Target Of Rapamycin (mTOR) protein level and increase of Beclin-1 expression levels to induce autophagy	Hoyer-Hansen et al. (2007), Wang et al. (2008), and Høyer-Hansen et al. (2010, 2005)
	Decreased $p19^{INK4D}$ → increased autophagy	Tavera-Mendoza et al. (2006)
	Decreased autophagic activity induced by TNF-α	Stubbs et al. (2010)
	Inhibition of IFN-γ release from macrophages and peripheral blood mononuclear cells. This phenomenon may results in an inhibition of IFN-γ induced activation and potentiation of lysosomal activity of macrophages, recruitment of autophagic proteins, and, ultimately, may lead to a decrease of autophagy	Wu and Sun (2011)
	NF-kB expression (?)	Bao et al. (2010), Krishnan and Feldman (2010), Tse et al. (2010), and Janjetovic et al. (2011)
	Induced expression of thioredoxin reductase 1 (TXNRD1)	Swami et al. (2003), Peehl et al. (2004), Kovalenko et al. (2010)
Antioxidant defense and DNA repair	Increased production of superoxide dismutase 1 and 2 (SOD1 and SOD2) in prostate epithelial cells (PECs) and in androgen-sensitive prostate cancer cells (LNCaP)	Peehl et al. (2004) and Lambert et al. (2006)
	Increase of glucose-6-phosphate dehydrogenase (G6PDH) expression levels	Zhang et al. (2005), Bao et al. (2008), and Kovalenko et al. (2010)
	Induction of nuclear factor erythroid-derived 2-like 2 (NFE2L2) transcription factor that controls the gene expression of several enzymes of the antioxidants systems such as glutathione peroxidase (GPX) 3, heme oxygenase 1 (HMOX-1), and aldo-keto reductase 1C2(AKR1C2)	
Regulation of proteins involved in DNA repair	Regulation of genes coding for DNA repair enzymes	Krishnan et al. (2004) and Nair-Shalliker et al. (2012)
	Overexpression of the (GADD45 α) gene	Akter et al. (1997) and Jiang et al. (2003a)
	Increased expression levels of P53 and proliferating cell nuclear antigen (PCNA) up-regulation of DNA repair genes, ATM, and RAD50	Swami et al. (2003) and Ting et al. (2012)
	Prevention of cathepsin L-mediated degradation of DNA repair protein 53BP1	Gonzalo (2014)
Prostaglandins synthesis and metabolism	Negative modulator of PGs synthesis and activity. Inverse correlation between COX-2 and VDR expression in breast cancer and ovarian cancer cells	Moreno et al. (2006) and Krishnan and Feldman (2010)
	Decreased the expression levels of COX-2, EP2, and FP. Increased expression of 15-hydroxyprostaglandin-D dehydrogenase (15-PGDH) a NAD+-dependent enzyme involved in the degradation of PGE2	Moreno et al. (2005), Krishnan and Feldman (2010), Cordes et al. (2012), Thill et al. (2012), and Yuan et al. (2012)
Angiogenesis	Inhibition VEGF expression and new blood vessels and the formation in mice transplanted with MCF7 human breast cancer	Mantell et al. (2000)
	Repression of hypoxia-inducible factor 1 (HIF-1)	Ben Shoshan et al. (2007)
	Suppression in SCC tumor cells of the expression of the proangiogenic factor IL-8 via NF-kB-dependent manner thus leading to the inhibition of endothelial cell migration and tube formation	Bao et al. (2006)
	Upregulation of thrombospondin-1 (*THSD1*) mRNA levels in SW480-ADH human colon tumor cells	Fernandez-Garcia et al. (2005)
	Increase of the intracellular levels of VDR and that of the pro-apoptotic protein p27 which reduces the expression level of signal molecules for angiogenesis, including angiopoietin-2	Bernardi et al. (2002) and Flynn et al. (2006)
	Cell-cycle arrest in the G0/G1 phase and a decrease of the number of cells in the S phase, due to the induction of p27 and the down-regulation of p21 in tumor-derived endothelial cells	Chung et al. (2006)
	Growth arrest of RWPE1 prostatic epithelial cells following decrease in the expression levels of genes coding for NF-kB, IGF-1; inhibition of the transcription of pro-	Kovalenko et al. (2010)

	inflammatory cytokines, including IL-1, IL-6, and IL-17; reduction of VEGF and VEGF receptors mRNA levels including the kinase insert domain receptor (KDR) and neuropilin 1 (NRP1). Induction of anti-angiogenic factors and that of molecules involved in the protection of cell from oxidative stress; induction of the expression of numerous isoforms of semaphorins, including SEMA 3B, 3F, and 6D	Aparna et al. (2008)
	Inhibition of COX-2 expression levels	Pendás-Franco et al. (2008b)
	Repressesion of the WNT antagonist DICKKOPF-4 (DKK4) which promotes invasion and angiogenesis in colorectal cancer cells	
Immune system	Modulation of the expression which encodes for proteins that are crucial for autophagy and for the antimicrobial activity in cells of the innate immune system	Hewison (2011)
	Induction of the expression of cathelicidin (CAMP) and, to a lessen exent, of defensin β2 (*DEFB2/HBD2*) in different normal and tumor cell types such as colon cancer, acute myeloid leukemia, keratinocytes, human bone marrow cells-derived macrophages, and bone marrow cell	Gombart et al. (2005)
	Alteration of the interrelationship between immune cells and cancer cells leading to a significant decrease in the pro-inflammatory cytokines TNF-α and IL-6	Bessler and Djaldetti (2012)
	Increased differentiation of blood-derived CD34+ cells into dendritic cells and decreased peripheral blood and intratumoral levels of immunosuppressive CD34+ cells in patients with head and neck cancer	Young and Day (2013)

factors. In particular, Vit D appears to be implicated in the regulation of insulin growth factor (IGF) and in that of certain IGF-binding proteins including the major binding protein IGFBP-3 (Boyle et al., 2001; Matilainen et al., 2005; Peng et al., 2004; Teegarden & Donkin, 2009). These observations are in line with the results from *in vitro* studies on MCF-7 and Hs578T human breast cancer cell lines showing that two Vit D analogues EB1089 and CB1093 may inhibit the stimulating effects of IGF-I on cell growth and may enhance the production of IGFBP-3 which, in turn, regulates the promoting activity of IGF-I and IGF-II on cell proliferation (Colston et al., 1998). Similar effects were observed in prostate cancer cells following their exposure to Vit D or its analogues (Huynh et al., 1998; Sprenger et al., 2001). The role of IGFBP-3 as a critical mediator of the antiproliferative activity of Vit D has been further highlighted by other studies which show that antisense oligonucleotides against IGFBP-3 antagonize the growth-inhibiting effects of Vit D in androgen-responsive LNCaP human prostate cancer cells (Boyle et al., 2001; Krishnan et al., 2004). On one hand, in agreement with these data, Peng et al. (2008) have recently shown that, in the LNCaP human prostate cancer cell line, high concentrations of androgens exert growth inhibitory effects at least partially through the IGFBP-3-p21/p27 pathway. On the other hand, *in vivo* studies by Nickerson and Huynh (1999) show that the administration of Vit D analog EB1089 to rats for 14 d increases the expression of several isoforms of IGFBPs, including IGFBP-3, in the prostatic tissue and that these effects were associated with a reduction of prostate volume. As IGFBP-3 has been shown to possess pro-apoptotic, antimetastatic, and anti-angiogenic activities against prostate cancer cells (Massoner et al., 2009), it is conceivable to hypothesize that the modulation of IGFP-3 expression by Vit D may be a possible effective therapeutic option in the clinical treatment of prostate cancer.

Transforming growth factor-β modulation by Vit D

Transforming growth factor-β (TGF-β) is a member of growth factor of the namesake superfamily of growth factors which is implicated in the regulation of several important biological processes such as cell proliferation, differentiation, motility, adhesion, organization, and programmed cell death (Massagué, 2008). TGF-β is known to inhibit the proliferation of normal epithelial cells and the early steps of carcinogenesis while it fosters the later steps of cancer progression, e.g., cell motility, invasion, and metastasis (Massagué, 2008). Vit D and TGF-β share similar effects on cell growth and differentiation (Daniel et al., 2007; Wu et al., 1998). Experimental studies stress that, according to the cell type, Vit D may increase the expression levels of TGF-β and that of its receptors (Chen et al., 2002; Daniel et al., 2007; Koli & Keski-Oja, 1995; Tu et al., 2013; Wu et al., 1997a,b, 1998; Yanagisawa al., 1999) or its secretion (Bizzarri et al., 2003; Koli & Keski-Oja, 1995). These effects, which may in part, account for the anti-proliferative effects of Vit D, were also described as occurring in various breast cancer cell lines such as MCF-7, MDA-MB-231, or MCF10CA (Lee et al., 2006; Swami et al., 2003; Wu et al., 1998; Yang et al., 2001) and in prostate cancer cells (Murthy & Weigel, 2004; Peehl et al.,

2004). In particular, some of these studies show that short-term exposure (<12 h) to $1,25(OH)_2D_3$ or to its analog EB1089 results in an increased expression level of TGF-β and/or TGF-β receptors in breast cancer cells (Yang et al., 2001). In addition, other *in vitro* observations on LNCaP prostate cancer cells show that the growth-inhibiting effects of $1,25(OH)_2D_3$ on these tumor cells appear to be associated with the increased expression and secretion of the growth differentiation factor-15 (GDF-15), another member of the TGF-β superfamily of growth factors (Lambert et al., 2006). Interestingly, the effects of a long-term treatment with $1,25(OH)_2D_3$ on the mRNA levels encoding different members of the family TGF-β are also reported on other tumor cell types such as colorectal cancer cells or squamous carcinoma cells (Lin et al., 2002; Pálmer et al., 2003).

Vit D interaction with Wnt/β-catenin-signaling pathways

Another possible mechanism by which Vit D may halt cell proliferation involves the inhibition of some of the numerous functions mediated by the Wnt/β-catenin-signaling pathway. To this end, *in vitro* observations show that in colon cancer cell lines $1,25(OH)_2D_3$ can block the transcriptional regulation mediated by β-catenin by decreasing the formation of the transcriptional complex TCF4-β-catenin (Larriba et al., 2013). Consistent with this hypothesis, Xu et al. (2010) have demonstrated that administration of $1,25(OH)_2D_3$ or that of its analogues for 12 weeks reduces the number of polyps in the colon mucosa and that, in the small intestine and in the colon, this effect is associated with a reduced expression of target genes for β-catenin. The effects mediated by Vit D may also indirectly affect the function of β-catenin through an increased production of E-cadherin, a membrane protein that binds β-catenin, thus preventing its nuclear localization and transactivation (Pálmer et al., 2001). However, there is evidence that $1,25(OH)_2D_3$ may also inhibit the growth of many different cells without affecting cadherin expression. These findings indicate that the up-regulation of E-cadherin is just one of the mechanisms by which Vit D may negatively affect the β-catenin signaling pathway (Shah et al., 2006). These observations also indicate that the effects of $1,25(OH)_2D_3$ on the growth and differentiation of many different epithelial cancer cells may be, in part, explained by its ability to differentially regulate the activity of VDR, E-cadherin, and β-catenin/TCF pathways (Beildeck et al., 2009; Shah et al., 2006). $1,25(OH)_2D_3$ may also interact with the Wnt/β-catenin-signaling pathway by affecting the expression of Wnt regulators, for instance, by up-regulating the expression of the Wnt antagonist Dickkopf-1 (DKK-1) protein (Pendás-Franco et al., 2008b). However, whether all the DNA binding sites for β-catenin are equally inhibited by VDR and whether the link of β-catenin in different sites is equally influenced by $1,25(OH)_2D_3$ and VDR remain still unraveled. Further studies may better define these interactions.

Vit D and apoptosis

The induction of apoptosis is an additional, important mechanism by which Vit D appears to exert its chemopreventive effects on cancer cell growth (Vanoirbeek et al., 2011). Vit D has been shown to promote apoptosis in breast cancer, prostate cancer, colon cancer, and SCC cells (Gocek & Studzinski, 2009). However, this phenomenon is not univocal. For instance, Zhang et al. (2005) have reported that, in ovarian cancer cells, Vit D may inhibit apoptosis. These findings are in agreement with the results of several studies showing that Vit D may positively or negatively modulate the expression of anti-apoptotic or pro-apoptotic factors according to the cell type (Díaz et al., 2000; Pereira et al., 2012). Although the mechanisms by which Vit D may promote apoptosis remain to be fully clarified, experimental evidence highlights the fact that $1,25(OH)_2D_3$ can trigger the intrinsic pathway of programmed cell death $1,25(OH)_2D_3$ (Guzey et al., 2002). In this context, *in vitro* studies on colorectal cancer cells show that $1,25(OH)_2D_3$ and its analogue EB1089 may promote apoptosis by a p53-independent mechanism (Díaz et al., 2000). These investigations also show that these molecules may inhibit apoptosis by down-regulating the expression of anti-apoptotic and pro-survival proteins such as Bcl-2, Bcl-XL, or by increasing the expression of pro-apoptotic proteins such as Bax, Bak, and Bad (Díaz et al., 2000). Additionally, these studies also show that the increased expression of Bak and the reduced expression of BCl-2 in response to EB1089 were more marked compared with that induced by $1,25(OH)_2D_3$ (Díaz et al., 2000). In line with these findings, Blutt et al. (2000) demonstrate that continuous 6-d exposure of LNCaP cells to $1,25(OH)_2D_3$ induces apoptosis and that this phenomenon was associated with the down-regulation of anti-apoptotic proteins Bcl-2 and Bcl-XL and with the up-regulation of pro-apoptotic protein Bax. More recently, Pan et al. (2010) have shown that $1,25(OH)_2D_3$ may promote apoptosis also in the HCG-27 gastric cancer cell line. This effect appears to be the result of the up-regulation of PTEN, a tumor suppressor gene that negatively regulates the anti-apoptotic activity of protein kinase B (Akt), mediated by VDR. Furthermore, in the MCF-7 cell line, the treatment with $1,25(OH)_2D_3$ induced an increase in the level of the pro-apoptotic Death Associated Protein-3 (DAP-3), Fas-Associated Death Domain (FADD), and the caspases-3, -4, -6, and -8 (Swami et al., 2003). Consistent with these observations *in vitro* studies on squamous cell carcinoma SCC25 cells and colon cancer SW480-ADH cells show that Vit D may potentiate its pro-apoptotic effects by increasing the gene expression of the pro-apoptotic protein G0–G1 switch 2 (*G0S2)* (Pálmer et al., 2003) or by activating caspase effector molecules (Pálmer et al., 2001). Furthermore, more recent *in vitro* studies from Sergeev (2012) suggest that, in breast cancer cells, Vit D can act as an apoptotic initiator that directly recruits $Ca^{(2+)}$-dependent apoptotic effectors such as $Ca^{(2+)}$-dependent μ-calpain and $Ca^{(2+)}$/calpain-dependent caspase-12 which are capable of executing apoptosis. Finally, Kasiappan et al. (2012) have recently reported that, in OVCAR3 ovarian cancer cells, $1,25(OH)_2D_3$ destabilizes telomerase reverse transcriptase (TERT) mRNA, inducing apoptosis through telomere attrition and the down-regulation of telomerase activity. The multiple mechanisms underlying Vit D-mediated apoptosis observed in different tumor cell lines may be, in part, explained by the need for tumor cells to develop different mechanisms that may be useful for escaping the pro-apoptotic effects induced by Vit D.

Vit D and autophagy

Autophagy or autophagocytosis is a catabolic process by which cells may degrade cytosolic macromolecules and intracellular components through the lysosomal machinery (Singletary & Milner, 2008). This process plays a key role in the regulation of several important biological processes such as cell growth, development, and homeostasis, by maintaining a balance among the synthesis, degradation, and subsequent recycling of cellular products. Although autophagy is generally regarded as a survival strategy or a mechanism that protects cells from stressful situations such in the case of lack of energy reserves or oxidative stress, it can also be modulated to determine the death of cancerous cells (Singletary & Milner, 2008). Therefore, unlike apoptosis, autophagy, in response to stressful stimuli, can contribute either to cell survival or to cell death (Morselli et al., 2009; Singletary & Milner, 2008). Many food components such as selenium, resveratrol, curcumin, and Vit D itself have been reported to promote autophagy (Singletary & Milner, 2008; Wu & Sun, 2011). The first evidence regarding the permissive effect of $1,25(OH)_2D_3$ on autophagy was reported by Mathiasen et al. (1999). These authors showed that $1,25(OH)_2D_3$ and two analogues EB1089 and CB1093 induced growth arrest in MCF-7 breast cancer cells expressing the tumor suppressor gene *p53* and in T47D breast cancer cell lines line lacking *p53*. Surprisingly, the same studies also highlighted the fact that the growth-inhibiting effects of Vit D and its analogues were also caspase independent and that the overexpression of the anti-apoptotic protein Bcl-2, completely protected tumor cells from autophagy induced by these molecules (Mathiasen et al., 1999). Consistent with these data, other *in vitro* studies reported that Vit D analog EB1089 induced tumor cell death by a mechanism not related to caspase activation and which consisted in the induction of chromatin condensation and DNA fragmentation (Høyer-Hansen et al., 2005). In particular, these investigations showed that, in MCF-7S1 tumor cells, autophagic activity could be increased by protein Beclin-1, also known as autophagy-related gene *ATG6*. Beclin-1 is a Bcl-2-interacting protein that promotes, in association with its binding partner class III phosphoinositide 3-kinase (PI3K), autophagosome formation (Høyer-Hansen et al., 2010). It may also function as a brake for autophagy and autophagic cell death when associated with Bcl-2 (Høyer-Hansen et al., 2010). Conversely, this phenomenon was inhibited by the mammalian target of rapamycin protein (mTOR) (Høyer-Hansen et al., 2007). These findings are consistent with those of Wang et al. (2008) showing that, in HL-60 human myeloid leukemia cells, Vit D triggered autophagy by up-regulating Beclin-1 and by down-regulating mTOR levels. Furthermore, additional evidence showed that Vit D-induced autophagy may be mediated by CDK inhibitors. For instance, Tavera-Mendoza et al. (2006) showed that $1,25(OH)_2D_3$ contribute to make SCC25 cells knocked down for *p19(INK4D)* gene expression more susceptible to cell death by autophagy as this gene protects cells from autophagy-induced death (Høyer-Hansen et al., 2005). This effect was also noted in MCF-7 human breast cancer cells (Høyer-Hansen et al., 2005). However, $1,25(OH)_2D_3$ has been shown to decrease the circulating levels of TNF-α, a phenomenon that may lead to a decreased autophagic activity induced by this molecule (Stubbs et al., 2010). In addition, Vit D may also inhibit the release of IFN-γ from macrophages and peripheral blood mononuclear cells (Wu & Sun, 2011). This effect may result in an inhibition of IFN-γ induced activation and potentiation of lysosomal activity of macrophages, recruitment of autophagic proteins and, ultimately, may lead to a decrease of autophagy (Wu & Sun, 2011). Another possible target for the chemopreventive activity of Vit D on cancer progression is the nuclear factor kappa B (NF-κB), a nuclear transcription factor involved in the regulation of many genes implicated in inflammation, growth regulation, apoptosis, autophagy, carcinogenesis, and malignant progression (Aggarwal, 2004; Baldwin, 2012). In this context, Tse et al. (2010) reported that Vit D_3 inhibited NF-κB activity in human breast cancer cells. Likewise, a similar effect was observed by other authors on colorectal cancer cells (Schwab et al., 2007) and prostate cancer cells (Krishnan & Feldman, 2010). Nevertheless, as opposing results have been also reported on the effects of Vit D on NF-kB expression levels (Bao et al., 2010; Janjetovic et al., 2011; Krishnan et al., 2007) further studies may better define the role of NF-kB in autophagy and, consequently, the potential therapeutic impact of Vit D in modulating this phenomenon in cancer cells.

Vit D and cell differentiation

Experimental studies show that Vit D may also induce differentiation in normal and neoplastic cells which, in some case, may be associated with a reduced proliferation rate (Gocek & Studzinski, 2009). The differentiating activity of Vit D is associated with the increased expression and/or activation of several intracellular signaling pathways. This may, in part explain, why the mechanisms underlying Vit D-induced differentiation may somehow differ according to the cell types (Gocek & Studzinski, 2009). For instance Geng et al. (2011) showed that CYP27B1/1α-hydroxylase is required for osteoblast differentiation of human marrow stromal cells. Recent studies suggest that the anti-apoptotic effects of $1,25(OH)_2D_3$ on osteoblasts and osteocytes are mediated by Src, PI3K, and JNK kinases (Gocek & Studzinski, 2009). The association of VDR with other proteins appears to be important in Vit D-induced osteoblast differentiation (Gocek & Studzinski, 2009; van Driel et al., 2006; Woeckel et al., 2013). $1,25(OH)_2D_3$ may also regulate keratinocytes differentiation by increasing intracellular calcium levels through the induction of the expression of calcium receptor (CaR) and phospholipase C (PLC) which are critical for calcium to stimulate keratinocyte differentiation (Bikle, 2012). Additionally, Vit D has been shown to increase, via AP-1 activation, the expression of several genes involved in the regulation of keratinocytes differentiation such as involucrin, transglutaminase, loricrin, and filaggrin and that of cornified envelope formation while inhibiting the proliferation of keratinocytes (Bikle, 2012). Moreover, time-dependent changes in the expression of VDR co-activators were noted during cell differentiation. It has been hypothesized that these changes may contribute to the temporal sequence of Vit D-mediated gene expression during keratinocytes differentiation (Bikle, 2012). $1,25(OH)_2D_3$ has also

been shown to facilitate myogenic differentiation by increasing the expression of IGF-II and Follistatin (Lee et al., 2010) and by decreasing the expression of the insulin growth factor I (IGF-I) and that of myostatin, a negative regulator of skeletal muscle mass (Garcia et al., 2011; Lee et al., 2010). In contrast, in human colon and breast cancer cells, Vit D appears to foster tumor cell differentiation by increasing the expression levels of proteins such as β-catenin and E-cadherin (Lopes et al., 2012; Pendás-Franco et al., 2008a). In particular, on one hand, the binding of β-catenin to VDR may cause the loss of this molecule from the transcriptional complex TCF-4-β-catenin in the nucleus. This phenomenon ultimately results in a decreased cell proliferation (Larriba et al., 2013). One of the proposed mechanisms that may account for the reduced cell proliferation associated with cell differentiation induced by Vit D may be related, as emphasized in *Caco-2* cells, to the marked inhibitory effects of this molecule on the expression of EGFR at both mRNA and protein levels (Gocek & Studzinski, 2009). On the other hand, *in vitro* studies on MDA-MB-453 human breast cancer cells have shown that their treatment with $1,25(OH)_2D_3$ resulted in accumulation of integrins, paxillin, and focal adhesion kinase and their phosphorylation (Pendás-Franco et al., 2007). Conversely, the mesenchymal marker N-cadherin and the myoepithelial marker P-cadherin resulted down-regulated. These findings suggest that $1,25(OH)_2D_3$ may revert the myoepithelial phenotype associated with more aggressive forms of human breast cancer. However, not all breast cancer cell lines show a similar response to $1,25(OH)_2D_3$. The difference appears, in part, to be due to the lack or decrease of VDR expression or function (Gocek & Studzinski, 2009; Valrance et al., 2007). However, alterations in $1,25(OH)_2D_3$ metabolizing enzymes, which can decrease Vit D levels below its effective concentration, cannot be ruled out (Byrne & Welsh, 2007; Gocek & Studzinski, 2009). For instance, in $ER^{(+)}$ breast cancer cell lines, $1,25(OH)_2D_3$ may facilitate cell differentiation by converging VDR and estrogen receptor pathways to regulate BRCA-1, a tumor suppressor gene that encodes a nuclear phosphoprotein that plays a role in maintaining genomic stability (Campbell et al., 2000; Roy et al., 2011). This effect contributes to regulating the balance between differentiation and proliferation signaling (Campbell et al., 2000; Gocek & Studzinski, 2009). Likewise breast cancer, experimental observations provide evidence that $1,25(OH)_2D_3$ may also induce differentiation in prostate cancer cells (Gocek & Studzinski, 2009). To this end, *in vitro* studies demonstrated that the treatment of LNCaP cells with $1,25(OH)_2D_3$ up-regulates the expression of the androgen receptor (AR) and increases the secretion of prostate-specific antigen (PSA), a differentiation marker for epithelial prostate cells (Gocek & Studzinski, 2009). The up-regulation of AR may cause, in turn, an increase in the expression levels of VDR which selectively enhances the AR-mediated androgenic pro-differentiating effects but not the proliferation activity. In contrast, microarray analysis by Krishnan et al. (2004) demonstrates that in LNCAP tumor cells $1,25(OH_)_2D_3$ increases the expression of insulin-like growth factor-binding protein-3 (IGFBP-3), which functions as an inhibitor of cell proliferation, by up-regulating p21/Cip1 (Boyle et al., 2001). In addition, Vit D treatment may also cause the up-regulation of a ''prostate differentiation factor,'' a member of the bone morphogenetic protein (BMP) family, which is generally involved in growth and differentiation of embryonic and adult tissues (Lambert et al., 2006). Interestingly, these studies also revealed that $1,25(OH)_2D_3$ regulates certain androgen-responsive genes as well as genes that encode enzymes involved in androgen catabolism. Prostate cancer cells are also known to undergo ''trans-differentiation'' to a neuroendocrine phenotype which is an aggressive form of prostate cancer. Recent evidence suggests a key role for NF-κB, as well as IL-6, in this process (Mori et al., 2009). In this context, Vit D up-regulates the expression of CCAAT/enhancer-binding protein beta (C/EBP β), a transcriptional activator that regulates genes involved in immune and inflammatory responses, and which cooperates with NF-κB in regulation of the secretion of IL-6 in neuroendocrine human prostate cancer cells (Xiao et al., 2004). These data suggest that $1,25(OH)_2D_3$ may be promising as a potential therapeutic agent in the treatment of this aggressive form of prostate cancer. Experimental findings show that Vit D may induce leukemic cells to differentiate. In particular, *in vitro* studies show that the exposure of human myeloid leukemia cells to physiological concentrations of $1,25(OH)_2D_3$ for 36–48 h induces their differentiation into functional monocytes (Hughes et al., 2010). The differentiating activity of Vit D is associated with the increased expression and/or activation of different intracellular pathways such as protein kinase C (PKC), PI3K/AKT pathway, p42 extracellular-regulated kinase (p42-ERK), p38-ERK, and the c-Jun N-terminal kinases (JNK) families of mitogen-activated protein kinases (MAPKs) (Hughes et al., 2010). Pharmacological or genetic blockade of these pathways may abrogate $1,25(OH)_2D_3$-driven monocytic differentiation.

Antioxidant defense and DNA

On one hand, free radicals, also known as reactive oxygen species (ROS), in concert with reactive nitrogen species (RNS) may play a dual role in cell homeostasis since they may function as a second messenger in controlling cell proliferation and differentiation. Furthermore, ROS may also foster cellular senescence (Dröge, 2003) and apoptosis (Circu & Aw, 2010). The cumulative production of ROS and RNS in response to endogenous or exogenous insults, i.e., the ''oxidative stress'', is a typical phenomenon that can be observed in many types of cancer cells (Valko et al., 2006). A redox imbalance occurring within these cells may ultimately facilitate oncogenic stimulation. On the other hand, the induction of antioxidant defense mechanisms may reduce the biological impact of ROS (Valko et al., 2006). In line with these observations, several *in vitro* and *in vivo* studies highlight the fact that $1,25(OH)_2D_3$ exerts antioxidative activities on colorectal cancer (Nair-Shalliker et al., 2012). In particular, it has been shown that DNA damage induced by oxidative stress, as measured by the amount of 8-hydroxy-2′-deoxyguanosine, is high in the epithelium of the distal colon of VDR-knockout mice and it is reduced in the epithelium of human colon after a daily supplement of 800 IU (international units) of Vit D (1 IU is the biological equivalent of 0.025 μg cholecalciferol or ergocalciferol) (Fedirko et al., 2010).

These findings further suggest that Vit D may protect against oxidative stress-induced DNA damage in humans. In line with this hypothesis, Banakar et al. (2004) report that the treatment of rats with calcitriol increases the expression of VDR and markedly reduces the levels of malondialdehyde. However, no substantial evidence has been obtained so far regarding a direct relationship between Vit D and prevention of DNA damage at a population level. Nevertheless, the clinical and epidemiological observations that suggest a correlation between deficient levels of calcidiol and increased incidence of diseases associated with increased levels of DNA damage in humans warrant further extensive investigation.

Induction of antioxidant enzymes

$1,25(OH)_2D_3$ is known to increase the expression of numerous enzymes of the antioxidant defense system in humans (Fleet et al., 2012). For instance, *in vitro* studies show that the exposure of prostate cancer cells and MCF-7 breast cancer cells to $1,25(OH)_2D_3$ or its analogues induce the expression of thioredoxin reductase 1 (TXNRD1), an enzyme that converts thioredoxin to its reduced form needed to perform its antioxidant function (Kovalenko et al., 2010; Peehl et al., 2004; Swami et al., 2003). In addition, $1,25(OH)_2D_3$ has been shown to increase the production of superoxide dismutase 1 (SOD1) and 2 (SOD2) in prostate epithelial cells (PECs) and in androgen-sensitive prostate cancer cells (LNCaP), respectively (Lambert et al., 2006; Peehl et al., 2004). Furthermore, other *in vitro* observations show that the treatment of the human prostate epithelial cell line RWPE-1, and that of BPH-1 benign prostatic hyperplasia (BPH) epithelial cell line or OVCAR3 ovarian carcinoma cell line, with $1,25(OH)_2D_3$, increases the intracellular levels of glucose-6-phosphate dehydrogenase (G6PDH), an enzyme which regulates the intracellular levels glutathione (Bao et al., 2008; Kovalenko et al., 2010; Zhang et al., 2005). This effect ultimately protects cells from apoptosis induced by H_2O_2. These findings are in line with experimental evidence showing that the expression levels of G6PDH in prostatic epithelial cells are modulated by $1,25(OH)_2D_3$ through VDRE located in the first intron of the gene coding for G6PDH (Bao et al., 2008). However, this phenomenon was not observed in DU145 and CWR22 prostate cancer cells (Bao et al., 2008). The different responses of these cell lines to $1,25(OH)_2D_3$ treatment may be, in part explained, with the loss of AR expression, which is a characteristic of tumor cells less susceptible to Vit D treatment (Stewart & Weigel, 2004; Ting et al., 2007a,b). Furthermore, the protection from oxidative stress mediated by Vit D, may also be indirectly due to the induction of the nuclear factor erythroid-derived 2-Like 2 (NFE2L2), a transcription factor that controls the gene expression of several enzymes of the antioxidant systems such as glutathione peroxidase 3 (GPX-3), heme oxygenase 1 (HMOX-1), and aldo-keto reductase 1C2 (AKR1C2) (Kovalenko et al., 2010). The effects of Vit D on the oxidative system further support the clinical benefit of this molecule in cancer chemoprevention.

Regulation of proteins involved in DNA repair

Experimental *in vivo* observations show that VDR-deficient mice are more susceptible to the development of skin tumors either induced by chemical carcinogens such as 7,12-dimethylbenzanthracene (DMBA) or by chronic UVR exposure (Bikle, 2012). These studies suggest that $1,25(OH)_2D_3$ may protect the skin from malignant transformation by controlling keratinocyte proliferation and differentiation, by facilitating DNA repair, and by suppressing the activation of the hedgehog (Hh) pathway following UVB exposure (Bikle, 2012). In particular, recent studies show that $1,25(OH)_2D_3$ may protect DNA by regulating the expression of genes coding for DNA repair enzymes (Krishnan et al., 2004; Nair-Shalliker et al., 2012). In this context, Akhter et al. (1997) have reported that in SCC cells, Vit D analog EB1089 induces the overexpression of the growth arrest and DNA-damage-inducible α (*GADD45α*) gene, a *p53* target gene whose products are involved in DNA repair. It has also been shown that the treatment of ovarian cancer cells with $1,25(OH)_2D_3$, causes cell-cycle arrest at the G2/M transition through p53-independent induction of *GADD45α* (Jiang et al., 2003a,b). The role of *GADD45α* induction in eliciting the chemopreventive effects of Vit D is supported by the findings that cell-cycle arrest in G2 or in M induced by $1,25(OH)_2D_3$ does not occur following *GADD45α* deletion (Akter et al., 1997; Jiang et al., 2003a). Furthermore, microarray analyses performed in MCF-7 breast cancer cells show that the treatment of these cells with $1,25(OH)_2D_3$ increases the mRNA expression levels of other molecules involved in DNA repair such as p53 and proliferating cell nuclear antigen (PCNA) (Swami et al., 2003). Additionally, more recent studies show that $1,25(OH)_2D_3$ treatment can protect BPH-1 human prostate epithelial cells from carcinogen-induced genotoxic stress via VDR-mediated transcriptional upregulation of DNA repair genes, *ATM* and *RAD50,* thereby facilitating DNA double-strand break repair (Ting et al., 2012). Interestingly, on one hand, recent findings stress that in *BRCA1*-deficient breast cancer cells, Vit D prevents the degradation of the DNA repair protein 53BP1 mediated by cysteine proteinases Cathepsin L (Gonzalo, 2014), a lysosomal endopeptidase which is involved in tumor cell proliferation, invasion, and metastasis (Gonzalo, 2014; Lankelma et al., 2010; Leto et al., 2010). On the other hand, recent observations report that the gene encoding the *BRCA1* protein is a critical downstream target of Vit D. Consistent with these data Campbell et al. (2000) show that treatment of MCF-7 cells with calcitriol results in a near 6-fold increase in BRCA1 protein and that VDR expression is directly correlated with induction of *BRCA1*.

Effects of Vit D on the synthesis and metabolism of prostaglandins

It is well established that prostaglandings (PGs) may promote cancer cell proliferation and progression (Wang & Dubois, 2010). Since experimental findings demonstrate that $1,25(OH)_2D_3$ may act as a negative modulator of the synthesis and activity of prostaglandins (PGs) (Krishnan & Feldman, 2010; Moreno et al., 2006), these effects may also, in part, account for the chemo-preventive activity of Vit D on tumor progression. In support of this hypothesis, recent studies have better defined the role of prostaglandin–endoperoxide synthase, a key enzyme of prostaglandin synthesis and more

widely known as cyclooxygenase (COX), on carcinogenesis (Wang & Dubois, 2010). Increasing experimental and clinical observations show that the inducible isoform of this enzyme, namely COX-2, is overexpressed in many human tumors and in cancer cell lines. Moreover, these findings also show positive relationship between COX-2 overexpression and tumor progression (Cordes et al., 2012; Krishnan & Feldman, 2011; Thill et al., 2012). Alterations in the expression of COX-2 and its product, prostaglandin E2 (PGE2), have been observed in breast cancer and colorectal cancer where these molecules appear to be involved in several key steps of malignant progression such as tumor initiation, tumor cell proliferation, and metastasis formation (Thill et al., 2012; Wang & Dubois, 2010). Thus, COX-2 may be considered an appropriate target for cancer chemoprevention and treatment. To this end, some *in vitro* studies carried out on LNCaP and PC-3 prostate cancer cells have shown that the treatment with $1,25(OH)_2D_3$ decreases the expression levels of COX-2 and that of prostaglandin receptors EP2 and FP, whereas it increases the expression of 15-hydroxyprostaglandin-D dehydrogenase (15-PGDH) a NAD^+-dependent enzyme involved in the degradation of PGE2 (Krishnan & Feldman, 2010; Moreno et al., 2005). Interestingly, Thill et al. (2012) have recently reported that VDR and COX-2 expressions are inversely correlated in malignant breast cell lines. This phenomenon has also been observed in ovarian cancer tissues (Cordes et al., 2012). These findings support the hypothesis that $1,25(OH)_2D_3$ may inhibit tumor cell proliferation by reducing the intracellular levels of biologically active prostaglandins. In line with these observations, more recent *in vitro* studies by Yuan et al. (2012) report that a 72-h exposure of MCF-7 breast cancer cell to $1,25(OH)_2D_3$ results in a significant decrease of COX-2 mRNA expression levels and in that of PGE2 in cell culture supernatant. These data suggest a possible therapeutic effectiveness of the association calcitriol with non-steroidal anti-inflammatory drugs (NSAIDs) in the prevention and treatment of breast and prostate cancers (Krishnan & Feldman, 2010; Moreno et al., 2005). This drug association may also have the vantage of lowering the dose of NSAIDs thus reducing their toxic effects (Moreno et al., 2005).

Target cells of Vit D

Effects of Vit D on cancer stem cells

Vit D is one of the molecules involved in the regulation of stem cell homeostasis. $1,25(OH)_2D_3$ exerts its important biological effects on both adult stem cells (ASC) (Cianferotti et al., 2007; Zhou et al., 2010) and cancer stem cells (CSCs) (Feldman et al., 2014; Maund et al., 2011; So et al., 2011). DNA repair and protection from oxidative damage are processes that mainly affect ASCs, while cell-cycle arrest and induction of apoptosis limit the expansion of the CSCs population. Extensive research has recently been carried out to evaluate the direct effects of $1,25(OH)_2D_3$ on stem cells (Fleet et al., 2012). Most of the experimental data regarding the molecular mechanisms underlying the inhibiting effects of $1,25(OH)_2D_3$ on CSC growth and differentiation have been obtained following investigations carried out on primary cancer cell cultures or on established cancer cell lines (Pervin et al., 2013). These studies highlighted the fact that in mice the proliferation of prostate stem cells was inhibited by $1,25(OH)_2D_3$. Further experiments performed to better clarify the possible mechanisms underlying this effect showed that the interaction between Vit D and VDR stimulates the production of interleukin-1α (IL-1α) (Maund et al., 2011). This pro-inflammatory cytokine, in turn, mediated the anti-proliferative effects of $1,25(OH)_2D_3$ in adult prostate progenitor/stem cells (PrP/SC) by promoting cell-cycle arrest and senescence (Maund et al., 2011). Furthermore, Fedirko et al. (2009) have recently shown that $1,25(OH)_2D_3$ treatment and calcium supplements decrease the expression of the human telomerase reverse transcriptase (hTERT) in cells of the upper portion of the colon. These observations indicate that Vit D may indirectly inhibit the expansion of this cell population and protect it from potential genetic mutations. In line with these observation, Kasiappan et al. (2012) described how $1,25(OH)_2D_3$ decreases the mRNA expression of hTERT by inducing the expression of non-coding small RNA microRNA-498 (miR-498) in ovarian tumor cells. These effects ultimately result in the suppression of ovarian cancer growth. Finally, $1,25(OH)_2D_3$, and its analogues have been reported to regulate the expression of CD44, a specific marker of breast cancer stem cells, in human breast cancer cells *in vitro* (Parvin et al., 2013; So et al., 2011). These studies provide a basis for preclinical and clinical evaluations of Vit D and its analogues for chemoprevention of cancer stem cells. These observations warrant more extensive studies to assess the impact of Vit D on cancer stem cells.

Effects of vitamin D on vascular cells and angiogenesis

Growing evidence indicates that Vit D may play an important role in the inhibition of tumor angiogenesis (Vanoirbeek et al., 2011; Xu et al., 2013). This peculiar function has been defined with the term ''angioprevention'' (Tosetti et al., 2002). On one hand, experimental studies suggest that the preventive activity of $1,25(OH)_2D_3$ on tumor angiogenesis might be the consequence of the effects of this molecule on vascular endothelial cells (EC) (Furigay & Swamy, 2004; Mantell et al., 2000). On the other hand, *in vitro* observations have highlighted the presence of VDR on cultured bovine aortic endothelial cells, in human capillary and in venous endothelial cells (Chung et al., 2006, 2009; Merke et al., 1989). In addition, the expression of the enzyme 1α-hydroxylase, a key enzyme involved in the biosynthesis of $1,25(OH)_2D_3$, has also been reported in these cells (Chung et al., 2009; Merke et al., 1989; Suzuki et al., 2009; Zehnder et al., 2002). Early experimental investigations by Mantell et al. (2000), aimed at evaluating the effect of Vit D on angiogenesis, show that $1,25(OH)_2D_3$ may inhibit the expression of the vascular endothelial growth factor (VEGF) and the formation of new blood vessels in mice transplanted with MCF7 human breast cancer, a tumor which expresses high levels of VEGF. In prostate cancer, $1,25(OH)_2D_3$ has been reported to decrease the expression of VEGF through transcriptional repression of the hypoxia-inducible factor 1(HIF-1) (Ben-Shoshan et al., 2007). Furthermore, in SCC tumor cells, $1,25(OH)_2D_3$ has been observed to suppress the expression of the proangiogenic factor IL-8 via NF-kB-dependent pathway thus leading to the inhibition of

endothelial cell migration and tube formation (Bao et al., 2006). The specific mechanisms underlying this phenomenon consist in a reduced translocation of the p65 subunit of NF-kB to the nucleus that results in a decreased transcription of *IL-8* gene mediated by NF-kB. Furthermore, Chung et al. (2009) have highlighted the fact that, in VDR wild-type (WT) or VDR knockout mice inoculated with transgenic adenocarcinoma of the mouse prostate (TRAMP), the tumor vessels are enlarged and their volume increased in KO mice thus suggesting a negative regulation of VDR-Vit D on tumor angiogenesis. These investigations additionally showed that VDR knockout mice had increased expression levels of pro-angiogenic factors such as HIF-1, VEGF, angiopoietin-1 (Ang-1), and platelet-derived growth factor (PDGF). Vit D can increase VEGF mRNA levels in vascular smooth muscle cells (Cardús et al., 2006) while in SW480-ADH human colon tumor cells, this molecule has been shown to upregulate the mRNA levels of thrombospondin-1 (THSD1), a potent anti-angiogenic factor (Fernandez-Garcia et al., 2005). Other studies aimed at investigating the effect of $1,25(OH)_2D_3$ on normal endothelial cells and tumor-derived endothelial cells (TDECs) showed effects of $1,25(OH)_2D_3$ greater than those elicited in the normal aortic endothelial cells or yolk sac endothelial cells (MYSECs) (Chung et al., 2009). Furthermore, Vit D analogues, EB1089, Ro 25-6760, and ILX23-7553, also showed a potent antiproliferative activity against TDECs (Bernardi et al., 2002). It has been also demonstrated that the Vit D-dexamethasone association was more effective in inhibiting TDECs growth than each single agent (Chung et al., 2009). Other *in vitro* observations reported that, in TDECs, Vit D increased the intracellular levels of VDR and that of the pro-apoptotic protein p27 which reduces the concentration of signal molecules for angiogenesis, including angiopoietin-2 (Bernardi et al., 2002; Flynn et al., 2006). Interestingly, on one hand, Chung et al. (2006) compared the effects of calcitriol on SCC TDECs and endothelial cells derived from matrigel (MDECs). These authors pointed out that both these cell types expressed VDR and the interaction with $1,25(OH)_2D_3$ resulted in a 47% growth inhibition of TDECs and in a 12.3% growth inhibition of MDECs. Furthermore, in TDECs, Vit D caused cell-cycle arrest in the G0/G1 phase and a decrease of the number of cells in the S phase, due to the induction of p27 and the down-regulation of p21. These data indicated that TDECs are more susceptible than MDECs to the anti-proliferative effects of $1,25(OH)_2D_3$. On the other hand, Flynn et al. (2006) showed that $1,25(OH)_2D_3$ regulated the expression of several proteins involved in TDEC differentiation and apoptosis. These authors reported that although VDR is present in TDECs and MYSEC and Vit D upregulated VDR in these cells, a 48-h exposure of the cells to dexamethasone further increased VDR expression. Finally, no increase in the intracellular levels of CYP24A4, the predominant enzyme involved in the catabolic inactivation of $1,25(OH)_2D_3$ in normal tissues, was found in TDECs. Conversely this phenomenon occurred in MYSECs (Flynn et al., 2006). In line with these findings, Chung et al. (2006) have demonstrated that TDECs may be more sensitive to calcitriol due to novel epigenetic silencing of CYP24A1. Therefore, the direct effects of calcitriol on endothelial cells may play a role in the calcitriol-mediated antitumor activity observed *in vivo* in animal tumor models. Furthermore, *in vitro* studies on RWPE1 prostatic epithelial cells highlighted treatment with $1,25(OH)_2D_3$ as causing growth arrest (Kovalenko et al., 2010). The subsequent genomic analysis revealed a decrease in the expression level of genes coding for NF-kB and IGF-1. Additionally, the inhibition of the transcription of pro-inflammatory cytokines, including IL-1, IL-6, and IL-17, was noted as occuring after about a 6-h exposure. The same studies also showed that $1,25(OH)_2D_3$ caused a reduction of VEGF and VEGF receptors mRNA levels, including the kinase insert domain receptor (KDR) and neuropilin 1 (NRP1) and the induction of anti-angiogenic factors and that of molecules involved in the protection of cells from oxidative stress and in the homeostasis of cellular redox (Kovalenko et al., 2010). Finally, it was shown that, in RWPE1 cells, $1,25(OH)_2D_3$ induced the expression of numerous isoforms of semaphorins, including SEMA 3B, 3F, and 6D (Kovalenko et al., 2010). As these molecules may antagonize the proangiogenetic effects of VEGF by a competitive binding to receptor NRP1, this may also partly account for the growth-inhibiting effects of Vit D on prostate cancer cells. An additional mechanism of the preventive effects of Vit D on tumor angiogenesis involves its ability to inhibit COX-2 expression levels (Aparna et al., 2008). COX-2 has been shown to exert indirectly its promoting effects on tumor angiogenesis by increasing the synthesis of HIF-1α protein in cancer cells (Sahin et al., 2009). Therefore, the inhibition of this enzyme may result in a decreased growth activity of tumors. Moreover, $1,25(OH)_2D_3$ strongly represses DICKKOPF-4 (DKK4), a weak WNT antagonist that promotes invasion and angiogenesis in cultured colorectal cancer (CRC) cells and that is up-regulated in human colon tumors (Pendás-Franco et al., 2008b). All these effects may also, in part, account for the significant inhibition of metastasis observed in murine models of prostate and lung cancer treated with Vit D.

Regulation of immune function by vitamin D

Vit D and the immune system

The role of Vit D, as an immunomodulator, was well established nearly 30 years ago (Rook et al., 1986). The effects of Vit D on immune system appear to be closely linked to the chemopreventive effects on tumors (Fleet et al., 2012). The possible role of Vit D in the regulation of immune responses is strongly supported by the findings that almost all immune cells, including T cells, B cells, monocytes, neutrophils, platelets, macrophages, and dendritic cells express Vit D receptors and that Vit D appears to modulate the activity of these cells (Hewison, 2011). The ligand for VDR also showed a synergic activity with Vitamin A, Vitamin K2, and certain chemotherapeutic agents (Funato et al., 2002; James et al., 1999). Interestingly, only naive T cells display very low VDR levels, while this receptor is abundantly present upon T cell activation (Hewison, 2011). However, the differentiation of monocyte into macrophages or dentritic cells (DCs) has been shown to be associated with a decrease in VDR-expression, making these cells less sensitive to $1,25(OH)_2D_3$ (Hewison, 2011). In this context, $1,25(OH)_2D_3$ has been recognized as an important mediator of innate immune responses, enhancing

the antimicrobial properties of immune cells such as monocytes and macrophages (Hewison, 2011). There is evidence that Vit D modulates the expression of many genes in cells of the innate immune system which encode for proteins that are crucial for autophagy and for the antimicrobial activity (Hewison, 2011). In particular, one of these studies reported that Vit D increased the expression levels of genes encoding for the antimicrobial peptides human cathelicidin (CAMP) and β defensin-1 (DEFB1) in isolated human keratinocytes, monocytes, and neutrophils (Wang et al., 2004). These molecules form the first line of host defence against microbial pathogens. Another study showed that $1,25(OH)_2D_3$ markedly induced the expression of CAMP and, to a lessen extent, that of defensin β2 (DEFB2/HBD2) in different cell types such as colon cancer, acute myeloid leukemia, keratinocytes, human bone marrow cells-derived macrophages, and bone marrow cells (Gombart et al., 2005). These effects occurred following the interaction of Vit D with a specific VDRE present in the promoter region. In particular, *in vitro* studied showed that $1,25(OH)_2D_3$ stimulates the expression of the pattern recognition receptor *NOD2/CARD15/IBD1* gene in primary human monocytic and epithelial cells. As a consequence of the *NOD2* downstream signaling activation, a stimulation of NF-κB transcription factor function occurs. This effect, in turn, induces the expression of the gene-encoding antimicrobial peptide defensin β2 (*DEFB2/HBD2*) (Wang et al., 2010a). Furthermore, numerous cytokines can modulate the metabolism of Vit D in macrophages, monocytes and dendritic cells (Hewison, 2011). The pro-inflammatory cytokines such as IFN-γ and TNF-α stimulate the synthesis of $1,25(OH)_2D_3$ by increasing the expression of CYP27B1 in monocytes (Zehnder et al., 2002). On the other hand, several inflammatory cytokines and agonists of toll-like receptor (TLR), which are transmembrane proteins involved in recognizing and defending against invading pathogens, may increase the expression of CYP27B1 and that of VDR in dendritic cells (Széles et al., 2009). In contrast, IL-4 produced by type-2 T-helper lymphocytes potentiates *CYP24* gene expression in monocytes, leading to the formation of inactive metabolite 24,25-dihydroxyvitamin D_3 (Edfeldt et al., 2010). These effects may alter the intracellular levels Vit D metabolites which, in turn, can modulate the function of other immune cells in the microenvironment. Numerous studies have demonstrated that $1,25(OH)_2D_3$ is also involved in the regulation of cell functions of the adaptive immune system (Hewison, 2011). Consistent with these findings, Vit D deficiency has been associated with the development of several autoimmune diseases, including ulcerative colitis, Crohn's disease, and also infectious diseases (Cantorna, 2012; Meeker et al., 2014). However, a very limited number of studies have been performed to assess the role of the immune regulatory function of Vit D in cancer. In this context, Krishnan et al. (2007) reported that calcitriol exhibits anti-inflammatory effects that may account for its inhibitory activity in prostate cancer. More recently, Bessler and Djaldetti (2012) showed that Vit D alters the relationship between immune and cancer cells leading to a significant decrease in the pro-inflammatory cytokines TNF-α and IL-6. On the basis of these results, these authors hypothesized that the reduced production of pro-inflammatory cytokines induced by Vit D may lead to a suppression of tumor development. Furthermore, recent studies by Young and Day (2013) showed that the time elapsing before cancer recurrence following surgical treatment was increased by over 3-fold in head and neck patients receiving $1,25(OH)_2D_3$ as compared with untreated patients. This phenomenon was associated with, and increased, differentiation of blood-derived CD34+ cells into dendritic cells and to a decrease in the peripheral blood and intratumoral levels of immunosuppressive CD34+ cells.

Chemopreventive effects of Vit D on specific tumors: possible mechanisms of action

Effects of vitamin D on breast cancer cells

In vitro and *in vivo* studies carried out in order to assess the effects of $1,25(OH)_2D_3$ and its semisynthetic analogues on breast cancer cell proliferation and malignant progression showed that different ligands of the VDR are equally effective in inhibiting the growth of $ER^{(+)}$ breast cancer cell lines such as MCF-7, T-47-D, ZR-75-1, SKBR-3 (Krishnan et al., 2012), and $ER^{(-)}$ breast cancer cell lines such as BT-20, MDA-MB-435, MDA-MB-231, and SUM-159PT (Flanagan et al., 2003; Mehta et al., 2012). These data are in agreement with the clinical observations that describe the therapeutic benefit of Vit D and its analogues in both $ER^{(+)}$ and $ER^{(-)}$ breast cancer (Krishnan et al., 2012; Lee et al., 2008; Li & Brown, 2009; Mehta et al., 2012). Although the exact mechanisms by which Vit D may exert its growth inhibitory activity on breast cancer cells has not yet been fully understood, *in vitro* observations indicate that this molecule may affect tumor cell proliferation by causing cell-cycle arrest in the G0/G1 phase, by promoting apoptosis and by inhibiting tumor angiogenesis (Li et al., 2005; Nagpal et al., 2005; Vanoirbeek et al., 2011). The inhibiting effects of Vit D on cell-cycle arrest in G0/G1 entry appears to be due to an increase of the expression of cyclin kinase inhibitors CDKIs, including p21 and p27 which inhibit cell-cycle progression by blocking the activity of CDK complexes with VDR ligands (Jensen et al., 2001; Krishnan et al., 2012). Furthermore, studies carried out on the MCF-7 cell line showed that $1,25(OH)_2D_3$ reduced, in a time-dependent fashion, the intracellular levels of CDK2, CDK4, cyclin D1, and cyclin A (Jensen, 2001; Lowe et al., 2003; Verlinden et al., 1998). In particular, $1,25(OH)_2D_3$ was shown to prevent the activation of the cyclin D1-CDK4 complex, to decrease cyclin D3 expression, and to inhibit the E2F transcription factor thus decreasing the expression of cyclin A (Jensen et al., 2001). However, on one hand, the antiproliferative effects of Vit D on breast cancer cells also appears to be mediated by the induction of TGF-β (Colston & Hansen, 2002; Koli & Keski-Oja, 1995; Proietti et al., 2011) and by the suppression of the protoncogene *c-myc* expression (Jensen et al., 2001; Lopes et al., 2012; Saunders et al., 1993). In addition, Vit D can block the proliferative activity of insulin and IGF-1, most likely by increasing the expression of IGFBP-3 and IGFBP-5 (Colston et al., 1998; Lee et al., 2006; Rozen et al., 1997). On the other hand, the promoting effect of Vit D on apoptosis in breast cancer cells appears to be the result of decreased levels of Bcl-2, a redistribution of Bax, a release of cytochrome c, and DNA fragmentation (Nagpal et al., 2005; van den Bemd & Chang, 2002). Furthermore, it

was demonstrated that $1\alpha,25(OH)_2D_3$ is involved in the inhibition of transcriptional activity of NF-κB in breast cancer cells (Tse et al., 2010). In addition to their direct growth-inhibiting effects, VDR ligands may also inhibit angiogenesis and decrease the invasive and metastatic potential of breast cancer cells *in vitro* and *in vivo* (Hansen et al., 1994; Mantell et al., 2000; van den Bemd & Chang, 2002; Vanoirbeek et al., 2011). These results support the concept that the combination of VDR ligands with the most common clinically available antitumor agents used in breast cancer treatment might result in a more effective therapeutic response (Krishnan & Feldmann, 2011). This hypothesis has been further sustained by *in vitro* studies which demonstrated that $1,25(OH)_2D_3$ enhances the cytotoxic effects of doxorubicin, paclitaxel, adriamycin, cisplatin, and those induced by irradiation on tumor cells (Chaudhry et al., 2001; Lawrence et al., 2013; Sundaram et al., 2000). Interestingly, recent observations show that Vit D prevents genomic instability due to the Cathepsin L-mediated degradation of DNA repair protein 53BP1 in BRCA-1-negative breast cancer cells (Gonzalo, 2014). These results suggest that Vit D may be of clinical relevance in the treatment of aggressive form of breast cancer such as triple-negative BRCA-1-deficient ones.

Vit D on breast cancer: clinical studies

The epidemiologic studies regarding the association between Vit D and breast cancer risk have generated conflicting results so far (Engel et al., 2010; Freedman et al., 2007; Khan et al., 2010; Maalmi et al., 2014; Mehta et al., 2012; Mohr et al., 2011; Rose et al., 2013). For instance recent clinical observations showed a significant inverse association between $1,25(OH)_2D_3$ serum concentration and risk of breast cancer with a more pronounced decrease in risk in premenopausal than in perimenopausal women (Engel et al., 2010; Maalmi et al., 2014; Mohr et al., 2011; Mehta et al., 2012). In line with these findings, other clinical investigations reported that women with $1,25(OH)_2D_3$ serum concentrations greater than 52 ng/ml showed a significant decrease (50%) in the risk of breast cancer compared with women with circulating levels of $1,25(OH)_2D_3$ lower than 13 ng/ml (Bertone-Johnson et al., 2005; Garland et al., 2007; Lowe et al., 2005). Furthermore, women with $1,25(OH)_2D_3$ serum concentrations above 27 ng/ml showed a decreased risk of breast cancer by 27%, compared with those with circulating levels of this molecule lower than 19.8 ng/ml (Engel et al., 2010). These data also showed that the preventive effects of a high plasma concentration of $1,25(OH)_2D_3$ on the onset of breast cancer were more pronounced in women with normal body mass index (BMI), i.e., lower than 25 kg/m^2 (Engel et al., 2010). These findings also indicate that, in order to maintain plasma concentrations of $1,25(OH)_2D_3$ higher than 30 ng/ml, women leading a sedentary life style and who are exposed for a short period of time to sunlight require an intake of at least 2000 IU/d (Garland et al., 2007). Alternatively, it was calculated that 12 min per day of continuous exposure to sunlight and the exposure of at least the 50% skin surface is approximately equivalent to an oral administration of 3000 IU of Vit D (Engel et al., 2010; Holick, 2013). However, the optimal cut-off levels of Vit D and the threshold values below which a subject may be considered Vit D deficient are still controversial. The optimal dose currently suggested to be administered in order to prevent deleterious consequences due to hypercalcemia is 30 ng/ml (Khan et al., 2010). Foods naturally rich in Vit D content more widely consumed by the population are fish (55%), meat (23.5%), cheese (6.8%), and eggs (5.6%). However, no consistent association of breast cancer risk with the consumption of meat, eggs, or dairy foods have been highlighted so far, while the correlation between fish intake and breast cancer incidence still remains debatable (Engeset et al., 2006; Kim et al., 2009; Pala et al., 2009). The geographical area of residence, such as in the case of northern and southern Italy, has been shown to be an important factor in influencing the endogenous synthesis of Vit D (Rossi et al., 2009). In fact, an inverse association between Vit D and breast cancer was observed to be much higher in women in southern Italy compared with women in the northern part of the country because of longer exposure to sunlight (Rossi et al., 2009). However, conflicting results on the relationship between skin pigmentation and Vit D synthesis were obtained following a study carried out in United States to assess the risk of breast cancer when comparing women of Latin American origin (Hispanic women) and non-Hispanic white women (John et al., 2007; Rollison et al., 2012). Overall these studies showed an inverse relationship between Vit D intake and incidence of breast cancer either in premenopausal women or in post-menopausal women (Anderson et al., 2010; Rollison et al., 2012; Shin et al., 2002). In addition, circulating levels of Vit D were shown to be associated with increased breast cancer mortality (Goodwin et al., 2009; Mohr et al., 2011; Yao & Ambrosone, 2013). On one hand, ethnic difference and the degree of skin pigmentation have provided important evidence that exposure to sunlight is the main source of Vit D and that this phenomenon stimulates the synthesis and increases the concentrations of circulating $1,25(OH)_2D_3$ (Armas et al., 2007; John et al., 2007; Knight et al., 2007; Rollison et al., 2012; Yao & Ambrosone, 2013). On the other hand, discrepant results regarding the relationship between existing pigmentation of the skin and ability to synthesize Vit D have been obtained from other studies (Bogh et al., 2010; John et al., 2007; Rollison et al., 2012). Increased skin pigmentation reduced the dose of UV exposure, consequently it may cause a decreased synthesis of Vit D. The Hispanic women showed lower levels of circulating Vit D than white women exposed to the same amount of sunlight and, consequently, an increased incidence of breast cancer and mortality (Mohr et al., 2011; Yao & Ambrosone, 2013). The integration of Vit D in one's diet, in particular among Hispanic women, had a considerable impact on Vit D circulating levels and on the incidence of breast cancer (John et al., 2007; Mohr et al., 2011; Rollison et al., 2012; Yao & Ambrosone, 2013). Overall these studies do not fully clarify whether Vit D is associated with a reduced risk of breast cancer. Further investigation may better define the clinical impact of vitamin supplementation in breast cancer development and treatment.

Effects of vitamin D on prostate cancer cells

Experimental and clinical studies provide evidence of a positive correlation between Vit D deficiency and prostate

cancer (Swami et al., 2011). The findings showing that VDRs are expressed in normal prostate tissue, in benign prostate hyperplasia (BPH), and in prostate cancer cells suggest that BPH and prostate cancer (PCa) may represent potential targets for VDR ligands (Adorini et al., 2007; Crescioli et al., 2002; Hendrickson et al., 2011; Krill et al., 2001; Munetsuna et al., 2011; Swami et al., 2011). Several experimental observations have highlighted the fact that VDR ligands may exert numerous effects on prostate cancer cells including cell-cycle arrest in the G0/G1 phase, apoptosis, and interaction with androgen-mediated signaling (Guzey et al., 2002; Nagpal et al., 2005; Zhuang & Burnstein, 1998). VDR has also been shown to repress COX-2 and enhance expression of hydroxyprostaglandin dehydrogenase 15-(NAD) (HPGD), the combined effects of which can serve to limit prostate cancer cell growth through an overall reduction in prostaglandin activity (Moreno et al., 2005). Consistent with this hypothesis, earlier studies by Zhao et al. (1997) showed that the androgen antagonist Casodex suppresses the antiproliferative effect of $1,25(OH)_2D_3$ in LNCaP cells, indicating that the AR is involved in $1,25(OH)_2D_3$-mediating signaling. Experimental evidence shows that the growth inhibition mediated by VDR ligands appears to be the result of a decrease in CDK2 activity and an increase in the expression of p21, p27, IGFBP-3, IGFBP-5, and E-cadherin (Drivdahl et al., 1995; Guzey et al., 2002; Huynh et al., 1998; Krishnan et al., 2004; Zhuang & Burnstein, 1998). In addition, in some prostate cancer cells, Vit D has been shown to down-regulate some anti-apoptotic genes such as Bcl-2 (Chen & Holick, 2003; Guzey et al., 2002). Recent cDNA analysis of normal prostate cells and LNCaP prostate cancer cells treated with $1,25(OH)_2D_3$ contributed to identifying the target genes and to clarifying, in part, the mechanism of Vit D-induced tumor cell growth inhibition (Krishnan et al., 2004; Peehl et al., 2004). These studied have reported the overexpression of the gene-encoding 24-hydroxylase, the enzyme which catalyzes the first step of the catabolic degradation of $1,25(OH)_2D_3$ (Chen et al., 2012; Krishnan et al., 2004; Muindi et al., 2007, 2010). These observations imply that the use of 24-hydroxylase inhibitors may increase the inhibitory activity of $1,25(OH)_2D_3$ and that of its synthetic analogues (Muindi et al., 2010). Other possible mechanisms underlying the growth-inhibiting activity of Vit D on prostate cancer cells include stimulation of differentiation, modulation of growth factor activity, and inhibition of tumor angiogenesis, invasion, and metastasis (Krishnan & Feldman, 2011; Marchiani et al., 2006; Stio et al., 2011). Moreover, it has been also reported that, in normal and malignant prostate cells, $1,25(OH)_2D_3$ may induce the expression of enzymes implicated in the maintenance of redox balance and protection of cells from oxidative damage such as TXNRD1 and SOD-2 (Peehl et al., 2004). Although a direct inhibiting effect of these enzymes in prostate cell growth inhibition appears unlikely, it is reasonable to hypothesize that they may indirectly mediate the chemopreventive effects of $1,25(OH)_2D_3$ by preventing DNA damage caused by ROS. Finally, recent finding by Hsu et al. (2011) show that Vit D may indirectly affect tumor cell invasion and metastasis by facilitating prostate cancer cell aggregation through the increase of E-cadherin expression. However, Ajibade et al. (2014) recently reported that prolonged treatments with calcitriol in homozygous male TRAMP mice resulted in the development of a resistant and more aggressive form of prostate cancer associated with increased distant organ metastasis. Although the possible mechanism(s) facilitating these effects were not defined, these results support the concept that Vit D compounds may be effective in slowing or preventing progression of prostate cancer of earlier stages.

Clinical studies with prostate cancer

The data obtained following epidemiological studies on the effects of $1,25(OH)_2D_3$ on prostate cancer are not univocal (Gilbert et al., 2012; Hendrickson et al., 2011; Krishnan & Feldman, 2011; Kristal et al., 2014; Swami et al., 2011). In fact, whereas some studies demonstrated a strong inverse association between Vit D serum concentrations and risk of prostate cancer (Gilbert et al., 2012; Tretli et al., 2009), other investigations show no significant correlation between Vit D circulating levels and serum PSA (Gilbert et al., 2012). More recently Kristal et al. (2014) reported that low and high Vit D concentrations were associated with increased risk of prostate cancer. This association resulted stronger for high-grade disease. Other clinical studies have highlighted the effective therapeutic potential of Vit D when administered alone or in combination with other cytostatic agents (Beer, 2008; Hershberger et al., 2001; Krishnan & Feldman, 2011; Ma et al., 2010; Nagpal et al., 2005; Swami et al., 2011; Wigington et al., 2004). In addition, these studies have provided the rationale for a combination calcitriol–taxanes therapy in patients with prostate cancer (Beer et al., 2008; Ting et al., 2007a,b). On the basis of these considerations, many clinical studies have been undertaken in androgen-independent prostate cancer patients where Vit D_3 was often combined with standard cancer therapies. However, although these drug associations were well tolerated and the addition of Vit D_3 did not result in any additional toxicity, when compared with the standard therapies alone, most of these investigations reported no beneficial effect of Vit D in these patients (Leyssens et al., 2013).

Effects of vitamin D on colon cancer cells

A consistent number of investigations provide evidence that $1,25(OH)_2D_3$ and its semisynthetic analogues may play a key role in the prevention and treatment of colorectal cancer (Byers et al., 2012; Leyssens et al., 2013; Pereira et al., 2012). Numerous studies have shown that colorectal cancer cells express VDR and the enzyme 1α-hydroxylase that converts 25-hydroxyvitamin D_3 [$25(OH)D_3$] into the active metabolite of vitamin D, $1,25(OH)_2D_3$ (Matusiak et al., 2005). The activation of VDR elicits antitumor effects by triggering apoptosis and by inhibiting cell proliferation, invasion, and angiogenesis (González-Sancho et al., 2006; Krishnan & Feldman, 2011; Pendás-Franco et al., 2008a; Samuel & Sitirin, 2008). In particular, *in vitro* studies showed that this molecule may inhibit the proliferation of colon tumor cells by blocking the cell cycle in the G1 phase, by promoting apoptosis and cell differentiation and by affecting tumor angiogenesis (Bettoun et al., 2002; Pálmer et al., 2001; Pendás-Franco et al., 2008a; Pereira et al., 2012).

Furthermore, on one hand, studies on different colon carcinoma cell lines showed that $1,25(OH)_2D_3$ may promote apoptosis by the up-regulation of the proapoptotic protein BAK1 and the down-regulation of the nuclear anti-apoptotic protein BAG1 and by preventing the formation of apoptotic heterodimers Bcl-2-Bax (Pereira et al., 2012; Welsh, 2012). The negative effects of $1,25(OH)_2D_3$ on cell proliferation and apoptosis are further underlined by the findings that this molecule promotes sensitivity to the chemotherapeutic agent 5-fluorouracil (5-FU) by down-regulating the expression of the anti-apoptotic protein survivin and that of thymidylate synthase, a key enzyme in the biosynthethic pathway of DNA. On the other hand, *in vitro* studies also reported that $1,25(OH)_2D_3$ promotes cell differentiation by increasing the expression of several components of cell adhesion that are essential for the maintenance of the epithelial phenotype and that of proteins associated with the actin cytoskeleton and intermediate filaments (Pálmer et al., 2001; Pereira et al., 2012). These findings are consistent with the observations that the treatment of *Caco-2* colon cancer cells with $1,25(OH)_2D_3$ induced an increase in the expression of p21, p27, E-cadherin, and other adhesion proteins (ZO-1, ZO-2, and vinculin) and promotes the translocation of β-catenin and ZO-1 from the nucleus to the plasma membrane (Gaschott et al., 2002). More specifically, it was shown that the VDR antagonist, ZK-191-732, modulates the differentiation process induced by butyrate on *Caco-2* cells (Gaschott et al., 2002). Furthermore, it was established that a decrease in the levels of cyclin D1 is essential for the anti-proliferative effects of Vit D (Hofer et al., 1999; Maier et al., 2009). *In vitro* studies aimed at assessing the effects of $1,25(OH)_2D_3$ on the gene expression in SW480-ADH cell line showed that Vit D increased the expression of *c-Jun, JunD, Jund, FREAC-1/Fox1, ZNF-44/Kox7, G0S2*, tumor suppressors normal epithelial-cell-specific gene 1 (*NES-1*), or kallikrein 10 and protease M (Pálmer et al., 2003). This phenomenon further stresses the concept that Vit D appears to play a role in cell growth inhibition, adhesion, differentiation, and apoptosis. These effects ultimately lead to the reversion of the neoplastic phenotype to a normal epithelial phenotype (Gocek & Studzinski, 2009; Nagpal et al., 2005). Finally, Meeker et al. (2014) by using a model of bacteria-driven colitis and colon cancer when infected with *Helicobacter bilis* (*H. bilis*) showed that mice fed high vitamin D diet had a significantly lower incidence of cancer compared with mice fed maintenance diet. These findings further suggest that increased dietary vitamin D is beneficial in preventing inflammation-associated colon cancer through the suppression of inflammatory responses during the initiation of neoplasia or early-stage carcinogenesis.

Colorectal cancer and vitamin D

Clinical studies

Colorectal cancer is the third most common type of cancer in men and women in western countries (Chan & Giovannucci, 2010). There is strong evidence on a significant relationship between lifestyle and diet and incidence-rate of this type of cancer (Chan & Giovannucci, 2010). Several case–control studies and cohort studies have examined the relationship between Vit D intake (total, with diet, or supplementary) and the risk of colorectal cancer (Giovannucci, 2010; Woolcott et al., 2010). The consistent body of investigation that analyzed the relationship between the serum 25-hydroxyvitamin D_3 [$25(OH)D_3$] level and colorectal cancer risk generally shows an inverse association (Freedman et al., 2007). In support of these observations, a large observational-nested case–control study undertaken within the European Prospective Investigation into Cancer and Nutrition showed a strong inverse association between $25(OH)D_3$ concentrations and colorectal tumor (Jenab et al., 2010). Furthermore, a recent meta-analysis of 35 independent studies further confirmed the inverse relationship between circulating levels of $25(OH)D_3$ and colorectal cancer risk (Gandini et al., 2011; Maalmi et al., 2014). Finally, a systematic review of 18 prospective studies carried out by Ma et al. (2011) undertaken to assess the association of Vit D intake or 25-hydroxyvitamin D_3 serum levels and the risk of colorectal tumor in about 1 000 000 individuals highlighted the fact that vitamin D intake and $25\text{-}(OH)D_3$ blood levels were inversely associated with the risk of colorectal cancer. In particular, some of these studies showed that the intake of 1000 IU/d of Vit D was associated with a 50% decrease in risk of colorectal tumor, while plasma concentrations of 20–29 ng/ml of 25-hydroxyvitamin D_3 were associated with an increased risk of developing colorectal cancer. Conversely, concentrations higher than 30–39 ng/ml were associated with a decreased risk (Jenab et al., 2010). However, some investigations have reported different results. For instance, on one hand, Wactawski-Wende et al. (2006) failed to show marked effects of Vit D on the incidence of colorectal cancer. Furthermore, the co-administration of calcium and Vit D in women taking estrogen resulted in an increased risk of colorectal cancer. The conclusion of the study was that Vit D supplementation may have a greater impact on mortality, but a lower incidence of colorectal cancer. On the other hand, Ishihara et al. (2008) did not highlight any statistically significant correlation between Vit D intake and risk of colorectal cancer. Only concentrations of $25\text{-}(OH)D_3$ lower than 80 nmol/L were inversely associated with mortality from colorectal cancer, but not with the incidence. The discrepancies in these results were, in part, explained by the fact that the subjects considered in these studies were often taking food high in calories and low in fiber. Moreover, these subjects did not carry out any physical activity, had a high body mass index, a positive family history of colorectal cancer, and were smokers. There was no difference in the amount of Vit D and sunlight exposure between cases and controls. The use of Vit D supplements was more common among the controls, and this was statistically significant among men. Because of these conflicting results further studies are needed to better define the chemopreventive effects of Vit D on colorectal cancer.

Effects of vitamin D on SCC

The presence of VDR in keratinocytes and the ability Vit D to induce differentiation and to inhibit cell proliferation may, in part, account for the therapeutic potential of this molecule and its semisynthetic analogues in the SCC of the head neck (H&NSCC) and aerodigestive tract (Bikle, 2012; Ma et al., 2013a,b; Nagpal et al., 2005). In particular, the

inhibition of p21 expression in keratinocytes has been reported to be an essential prerequisite for the induction of cell differentiation (Di Cunto et al., 1998). $1,25(OH)_2D_3$ has been shown to inhibit SCC growth *in vitro* and *in vivo* (Hershberger et al., 2001; Ma et al., 2013b). Interestingly, recent *in vitro* studies have highlighted the additive inhibitory effects of Vit D in combination with 5-FU and or 13-*cis*-retinoic acid on human oral squamous carcinoma cell growth (Dalirsani et al., 2012). In contrast, Gedlicka et al. (2006) have shown that Vit D induced growth inhibition in SCC cell lines of the head and neck by arresting cells in the G0/G1 phase of the cell cycle and that this effect was associated with an upregulation of p18 cell-cycle inhibitor. Further studies demonstrated that $1,25(OH)_2D_3$ inhibits, *in vitro*, cell motility and invasion of SCC cells while, *in vivo*, this molecule markedly suppresses the ability of SCC cells to establish pulmonary metastases in tumor-bearing mice (Ma et al., 2013a). The potential target genes for Vit D have been identified in cell line SCC25 following treatment with the analogue EB1089 (Lin et al., 2002). The expression of genes such as 24-hydroxylase, protease M, cystatin M, amphiregulin, stromelysin, and collagenase I resulted up-regulated whereas the expression levels of CRABP-II, N-cadherin, and SCC antigen were down-regulated (Lin et al., 2002). The effects of Vit D and its analogues on the expression of genes involved in cell differentiation, growth inhibition, and immunomodulation, further indicate that SCC cell lines can be driven to differentiation by these compounds (Goceck & Studzinski, 2009). To date, limited epidemiologic studies on the effect of Vit D or its metabolites on SCC prevention or treatment in humans have generated conflicting data. Recently, Eide et al. (2011) reported a positive relationship between plasma levels of $25(OH)D_3$ and non-melanoma skin cancer (NMSC) including SCC and basal cell carcinoma (BCC), in a study of 3223 white health maintenance organization patients who sought advice about the risk of osteoporosis or low bone density. These findings have been confirmed by a recent nested case–control study among women by Liang et al. (2012). Conversely, Tang et al. (2010) highlighted higher serum $25(OH)D_3$ levels as being associated with a decreased risk of NMSC in older Caucasian men. The discrepancies in these results were explained by the different observation periods and the different types of subject enrolled in these studies. Overall, there is some evidence that Vit D may be of clinical interest in SCC and melanoma prevention. However, additional studies are needed to assess the suitability of topical or oral Vit D for chemoprevention of SCC and BCC in humans (Tang et al., 2012).

Effects of vitamin D on hematological malignancies

There is increasing interest in the possible use of Vit D to combat hematological diseases including leukemias, myelodysplastic syndrome (MDS), lymphomas, and multiple myeloma (MM) (Kim et al., 2012; Motomura et al., 1991; Shanafelt et al., 2011). The potential therapeutic role of $1,25(OH)_2D_3$ in the treatment of hematologic malignancies was first highlighted by Abe et al. (2004) who reported that Vit D was able to induce *in vitro* differentiation of M1 murine myeloid cells. These findings were further confirmed by other experimental *in vivo* studies which showed that the administration of Vit D increased the survival time of mice inoculated with leukemic cells (Honma et al., 1983). The possible therapeutic effectiveness of $1,25(OH)_2D_3$ in the clinical treatment of hematological malignancies relies on the observations that this molecule appears to inhibit the proliferation of hematopoietic precursor cells and to promote their maturation and, ultimately, cell differentiation (Abe et al., 2004; Gocek & Studzinski, 2009; Honma et al., 1983; Kim et al., 2012; Shanafelt et al., 2011). The biochemical mechanisms through which Vit D and its derivatives induce these effects have been, only in part, elucidated (Kim et al., 2012). Experimental observations have highlighted the fact that these mechanisms may be different according to the cell type (Bhatia et al., 1995; Hughes et al., 2010; Kim et al., 2012). For instance, Vit D may induce monocytic differentiation of myeloid leukemia cells. This phenomenon may result in the G1 phase cell-cycle block and, consequently in the cessation of cell proliferation (Bhatia et al., 1995). In acute promyelocitic leukemic cells, Vit D appears to activate three types of intracellular signaling pathways, namely PKC pathway, PI3K-AKT pathway, and three different MAPK pathways which have been suggested to intersect at a common nodal point (Raf-1) (Bhatia et al., 1995; Goceck & Studzinski, 2009; Hughes et al., 2010; Kim et al., 2012; Wang & Studzinski, 1997). Activation of the MAPK and PI3K-AKT pathways has also been implicated in Vit D-mediated VDR synthesis and nuclear translocation (Goceck & Studzinski, 2009; Kim et al., 2012). However, in the acute promyelocytic leukemia cell line NB4, the monocytic differentiation was induced independently of any VDR/VDRE interaction (Bhatia et al., 1996). On the other hand, in U937 leukemic cells, Vit D was shown to activate the transcription of cycline-dependent kinase inhibitors p21Waf1/Cip1 and p27Kip1 and that of the protein HOXA10 (Liu et al., 2010). Overexpression of p21 and HOXA10 facilitates the differentiation of U937 cells into monocytes/macrophages cell lineage (Kim et al., 2012; Rots et al., 1998). Therefore, the therapeutic strategies currently available in the clinical treatment of leukemias and MDS include agents that induce differentiation of hematopoietic precursors (Harrison & Bershadskiy, 2012; Kim et al., 2012; Nagpal et al., 2005; Shanafelt et al., 2011). The main hematologic responses were observed in patients with MDS treated with calcitriol and alfacalcidol (Kim et al., 2012; Mellibovsky et al., 1998). Although the range of response in these studies varied from 44 to 100%, with complete remission in only 6% of patients, the prevention of the progression of MDS is significant. Recent findings have shown that Vit D may induce antileukemic effects by promoting autophagy in leukemic cells via the increase of the intracellular levels of beclin-1 which is known to induce the formation of autophagosomes in mammalian systems (Kim et al., 2012; Wang et al., 2008). On the contrary, in the K562 chronic myeloid leukemia cell line, which is characterized by a rapid growth rate and lack of differentiation, Vit D was found to induce apoptosis (Kim et al., 2012; Wang & Studzinski, 1997). Similarly, the treatment of HL-60 cells with Vit D induced an increase in Mcl-1, an anti-apoptotic protein that blocks cytochrome c release in the apoptosis pathway and may also target Raf-1 (Kim et al., 2012; Wang & Studzinski, 1997). Although experimental studies support a potential

clinical benefit of the use of Vit D derivatives in the treatment of hematological malignancies, the partial validation observed in clinical trials against acute myeloid leukemia and MDS implies that the therapeutic effectiveness of Vit D in these malignancies deserves further extensive investigation (Kim et al., 2012).

Effects of cancer on vitamin D metabolism

Effects of cancer on VDR levels

Although a consistent body of experimental and clinical research have been undertaken in order to define the therapeutic effectiveness of Vit D on cancer, other studies have been directed towards investigating the impact of cancer on the Vit D system. Some of these studies have shown that VDR may be overexpressed or down-regulated in various human cancers (Friedrich et al., 2006; Kure et al., 2009; Lopes et al., 2012; Motomura et al., 1991; Matusiak et al., 2005; Reichrath et al., 2004). For instance, Matusiak et al. (2005) demonstrated that the expression levels of VDR were low in normal epithelial cells of the colon while they were increased in aberrant crypts foci, in polyps, and in well-differentiated cancer cells. These findings are in line with those of Kure et al. (2009) who showed that in 233 out of 619 patients the overexpression of VDR was associated with mutations of *Ras*-MAPK and PI3K-AKT. Conversely, clinical studies in 82 patients with melanoma highlighted a remarkable reduction, or even the absence, of VDR expression in tumor tissue in comparison with normal skin. This phenomenon was correlated with the progression of melanocytic lesions (Brożyna et al., 2011). These data suggest that altered expression levels of VDR may be regarded as a potentially useful marker in the follow-up of melanoma patients treated with Vit D or its semisynthetic analogues. Furthermore, studies undertaken to assess the expression level of VDRs in normal mammary cells, in benign lesions, in localized breast cancer, and invasive breast cancer highlighted the presence of these receptors in a variety of breast tissues with some quantitative differences (Lopes et al., 2010; Zhang et al., 2014). In particular, VDRs were frequently expressed in benign lesions (93.5%) while in carcinoma *in situ* or in metastatic tumor the rate of expression was lower (56.2%) (Lopes et al., 2010). Similar results were obtained from studies which evaluated the expression level of VDR in benign and malignant ovarian tissues (Thill et al., 2010). These studies showed that VDR expression levels were significantly lower in malignant tissue as compared with normal tissue. On one hand, it is worth noting that Zhang et al. (2014) recently demonstrated a negative correlation between VDR expression in human breast cancer tissue and metastasis in breast cancer. Furthermore, coculture of VDR-overexpressing breast cancer cells with a macrophage cell line demonstrated that overexpression of VDR alleviated the prometastatic effect of cocultured macrophages on breast cancer cells. On the other hand, Hendrickson et al. (2011), by assessing the level of expression of VDR in tumor tissue from 841 patients with prostate cancer, showed that the high expression of VDR in tumor tissue was associated with a reduced risk of cancer death suggesting an important role of Vit D on prostate cancer progression. Interestingly, Srinivasan et al. (2011) recently described the presence of VDR at nuclear and cytoplasmic levels in lung cancer cells. These authors additionally showed that while the high levels of nuclear VDR were associated with increased survival, no correlation was observed between the survival and the expression levels of cytoplasmic VDR. Furthermore, it has been reported that some gene mutations are strongly associated to cancer progression such as observed in the case of *Ha-Ras*, in HC-11 mouse mammary epithelial cells (Escaleira & Brentani, 1999) or *K-Ras* in RWPE-2 cells (Zhang et al., 2010) or Simian Virus 40 (*SV40*) in human mammary epithelial (HME) cells (Kemmis & Welsh, 2008), and that this phenomenon may result in a decreased expression of VDR. On the other hand, several studies have identified many factors that may influence the expression of VDR in cancer. For instance, it has been shown that the overexpression of SNAIL transcription factors can reduce the expression of the gene encoding VDR in SW-480-ADH, HCT116, *Caco-2*, LS174T, and HT29 colon cancer cell lines (Pálmer et al., 2004). Furthermore, Larriba et al. (2013) showed that SNAIL1 repressed the expression of VDR and also inhibited the migration of nuclear β-catenin induced by $1,25(OH)_2D_3$ in SW-480-ADH colon cancer cells. In addition, SNAIL1 thwarted the inhibitory effects of $1,25(OH)_2D_3$ on cell proliferation. In colon and breast cancer cell lines, SNAIL1 and SNAIL2 can bind E-boxes in the proximal promoter of the gene for VDR and enhance the recruitment of co-repressors that reduce the expression of VDR (Mittal et al., 2008; Peña et al., 2005). Another protein that regulates the expression of VDR gene is *p53*. It has been shown that in Saos-2 osteosarcoma cells and human non-small cell lung cancer cells H1299, the overexpression of *p53*, resulted in an increased expression of VDR (Maruyama et al., 2006). Unfortunately, *p53* gene is mutated in many human tumors and this may account for the reduced expression of *VDR* gene in breast and lung cancer cells. Interestingly, the *p53* mutant form has been shown to be able to interact with VDR, to increase its accumulation in the nucleus, and to convert Vit D into an antiapoptotic agent (Stambolsky et al., 2010). These findings indicate that in tumors with *p53* mutant, the therapeutic potential of Vit D or its analogues may be limited (Stambolsky et al., 2010). In addition, there is also some evidence that *ras* activation can decrease the transcriptional activity mediated by Vit D (Fleet et al., 2012). This phenomenon was first described by Solomon et al. (1999), who observed that in *ras*-transformed keratinocytes, the transcriptional activity mediated by VDRs was reduced as a result of phosphorylation on serine 260 of the heterodimeric partners of the VDR, namely RXR. A reduction in the transcriptional activity of the VDR after phosphorylation within the domain of RXR AF-1 was also observed in the RWPE2 cell line (Zhang et al., 2010). Overall, these studies suggest that the development of cancer can lead to a reduction of the responses mediated from $1,25(OH)_2D_3$ thus weakening VDR-mediated signaling pathways.

Effects of malignant transformation on 1-α-hydroxylase (CYP27B1) expression levels

Although the presence of the enzyme 1-α-hydroxylase, in many tumor tissues, has been well recognized, studies on the

expression of this enzyme during cancer development have had discrepant results (Cross et al., 2005, 2009; Höbaus et al., 2013). In fact, the expression and activity of CYP27B1 have been shown to increase, decrease, or remain unchanged according to the organ, the tumor grade, and the reference tissue (Cross et al., 2005; Höbaus et al., 2013). In this context, several *in vitro* studies have shown that the activity of CYP27B1 and its contribution to $1,25(OH)_2D_3$ production is lost in tumors with an aggressive phenotype (Fleet et al., 2012). On the other hand, Hsu et al. (2011) reported that CYP27B1 was present in normal prostate epithelia cells (PECs). However, its enzyme activity was reduced in cells isolated from BPH while it was virtually absent in cells isolated from prostate cancer patients. Further, Segersten et al. (2002) reported that this enzyme was overexpressed in a number of parathyroid adenomas of primary hyperthyroidism (HTP) and in hyperplastic glands of secondary HPT while it resulted underexpressed in parathyroid carcinomas, compared with normal parathyroid glands. These findings are consistent with the observations that, unlike cancer cells, normal cells were susceptible to the growth inhibiting effects of $1,25(OH)_2D_3$ treatments. These results were confirmed by another study which showed that the reduced expression of CYP27B1 in LNCaP cells resulted in a reduced growth inhibition induced by $1,25(OH)_2D_3$ (Chen et al., 2003). Moreover, CYP27B1 expression was undetectable in metastases from human colon cancer (Matusiak & Benya, 2007). More recently, Brożyna et al. (2011) showed an inverse correlation between CYP27B1 expression levels and melanoma progression. However, the relationship between decreased levels of CYP27B1 and malignant phenotype was not univocally observed in all types of cancer (Fleet et al., 2012). For instance, Friedrich et al. (2006) showed that the mRNA coding for CYP27B1 increased in breast cancer when compared with normal tissue. Furthermore, Clinckspoor et al. (2012) recently highlighted CYP27B1 expression levels as increasing in malignant thyroid tumors. Conversely, immunohistochemical observations by Lopes et al. (2010, 2012) failed to highlight significant differences in the expression of CYP27B1 between normal breast tissue and tumor tissue while discrepant results were obtained with renal carcinoma (Blomberg Jensen et al., 2010; Urbschat et al., 2013). Because of these conflicting data, no definitive conclusion can be drawn on the consequences of malignant transformation on the expression of CYP27B1.

Effects of malignant transformation on 24-hydroxylase (CYP24A1) expression levels

Similarly, as described for CYP27B1, the level of CYP24A1 expression, an enzyme involved in the degradation of metabolic products of Vit D, may be influenced by the malignant transformation (Cross et al., 2011; Hobaus et al., 2013). In fact, aberrantly high basal expressions of CYP24A1 have been observed in various tumors (Hobaus et al., 2013; Horváth et al., 2010). Furthermore, epidemiological studies showed that serum levels of $25(OH)D_3$, the precursor of $1,25(OH)_2D_3$, below 30 nM were strongly associated with an increased incidence of colorectal cancer (Cross et al., 2011). In this context, recent experimental and clinical observations by Cross et al. (2005) and Brozek et al. (2012) reported that the expression levels of CYP24A1 increased dramatically during colorectal cancer progression to a poorly differentiated stage (G3–G4). Furthermore, studies on breast cancer showed that the *CYP24A1* gene was overexpressed in this type of tumor and that this phenomenon also accounted for the inhibition of the antiproliferative effects of $1,25\text{-}(OH)_2D_3$ on tumor cells (Lopes et al., 2010). Consistent with these observations, Anderson et al. (2006) reported an increased expression of CYP24A1 levels in MCF-7, SW-620 breast cancer cells, in breast tumor tissue and in ovary, colon, and lung cancer. Subsequent studies showed that CYP24A1 was present in the nuclei of normal colonic epithelial cells, aberrant cryptic foci and adenomatous polyps (Matusiak & Benya, 2007). However, following malignant transformation, the location of this enzyme shifted almost entirely from the nuclear compartment to the cytoplasmic compartment (Matusiak & Benya, 2007). Overexpression of CYP24A1 was also demonstrated in prostate cancer, ovarian cancer, cervical cancer, lung cancer, SCC, and BCC (Friedrich et al., 2006; Mitschele et al., 2004; Muindi et al., 2007). In esophageal cancer, high CYP24A1 expression was reported to correlate with a poor prognosis (Mimori et al., 2004). Overall, these data highlight the altered metabolism of $1,25\text{-}(OH)_2D_3$ as appearing to be a typical feature of advanced cancer and also suggest that one major mechanism responsible for Vit D resistance or reduced sensitivity to calcitriol in VDR-positive cells may be dependent on an increase of $1,25\text{-}(OH)_2D_3$ and $25(OH)D_3$, catabolism via the C-24 hydroxylation pathway. In line with these observations, Muindi et al. (2007) have demonstrated that ketoconazole, an inhibitor of CYP24A1, can restore the activity of growth inhibition exerted by $1,25\text{-}(OH)_2D_3$ in prostate cancer cells. More recently, Komagata et al. (2009) found that the levels of CYP24A1 can be reduced at post-transcriptional levels by miR125b, a micro-RNA that can bind the 3′-UTR (untranslated region) mRNA for CYP24A1. In addition, these authors demonstrated that, in breast cancer, CYP24A1 levels were inversely related to miR125b levels suggesting that the lack of this regulatory RNA may account for the increased expression levels of CYP24A1 in cancer cells. These findings might, in part, account for the limited therapeutic effects of $1,25(OH)_2D_3$ observed in some clinical trials. Thus the inhibition of CYP24A1 by pharmacological means may lead to a new approach in Vit D-based treatment of neoplastic diseases.

The semi-synthetic analogues of Vit D

The numerous epidemiological and preclinical investigations suggesting a role of Vit D in the prevention and treatment of several human tumors support the clinical use of $1\alpha,25(OH)_2D_3$ and Vit D analogues as potential preventive and therapeutic anticancer agents (Brown & Slatopolsky, 2008; Trump et al., 2010). However, the hypercalcemic effects induced by $1,25(OH)_2D_3$ have strongly limited its therapeutic use (Ma et al., 2010; Mehta, 2012). Therefore, many efforts are currently being directed towards synthesizing new Vit D analogues with the goal of improving the biological profile of the natural hormone, which retains the

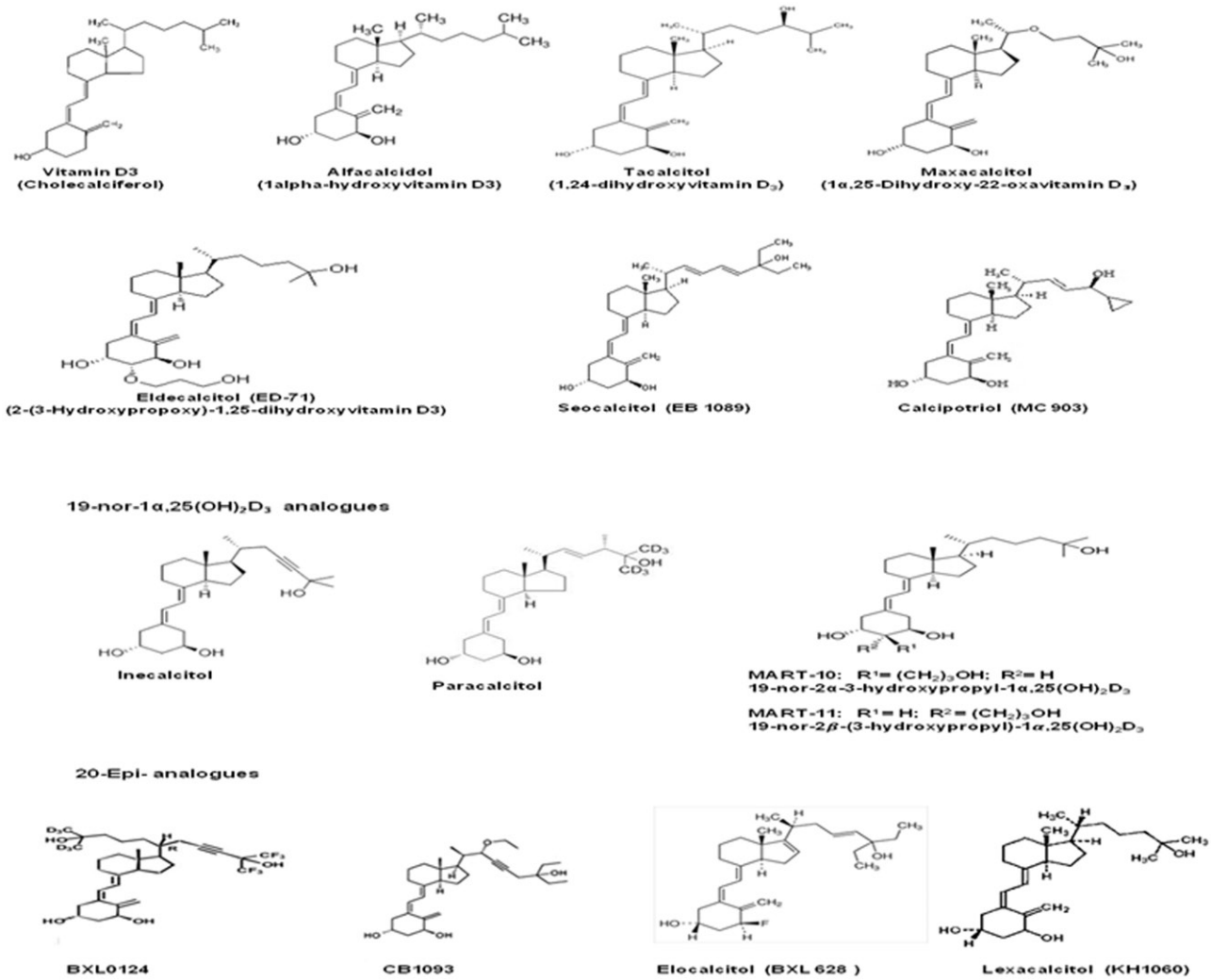

Figure 2. The chemical structure of Vitamin D analogues endowed with cancer chemo-preventive activity.

therapeutic activity the analogues being endowed with a lower calcemic effect (Byers et al., 2012; Chen & Kittaka, 2011; Fleet et al., 2012; Trump et al., 2010). Vit D analogues are semi-synthetic molecules, since they share the basic structure of $1,25(OH)_2D_3$ but differ in the presence of a characteristic functional group (R) (Brown & Slatopolsky, 2008; Trump et al., 2010; Unten et al., 2004) (Figure 2). To date, nearly 1500 Vit D analogues have been synthesized and evaluated, *in vitro* and *in vivo*, for their therapeutic effectiveness, in a variety of carcinogenesis and human cancer models (Leyssens et al., 2014; Mehta, 2012). However, of these compounds, only a few have been approved for further evaluation in clinical trials in leukemia patients, breast cancer patients, prostate cancer patients, and colon cancer patients (Agoston et al., 2006; Hussain et al., 2003; Kim et al., 2012; Leyssens et al., 2014; Mehta, 2012). The chemical structure of some of the Vit D analogues endowed with cancer chemo-preventive activity is shown in Figure 2. Analogues that are structurally unrelated to Vit D but are able to interact with VDR have also been synthesized (Byers et al., 2012; Choi & Makishima, 2009). More recently a new class of Vit D analogues, characterized by two side-chains linked to carbon-20 (Gemini) and with deuterium substituted on one side-chain, have been synthesized (Maehr et al., 2013; Okamoto et al., 2014; Spina et al., 2007). Although these analogs do not show any adverse calcemic effects from Vit D, they may induce other toxic effects unrelated to calcemia (Mehta et al., 2012).

Alfacalcidol and doxercalciferol

Alfacalcidol ($1\alpha,25(OH)D_3$) and doxercalciferol ($1\alpha,25OHD_2$) (Figure 2) are Vit D analogues endowed with chemopreventive effects. Early reports from Iseki et al. (1999) showed that a long-term administration of high doses of alfacalcidol significantly reduced the incidence of colon cancer in rats. In line with these observations, Kikuchi et al. (2007) showed that treatment with alfacalcidol prevented the ulcerative colitis and the development of colon cancer in mice. Similar results were reported from experiments on female Sprague–Dawley rats with 7,12-dimethylbenz[*a*]anthracene(DMBA)-induced mammary tumor where the administration of alfacalcidol suppressed the growth of tumors in a dose-dependent fashion (Iino et al., 1992). Hara et al. (2001) showed that the oral administration of alfacalcidol to mice transplanted with Dunn osteosarcoma significantly suppressed tumor growth and metastasis formation. The same study reported that the co-

administration of alfacalcidol and doxorubicin resulted in additive antitumor and antimetastatic effects. Although the mechanisms of the antitumor effects of alfacalcidol have not yet been fully elucidated, experimental evidence indicates that, in particular, this molecule may inhibit tumor angiogenesis (Iseki et al., 1999). These findings suggest that alfacalcidol might be of therapeutic value as a chemopreventive agent in the clinical management of cancer patients. Consistent with these results, clinical studies by Tałalaj et al. (2005) reported that, in patients with advanced prostatic carcinoma treated with complete androgenic blockade, the administration of alfacalcidol and calcium supplement ($CaCO_3$) prevented both trabecular and compact bone loss. On one hand, more recent clinical observations by Obara et al. (2008) have highlighted the fact that in Japanese patients with metastatic renal carcinoma (RCC), the combination of alfacalcidol and interferon-α may prolong the survival of these patients without causing severe adverse events. On the other hand, Trouillas et al. (2001) showed that alfacalcidol may induce in some patients, in synergy with classical surgery–radiotherapy–chemotherapy treatment, a particular progressive and durable regression of the tumor. Cunningham et al. (1985) reported that the administration of alfacalcidol induced a partial remission in patients with lymphoma. On one hand, more recently Akiyama et al. (2010) showed that the addition of alfacalcidol to Vitamin K appears to improve anemia and thrombocytopenia in about one-third of patients with low/intermediate-1 MDS who had not responded to Vitamin K2 monotherapy for 16 weeks. On the other hand, recent findings by van Ginkel et al. (2007) show that doxercalciferol can inhibit human neuroblastoma growth *in vivo* with relatively low toxicity. However, recent clinical investigation by Gee et al. (2013) reported no obvious beneficial effects in men undergoing prostatectomy for early-stage prostatic neoplasia, while Petrich et al. (2008) showed that a short-term treatment with doxercalciferol has limited activity in patients with MDS. These conflicting results suggest that further clinical studies are needed to better define the clinical effectiveness of this molecule in cancer chemoprevention.

Calcipotriol

Calcipotriol, or calcipotriene (Figure 2), is a synthetic Vit D analogue that exhibits a vitamin D-like effect by competing for VDR displaying minimal effects on calcium homeostasis (Binderup & Bramm, 1988). The intraperitoneal or oral administration of calcipotriol to rats showed that the compound was at least 100 times less active than $1,25(OH)_2D_3$ in determining hypercalcemia and hypercalciuria (Binderup & Bramm, 1988; Kissmeyer & Binderup, 1991). Experimental observations show that this molecule exerts important effects on cellular differentiation and proliferation *in vitro* while its effect on calcium metabolism *in vivo* is negligible (Binderup & Bramm, 1988; Cho et al., 1996). *In vitro* studies showed that this molecule may regulate cell differentiation and proliferation and exhibits growth-inhibiting effects against several human cancer cell lines including HL-60 and HL60/MX2 promyelocytic leukemia, U937 histiocytic lymphoma, MCF-7, T47D human breast cancer, HT-29 human colon cancer, and human SCC (Colston et al., 1992; Meephansan et al., 2012; Milczarek et al., 2013a, 2014). Regarding the possible mechanisms underlying the growth inhibiting and pro-differentiating effects of calcipotriol on tumor cells, *in vitro* studies highlighted the fact that a 20-h exposure of LNCaP prostate cancer cells and MCF-7 breast cancer cells to this analogue or to BGP-15, a new calcipotriene-derived vitamin D_3 analogue, generated procaspase-3 cleavage and ultimately, apoptosis (Berkovic et al., 2010). In addition, Wang et al. (2010b) recently reported that calcipotriol significantly suppressed *in vitro* colon carcinoma cell invasion and enhanced the cytotoxicity of the anticancer regimen FOLFIRI (folinic acid, 5-FU, irinotecan) to cells in culture or in anchorage-independent growth. These effects appeared to be the consequence of a suppression of gene transcriptional activities and protein expression of survivin and thymidylate synthase, to the enhanced E-cadherin localization in cell membranes and the complex formation of E-cadherin and β-catenin, and repression TCF4 transcriptional activation. Consistent with these observations, on one hand, recent findings by Milczarek et al. (2014) showed that calcipotriol may potentiate the antitumor activity of 5-FU in HT-29 colon tumor-bearing mice. These studies also showed that the mechanism of potentiation of 5-FU antitumor was related to the increased expression of *p21* and decreased expression of pERK1/2 level which may lead to a decreased expression of thymidylate synthase. On the other hand, Meephansan et al. (2012) highlighted the fact that calcipotriol affects *in vitro* the invasive potential of DJM human squamous carcinoma cells by reducing the production of matrix-metalloproteinases MMP-9 and MMP-13 through inhibition of the ERK and p38 phosphorylation. The antitumor activity of calcipotriol was also investigated *in vivo* using rats transplanted with breast cancer induced by *N*-methyl-nitrosourea (Colston et al., 1992a). These studies showed how the administration of calcipotriol (50 μg/kg) resulted in an inhibition of tumor progression without development of severe hypercalcemia. On one hand, more recent *in vivo* investigation by Pommergaard et al. (2013) demonstrated that in mice with UVB-induced non-melanoma skin cancer (NMSC), the topical treatment with calcipotriol combined with diclofenac and difluoromethylornithine for 17 weeks significantly reduced the number of mice with tumors as well as tumor area size compared with placebo. On the other hand, early clinical studies reported that in 15 out of 19 patients with localized breast cancer or skin metastases, the topical treatment with calcipotriol (100 mg/d for 6 weeks) caused a reduction of 65% of the diameter of the lesions, a reduction of 50%, in 3/19 patients while one patient showed a minimal response (Bower et al., 1991). It is worth noting that only two patients developed hypercalcemia during treatment. Interestingly, recent observations by Al-Jaderi and Maghazachi (2013) suggested that calcipotriol may influence the activity of cells of the innate immune system. In fact, these authors showed that calcipotriol may increase IL-2-activated NK cell lysis of K562 and RAJI tumor cell lines as well as immature and mature dendritic cells and may down-regulate the expression of the killer inhibitory receptor CD158. These findings make calcipotriol of potential interest as another novel chemopreventive agent in cancer treatment.

Maxacalcitol (22-oxa-1α,25-D3)

Maxacalcitol (1α,25-dihydroxy-22-oxacalcitriol) (OCT) (Figure 2) is another non-calcemic Vit D analogue and VDR ligand that has been shown to promote cell differentiation and to inhibit cell proliferation, without inducing hypercalcemia (Abe et al., 1991; Nishii & Okano, 2001; Funato et al., 2002). This molecule contains an oxygen in place of a carbon in position 22 of the side chain (Figure 2) and is less calcemic than $1,25(OH)_2D_3$. However, it retains considerable potency to suppress PTH *in vitro* and *in vivo* (Nishii & Okano, 2001). *In vitro* studies show that maxacalcitol could block the G1/S transition and induce tumor cell growth inhibition in responsive pancreatic tumor cells (Kawa et al., 2005). The antiproliferative effect of maxacalcitol on these cells appears to be due to the up-regulation of p21 and p27 induced by this molecule (Kawa et al., 2005). Interestingly, Funato et al. (2002) also report that the co-administration of OTC with Vitamin K2 induces an accumulation of the cells in the G0/G1 phase but suppresses apoptosis. The growth inhibitory effects of 22-oxa-calcitriol were also reported in breast cancer cells. In fact, *in vitro* studies showed that this molecule inhibited, in a dose- and time-dependent fashion, the proliferation of $ER^{(+)}$ breast cancer cells (MCF-7, T-47D, and ZR-75-1) and $ER^{(-)}$ breast cancer cells (MDA-MB-231 and BT-20) (Abe et al., 1991). In line with these findings, *in vivo* studies on athymic mice transplanted with MCF-7/$ER^{(+)}$ or MX-1/$ER^{(-)}$ breast cancer cells showed that the administration of OCT elicited a potent antitumor effect in both $ER^{(+)}$ and $ER^{(-)}$ tumor cells (Abe-Hashimoto et al., 1993). These studies also demonstrated that the degree of therapeutic effectiveness of OCT was comparable with that of tamoxifen in $ER^{(+)}$ tumors or to that of adriamycin in $ER^{(-)}$ tumors. Furthermore, in MCF7/$ER^{(+)}$ tumors, the co-administration of OCT and tamoxifen resulted in a synergistic antitumor effect (Abe et al., 1991; Abe-Hashimoto et al., 1993). Interestingly, on one hand, other *in vivo* studies reported that in mice transplanted intravenously with Lewis Lung Carcinoma cells (LLC), the administration of OTC was highly effective in reducing lung metastasis formation (Nakagawa et al., 2005). These authors suggested that this effect was likely related to ability of this compound to affect tumor-induced angiogenesis *in vitro* and *in vivo* (Nakagawa et al., 2005). On the other hand, the results of these studies are consistent with those of Matsumoto et al. (1999) reporting that the administration of OTC to mice transplanted with MDA-MB-231 $ER^{(-)}$ human breast cancer cells partially suppresses tumor growth by inhibiting the expression levels of the vascular endothelial growth factor (VEGF) and, ultimately, tumor neovascularization. More recent observations by Seubwai et al. (2010) showed that OTC effectively suppressed the growth of cholangiocarcinoma (CCA) cell lines in a time-dependent and dose-dependent manner by blocking tumor cells in the G1 phase of the cell cycle and the transition of CCA cells from G1 to S phase by suppressing or up-regulating the expression of genes, i.e., cyclin D1 and p21, respectively, which regulate this transition. Moreover, supplementation of OTC to CCA-inoculated NOD/Scid/Jak3-deficient mice (NOJ) significantly inhibited tumor growth without hypercalcemia or other serious side effects. This treatment also induced cellular apoptosis in tissue samples from patients with CCA. The effects of maxacalcitol were also investigated in rats treated with five carcinogens (Otoshi et al., 1995). In this study, 25 rats were administered intraperitoneally with maxacalcitol (30 μg/kg), three times a week for 24 weeks from the initial exposure to carcinogens. At the end of a 30-week observation period, none of the rats that had received maxacalcitol after the carcinogens developed cancerous lesions in the small intestine while the incidence was higher in control animals. The incidence of colon carcinoma in the group that received only maxacalcitol showed a great tendency to decrease (Otoshi et al., 1995). These studies, although not clarifying the mechanisms of action, suggest that the maxacalcitol may be of potential clinical value in the prevention of breast cancer and colorectal cancer.

EB1089 (seocalcitol)

EB1089, also known as seocalcitol (Figure 2), is a Vit D analogue which has been shown to inhibit either *in vitro* or *in vivo* the growth of several types of tumor such as breast cancer (Colston et al., 1992a; Macejová et al., 2011; Valrance et al., 2007; VanWeelden et al., 1998), prostate cancer (Bhatia et al., 2009; Chen et al., 2003; Oades et al., 2002), colon cancer (Akhter et al., 1997; Oh et al., 2001), and hepatocellular carcinoma (Ghous et al., 2008; Zhang et al., 2013). *In vitro* observations showed that EB1089 was more effective than $1,25(OH)_2D_3$ in inhibiting the cell proliferation of MCF-7 breast cancer cells (VanWeelden et al., 1998). *In vivo*, the antitumor effects of EB1089 were investigated in rats with mammary cancer induced by *N*-methyl-nitrosourea (Colston et al., 1992) orally administered with two different dose levels. The administration of the lower dose resulted in a significant inhibition of tumor growth while, an equivalent dose of $1,25(OH)_2D_3$ had no effect on tumor growth but induced hypercalcemia. Conversely, at the higher dose, EB1089 determined tumor regression. In addition, on one hand, experimental evidence showed that the combination of EB1089 with cytotoxic agents and/or ionizing radiation resulted in additive antitumor effects on breast cancer tumor cells either *in vitro* or *in vivo* (Demasters et al., 2006; Koshizuka et al., 1999; Sundaram et al., 2003; Valrance et al., 2007). On the other hand, Oades et al. (2002) showed that EB1089 also inhibited the growth of prostate adenocarcinoma in the Dunning prostate model and in athymic nude mice transplanted with LNCaP. Similar effects were recently reported in an *in vivo* study by Bhatia et al. (2009). These authors showed that EB1089 inhibited prostate cancer cell proliferation and reduced tumorigenesis as well as tumor metastases. Furthermore, *in vitro* studies on U937 histiocytic lymphoma cells showed that EB1089 was 50–100 times more effective in inhibiting cell proliferation and in inducing cell differentiation than $1,25(OH)_2D_3$ but less effective than calcitriol in affecting *in vivo* calcium metabolism in rats (Mathiasen et al., 1993). These data match those reported by Gulliford et al. (1998) showing that EB1089 was significantly less calcemic than 1,25-dihydroxyvitamin D_3 when administered at a maximum-tolerated dose (7 $\mu g/m^2$) to patients with breast cancer or colorectal cancer. Other *in vitro* observations

on HT-29 colon adenocarcinoma cells showed that EB1089 was more effective than Vit D in inhibiting HT-29 proliferation (Oh et al., 2001). In particular, immunoblot analysis showed that EB1089 inhibited the secretion of IGF-2 and stimulated the production IGFBP-6. More recently Lu et al. (2008) reported that EB1089 inhibits the proliferation of Hep-2 human laryngeal squamous carcinoma cells. The growth-inhibiting activity of this molecule appeared to be due to an increase of p57 cyclin-dependent kinase inhibitor at mRNA and protein levels induced by this molecule. EB1089 brought about tumor cell death by a mechanism, not related to caspase activation, which consisted in the induction of chromatin condensation and DNA fragmentation (Høyer-Hansen et al., 2005). Furthermore, recent findings by Ghous et al. (2008) showed that EB1089 may inhibit either *in vitro* or *in vivo* the growth of hepatocellular carcinoma (HCC). Interestingly, Zhang et al. (2013) recently reported that a combination of retinoic acid (RA) and EB1089 exerted a synergistic growth inhibition and apoptosis induction in HCC cells. Furthermore, *in vivo* studies carried out in rats with prostate cancer showed that the administration of EB1089 decreased the tumor size and the number of lung metastases (Chen et al., 2003). Although these experimental observations are encouraging, clinical studies aimed at evaluating the therapeutic effectiveness of this molecule in cancer patients have generated controversial results. In particular, phase I and II studies in patients with breast cancer, colorectal cancer, hepatocellular carcinoma, or pancreatic cancer failed to demonstrate any therapeutic response of patients to EB1089 treatment (Dalhoff et al., 2003; Evans et al., 2002; Gulliford et al., 1998). However, it cannot be ruled out that patients' characteristics (i.e., presence/absence of VDR in tumor tissue, previous treatments, low number of enrolled patients, dose-limiting hypercalcemia, etc.) might, in part, account for this phenomenon. Therefore, further studies are needed to better define the potential clinical effectiveness and toxicity of EB1089.

Analogues of 20-epi vitamin D

The 20-epi-vitamin D_3 analogues including CB-1093, KH-1060 (lexicalcitol), BXL-628 (elocalcitol), and 2MD are molecules characterized by an altered stereochemistry at carbon 20 in the side-chain (Binderup et al., 1991) (Figure 2). These molecules except 2MD (2-methylene-19-nor-(20S)-1-,25$(OH)_2D_3$) have been shown to possess chemopreventive activity. 2MD has been shown to stimulate bone formation *in vivo* and *in vitro* (Ke et al., 2005; Mäenpää et al., 2001) but it appears to be devoid of antitumor activity (Ke et al., 2005). Therefore, this compound has been proposed for the treatment of osteoporosis (Plum et al., 2006). *In vitro* observation showed that KH1060 and CB1093 may inhibit cell proliferation, at lower concentrations and earlier points in time than calcitriol, by blocking the cell cycle in the G0/G1 phase (Mäenpää et al., 2001; Ryhänen et al., 2003). This phenomenon appeared to be the consequence of an increase in the p27 level and a marked decrease of Cdk2. These phenomena ultimately contribute to keeping retinoblastoma (Rb) protein in its hypophosphorylated, i.e., growth suppressing, form thus preventing cell-cycle progression through the restriction point (Elstner et al., 1999; Mäenpää et al., 2001; Ryhänen et al., 2003). These data are consistent with other *in vitro* studies showing that CB1093 and KH1060, alone or in combination with 9-*cis*-retinoic acid, inhibited the growth of LNCaP human prostate cancer cells (Elstner et al., 1999) and human neuroblastoma cells (Gumireddy et al., 2003). It was also demonstrated that these effects were associated with increased levels of p21(waf-1) and p27(kip1) protein. The inhibiting effects of CB1093 on the growth of prostate adenocarcinoma was also confirmed *in vivo* by some experimental studies showing that this analogue inhibited tumor growth in the Dunning prostate model and in athymic nude mice transplanted with LNCaP tumor cells (Oades et al., 2002). Other studies, performed on MCF-7, T47D, and Hs578T breast cancer cell lines, showed that CB1093 enhanced the response of breast cancer cells to TNF-α and the intracellular production of ceramide which appeared to act as downstream effectors in Vit D-mediated caspase-independent cell death (Mathiasen et al., 1993; Pirianov & Colston, 2001). Interestingly, Danielsson et al. (1998) reported that CB1093 appeared to be very effective in inducing apoptosis in the early stage in the WM1341 melanoma cell line, but not in the advanced stage in MeWo melanoma cell line. Other studies examined the effects induced by BXL-628 analogue or Elocalcitol in benign prostatic hyperplasia and in prostate cancer cells (Adorini et al., 2007; Penna et al., 2009; Tiwari, 2009). The results of these studies indicated that the main mechanism appears to be related to the inhibition of growth factors and to the interleukin-8 (IL-8) production via a decrease in COX-2 and PGE2 synthesis (Adorini et al., 2007; Penna et al., 2009). Elocalcitol was also able to inhibit the proliferation and invasiveness of prostate cancer cell line, DU145 by interfering with keratinocytes growth factor (KGF)-induced proliferation (Marchiani et al., 2006). Based on these results, these molecules may be of potential clinical interest as novel chemopreventive agents. Further clinical studies may better assess their clinical effectiveness in cancer prevention and treatment.

Tacalcitol and eldecalcitol

Tacalcitol (1,24-dihydroxyvitamin D3, PRI-2191) is an active metabolite of 1α,25$(OH)_2D_3$ (Figure 2) that does not exhibit the high calcemic activity of the original compound (Wietrzyk et al., 2004). Studies aimed at assessing the antitumor activity and toxicity of tacalcitol showed a lower toxicity of this molecule after its subcutaneous administration compared with that of calcitriol (Wietrzyk et al., 2004). Furthermore, the oral administration of tacalcitol increased calcium serum levels by 47%, while calcitriol increased these levels by 78%. Interestingly, the treatment of mice with breast cancer with tacalcitol caused a reduction of the tumor volume by 41% (Wietrzyk et al., 2004). Moreover, the co-administration of tacalcitol with antitumor drugs such as 5-FU and cisplatin or oxaliplatin and irinotecan to mice transplanted with MC38 (mouse) or HT-29 human colon cancer induced a tumor growth inhibition significantly greater than tacalcitol alone and a significant prolongation of the survival time of mice (Milczarek et al., 2013b,c). Another study carried out on different cell lines, namely A549 (lung cancer), B16 (murine melanoma), HL-60 (human leukemia),

SW707 (human colon cancer), MCF-7 and T47D (breast cancer), WEHI-3 (murine leukemia), and on normal murine fibroblasts BALB-3T3 cells, demonstrated that the administration of tacalcitol in association with cisplatin or doxorubicin resulted in a significant decrease in the IC_{50} values of these antitumor agents (Pelczynska et al., 2006). Furthermore, Switalska et al. (2012) recently reported that the administration of tacalcitol enhanced the antiproliferative activity of imatinib demesilate on HL-60 leukemia cells. Eldecalcitol (1α, 25-dihydroxy-2β-[3-hydroxypropyloxy] vitamin D_3), (ED-71), is an orally administered analogue of active Vit D that is mainly used in the clinical treatment of osteoporosis (Sanford & McCormack, 2011) (Figure 2). Further experimental studies showed that ED-71 is endowed with chemopreventive activity against tumors and with reduced hypercalcemic effects (Hatakeyama et al., 2010). Studies on myeloid leukemia demonstrated the ability of this molecule to induce tumor cells to differentiate into normal monocyte-macrophages (Gocek & Studzinski, 2009; Hatakeyama et al., 2010; Nishii & Okano, 2001). This molecule also inhibited the proliferation of U937 human, histiocytic lymphoma cells, and increased osteocalcin concentrations in the human osteosarcoma cells (MG-63) (Hatakeyama et al., 2010). Eldecalcitol is a CYP24A1-resistant analogue (Ritter & Brown, 2011). CYP24A1 is expressed in many tissues and cells, including the prostate, and appear to be implicated in the resistance of prostate cancer to Vit D (Tannour-Louet, 2014). Therefore, ED-71 may be of clinical usefulness in the prevention and treatment of prostate cancer (Chen et al., 2012; Sakaki et al., 2014).

BXL 0124

BXL0124, a new analogue of Vit D, belongs to the deuterated Gemini Vit D compounds (Figure 2). These compounds have a C-20 methyl group, a deuterium-substituted side chain, and a second side chain that has a double or triple bond and a fluorine. BXL0124 has been shown to possess a chemopreventive effect on breast cancer and prostate cancer (Lee et al., 2008; Spina et al., 2007; Wahler et al., 2014). Interestingly, *in vitro s*tudies on MCF10DCIS cells showed that this molecule had antiproliferative effects on breast cancer and markedly decreased the expression of CD44 (So et al., 2011). Intriguingly, recent observations showed that BXL0124 inhibited breast cancer cell invasion by targeting CD44-STAT3 signaling (So et al., 2013a). These findings are consistent with the observations that the JAK2/STAT3 signaling pathway is essential for growth of the $CD44^+$/$CD24^-$ stem cell-like breast (Marotta et al., 2011). In contrast, the inhibiting activity of this molecule on tumor growth observed in *vitro* was further confirmed by *in vivo* studies showing that either the oral (0.03 or 0.1 μg/kg) or intraperitoneal (0.1 μg/kg) administration of Gemini 6d a week for 5 weeks to mice-bearing tumor caused a reduction in the growth of breast cancer and a consistent decrease in the expression of CD44 protein, without causing hypercalcemia (So et al., 2011). Consistent with these findings, Wahler et al. (2014) showed that, in mice inoculated with ductal carcinoma *in situ* (DCIS) MCF10DCIS com. cells, the administration of BXL0124 resulted in a 43% reduction in tumor volume. Moreover, BXL0124 treatment also decreased the mRNA levels of MMPs starting at week 3, thus contributing to the inhibition of invasive transition. These findings indicate that this molecule may be an important target for the chemoprevention and treatment of breast cancer. Consistent with these findings, a more recent study by So et al. (2013b) reported that BXL0124 may be effective, in combination with other molecules, as potential chemopreventive agent, but not for the treatment, against the tumorigenesis of ErbB2-overexpressing breast cancer. The same authors highlighted the fact that BXL0124 (10 nM) induced expression of mRNA coding for osteopontin, one of the genes regulated by Vit D, thus contributing to the regulation of cell proliferation (So et al., 2011). BXL0124 has also been shown to reduce tumor growth by 50% and to prevent metastasis formation in the MC-26 colon cancer xenograft model while no effect was noted on calcium homeostasis (Spina et al., 2007). These data warrant future clinical studies to assess the pharmacological profile of this analog in humans.

19-nor-1α,25(OH)$_2$D$_3$ analogs: MART-10, MART-11, paracalcitol, and inecalcitol

MART-10 MART-11

19-nor-Vit D compounds are Vit D analogs in which the ring A methylene group on C-19 is replaced with two hydrogen atoms (Chen & Kittaka, 2011) (Figure 2). MART-10 (19-nor-2α-(3-hydroxypropyl)-1α,25(OH)$_2$D$_3$) and MART-11 (19-nor-2β-3-hydroxypropyl-1α,25(OH)$_2$D$_3$) are two new synthetic C2-substituted 19-nor-1α,25(OH)$_2$D$_3$ analogs of Vit D that have negligible effects on calcium plasma levels and that appear to be effective in the prevention and treatment of prostate cancer (Chen & Kittaka, 2011; Kittaka et al., 2012). *In vitro* studies on the HL-60 cell line showed that compared with 1,25(OH)$_2$D$_3$, MART-10, and MART-11 are endowed with a different degree of binding affinity for VDR (100 and 3%). In addition, both analogues were more effective in inducing cell differentiation, compared with 1,25(OH)$_2$D$_3$ (Arai & Kittaka, 2006; Ono et al., 2003). The discrepancy between the rate of VDR binding and differentiation activity was explained, at least in part, by their remarkable ability to recruit co-activators, such as hTIF-2 and HSRC-1 (Arai et al., 2007). The antiproliferative activity of MART-10 and MART-11 was investigated in LNCaP and PC3 human prostate cancer cells (Chen et al., 2003; Flanagan et al., 2009). These investigations have shown that both analogues possess antiproliferative activity comparable with that of 1,25(OH)$_2$D$_3$. However, MART-10 proves about 1000-fold more active than 1α,25(OH)$_2$D$_3$ in inhibiting LNCaP and PC-3 prostate cancer cell proliferation (Chen & Kittaka, 2011; Iglesias-Gato et al., 2011). Furthermore, *in vitro* studies which compared the expression level of the enzyme CYP24A1 in LNCaP and PC-3 in response to treatment with 1,25(OH)$_2$D$_3$ and MART-10 showed that this latter molecule was able to induce the expression of CYP24A1, one of the three major enzymes involved in the metabolism of Vit D (Jones et al., 2012), to a lower concentration than calcitriol (Flanagan et al., 2009). *In vivo*, the subcutaneous administration of MART-10 was reported to up-regulate CYP24A1 mRNA expression in rat kidneys without affecting their

plasma calcium levels (Iglesias-Gato et al., 2011). These findings demonstrated that MART-10 is biologically active *in vivo* and may be of clinical usefulness in treating prostate cancer. Therefore, the induction of the *CYP24A1* gene was used as an index for evaluating the biological potency of new Vit D analogs (Chen & Kittaka, 2011). These studies additionally highlighted the fact that MART-10 induced CYP24A1 gene expression at a lower concentration with a longer duration compared with $1\alpha,25(OH)_2D_3$, suggesting that MART-10 is less susceptible to CYP24A1 degradation (Flanagan et al., 2009; Iglesias-Gato et al., 2011). Furthermore, the effects induced by MART-10 lasted for a longer period of time. This phenomenon indicated a low susceptibility of this molecule to being degraded by CYP24A1 (Flanagan et al., 2009). Interestingly, MART-10 has been shown to be a potent inhibitor of cancer cell invasiveness (Chen & Kittaka, 2011). In fact, *in vitro* studies on PC-3 prostate cancer cells showed that MART-10 determined down-regulation of matrix metalloproteinase-9 (MMP-9), an enzyme which fosters tumor invasion, angiogenesis, and metastasis (Chen & Kittaka, 2011). More recent observations by Chiang et al. (2014) show that MART-10 is more active than $1\alpha,25(OH)_2D_3$ in preventing MCF-7 cell invasion and migration, probably mediated through the up-regulation of E-cadherin, and that of the transcription factors implicated in epithelial–mesenchymal transition (EMT) down-regulation, e.g., Snail, Slug, and Twist, as well as MMP-13. These data further confirm and extend previous finding of the same authors showing that MART-10 is also able to inhibit cell proliferation and to induce apoptosis in MCF-7/ER$^{(+)}$ breast cancer cells (Chiang et al., 2012). These findings suggest that these analogues and their structurally related analogues may be good candidates for the treatment of different human tumors including breast, prostate, and liver cancers (Kittaka et al., 2012). These observations warrant further *in vivo* animal study to better assess the pharmacological profile and the therapeutic effectiveness of these molecules in humans.

Paricalcitol

Paricalcitol (19-nor-1α,25-dihydroxyvitamin D_2) is a synthetic analog of calcitriol, the active form of Vit D (Figure 2). Experimental *in vitro* and *in vivo* studies have reported that paricalcitol presents anticancer activity against several hematological and solid tumors including myeloid leukemia, myeloma, gastric cancer, colon cancer, and pancreatic cancer and that these effects may be mediated through the VDR (Kumagai et al., 2003, 2005; Park et al., 2012; Schwartz et al., 2005, 2008). Interestingly, recent clinical observation by Lawrence et al. (2013) has shown that paricalcitol, in combination with taxane-based chemotherapy, appears to be safe and feasible and may have a clinical benefit for women with metastatic breast cancer. Unlike these findings, Schwartz et al. (2005) did not observe any clear response in patients with advanced prostate cancer. On the other hand, in HL-60 and NB4 myeloid leukemia cell lines, paricalcitol was noted to suppress proliferation and induce differentiation (Kumagai et al., 2005; Molnar et al., 2004). In human NCI-H929 myeloma cells, this molecule inhibited cell growth by causing cell-cycle block and apoptosis indicating its potential as an antileukemic agent (Molnar et al., 2004). Furthermore, in patients with all-*trans*-retinoic acid (ATRA)-resistant myeloid leukemia a combination therapy consisting of paricalcitol and arsenic trioxide also appears to be promising (Kumagai et al., 2005). These findings together with its low calcemic activity and achievable therapeutic doses strongly support its use in clinical trials regarding hematological diseases such as MDS and acute myeloid leukemia.

Inecalcitol

Inecalcitol is a novel Vit D 19-nor analogue (19-nor-14-epi-23-yne-1,25 dihydroxyvitamin D_3) that differs from $1,25(OH)_2D_3$ through epimerization of C14, deletion of C19, and 23-yne modification in the side chain (Figure 2) (Verlinden et al., 2000). This analogue appears to be less inclined to induce hypercalcemia while it remains a potent stimulant of VDR (Verlinden et al., 2000). Inecalcitol has been shown to suppress both *in vitro* and *in vivo* the growth of human LNCaP prostate cancer and that of SCC (Ma et al., 2013a,b; Okamoto et al., 2012). The antitumor activity of inecalcitol, at least in SCC, appears to be the consequence of (a) the arrest of tumor cells in G0/G1 transition phase of the cell cycle, (b) the triggering of the apoptosis cascade through the activation of caspase 8/10–caspase 3 pathway, and (c) the inhibition of expression of c-IAP1 and XIAP. Inecalcitol has also been shown to repress cyclin D1 and cyclin C gene expression and to induce *p21* and *p27* gene expression more efficiently than calcitriol (Ma et al., 2013a). Inecalcitol has been utilized in clinical studies in association with the classical antitumor agent docetaxel (Medioni et al., 2014). The results of the phase II trial in castration-resistant prostate cancer showed that this drug combination had a better PSA response than docetaxel alone. These data provide support for further evaluation of inecalcitol in cancer treatment.

Conclusions

On one hand, epidemiological observations highlight the fact that high levels of vitamin D may offer protection against many types of cancer (Leyssens et al., 2013; Pilz et al., 2013). On the other hand, many experimental studies provide evidence for the growth inhibiting, anti-inflammatory, and pro-differentiation effects of Vit D *in vitro* on human cancer cell lines and *in vivo* on tumor-bearing animals (Krishnan & Feldman, 2011; Meeker et al., 2014; Trump et al., 2010; Vanoirbeek et al., 2011). Therefore, the use of vitamin D or its semisynthetic analogues in cancer therapy could provide effective chemopreventive effects against tumor progression (Byers et al., 2012; Krishnan & Feldman, 2011; Meeker et al., 2014; Trump et al., 2010; Vanoirbeek et al., 2011). The possible mechanisms by which vitamin D mediates these effects have been, only in part, identified. Although the preclinical data are striking and the epidemiologic data are encouraging, no well-designed clinical trial on the optimal administration of vitamin D as a cancer therapy has ever been undertaken (Krishnan & Feldman, 2010; Leyssens et al., 2014). Future clinical investigations may better define the clinical role of Vit D or its analogues in cancer prevention and treatment. Another relevant feature of the paradigm Vit D/

cancer needing further investigation is related to tumor resistance as this phenomenon may affect the synthesis of the active metabolite of Vit D and, consequently, its potential therapeutic activity (Ajibade et al., 2014; Giardina et al., 2012; Larriba & Muñoz, 2010; Tannour-Louet, 2014). Therefore, many studies are currently directed to find new molecules which may circumvent tumor resistance to Vit D analogues (Deeb et al., 2007; Fischer et al., 2012; Ritter & Brown, 2011; Solomon et al., 2014).

Declaration of interest

The authors report that there are no declarations of interest.

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