

## Mini Review

**Role of Dihydroxyvitamin D<sub>3</sub> and Its Nuclear Receptor in Novel Directed Therapies for Cancer**

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**Abstract.** Dihydroxyvitamin D<sub>3</sub> is known to affect broad spectrum of various biochemical and molecular biological reactions in organisms. Research on the role and function of nuclear vitamin D receptors (VDR) playing a role as dihydroxyvitamin D<sub>3</sub> inducible transcription factor belongs to dynamically developing branches of molecular endocrinology. In higher organisms, full functionality of VDR in the form of heterodimer with nuclear 9-cis retinoic acid receptor is essential for biological effects of dihydroxyvitamin D<sub>3</sub>.

This article summarizes selected effects of biologically active vitamin D<sub>3</sub> acting through their cognate nuclear receptors, and also its potential use in therapy and prevention of various types of cancer.

**Key words:** Dihydroxyvitamin D<sub>3</sub> — Metabolism — Nuclear receptor — 9-cis retinoic acid — Cancer therapy

Vitamin D family consists of 9,10-secosteroids which differ in their side-chain structures. They are classified into five forms: D<sub>2</sub>, ergocalciferol; D<sub>3</sub>, cholecalciferol; D<sub>4</sub>, 22,23-dihydroergocalciferol; D<sub>5</sub>, sitosterol (24-ethylcholecalciferol) and D<sub>6</sub>, stigmaterol (Napoli et al. 1979). The main forms are vitamin D<sub>2</sub> (ergocalciferol: plant origin) and vitamin D<sub>3</sub> (cholecalciferol: animal origin). Both 25-hydroxyvitamin D<sub>2</sub> and 1 $\alpha$ ,25-dihydroxyvitamin D<sub>2</sub> have been evaluated for their biological functions. Vitamin D itself is a prohormone that is metabolically converted to the biologically active metabolite, 1,25-dihydroxyvitamin D<sub>3</sub> in kidney. This vitamin D<sub>3</sub>, currently considered a steroid hormone, activates its cognate nuclear receptor (vitamin D receptor or VDR) which alter transcription rates of the target genes responsible for its biological responses. In general, vitamin D is essential for mineral homeostasis, for absorption and utilization of both calcium and phosphate and it aids in the mobilization of bone calcium and maintenance of serum calcium concentrations. Through these function, it plays an important role in ensuring proper

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functioning of muscles, nerves, blood clotting, cell growth and energy utilization. It has been proposed that vitamin D is also important for insulin and prolactin secretion, immune and stress responses, melanin synthesis and for differentiation of skin and blood cells (Lips 2006). Vitamin D metabolites also play a role in the prevention of auto-immune diseases and cancer (Pinette et al. 2003; Dusso et al. 2005). The steroid hormone  $1\alpha,25$ -dihydroxyvitamin  $D_3$  (calcitriol) exerts biological responses by interaction with both the well-characterized nuclear receptor ( $VDR_{nuc}$ ) responsible for activation gene transcription and not fully characterized membrane-associated protein/receptor ( $VDR_{mem}$ ) involved in generating a variety of rapid, non-genotropic responses (Evans 1988; Norman et al. 2002).

### Vitamin D metabolism

Vitamin D, the “sunshine” vitamin, is synthesized under the influence of ultraviolet light in the skin. Many mammals have provitamin D (7-dehydrocholesterol) which is converted to provitamin  $D_3$  in their skin. When human skin is exposed to sunlight, the UV-B photons (wavelengths 290–315 nm) interact with 7-dehydrocholesterol causing photolysis and cleavage of the B-ring of the steroid structure, which upon thermoisomerization yields a secosteroid. Thus, provitamin  $D_3$  which is inherently unstable rapidly converts by a temperature-dependent process to vitamin  $D_3$  (MacLaughlin et al. 1982; Holick 1994). 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 liver and kidney for bioactivation (Wikvall 2001). In the first activation step, vitamin  $D_3$  is hydroxylated by the enzyme 25-hydroxylase to 25-hydroxyvitamin  $D_3$  mainly in the liver. This metabolite is present in the circulation at the concentration of more than  $0.05 \mu\text{mol/l}$  (20 ng/ml). In the second step, the biologically active hormone  $1\alpha,25$ -dihydroxyvitamin  $D_3$  is generated by hydroxylation of 25-hydroxyvitamin  $D_3$  at  $1\alpha$ -position in kidney. The enzyme  $1\alpha$ -hydroxylase has been shown to be also present in keratinocytes and prostate epithelial cells, suggesting that those organs may also be able to generate  $1\alpha,25$ -dihydroxyvitamin  $D_3$  from 25-dihydroxyvitamin  $D_3$  (Schwartz et al. 1998). The activity of  $1\alpha$ -hydroxylase in the kidney serves as the major control point in production of the active hormone. The active metabolite  $1\alpha,25$ -dihydroxyvitamin  $D_3$  is present in human plasma at the concentration ranging from 0.05 to 0.15 nmol/l (20–60 pg/ml) (Hartwell et al. 1987; Gross et al. 1996). In general, 90 to 100% of the most human being vitamin D requirement comes from exposure to sunlight (Holick 2003) and the rest of the vitamin  $D_3$  content is obtained from diet (Malloy and Feldman 1999). The catabolism of vitamin D occurs by further hydroxylation of 25-dihydroxyvitamin  $D_3$  by 24-hydroxylase to yield 24,25-dihydroxyvitamin  $D_3$ . The 24-hydroxylase is ubiquitous enzyme and is expressed in all the cells expressing VDR. This enzyme is regulated by parathyroid hormone and  $1\alpha,25$ -dihydroxyvitamin  $D_3$ . The major significance of 24-hydroxylation is inactivation of vitamin D (Nishimura et al. 1994; Brenza and DeLuca 2000). The combinations of 1,25-dihydroxyvitamin  $D_3$

with inhibitors of 24-hydroxylase such as ketoconazole or liarozole may enhance its antitumour effects in prostate cancer therapy.

### Vitamin D<sub>3</sub> receptor

More than 2000 synthetic analogues of the biological active form of vitamin D, 1 $\alpha$ ,25-dihydroxyvitamin D<sub>3</sub>, are presently known. Basically, all of them interfere with the molecular switch of nuclear 1 $\alpha$ ,25-dihydroxyvitamin D<sub>3</sub> signalling, which is the complex of the VDR, the retinoid X receptor (RXR), and a 1 $\alpha$ ,25-dihydroxyvitamin D<sub>3</sub> response element (VDRE) (Carlberg 2003).

VDR is the only nuclear protein that binds the biologically most active vitamin D metabolite, 1 $\alpha$ ,25-dihydroxyvitamin D<sub>3</sub>, with high affinity ( $K_d = 0.1$  nmol/l). This classifies the VDR into the classical endocrine receptor subgroup of the nuclear receptor superfamily, which also contains the nuclear receptors for hormones as retinoic acid, thyroid hormone, estradiol, progesterone, testosterone, cortisol, and aldosterol (Carlberg 1995). Similarly, like other biologically active ligand for nuclear hormone receptors, 1,25-dihydroxyvitamin D<sub>3</sub> can modulate expression of selected ion transport protein genes (Van Baal et al. 1996; Hudecova et al. 2004).

The VDR was first isolated after transfection of COS-1 cells with cloned sequences of complementary DNA that was isolated from human intestine (Baker et al. 1988). VDR has been found in more than 30 tissues including intestine, colon, breast, lung, ovary, bone, kidney, parathyroid gland, pancreatic  $\beta$ -cells, monocytes, keratinocytes, and many cancer cells, suggesting that the vitamin D endocrine system may also be involved in regulating the immune systems, cellular growth, differentiation and apoptosis (Jones et al. 1998). The active form of vitamin D binds to intracellular receptors that then function as transcription factors to modulate gene expression. Like the receptors for other steroid hormones and thyroid hormones, the VDR has specific hormone-binding and DNA-binding domains. It contains two zinc finger structures forming a characteristic DNA-binding domain (DBD) of 66 amino acids and a carboxy-terminal ligand-binding domain (LBD) of approximately 300 amino acids, which is formed by 12  $\alpha$ -helices. Ligand binding causes a conformational change within the LBD, in which helix 12, the most carboxy-terminal  $\alpha$ -helix, closes the ligand-binding pocket *via* a “mouse-trap like” intramolecular folding (Moras and Gronemeyer 1998). Moreover, the LBD is involved in a variety of interactions with nuclear proteins, such as other nuclear receptors, corepressor and coactivator proteins. These ligand-triggered protein-protein interactions are the central molecular event of nuclear 1 $\alpha$ ,25-dihydroxyvitamin D<sub>3</sub> signalling.

The VDR is a high-affinity, low-capacity receptor protein of 48 to 55 kD, primarily located in the nucleus, although evidence exists for the presence of cytoplasmic receptors (Darwish and DeLuca 1996). The human VDR (hVDR) 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 span approximately 75 kb (Miyamoto et al. 1997).

From a study carried out on cDNA clones obtained from intestinal VDR, it was shown that VDR belongs to the steroid-receptor gene family and is closest in size and sequence to the thyroid hormone receptor (Baker et al. 1988). VDR possess five functional domains. Its 427 amino acids encompass a short N-terminal activation-function 1 (AF-1) domain (A/B), a DBD containing two Zn<sup>2+</sup>-fingers (domain C), a flexible "hinge" region (domain D) that includes localization signals and a HSP-70 site, and finally the LBD (domain E) whose C-terminal end also has transcriptional activation function(s) (AF-2).

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).

The DBD of VDR has been mapped to amino acid residues 22–114 (Sone et al. 1991). It is highly conserved throughout all nuclear receptors and is mainly made up of two zinc finger motifs.  $\alpha$ -helix is 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 is found. 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).

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

The LBD varies considerably between the nuclear hormone receptors. The structure of the VDR LBD is similar to that of other nuclear receptors being most closely related to that of the RAR $\gamma$  LBD (Rochel et al. 2000). The hormonal binding domain is made up of 12  $\alpha$ -helices with several short  $\beta$ -strands that are organized in a three dimensional lipophilic hormone binding pocket, to which vitamin D is attached. Specific amino acids in this domain as well as in the DNA binding domain are important for heterodimerization with the RXR. A tryptophan residue in position 286 of the human VDR is very important for specific ligand (vitamin D) interactions and also for the interaction with the RXR (Solomon et al. 2001).

The RXR-VDR heterodimeric complex is weakly associated with DNA until occupied by 1 $\alpha$ ,25-dihydroxyvitamin D<sub>3</sub> at which time it penetrates the major groove to recognize a hexanucleotide direct repeat VDRE. 1 $\alpha$ ,25-dihydroxyvitamin D<sub>3</sub> also conformationally activates VDR by positioning its transcriptional AF-2 for productive interactions with coactivators.

The mechanism of action of the VDR is very similar to that of other steroid receptor. As 1 $\alpha$ ,25-dihydroxyvitamin D<sub>3</sub> is lipid soluble, it enters the cell through the plasma membrane and interacts noncovalently but stereospecifically with the VDR (Reichel et al. 1989). The VDR will then bind to the RXR forming a heterodimer that will in turn bind to short DNA sequences initiating transcription of genes (Haussler et al. 1998). These specific DNA sequences are known as the VDREs. A VDRE consists of two 6 base pair half elements that are separated by a spacer of 3 nucleotides (DR3). A typical VDRE sequences for human parathor-

none is GGTTCA AAG CAGACA. The nucleotide guanine in the third position of the spacer is considered to be very important for receptor binding, since it is the DR3 recognition site in the DNA that attracts the RXR-VDR complex (Haussler et al. 1998).

### Role of vitamin D<sub>3</sub> in cancer

Some of biologically active ligands for nuclear receptors exert tumour-suppressive activity, and they have therapeutical exploitation due to their antiproliferative and apoptosis-inducing effects (Brtko and Thalhamer 2003).

### Prostate cancer

Epidemiological studies suggest that vitamin D has the ability to inhibit growth, induce differentiation and apoptosis, and inhibit invasion by cancer cells. Vitamin D, its precursors, and analogues are being explored as potential agents for prostate cancer prevention and treatment.

Adenocarcinoma of the prostate gland is the most common diagnosed malignancy in American men and is the second leading cause of cancer deaths in the USA (Hellerstedt and Pienta 2002). Prostate intraepithelial neoplasia (PIN), a precursor of prostate cancer (PCa), has been observed in young men in age ranging from 20 to 30 years (Sakr et al. 1993). Progression of PIN to high grade PIN (HGPIN) may take another 10 years, followed by the development of metastatic cancer several years later. HGPIN occurs predominantly in the peripheral zone of the prostate where 70% of prostate cancers arise (Bostwick 1995). There are several risk factors for PCa have been identified including age, race, and genetic influence (Gross et al. 1997; Blutt and Weigel 1999; Konety et al. 1999; Miller 1999; Feldman et al. 2000). The hypothesis that vitamin D deficiency increases the risk of PCa (Schwartz and Hulka 1990) was based on the observations that mortality rates due to PCa in the USA are inversely related to sunlight exposure and that UV light is essential for the synthesis of vitamin D in the skin. High serum vitamin D levels have also been related to reduced risk and better prognosis for colon, breast, and prostate cancer (Robsahm et al. 2004). Although some studies suggest that lower serum 1 $\alpha$ ,25-dihydroxyvitamin D<sub>3</sub> levels are a risk factor for PCa, others do not agree on these findings (Gross et al. 1997; Blutt and Weigel 1999; Konety et al. 1999; Miller 1999; Feldman et al. 2000).

Human prostate epithelial cells express VDR with high rate of expression in epithelial cells of the peripheral zone, consequently, growth was found to be inhibited by calcitriol *in vitro* (Barreto et al. 2000). Calcitriol also inhibits growth and invasion and induces differentiation in prostate cancer cells (Tokar and Webber 2005). 1 $\alpha$ -hydroxylase is also present in human keratinocytes and many other cell types including prostate epithelial cells (Schwartz et al. 1998). In contrast, it is not known if 25-hydroxylase, which catalyzes the conversion of vitamin D<sub>3</sub> to 25-hydroxyvitamin D<sub>3</sub> is expressed in prostate epithelial cells.

25-hydroxyvitamin D<sub>3</sub> 24-hydroxylase (CYP24) catalyzes the initial step in the conversion of the active molecule 1 $\alpha$ ,25-dihydroxyvitamin D<sub>3</sub> into less active metabolites. Among the human PCa cell lines DU 145, PC-3, and LNCaP, DU 145 cells exhibit a high level of 24-hydroxylase induction and are least responsive to 1 $\alpha$ ,25-dihydroxyvitamin D<sub>3</sub> in terms of growth inhibition. On the other hand, the basal and induced expression of 24-hydroxylase is very low in LNCaP cells and growth inhibition by 1 $\alpha$ ,25-dihydroxyvitamin D<sub>3</sub> is substantial. It has shown that in DU 145 cells, liarozole (an imidazole derivative which inhibits P450 hydroxylases) causes significant inhibition of 24-hydroxylase activity leading to increased 1 $\alpha$ ,25-dihydroxyvitamin D<sub>3</sub> half-life in these cells (Ly et al. 1999). It has also been demonstrated that the differences in 1 $\alpha$ ,25-dihydroxyvitamin D<sub>3</sub> mediated growth inhibition between various PCa cell lines correlate inversely to 24-hydroxylase expression in these cells (Miller et al. 1995). A recent study has shown that in primary human PCa cells, the P450 inhibitor ketoconazole potentiates the growth inhibitory effects of 1 $\alpha$ ,25-dihydroxyvitamin D<sub>3</sub> or its structural analogue EB 1089 by inhibiting the 24-hydroxylase activity in these cells (Peehl et al. 2002).

Levels of active vitamin D are controlled by synthesis *via* 25-hydroxyvitamin D<sub>3</sub> 1 $\alpha$ -hydroxylase (CYP27B1). CYP27B1 is a cytochrome P450-containing hydroxylase expressed in kidney and other tissues that generates 1 $\alpha$ ,25-dihydroxyvitamin D<sub>3</sub> from an inactive vitamin D precursor 25-hydroxyvitamin D<sub>3</sub> (Kemmis et al. 2006; Schuster et al. 2006). The activity of the CYP27B1 in primary cancer cells is lower than that of benign prostatic hyperplasia (BPH) and the PCa cell lines express the lowest 1 $\alpha$ -hydroxylase activity. The antiproliferative effect of 25-hydroxyvitamin D<sub>3</sub> correlates with the endogenous 1 $\alpha$ -hydroxylase activity in these cells. On the other hand, in primary epithelial cells from cancer or in the PCa cell line LNCaP with very low endogenous 1 $\alpha$ -hydroxylase activity, the antiproliferative action of 25-hydroxyvitamin D<sub>3</sub> is much less pronounced in comparison with 1 $\alpha$ ,25-dihydroxyvitamin D<sub>3</sub>. It was concluded that a decrease in 1 $\alpha$ -hydroxylase activity may represent an important mechanism in PCa development and/or progression. While 1 $\alpha$ -hydroxylase was initially found still high within the prostate, it has been suggested that the administration of 25-hydroxyvitamin D<sub>3</sub> might be one of effective chemopreventive approaches (Krishnan et al. 2003).

Several polymorphisms have been identified in the VDR gene (Uitterlinden et al. 2001). Some of these polymorphisms may contribute to PCa risk, although not all studies could confirm this finding (Feldman 1997; Ingles et al. 1998). The role of VDR polymorphisms in diseases such as osteoporosis and PCa is being actively investigated and it was proposed that differences in the functional activity of the different VDR alleles might contribute to the risk of these diseases (Jurutka et al. 2000). Both the epithelial cells of the peripheral zone of the prostate and the primary site of prostate cancer show the highest expression of VDR (Krill et al. 2001). The level of RXR expression and also interaction of VDR with RXRs are crucial for transcriptional effects and vitamin D effects. Elevated levels of RXR $\alpha$  increase the antiproliferative effects of 1 $\alpha$ ,25-dihydroxyvitamin D<sub>3</sub> (Prufer and Barsony 2002). The non-tumorigenic human epithelial cell line (RWPE-1) constitutively ex-

presses all three subtypes of RXRs, where  $\text{RXR}\alpha$  shows the highest and  $\text{RXR}\beta$  the lowest expression levels (Tokar and Webber 2005). It has been previously shown that human prostate cells produce transforming growth factor- $\beta$  ( $\text{TGF}\beta$ ), express  $\text{TGF}\beta$  receptors, and that  $\text{TGF}\beta$  inhibits the growth of both RWPE-1 cells and of RWPE-2 human prostate carcinoma cells (Bello et al. 1997). The mechanisms by which vitamin  $\text{D}_3$  inhibits growth are not known yet, but it is believed that the effects may be either direct or indirect *via* its conversion to  $1\alpha,25$ -dihydroxyvitamin  $\text{D}_3$ . Vitamin  $\text{D}_3$  can bind directly to VDR but it has a considerably lower affinity than calcitriol (Chen et al. 2000).

Recent studies show that human mitochondrial 25-hydroxylase (CYP27A1) can not only catalyze the conversion of vitamin  $\text{D}_3$  to 25-hydroxyvitamin  $\text{D}_3$  but also the further conversion of 25-hydroxyvitamin  $\text{D}_3$  to the most biologically active hormone calcitriol (Axen et al. 1994; Sawada et al. 2000; Wikvall 2001). Tokar and Webber (2005) explored the expression of the CYP27A1 in RWPE-1 cells. It has been demonstrated that human prostate epithelial cells constitutively express appreciable levels of 25-hydroxylase CYP27A1 protein which is up-regulated by vitamin  $\text{D}_3$ .

Prostate-specific antigen secretion and androgen receptor expression are enhanced in LNCaP cells in response to  $1\alpha,25$ -dihydroxyvitamin  $\text{D}_3$ , which also inhibits proliferation of LNCaP, a well established human prostate cancer cell line, in a dose-dependent manner (Miller et al. 1992; Skowronski et al. 1993). Dexamethasone significantly increases VDR concentration without changing its affinity ( $K_d$ ) for ligand (Yu et al. 1998). The combination of  $1\alpha,25$ -dihydroxyvitamin  $\text{D}_3$  and dexamethasone causes a significant increase in VDR protein concentration when compared to either  $1\alpha,25$ -dihydroxyvitamin  $\text{D}_3$  alone or dexamethasone alone. High-dose intermittent  $1\alpha,25$ -dihydroxyvitamin  $\text{D}_3$  plus dexamethasone appears to be safe, feasible, and has marked antitumour activity. Subjective clinical improvement occurred in some patients where the majority of them suffered with bone metastases and demonstrated rising prostate-specific antigen levels (Trump et al. 2006).

### Breast cancer

Breast cancer is considered the most frequent malignancy of women in the world. Its development is associated with deregulation of cell growth and cell death. It has been shown that retinoids, biologically active ligands of nuclear receptors of the steroid/thyroid/retinoid superfamily are able to inhibit mammary gland cancer in animal models and human breast cancer (Brtko and Thalhamer 2003). A strong antiproliferative effect of  $1\alpha,25$ -dihydroxyvitamin  $\text{D}_3$  has been demonstrated for breast tumour cell cultures *in vitro* (Wu et al. 1997) as well as *in vivo* (Frampton et al. 1983). Low non-calcemic doses of vitamin  $\text{D}_3$  ( $10^{-9}$  mol/l), in combination with melatonin, have the same growth inhibitory actions as  $1\alpha,25$ -dihydroxyvitamin  $\text{D}_3$  at up to 100-fold higher concentrations ( $10^{-7}$  mol/l and  $10^{-8}$  mol/l). A similar effect was observed with a selective estrogen receptor modulator tamoxifen or

other antiestrogens associated with vitamin D<sub>3</sub> or its synthetic analogues (Abe-Hashimoto et al. 1993; Vink-van Wijngaarden et al. 1994; Love-Schimenti et al. 1996). Melatonin increases the sensitivity to vitamin D<sub>3</sub> and induces a marked increase in TGF $\beta$ <sub>1</sub> release, even if the cancer cells are not stimulated by estrogens. This combined approach is an interesting candidate for clinical trials on breast cancer therapy (Bizzarri et al. 2003). Vitamin D might also reduce the invasiveness of cancer cells and act as an anti-angiogenesis agent. All of these antitumour features suggest that the properties of vitamin D could be explored for chemopreventive and therapeutic purposes in cancer (Bortman et al. 2002). Regarding CYP24A1 expression in breast tumours, one study is showing that a region of amplification within chromosomal region 20q13.2 was mapped to the CYP24A1 gene. When breast tumour samples were examined, relative levels of CYP24A1 mRNA were found to be higher in tumours with higher amplification at the CYP24 locus (Albertson et al. 2000), suggesting that CYP24A1 may be up-regulated in breast cancer. However, in other current study, it was observed that the mean value of CYP24A1 mRNA in the breast tumour samples was significantly lower than that in the normal breast tissue samples (Anderson et al. 2006). More studies comparing CYP24A1 expression in breast tumour will be needed to understand the role of CYP24A1 in breast tumour pathogenesis. Other study has shown that the 24-hydroxylase mRNA level was overall two-fold higher in breast carcinoma as compared to normal tissue. Notably, the CYP24 gene has been described as a breast candidate oncogene (Albertson et al. 2000). A problem is linked with rapid 24-hydroxylation and subsequent degradation of 25-hydroxyvitamin D<sub>3</sub> of locally synthesized 1 $\alpha$ ,25-dihydroxyvitamin D<sub>3</sub>. Exploitation of more specific 24-hydroxylase inhibitors (Schuster et al. 2003) than liarozole and ketoconazole (Ly et al. 1999; Peehl et al. 2002) may hopefully present future therapeutic alternatives.

Studies on experimental animals have demonstrated that the VDR is dynamically regulated during pregnancy and lactation, however, little is known about its specific functions. Highest levels of VDR in mammary gland have been observed during lactation, being maximal at 3 days post partum when the concentration of calcium in milk is of the highest value (Colston et al. 1988). Addition of 1 $\alpha$ ,25-dihydroxyvitamin D<sub>3</sub> to mammary gland explants caused an increase in VDR expression and also enhanced calcium uptake (Mezzetti et al. 1988).

1-methyl-1-nitrosourea is a well characterized carcinogen that induces adenocarcinomas in rat mammary gland with high specificity. This model has proven to be of resemblance to human breast cancer and is therefore of great interest for mammary gland tumour studies (Mehta 2000; Macejová and Brtko 2001; Macejová et al. 2005).

### **The other cancers**

During the last decade, evidence for vitamin D<sub>3</sub> effects has been accumulating not only for prostate cancer (Feldman et al. 1995; Ma et al. 2004) but also for colon cancer (Cross et al. 1997; Bischof et al. 1998). 1 $\alpha$ -hydroxylase was found to be



expressed and active in colorectal cancer (Bareis et al. 2001; Cross et al. 2001; Tangpricha et al. 2001; Ogunkolade et al. 2002) and ovarian cancer (Miettinen et al. 2004). In both colon and also lung tumours, CYP24A1 mRNA was significantly up-regulated, while VDR mRNA was generally down-regulated when compared to respective normal tissues. When the level of VDR in 12 malignant colonic tumours was compared with that of adjacent normal tissue, in 9 cases out of 12, expression of VDR in tumours was decreased. However, in that study, the expression of CYP24A1 was not assessed. It has also been shown that, at least in human colon cancer cell lines, the level of VDR correlates with the degree of cell differentiation (Shabahang et al. 1993; Anderson et al. 2006).

Recently, it has been suggested that actually 20–30% of colorectal cancer incidence might be due to insufficient exposure to sunlight. This fact was strengthened by correlation between reduced colorectal cancer incidence and sunlight exposure, low skin pigmentation, nutritional vitamin D intake and high serum levels of 25-hydroxyvitamin D<sub>3</sub> (Grant and Garland 2003). In the colon at least, CYP27B1 and VDR expression was described to be actually elevated during early tumour progression and that described dual positivity was found in many, but not all the tumour cells. In human colon tumours, CYP24 mRNA is quite highly expressed and the studies also demonstrated that with the exception of differentiated Caco-2 cells, CYP24 activity is constitutively present or can be induced by 1 $\alpha$ ,25-dihydroxyvitamin D<sub>3</sub>. During tumour progression in the colon, not only VDR but CYP27B1 and CYP24 expression were found to be increased in tumour tissues (Bareis et al. 2001; Bises et al. 2004).

Androgens, retinoids, glucocorticoids, estrogens and agonists of peroxisome proliferator-activated receptor directly or indirectly have reasonable impact on vitamin D signalling pathways, and *vice versa*. It was proposed that sex hormones might reduce colorectal cancer risk (McMichael and Potter 1980). The studies suggested that current and long-term use of estrogens is associated with a substantial decrease in risk of fatal colon cancer. The mechanism, however, by which estrogens could inhibit colonic tumour growth, remains an enigma. There are at least two distinct estrogen receptors in the human body: ER $\alpha$  and ER $\beta$ . In the normal human colon, ER $\beta$  is widely regarded to be the predominant subtype (Campbell-Thompson et al. 2001). In a recently terminated pilot study together with Strang Cancer Prevention Centre at Rockefeller University (NY, USA), tissues from postmenopausal women receiving 17 $\beta$ -estradiol for expression of CYP27B1 by real time RT-PCR were examined. CYP27B1 was found to be elevated significantly in all subjects after receiving 17 $\beta$ -estradiol for 4 weeks.

Amplification of chromosomal region 20q12q13 containing the CYP24A1 gene has been reported in ovarian cancer, as well (Tanner et al. 2000). Although inhibition of ovarian cancer cell growth by 1 $\alpha$ ,25-dihydroxyvitamin D<sub>3</sub> has been reported (Saunders et al. 1992, 1995), a clinical trial testing the efficacy of 1 $\alpha$ ,25-dihydroxyvitamin D<sub>3</sub> combined with isotretinoin in treating 22 epithelial ovarian cancer patients for 74 weeks has not produced positive results (Rustin et al. 1996).

In the coming decade, sophisticated molecular biology approaches will very

likely open new possibilities for vitamin D<sub>3</sub> and its synthetic analogues exploitation in novel directed therapies for cancer. This will further increase our knowledge about the relationship between tumour development and role of VDR in cancer and thus help to build up innovative and rational approaches for future cancer treatment.

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