Review

The Roles of Cytochrome P450 Enzymes in Prostate Cancer Development and Treatment

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Abstract. *The active form of vitamin D, 1α,25-dihydroxyvitamin D [1α,25(OH)$_2$D], interacts with vitamin D receptor (VDR) and induces antiproliferative, anti-invasive, proapoptotic and pro-differentiation activities in prostate cancer cells. Three cytochrome P-450 (CYP) hydroxylases are responsible for vitamin D synthesis and degradation, including vitamin D-25-hydroxylase (25-OHase) in the liver, and 25(OH)D-1α-hydroxylase (1α-OHase) or CYP27B1, and 25(OH)D-24-hydroxylase (24-OHase) or CYP24A1 in the kidneys. However, it is now recognized that CYP27B1 and CYP24A1 are also expressed in many tissues and cells, including the prostate. Although at least six CYP enzymes have been identified with 25-OHase activity, the two major ones are CYP27A1 and CYP2R1, and both are expressed in the prostate, with CYP2R1 as the predominate type. This indicates that prostate tissue has the ability to activate and inactivate vitamin D in an autocrine/paracrine fashion. Recent evidence indicates that 25-hydroxyvitamin D [25(OH)D] and its analogs can bind to VDR as agonists, without converting them to 1α,25(OH)$_2$D or the corresponding 1α-hydroxylated metabolites, to modulate gene expressions that will lead to cell growth arrest and other antitumor activities. This finding suggests that the circulating levels of 25(OH)D, and the autocrine synthesis of 25(OH)D may play an important role in regulating the growth of prostate cancer. Thus, in addition to 1α,25(OH)$_2$D analogs, the presence of CYP2R1, CYP27B1 and CYP24A1 in the prostate suggests that the analogs of vitamin D and 25(OH)D, especially those that are resistant to CYP24A1 degradation, can be developed and used for the prevention and treatment of prostate cancer.*

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Key Words: Vitamin D analog, proliferation, CYP enzymes, prostate cancer, review.

Human Cytochrome P450 Enzymes

Cytochrome P (CYP) 450 enzymes are a superfamily of isoenzymes. These enzymes catalyze the metabolism of a large number of compounds of both exogenous and endogenous origin, including steroids, vitamins, fatty acids, prostaglandins and leukotrienes, and are involved in drug metabolism and detoxification (1). In some cases, the enzymes may activate exogenous compounds to toxins or carcinogens (2). In the human genome, there are 57 genes involved in cytochrome P450 enzyme synthesis. There are a total of 57 enzymes classified into 18 families (1). Among them, 51 are present in microsomes and 6 are in mitochondria. The tertiary structures of microsomal CYP1A2 (3), 2A6 (4), 2C5 (5), 2C8 (6), 2C9 (7), 2D6 (8), 3A4 (9), 19A1 (10), 46A1 (11), 2R1(12) and 51 (13) are known, whereas the crystal structures of only two of the mitochondrial enzymes have been revealed; they are CYP24A1 (14) and 11A1 (15). Because the CYP enzymes play such versatile and important roles in the body, any mutations in the *CYP* genes can cause serious health problems. For example, mutations in *CYP17A1* lead to mineral corticoid excess syndromes, glucocorticoid and sex hormone deficiencies, and increased risk of prostate cancer and benign prostatic hypertrophy (1). Likewise, mutations in *CYP2R1*, responsible for the synthesis of 25-hydroxyvitamin

Table I. *Functions and substrates of vitamin D cytochrome P450 enzymes, and disorders resulting from their mutation.*

Gene	Function of the gene product/enzyme	Enzyme substrate	Disorder resulting from gene mutation
CYP2R1	C-25 hydroxylation	Vitamin D_3	Vitamin D-dependent
		Vitamin D_2	ricket type 1
CYP27A1	C-25 hydroxylation	Vitamin D_3	Cerebrotendinous xanthomatosis
CYP27B1	C-1 hydroxylation	$25(OH)D_3$	Vitamin D-dependent
		$25(OH)D_2$	ricket type 1
CYP24A1	C-24 hydroxylation	$25(OH)D_3$	Idiopathic infantile hypercalcemia
		$25(OH)D_2$*	
		$1\alpha,25(OH)_2D_3$	
		$1\alpha,25(OH)_2D_2$	
CYP3A4	C-25 hydroxylation	Vitamin D_3	
		Vitamin D_2	Unknown
CYP2D25 (Pig)	C-25 hydroxylation	Vitamin D_3	Unknown
CYP2J3 (Rat)	C-25 hydroxylation	Vitamin D_3	Unknown
CYP2C11 (Male rat)	C-25 hydroxylation	Vitamin D_3	Unknown

*Although no study has been reported, a metabolic pathway similar to that of $1\alpha,25(OH)_2D_2$ C-24 hydroxylation can be assumed.

D [25(OH)D] from vitamin D, and *CYP27B1*, responsible for the 1α-hydroxylation of 25(OH)D to produce the active form 1α,25-dihydroxyvitamin D [$1\alpha,25(OH)_2D$], causes vitamin D deficiency-induced rickets and vitamin D-dependent rickets type 1, respectively (16, 17). On the other hand, mutations in *CYP24A1* induces idiopathic infantile hypercalcemia because CYP24A1 is responsible for the degradation of 25(OH)D, the circulating form of vitamin D, and $1\alpha,25(OH)_2D$, the active form (18) (Table I).

Historical Review of Vitamin D Metabolism

The modern understanding of vitamin D metabolism began in 1964 with the publication by Norman, Lund and DeLuca (19) entitled "Biological Active Forms of Vitamin D_3 in Kidney and Intestine". During the subsequent several years, intensive efforts were carried out by DeLuca and associates to isolate and identify the first metabolite of vitamin D_3, $25(OH)D_3$ (20). The *in vivo* and *in vitro* synthesis of $25(OH)D_3$ in liver cells was demonstrated a year later by Ponchon and DeLuca (21) and by Horsting and DeLuca (22), respectively. In the same year, Lawson *et al*. described a new cholecalciferol metabolite with a loss of hydrogen at C-1 in chick intestinal nuclei (23, 24), which was followed by the identification of a unique biological active vitamin D metabolite synthesized in the kidneys (25). The structure of this active metabolite was later identified as 1α,25-dihydroxycholecalciferol (26-28), and chemically synthesized (29). One additional metabolite of vitamin D, namely 21,25-dihydroxycholecalciferol, was described by Suda *et al*. in 1970 (30). However, the structure of this metabolite was later revised as 24,25-dihydroxycholecalciferol (31). A reciprocal synthesis of 24,25-dihydroxycholecalciferol and 1α,25-dihydroxycholecalciferol was observed after infusion of parathyroid hormone (PTH), suggesting an inverse regulation of these two enzymes (32).

At the present time, there is only one enzyme (CYP27B1) known to be involved in the 1α-hydroxylation, and one enzyme (CYP24A1) in the 24-hydroxylation of 25(OH)D. However, there are at least six CYP enzymes which have been implicated in the synthesis of 25(OH)D (33): CYP2C11, CYP27A1, CYP3A4, CYP2J3, CYP2D25 and CYP2R1. The inclusion of CYP2R1 as a 25-OHase did not occur until 2003 when Russell and co-workers screened a cDNA library made from hepatic mRNA of mice deficient in the gene encoding the mitochondrial CYP27A1 using a VDR-based ligand activation assay (16). Later, they confirmed that a mutation of *CYP2R1* gene in an individual caused vitamin D-deficient rickets (17). Using a cell-free reconstituted enzyme system, Sakaki and co-workers demonstrated that CYP2R1 was able to hydroxylate both vitamin D_3 and vitamin D_2 to their corresponding 25-hydroxylated metabolites (34). However, this is not the case for CYP27A1. This enzyme can hydroxylate vitamin D_3 to $25(OH)D_3$, but cannot hydroxylate vitamin D_2 to $25(OH)D_2$. Instead, the enzyme produces the C-24 or C-27 hydroxylated metabolite (Figure 1). In addition, Sakaki *et al*. demonstrated that CYP24A1 is able to convert $1\alpha,25(OH)_2D_3$ to calcitroic acid by a six-step monooxygenation including C-24 hydroxylation as the first step of $1\alpha,25(OH)_2D_3$ catabolism (35). A similar CYP24A1-dependent C-24 hydroxylation of $1\alpha,25(OH)_2D_2$ has been observed as the first step of the $1\alpha,25(OH)_2D_2$ degradative pathway (Figure 1) (36).

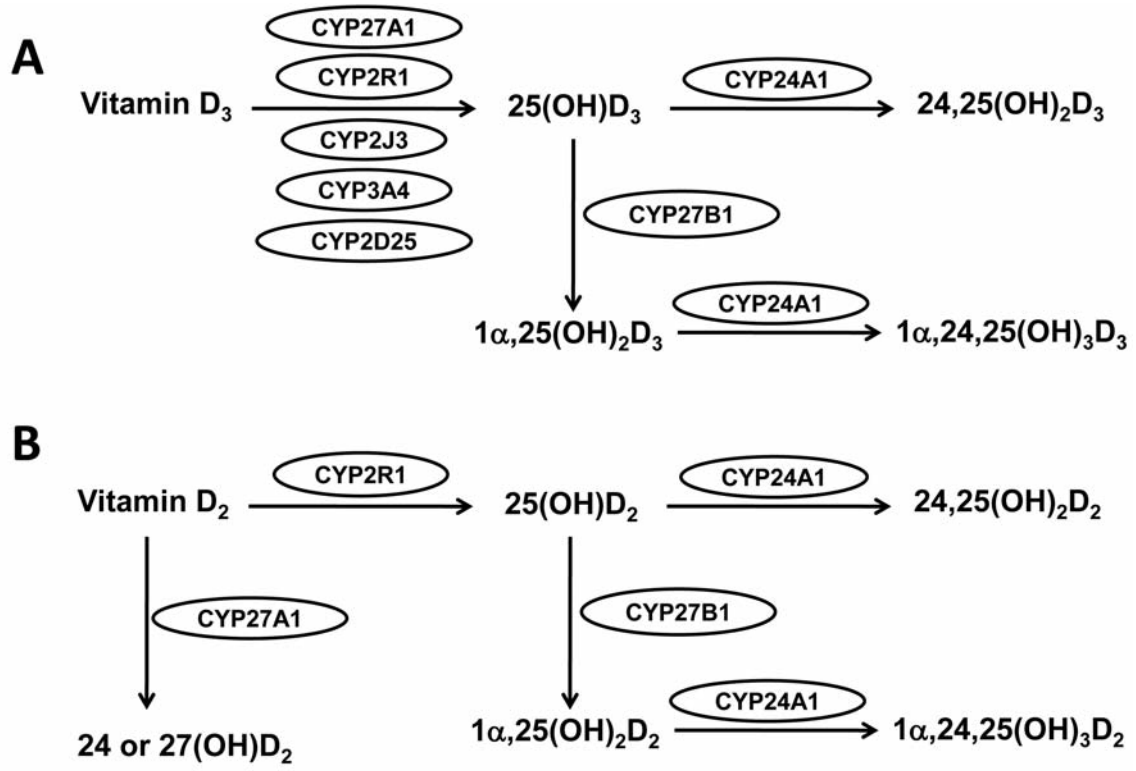

Figure 1. *Metabolism of vitamin D_3 (A) and D_2 (B) involving different cytochrome P450 (CYP) enzymes.*

Study of 25-Hydroxylases in Prostate Cells

The demonstration of 25-OHase in prostate cells was accomplished by (i) three functional assays, (ii) direct determination of the presence of 1α,25(OH)$_2D_3$ after the addition of vitamin D_3 to prostate cells, and (iii) determination of the expression of *CYP2R1* and *CYP27A1* mRNA in prostate cells (37).

There are two genes in prostate cells which are highly responsive to the stimulation by 1α,25(OH)$_2D_3$. They are *CYP24A1* (or 24-OHase) and insulin-like growth factor-binding protein 3 (IGF-BP3). When prostate cell cultures were treated with vitamin D_3, the effects on these two genes would only occur when 25(OH)D_3 was first synthesized from vitamin D_3 through 25-OHase catalysis prior to the synthesis of 1α,25(OH)$_2D_3$. Using this approach, it was shown that vitamin D_3 caused a dose-dependent up-regulation of these two genes in PZ-HPV-7 prostate cells (37). Similarly, the ability of vitamin D_3 to inhibit [^{3}H]-thymidine incorporation into prostate cells as an index of antiproliferation was used to demonstrate the presence of 25-OHase activity. As shown in Figure 2, vitamin D_3 at 10^{-6} M caused about 40% inhibition of [^{3}H]-thymidine incorporation into DNA. The presence of 1α,25(OH)$_2D_3$ was also confirmed by thymus receptor binding assay (Figure 3). Most interestingly, when the expression of three human-related 25-OHases was examined in human normal prostate and liver tissues, and in prostate cancer cell lines, CYP2R1 was found to be more prominently expressed in prostate tissue and cell lines than in liver tissue. Very little CYP27A1 or CYP3A4 was expressed in normal prostate tissue and cell lines, whereas they were highly expressed in liver tissue. Therefore, the results suggest that CYP2R1 is more likely the 25-OHase responsible for the hydroxylation of vitamin D_3 to 25(OH)D_3 in the prostate (37). Very little is known about the regulation of CYP2R1, except that it can be down-regulated by 1α,25(OH)$_2D_3$ (38). It would be interesting to see whether the promoter of CYP2R1 gene has negative vitamin D response element (VDRE).

Study of 1α-Hydroxylase in Prostate Cells

The autocrine/paracrine action of vitamin D in prostate cells (39, 40) was proposed after the demonstration of 1α-hydroxylase activity in certain prostate cells (41), which was in agreement with the expression of *CYP27B1* mRNA as analyzed by real-time quantitative polymerase chain reaction (qPCR), and promoter activity in different prostate cell lines.

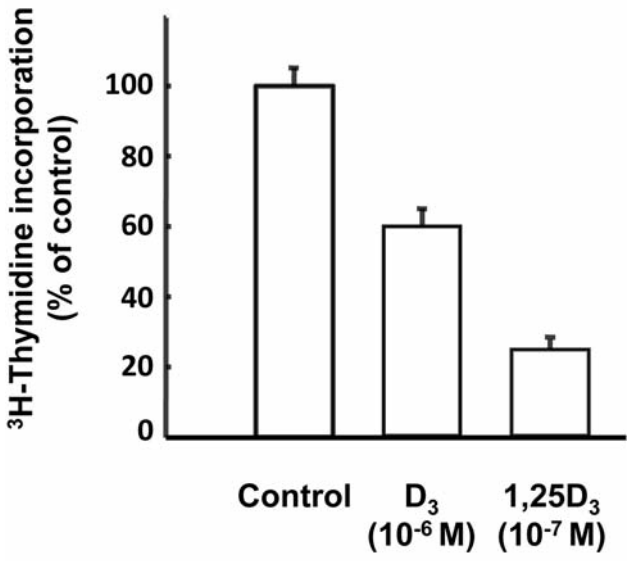

Figure 2. *Inhibition of cell proliferation in the presence of vitamin D_3 and 1α,25(OH)$_2$D$_3$ in PZ-HPV-7 prostate cells. Vitamin D_3 (D_3) at 10^{-6} M inhibited [^{3}H]-thymidine incorporation by 40%, whereas an inhibition of 80% was observed with 1α,25(OH)$_2$D$_3$ (1,25D$_3$) at 10^{-7} M. Cell culture and [^{3}H]-thymidine incorporation into DNA were performed as described elsewhere (37). The data represent the means±SD of 6-8 determinations.*

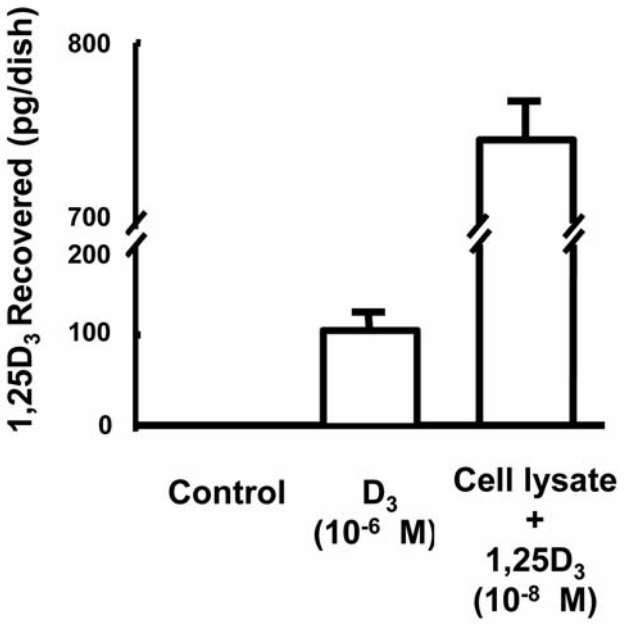

Figure 3. *The synthesis of 1α,25(OH)$_2$D$_3$ from vitamin D_3 in prostate cells. PZ-HPV-7 prostate cells were incubated with vitamin D_3 (D_3) at 10^{-6}M as described elsewhere (37). The concentration of 1α,25(OH)$_2$D$_3$ (1,25D$_3$) produced was measured by the thymus receptor binding assay as described (37). Cell lysate spiked with 10^{-8} M 1α,25(OH)$_2$D$_3$ was used as a positive control for the assay. The data represent the means±SD of 3 separate dishes.*

No or very little 1α-OHase activity and mRNA expression were found in LNCaP cells, which supports the data showing that LNCaP cells were not responsive to the addition of 25(OH)D$_3$ (42, 43) because the cells could not convert 25(OH)D$_3$ to 1α,25(OH)$_2$D$_3$. Transfection of LNCaP cells with *CYP27B1* cDNA restored their responsiveness to 25(OH)D$_3$. Unlike *CYP27B1* in the kidneys (44), prostate *CYP27B1* was not regulated by PTH or calcium (45). However, the enzyme was down-regulated by its own product, 1α,25(OH)$_2$D$_3$, at the promoter and enzyme activity levels (46). Moreover, it was shown that epidermal growth factor (EGF) up-regulated *CYP27B1* at both the transcriptional and translational levels as evident from the luciferase promoter assay, real-time quantitative RT-PCR analysis and enzyme activity measurement using high performance liquid chromatography. The EGF-dependent up-regulation of the promoter activity is likely mediated through the mitogen-activated-protein-kinase (MAPK) pathway as the activity was inhibited by MAPK kinase inhibitor, PD98059 (46). Preliminary data using the Chip assay indicate that EGF/EGFR complex may directly bind to the promoter of *CYP27B1* in PZ-HPV7 cells. Overall, the data suggest that EGF may play an important role in the development of prostate cancer (Figure 4), and CYP27B1 is likely a tumor suppressor in the prostate (47).

Study of 24-Hydroxylation by CYP24A1 in Prostate Cells

The enzyme, CYP24A1, is known to be expressed in many tissues, including the prostate (48-51). This enzyme is responsible for the degradation of 1α,25(OH)$_2$D$_3$ through a six-step monooxygenation pathway (35), leading to the formation of water-soluble calcitroic acid which is excreted into the urine (52). Therefore, one mechanism to enhance the biological activity of vitamin D analogs is to make them resistant to hydroxylation by CYP24A1. Several structural modifications of the 1α,25(OH)$_2$D$_3$ molecule have been accomplished to achieve this goal. For example, ED-71, a well-studied 1α,25(OH)$_2$D$_3$ analog with an addition of 3-hydroxypropoxy group attached to the C-2 position of the 1α,25(OH)$_2$D$_3$ molecule in β-configuration, is a poor substrate for CYP24A1 (Dr. Noboru Kubodera, personal communication). Likewise, O2C3, the C-2 epimer of ED-71, is also resistant to CYP24A1 hydroxylation as determined by a cell-free reconstituted enzyme system (53). We have studied a list of 19-nor-1α,25(OH)$_2$D$_3$ analogs with a modification at the C-2 of this molecule (54, 55). We found that one of these compounds, 19-nor-2α-(3-hydroxypropyl)-1α,25(OH)$_2$D$_3$ (MART-10), was 500-1000 times more active in inhibiting prostate cell proliferation and about 300-500 times less susceptible to

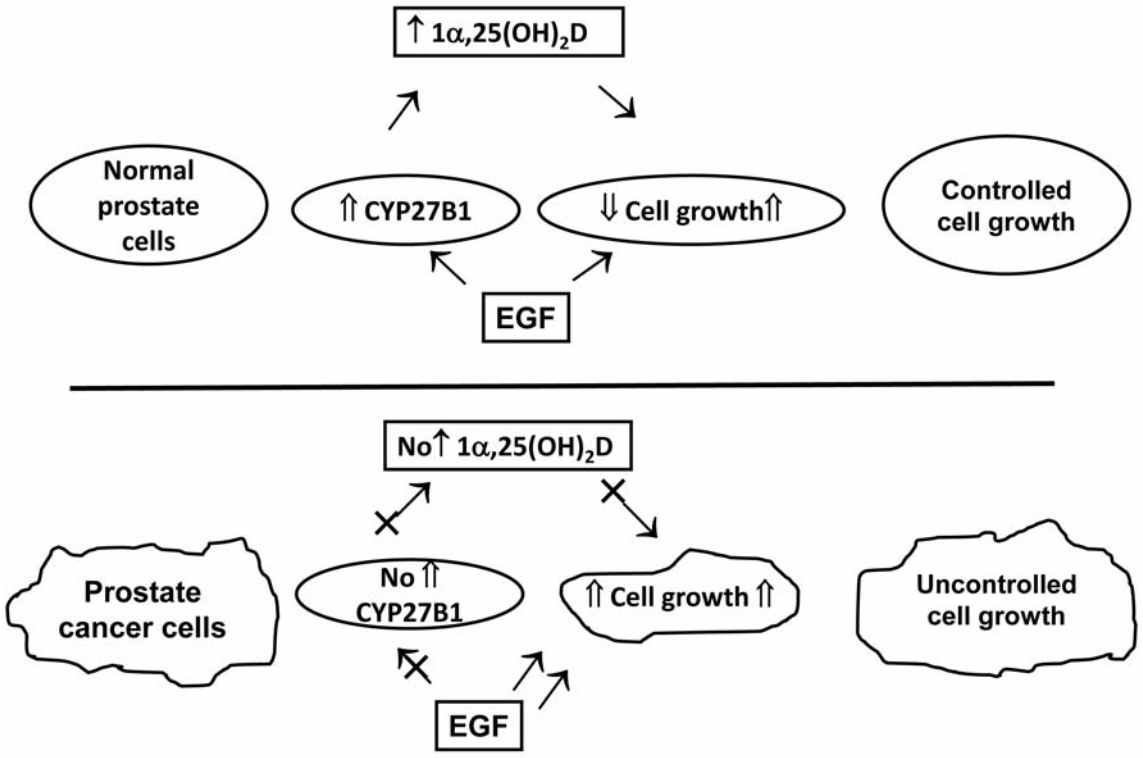

Figure 4. *Interactions among epidermal growth factor (EGF), CYP27B1 and prostate cell growth. An up-regulation of CYP27B1 by EGF is hypothesized to be responsible for the normal growth of prostate cells (upper panel), whereas dysregulation of CYP27B1 by EGF may cause uncontrollable prostate cell growth (lower panel).*

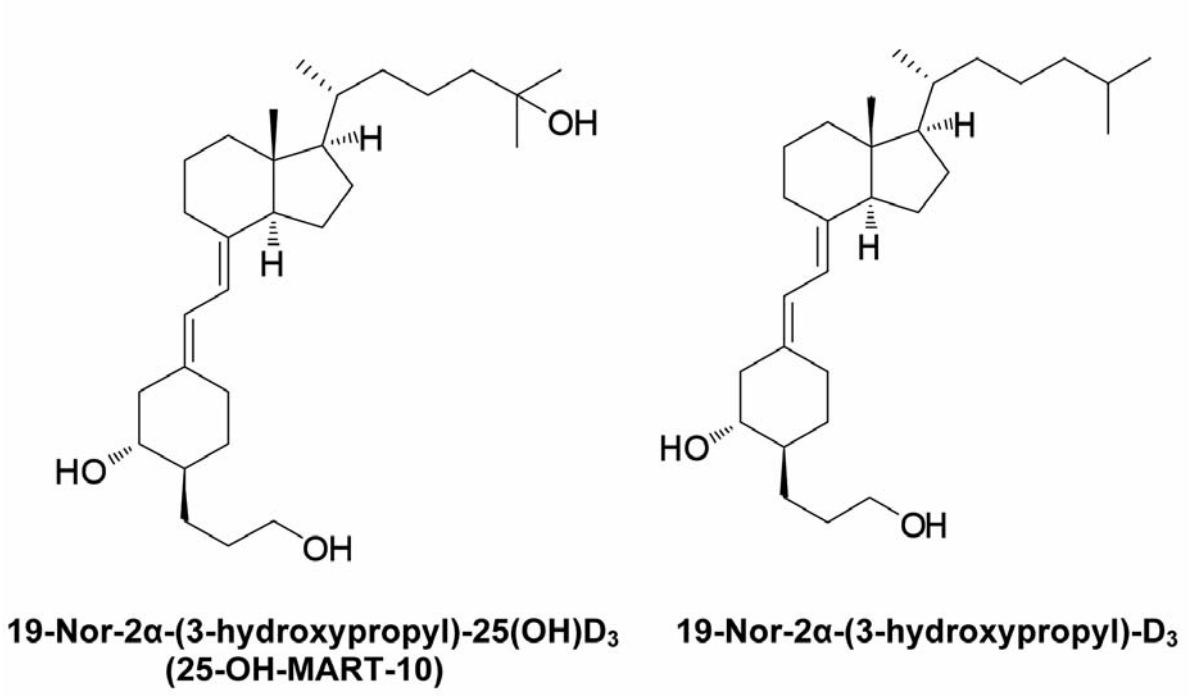

Figure 5. *Chemical structures of 19-nor-2α-(3-hydroxypropyl)-D_3 and (3-hydroxypropyl)-25(OH)D_3.*

CYP24A1 degradation than 1α,25(OH)$_2$D$_3$ (51, 53). To study the docking of MART-10 into CYP24A1, we generated a human CYP24A1 substrate binding site based on the published crystal structure of rat CYP24A1 (14). Using this model, we have found that the A-ring of MART-10 is positioned over the heme group and the 3-hydroxypropyl group on the A-ring is located on the groove of I-helix kink forming hydrogen bonds with the backbone of L325 and E329 and blocking the groove. Consequently, the side-chain of MART-10 is far away from the heme group and is unavailable for 24-hydroxylation, suggesting that MART-10 will have a longer half-life in the prostate. Thus, the results further suggest that analogs with modification at the C-2 position, such as MART-10, could be developed for the treatment of prostate cancer due to their longer bioavailability and greater potency (56).

25(OH)D Can Be Active without 1α-Hydroxylation

After the discovery of 25(OH)D$_3$ in 1968, Olson and DeLuca used isolated small intestine loop to evaluate the biological activity of 25(OH)D$_3$ and reported that 25(OH)D$_3$ was capable of enhancing calcium absorption from the lumen. They concluded that 25(OH)D$_3$ was the metabolically active form of vitamin D$_3$ and had direct effect on calcium transport (57). The direct effect of 25(OH)D$_3$ was observed three decades later by Ritter *et al*. who used the CYP450 inhibitor clotrimazole to block the conversion of 25(OH)D$_3$ to 1α,25(OH)$_2$D$_3$ in bovine parathyroid cells (bPTC). They reported that the blockage did not prevent PTH secretion in the presence of 25(OH)D$_3$ (58). The direct effect caused by 25(OH)D$_3$ in the bPTCs was subsequently shown to be VDR dependent (59). Along this line, Lou *et al*. demonstrated the antiproliferative action of 25(OH)D$_3$ on human MCF-7 breast cancer cells and in the primary cultures of kidney, skin and prostate cells prepared from Cyp27b1 knock-out mice (60). The authors reported that the action induced by 25(OH)D$_3$ was dependent on VDR, and 25(OH)D$_3$ had an identical binding mode to 1α,25(OH)$_2$D$_3$ in these cells. Furthermore, a synergistic effect of 25(OH)D$_3$ with 1α,25(OH)$_2$D$_3$ in *Cyp27b1*$^{-/-}$ cells was observed. The authors suggest that a synergism between 25(OH)D$_3$ and 1α,25(OH)$_2$D$_3$ might be physiologically important. Similarly, DeLuca *et al*. (61) used the VDR knockout model to evaluate the hypercalcemic toxicity induced by high doses of vitamin D$_3$ and 25(OH)D$_3$. The authors demonstrated that high concentrations of 25(OH)D$_3$ were able to bind to VDR and induce gene transcription in the *Cyp27B1*$^{-/-}$ mice. Since no 1α,25(OH)$_2$D$_3$ was detected in the serum of these *Cyp27B1*$^{-/-}$ mice, they concluded that 25(OH)D$_3$, not 1α,25(OH)$_2$D$_3$, was likely responsible for the toxicity of vitamin D excess. Using a different approach, recently Munetsuna *et al*. (62) also demonstrated that 1α-hydroxylation of 25-hydroxy-19-nor-vitamin D$_3$ was not required for its biological activity in PZ-HPV-7 prostate cells. Overall, the results from these five studies suggest that less calcemic vitamin D$_3$ and 25(OH)D$_3$ analogs could be useful for treating cancer, including prostate cancer.

Summary and Conclusion

In this brief review, we primarily presented the data obtained from our laboratories regarding the expresion of three cytochrome P450 enzymes, CYP27B1, CYP2R1, and CYP24A1, in the prostate, and their roles in the activation and inactivation of vitamin D$_3$ in prostate cells. We propose that a dysregulation of CYP27B1 expression by EGF in prostate cells may play a role in the development of prostate cancer. In addition, because prostate cells are capable of synthesizing 25(OH)D$_3$ and 1α,25(OH)$_2$D$_3$ from vitamin D$_3$, not only the analogs of 1α,25(OH)$_2$D$_3$, such as MART-10 which is resistant to CYP24A1 hydroxylation, but also the analogs of vitamin D$_3$ and 25(OH)D$_3$, such as 19-nor-2α-(3-hydroxypropyl)-D$_3$ and 19-nor-2a-(3-hydroxypropyl)-25(OH)D$_3$ (Figure 5), could be developed for the treatment of prostate cancer.

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Received August 3, 2011
Revised September 21, 2011
Accepted September 22, 2011