1α,25-DIHYDROXYVITAMIN D_3 INDUCIBLE TRANSCRIPTION FACTOR AND ITS ROLE IN THE VITAMIN D ACTION

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Vitamin D is considered multifunctional steroid hormone that modulates calcium homeostasis through actions predominantly in kidney, bone and the intestinal tract. Nuclear vitamin D receptor (VDR) is a specific nuclear protein, a member of steroid hormone receptor superfamily. The amino acid sequence of the VDR shows a significant homology with other members of the nuclear hormone receptor superfamily, including receptors for glucocorticoids (GR), oestrogen (ER), androgen (AR), progesteron (PR), thyroid hormone (T_3R), retinoic acid (RAR), retinoid X (RXR) and over 150 orphan receptors. VDR is known to mediate the pleiotropic biological actions of 1α,25-dihydroxyvitamin D_3 through its ability to modulate the expression of target genes. VDR upon binding 1α,25-dihydroxyvitamin D_3 regulates specific gene transcription predominantly by binding as a heterodimer with the retinoid X receptor (RXR) to DNA enhancer sequence, termed the vitamin D-responsive element (VDRE) that is present within the promoter region of vitamin D-controlled genes. The VDR has been shown to associate with several additional molecules to form the active transcriptional complex required for gene regulation. The regulation of this ligand-activated cellular transcription factor occurs at both transcriptional and posttranslational levels. This article summarizes a variety of effects of 1α,25-dihydroxyvitamin D_3, acting through its cognate nuclear receptor, and its use in chemotherapy and chemoprevention of cancer.

Key words: Vitamin D_3 – Nuclear receptors – Mechanism of action – Gene expression

Although little is known about when vitamin D has been discovered (Holick 2003), it is commonly suggested that it was discovered by Edward Mellanby in 1919 during his classic experiments with rickets (Mellanby 1976). The biologically active form of vitamin D_3, a fat-soluble vitamin was identified as an anti-rachitic factor in the early 1920s (Welsh et al. 2002). Vitamin D deficiency belongs to one of major unrecognized health problems.

1α,25-dihydroxyvitamin D_3, recently known as "vitamin D hormone" or simple calcitriol are the possible names of biologically active form of vitamin D (cholecalciferol). Vitamin D is synthesized from its cholesterol-like precursor 7-dehydrocholesterol in response to ultraviolet light in the skin and after that is converted to 25-hydroxycholecalciferol in the liver. The active form of vitamin D (1α,25-dihydroxyvitamin D_3) was shown to be metabolized in the kidney (Reichel et al. 1989; Bikle and Pillai 1993). However, a smaller part of vitamin D_3 content of human body is obtained from the diet (Malloy and Feldman 1999). First chemical synthesis of vitamin D took 2 years and it was 21 steps synthesis. Semmler started with a kilogram of starting material and yielding less than 1 mg of the final product (Semmler et al. 1972).

Vitamin D is considered multifunctional steroid hormone that modulates calcium homeostasis through actions predominantly in kidney, bone and the intestinal tract (Bikle and Pillai 1993). These are effected in two ways: at the level of gene transcription through a nuclear receptor (VDR_{nuc}) or at level of the plasma membrane via membrane-associated receptor (VDR_{mem}) which do not require any protein synthesis as genomic actions do (Evans 1988; Norman et al. 2002). Vitamin D receptors (VDR) are present in the intestine, kidney and bone and also in a wide variety of other tissues, including the brain, heart, stomach, pancreas, activated T and B lymphocytes, skin, gonads, liver etc. (Gas-

con-Barre et al. 2003; Holick 2003). Physiological and pharmacological actions of vitamin D in various systems have indicated potential applications through VDR in inflammation, dermatological indications, osteoporosis, cancers and autoimmune diseases (Pinette et al. 2003). The effects exerted via the VDR are called genomic effects, in contrast to non-genomic effects (VDR in membrane), which means that 1α,25-$(OH)_2D_3$ acts within a short period without transcription of target genes (Norman 1998). Nuclear vitamin D receptor is specific nuclear protein, a member of steroid hormone receptor superfamily (Evans 1988). The vitamin D receptor is known to mediate the pleiotropic biological actions of 1α,25-dihydroxyvitamin D_3 through its ability to modulate the expression of target genes. The regulation of this ligand-activated cellular transcription factor is reported to occur at both transcriptional and posttranslational levels (Miyamoto et al. 1997).

In general, 1α,25-dihydroxyvitamin D_3 produces biological responses by interaction with a well-characterized nuclear receptor to regulate gene transcription (Norman et al. 2002). VDR regulates gene transcription both positively and negatively by binding to hexameric binding motifs in directly repeated or inverted palindromic arrangement in the promoter regions of target genes, designated vitamin D response elements, or VDREs (Issa et al. 1998). VDR acts as a ligand-dependent transcription factor and binds to the vitamin D response elements (VDRE) as a VDR : retinoid-X receptors heterodimer or VDR homodimers. This interaction results in a ligand-dependent activation or repression of target genes (Darwish and DeLuca 1993; Christakos et al. 1996).

Structure of VDR

From a study carried out on cDNA clones obtained from intestinal VDR, it was shown that the VDR belongs to the steroid-receptor gene family and is the closest in size and sequence to the thyroid hormone receptor (Baker et al. 1988).

The VDR is high-affinity, low-capacity receptor about 48 to 55 kDa, primarily located in the nucleus, although evidence exists for the presence of cytoplasmic receptors (Darwish and DeLuca 1996). The human VDR gene has been localized to human chromosome 12q13-14 (Faraco et al. 1989) (Szpirer et al. 1991). The hVDR gene consists of 11 exons, which spam approximately 75kb. The 5'non-coding region of the gene includes 3 exons, 1A, 1B and 1C, while remaining eight exons (exons 2-9) encode the translation product (Miyamoto et al. 1997). A single 4.6 kb human transcript, found in most human tissues tested, contains a 1281 nucleotide open reading frame. This transcript codes for the full length VDR protein of 427 amino acids, a 115 bp non-coding leader sequence and a 3.2 kb 3' untranslated region (Haussler and Norman 1969). The promoter is characterized by the lack of TATA box initiator, its GC-rich nature, and the presence of putative binding sites for SP1 and a variety of transcription factors (Miyamoto et al. 1997).

The amino acid sequence of the VDR shows a significant homology with other members of the nuclear hormone receptor superfamily, including the receptors for glucocorticoids (GR), oestrogen (ER), androgen (AR), progesteron (PR), thyroid hormone (T_3R), retinoic acid (RAR), retinoid X (RXR) and over 150 orphan receptors (Evans 1988; Mangelsdorf et al. 1995).

VDR is a phosphoprotein and its function is regulated by phosphorylation on serine residues in the ligand/hinge domains. The binding of calcitriol to the VDR induces receptor phosphorylation and ligand-bound phosphorylated receptor stimulates transcription. The details of this process are not completely understood (Weigel 1996).

The nuclear receptors (NRs) function by regulating the transcription of target genes and generally require their cognate ligands to express their function (Wurtz et al. 1996). When bound to the ligand, the NRs change their conformation to the active form, thereby acting as molecular switches of target gene transactivation (Wurtz et al. 1996; Yamada et al. 2000). In general, all members of the nuclear hormone receptor superfamily possess five functional domains (Issa et al. 1998). There are: a short N-terminal activation-function 1 (AF-1), domain (A/B), a DNA-binding domain containing two Zn^{2+}-fingers (C), a flexible "hinge" region (D), and finally the ligand-binding domain (LBD or "E") whose C-terminal end also has transcriptional activation function(s) (AF-2).

The A/B domain. The presence of transcriptional activation function (AF-1) in VDR A/B domain of the VDR is uncertain when compared to other nuclear receptors. The A/B domain of VDR is short, consisting of 21 amino acids. The exact mechanism by which protein modification of AF-1 region alters ligand-dependent function at the AF-2 region remains unclear (Sone et al. 1991).

Hormone dependent phosphorylation has been reported for most nuclear hormone receptors and may be involved in regulation of DNA binding, hormone bind-

ing, nuclear localization and gene transactivation. The major sites for phosphorylation are serine residues.

DNA binding domain C (DBD) of VDR has been mapped to amino acid residues 22-114 (Sone et al. 1991). The DNA binding domain is highly conserved throughout all nuclear receptors and is mainly made up of two zinc fingers. α-helix was found on the carboxyl terminal side of each zinc finger, with helix A and B constituting the DNA recognition and phosphate backbone binding helices. Between these two zinc fingers a cluster of five basic amino acids was identified. This region is also important for nuclear localization of the receptor and for binding to the DNA. Phosphorylation of a serine residue in this segment, by protein kinase C, would affect DNA binding (Haussler et al. 1998). It has been shown that DBD is rich in positively charged amino acids favouring electrostatic interactions with the negatively charged phosphate backbone of the DNA helix (Freedman 1992).

The hinge D region is the stretch of amino acids between the C and the E. This hinge region confers flexibility to the protein and changes in structural conformation upon ligand activation (Hsieh et al. 1998).

The E domain (ligand binding domain, LBD) varies considerably between the nuclear hormone receptors. Homology is highest between the VDR and T_3R (23 %). Deletion mutation analysis has defined the N-terminal boundary of the LBD to lie between residues 144 and 166. Whole fragment of LBD lies between 115-423 position of amino acids. Subdomain within the LBD domain appear to be involved in nuclear import signalling, dimerization, transcriptional inhibition, transactivation and interaction with the transcriptional machinery (Issa et al. 1998). The hormonal binding domain is made up of 12 α-helices with several short β-strands that are organized in a three-dimensional lipophilic hormone binding pocket, to which vitamin D is attached. Specific amino acids in this domain (E1, E2, E3 regions) as well as in the DNA binding domain are important for heterodimerization with the retinoid X-receptor (RXR). A tryptophan residue in position 286 of the hVDR was found to be very important for specific ligand (vitamin D_3) interactions and also for the interaction with the RXR (Solomon et al. 2001). AF-2 transcriptional activation function domain is supposed to provide an interactive surface for transcriptional corepressors and coactivators which link nuclear receptor activity with the preinitiation complex (PIC). The VDR AF-2 domain has been mapped at the C-end of VDR between amino acids Arg-402 to Gln-423 (Jurutka et al. 1997).

Until recently, there has been limited evidence for human VDR isoforms. The VDR gene harbours with several polymorphisms, both in the coding and non-coding portions of the gene (Haussler et al. 1998; Wood and Fleet 1998). The vast majority of these polymorphisms do not result in a structural alteration in the VDR protein with the exception of the *Fok I* variant (Gross et al. 1996). This polymorphism occurs within the first ATG start codon of human VDR (hVDR) and contains a *Fok I* restriction endonuclease site. The absence of *Fok I* site causes that a shorter 424 amino acid protein will be formed (Haussler et al. 1998).

Mechanism of action

Vitamin D is synthesized in response to ultraviolet light in the skin. Many mammals and humans, fish have provitamin D (7-dehydrocholesterol) in their skin. 7-dehydrocholesterol is upon UV irradiation with wavelengths 290-315 nm (UVB) converted to provitamin D_3. During exposure to ultraviolet radiation is 7-dehydrocholesterol incorporated to the lipid bilayer (Holick 2003). Only thermodynamically less stable cis, cis conformer of provitamin D_3 could be converted to vitamin D_3 (Tian et al. 1993). Vitamin D_3 enters the blood circulation and binds to vitamin D binding protein (DBP) (Haddad et al. 1993) which carries vitamin D_3 to the liver and kidney for bioactivation (Wikvall 2001). It has been shown that provitamin D_3 is metabolized by 25-hydroxylase to 25-hydroxycholecalciferol mainly in the liver. This metabolite is present in the circulation at a concentration of more than 0.05 mmol/l (20 ng/ml). The active metabolite of vitamin D is generated by hydroxylation at 1α-position in kidney. There is an evidence that 1α-hydroxylase has been shown to be present in keratinocytes and prostate epithelial cells, suggesting that target organs may also be able to generate 1α,25-dihydroxyvitamin D_3 from 25-hydroxyvitamin D_3 locally (Schwartz et al. 1998). The active metabolite 1α,25-dihydroxyvitamin D_3 is present in the human plasma at a concentration of 0.05-0.15 nmol/l (20-60 pg/ml) (Hartwell et al. 1987; Cross et al. 1997). Ninety to 100 % of most human being's vitamin D requirement comes from exposure to sunlight. This is the best and the most reliable source of vitamin D for most humans (Holick 2003). The rest of vitamin D_3 content is obtained from diet (Malloy and Feldman 1999). The skin's capacity to produce vitamin D_3 is reduced as the skin ages (MacLaughlin and Holick 1985) and also with increasing skin pigmentation as much as 50-fold (Clemens et al. 1982). The active hormone stays in blood circulation for

about 7 hours. $1\alpha,25(OH)_2D_3$, as fat soluble molecule, penetrates easily the plasma membrane of its target cells, where it is catabolized (Dusso et al. 1991).

It is well established that the molecular recognition and subsequent binding process between 1α,25-dihydroxyvitamin D_3 and VDR include hydrophobic, van der Walls and H-bonding interactions between VDR and $1\alpha,25(OH)_2D_3$ (Mohr et al. 2001). The nuclear VDR_{nuc} and the putative membrane VDR_{mem} both bind $1\alpha,25(OH)_2D_3$ with high affinity (Mehta and Mehta 2002; Norman et al. 2002). The ligand binding affinity of the VDR (especially LBD) varies in dissociation constant (K_D) values. The full length receptor has been reported to have K_D values ranging between 0.1 to 0.7 nmol/l, but there is an evidence of published K_D values 0.5-2 nmol/l (Strugnell et al. 1999; Norman et al. 2002). The reason of the variation in K_D values is unclear. It may depend on the way of expression of protein (Strugnell et al. 1999)**.** The vitamin D receptor may bind several forms of cholecalciferol. Affinity of 25-hydroxycholecalciferol roughly 1000 times less than affinity of $1\alpha,25\text{-}(OH)_2D_3$, which explains their relative biological potencies. VDR can function as a homodimer, but heterodimerization with the retinoid X receptor, retinoic acid receptor, or thyroid hormone receptor increases its affinity for response elements in the promotes of target genes. Upon binding $1\alpha,25\text{-}(OH)_2D_3$, VDR regulates specific gene transcription predominantly by binding as a heterodimer with the retinoid X receptor (RXR) to DNA enhancer sequence, termed the vitamin D-responsive element (VDRE), that is present within the promoter region of vitamin D-controlled genes in the nuclear and mitochondrial genome (Haussler et al. 1998; Jurutka et al. 2000). Transcriptional activity can be enhanced by co-stimulation with 9-*cis* retinoic acid. The VDR can recognize and bind hexameric half sites with (A/G)GGTGA and AGTTCA sequences (Freedman et al. 1994; Issa et al. 1998). VDRE consists of two 6 base pair half elements that are separated by a spacer of 3, or 6 nucleotides (DR3 or DR6) (Umesono et al. 1991; Mangelsdorf and Evans 1995). VDR homodimers show binding specificity for those elements in both a ligand-dependent and independent manner. VDR:RXR heterodimers bind to DR3 elements, possibly due to conformational differences arising from rotation of the DBD and LBD about the hinge region (Freedman et al. 1994). VDR-T3R heterodimers act differently on the two types of VDREs. On the DR3-type the highest induction was by thyroid hormone alone whereas on a DR6-type VDRE vitamin D and thyroid hormone together provided maximal gene activity. The VDR-RXR complex shows a ~3-fold higher binding affinity for the DR3-type VDRE than any other complexes and the stimulation with both vitamin D and 9-cis-RA provides also a ~3-fold higher absolute induction of gene activity. In contrast, on the DR6-type VDRE, the VDR-VDR, the VDR-T3R and the VDR-RAR complexes vary in both their DNA binding affinity and their induction of absolute ligand-dependent gene activity by less than a factor of 2 (Schrader et al. 1994).

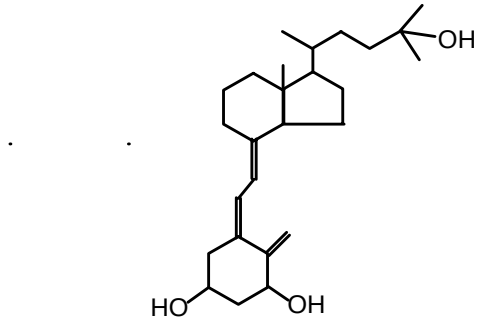

Fig.1 Chemical structure of vitamin D3 (1α,25-dihydroxyvitamin D3)

The specific recognition of hormone response elements is a central problem in understanding how the nuclear receptors function to differentially regulate gene expression (Glass 1994). The VDR has been shown to associate with several additional molecules to form the active transcriptional complex required for gene regulation (Haussler et al. 1998). The conformational change of VDR, caused by binding a ligand, induces releasing of corepressor molecules from the surface of VDR and allows receptor to interact with coactivator molecules. Coactivators modulate the local structure of chromatin and mediate the receptor interaction with basal transcription machinery in order to activate transcription of the target gene. These molecules include proteins of the p160 class that possess histone acetyl transferase (HAT) activity such as SRC-1 (Masuyama et al. 1997), GRIP1 (Hong et al. 1997) and ACTR (Chen et al. 1997), then SRC-1, RAC-3 and GRIP-1, DRIP205/TRAP220 (Yuan et al. 1998; Rachez et al. 1999). Among protein-coactivators postulated to stimulate VDR-mediated transactivation are TIF1 (Le Douarin et al. 1995), NCoA-62 (Baudino et al. 1998), p65 (Nakajima et al. 1997), and components of the transforming growth factor-ß (TGF-ß) signalling pathway, including Smad3 (Yanagi et al. 1999; Yanagisawa et al. 1999). Protein that negatively regulates VDR function is ubiquitous regulator Ying-Yang 1 (YY1).

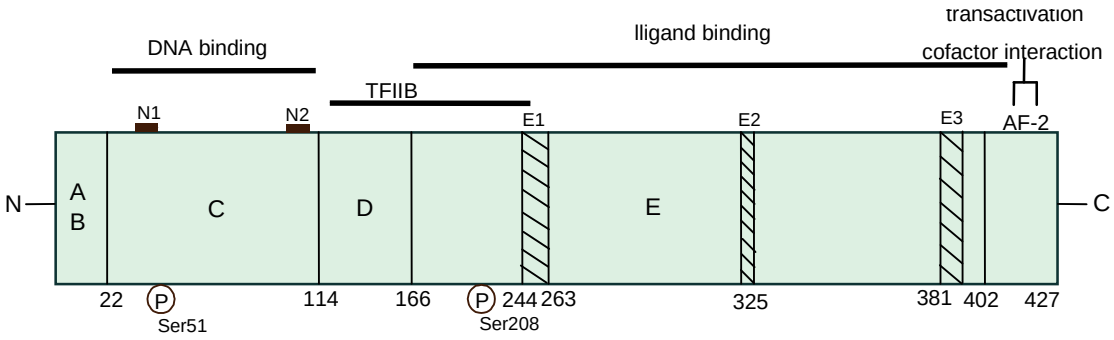

Fig.2 Modular structure of the VDR with its 5 functional domains.

YY1 binds specifically to YY1 recognition sequences (5'-CAT-3') within the VDRE in the bone-specific osteocalcin promoter, and may compete with VDR:RXR heterodimers for binding to their HRE (hormone responsive elements) (Guo et al. 1997), as well as competing for VDR binding with the general transcription factor, TFIIB (Usheva and Shenk 1994).

Other specific non-protein regulators of the VDR are 17ß-estradiol, dexamethasone and retinoic acid, all of which have been shown to up-regulate the VDR protein and/or mRNA *in vitro* (Krishnan and Feldman 1997).

Possible physiological effects mediated via VDR signalling pathways

Except the role of vitamin D affecting through vitamin D receptors in calcium homeostasis and bone metabolism, calcitriol exhibits anti-inflammatory and immunomodulatory properties. The crucial effect of vitamin D on bone is to provide the proper balance of calcium and phosphorus to support mineralization. 1α,25$(OH)_2D_3$ play a key role in the day-to-day maintenance of calcium balance. Through VDREs, located in the gene promoter regions of human sodium/phosphate cotransporter, VDR regulates the cellular expression of the molecules involved in calcium and phosphate (Taketani et al. 1998), calbindin (Haussler et al. 1998), human epithelial calcium channel (Hoenderop et al. 2001) and plasma membrane calcium pump isoform (Glendennig et al. 2000).

VDR is found not only in the classic target organs such as intestinal tract, kidney and bone, but also in many other epithelial and mesenchymal cells as well as leukemic cells, osteosarcoma, breast and colon carcinoma, melanoma, glioma, lung and prostate carcinoma and other malignant cell types (Reichel et al. 1989; Bikle and Pillai 1993). Vitamin D_3 and its analogues are potential therapeutics in psoriasis, multiple sclerosis, rheumatoid arthritis, diabetes and transplantation (Issa et al. 1998). It was shown that VDR signalling could modulate mammary gland development, function or sensitivity to carcinogenesis (Zinser et al. 2002). There is an evidence that T and B lymphocytes as well as monocytes have a VDR and thereby 1α,25$(OH)_2D_3$ is an effective immune modulator (Manolagas et al. 1985).

Vitamin D and cancer

In breast cancer cells, 1α,25$(OH)_2D_3$ causes growth arrest and apoptosis in vitro and in vivo, suggesting that vitamin D_3 based therapeutics may be useful for human cancer (Simboli-Campbell et al. 1996). Cancer cell replication is stimulated by low physiological concentrations of calcitriol but inhibited by higher concentrations. Low exposure to vitamin D or 1α,25-dihydroxycholecalciferol (calcitriol) increases the risk of prostate cancer (Schwartz and Hulka 1990; Corder et al. 1993). The anti-proliferative effects of vitamin D on breast cancer cells cause an accumulation of cells in the G_0/G_1 phase of the cell cycle (Frampton et al. 1983; Eisman et al. 1989). The dose required to achieve suppression of solid tumours in vivo is accompanied by hypercalcaemia, unless accompanied by marked restriction of calcium intake (Eisman et al. 1986). Newly synthesized non-hypercalcaemic analogues have potent anti-proliferative effects in vitro and chemopreventive and chemotherapeutic effects in vivo, making them useful for cancer therapy (Issa et al. 1998).

More than 2000 synthetic analogues of the biological active form of vitamin D, 1α,25-dihydroxyvitamin D_3 are presently known (Carlberg 2003). Structural analogs such as EB 1089, RO24-5531, 1α-hydroxyvi-

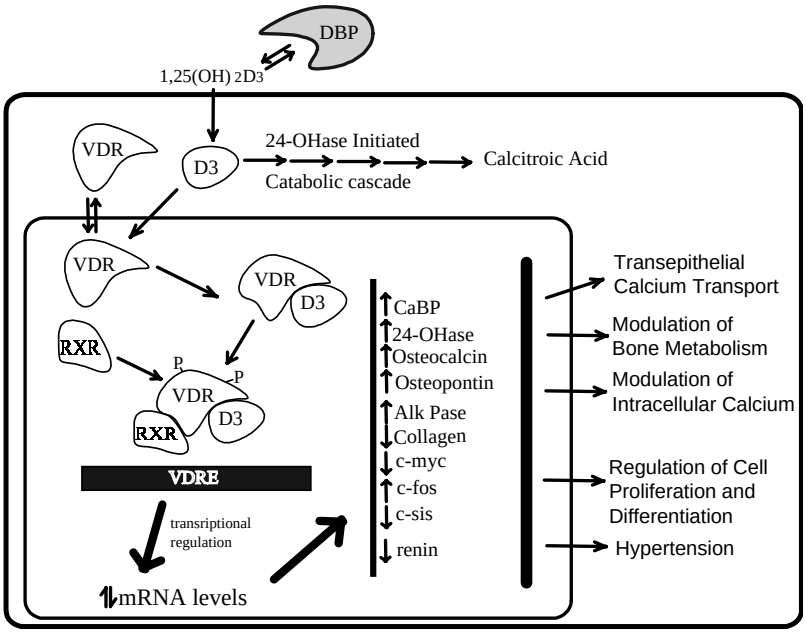

Fig. 3 Selected effects of 1α,25-dihydroxyvitamin D_3 through its cognate nuclear receptor and its co-regulators on the gene expression

tamin D_5 and a few others have been developed which display increased cell regulatory effects with minimal side effects (Simboli-Campbell et al. 1997). It is known that 1α,25-$(OH)_2D_3$ is a negative growth regulator of various cancer cells, and vitamin D_3 analogs, similarly as retinoids and rexinoids represent a novel approach for treatment human cancer (Simboli-Campbell et al. 1996; Brtko and Thalhamer 2003). It has been shown that 80 % of human breast tumours express VDR, but the expression of VDR may be at low levels (Eisman et al. 1981). Therefore, the strategies that enhance VDR expression might enhance the effectiveness of vitamin D_3 based therapies. Recently, phytoestrogens like genistein (present in soy) and resveratrol (present in red wine) are able to upregulate the exon 1c VDR promoter and enhance steady state VDR protein expression in an estrogen receptor-dependent manner, and thus they enhance VDR mediated transcriptional activation and sensitize cells to the growth inhibitory effects of 1α,25$(OH)_2D_3$ (Wietzke and Welsh 2003).

It was shown that pharmacological doses of the functional metabolite of vitamin D, 1α,25-dihydroxyvitamin D_3, reduce or eliminate the incidence of disease in the multiple sclerosis (MS) mouse model called experimental autoimmune encephalomyelitis (EAE) (Lemire and Archer 1991).

While the interaction between 1α,25-$(OH)_2D_3$ and the VDR is well characterized, several groups claim that 1α,25-$(OH)_2D_3$ has a second "nongenomic" mode of action and that nonhypercalcemic analogues may preferentially act through this latter mode (Meehan and DeLuca 2002). The major hormones driving pubertal mammary gland development are estrogen and progesterone. Estrogen stimulates ductal elongation and progesterone mediates branching (Silberstein 2001). It was shown the also VDR has a big impact on mammary gland development. VDR has been implicated in control of differentiation, cell cycle and apoptosis of mammary cells in culture, but little is known about the physiological relevance of vitamin D_3 endocrine system in the developing gland (Zinser et al. 2002). It was shown that mammary glands from vitamin D_3-deficient mice exhibit impaired Ca^{2+} transport and casein production, supporting a functional role for the vitamin D_3 signalling pathway in lactation (Bhattacharjee et al. 1987; Mezzetti et al. 1988). Mammary glands that lack VDR exhibit enhanced ductal extension and branching during puberty in vivo and in response to growth-promoting hormones in vitro, suggesting that the vitamin D_3 signalling pathway participates in negative growth regulation of the mammary gland (Zinser et al. 2002). Calcitriol significantly decreases P-ERK (phosphorylated extracellular signal-related kinase) and MEK (mitogen-activated protein kinase), induces MEK cleavage and uniquely induces MEKK-1 (mitogen-activated protein kinase). These changes in the signal-

ling pathway result in a significant antitumour activity both in vitro and in vivo. Synergistic antitumour activity can be observed when calcitriol is combined with glucocorticoids or chemotherapeutics agents, especially taxanes. These findings have demonstrated that vitamin D_3 acting via VDR has significant antitumour activities (Johnson et al. 2002).

Several clinical studies have proposed that $1\alpha,25(OH)_2D_3$ may also be beneficial to the cardiovascular system by decreasing blood pressure (Lind et al. 1995; Kristal-Boneh et al. 1997). It is suggested that VDR negatively regulates the expression of renin, allowing for decreased angiotensin production and lower blood pressure (Li et al. 2002; Sutton and MacDonald 2003).

The Food and Nutrition Board of the US National Research Council recommend a daily intake of 5 mg (200 IU) of vitamin D for adults, 7.5 mg (300 IU) for infants under 6 months, and 10 mg (400 IU) for children over 6 months, pregnant and lactating women. These recommendations can vary between countries. Hypervitaminosis D is a potentially serious problem as it can cause permanent kidney damage, growth retardation, calcification of soft tissues and death. Mild symptoms of intoxication are nausea, weakness, constipation and irritability. Lack of either function VDR or active $1\alpha,25(OH)_2D_3$ leads to profound, life-threatening hypocalcaemia and undermineralized skeletal tissue (Sutton and MacDonald 2003).

In conclusion, there is considerable interest in many laboratories and the pharmaceutical industry over the world in investigating vitamin D-related drugs that might exhibit specific actions applicable to the treatment of disorders of bone formation, malignant tumours and immune disorders (Choi et al. 2003).

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