The Function of Vitamin D Receptor in Vitamin D Action

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Vitamin D has roles in a variety of biological actions such as calcium homeostasis, cell proliferation and cell differentiation to many target tissues. Most of these biological actions of vitamin D are now considered to be exerted through the nuclear vitamin D receptor (VDR)-mediated control of target genes. VDR belongs to the nuclear hormone receptor superfamily and acts as a ligand-inducible transcription factor. For the ligand-induced transactivation of VDR, coactivator complexes have recently been shown to be essential. The function of VDR as a ligand-induced transcription factor is overviewed, and the phenotype of VDR gene knock-out mice and the VDR-mediated transcriptional and negative regulation of the key enzyme in vitamin D biosynthesis are also described, based mainly on our recent findings, to gain a better understanding of the function of VDR in the transcriptional control of vitamin D target genes.

Key words: nuclear receptor, VDR KO mice, vitamin D, vitamin D 1α-hydroxylase, transcription factor.

1. Introduction

Vitamin D has roles in a variety of biological actions such as calcium homeostasis, cell proliferation, and cell differentiation to many target tissues. Especially in calcium homeostasis and bone metabolism, vitamin D has long been believed to be a prime regulatory factor (1, 2), since it is well known that vitamin D deficiency causes rickets with growth retardation, impaired bone formation, and hypocalcemia. Rickets is caused by a dietary vitamin D deficiency, and is also a hereditary disease. Up until now, hereditary rickets has been classified into three types. The genes responsible for vitamin D-dependent rickets type II and X-linked hypophosphatemic vitamin D-resistant rickets (HYD) have been identified as the VDR gene (3) and the PEX gene (4), respectively. Recently, we found that the third kind of hereditary rickets, the vitamin D-dependent rickets type I, is caused by genetic mutations in the gene for 25-hydroxyvitamin D, 1α-hydroxylase [1α(OH)ase] (5, 6), as described below (Fig. 1).

Most of the biological actions of vitamin D are now thought to be exerted through the nuclear vitamin D receptor (VDR)-mediated control of target genes. VDR belongs to the nuclear hormone receptor superfamily and acts as a ligand-inducible transcription factor (7). This superfamily comprises more than 60 nuclear receptors for lipophilic ligands such as steroid/thyroid hormones, vitamin A and vitamin D. In this review, the function of VDR is described based mainly on our findings and those of others concerning transcriptional control.

2. Biosynthesis of vitamin D

The precursor of vitamin D, 7-dehydrocholesterol, is biosynthesized from cholesterol, then converted to vitamin D₃ by UV light on the skin. Vitamin D is also ingested in the diet as Vitamin D₂ (ergocalciferol), mainly from plants, and vitamin D₃ (cholecalciferol) from animals (8, 9). A hormonal form of vitamin D, 1α,25(OH)₂D₃, is metabolically formed through two hydroxylation steps at the final stage (Fig. 1) (see references in Refs. 5 and 6). First, vitamin D is hydroxylated in the liver to 25-dihydroxyvitamin D₃ [25(OH)D₃], which is subsequently hydroxylated in the kidney to 1α,25(OH)₂D₃. For the metabolic inactivation of 25(OH)D₃ or 1α,25(OH)₂D₃, the 24-hydroxylation to form 24,25-(OH)₂D₃ or 1α,24,25(OH)₃D₃ is the first step in the degradation of vitamin D. The serum level of 1α,25(OH)₂D₃ is held constant in the normal state, and is strictly regulated in response to factors controlling calcium homeostasis. The regulation of 1α,25(OH)₂D₃ and 24,25(OH)₂D₃ production by these factors is conducted by altering the activities of the enzymes that hydroxylate vitamin D derivatives. Vitamin D₃-25-hydroxylase (CYP27) catalyzes hepatic 25-hydroxylation, and renal 1α-hydroxylation is catalyzed by 25-hydroxyvitamin D₃ 1α-hydroxylase [1α(OH)ase]. The first step in the metabolic inactivation of vitamin D metabolites by 24-hydroxylation is catalyzed by 25(OH)D₃-24-hydroxylase (CYP24) (6).

3. Molecular mechanism of transcriptional control by the vitamin D receptor

The hormonal form of vitamin D, 1α,25-dihydroxyvitamin D₃ [1α,25(OH)₂D₃], acts as a ligand for the vitamin D receptor (VDR), and the liganded VDR activates target gene expression at the transcriptional level (Fig. 2) (10, 11). VDR forms homodimers or heterodimers with one of three retinoid X receptors (RXRα, RXRβ, RXRγ). The VDR homodimer or VDR-RXR heterodimer binds to specific enhancer elements, referred to as vitamin D response elements.
Rickets related to vitamin D

Vitamin D3 → Vitamin D deficiency

 Liver → 25(OH)ase → 25(OH)D3

 Kidney → 1α(OH)ase → VDDR I

 24(OH)ase → 24,25(OH)2D3

 [inactive form] → 1α,25(OH)2D3

 [active form] → target tissue → VDR

 Calcium homeostasis etc. → Hypocalcemia

Rickets

Fig. 1. Vitamin D-related rickets. The biosynthetic pathway of 1α,25(OH)2D3, and the mode of 1α,25(OH)2D3 action are illustrated. Defects in these processes cause rickets. Nutritional vitamin D deficiency and the defect in renal 1α(OH)ase activity by genetic mutations (VDDRII patients) result in a short supply of vitamin D. The mutated VDR in VDDRII patients is unable to respond to 1α,25(OH)2D3, resulting in rickets. The precursor of vitamin D, 7-dehydrocholesterol, is biosynthesized from cholesterol, then converted into vitamin D by UV light on the skin. Vitamin D is also ingested from the diet as vitamin D3 (cholecalciferol) and vitamin D2 (ergocalciferol) from plants and vitamin D3 (7-dehydrocholesterol) from animals. A hormonal form of vitamin D that acts as a ligand for VDR, 1α,25(OH)2D3, is formed metabolically through two hydroxylation steps at the final stage. First, vitamin D is hydroxylated in the liver to 25-hydroxyvitamin D3, 1α,25(OH)2D3, by the vitamin D3-25-hydroxylase (CYP27). Subsequently, 25-hydroxyvitamin D3 is hydroxylated (1α,25(OH)ase) in the kidney to undergoes conversion into 1α,25(OH)2D3.

(VDREs), for 1α,25(OH)2D3-induced transactivation (12). For ligand-induced transactivation by VDR, coactivators that interact with VDR in a ligand-dependent way have recently been shown to be essential for the formation of the initial transcription complex with RNA polymerase II (Figs. 2 and 3) (13). They include the SRC-1/TIF2 160 kDa protein family, CBP/p300 protein family, SRA (a RNA coactivator), and others (see references in Refs. 13–16). Most interestingly, these coactivators themselves are histone acetylases (HATs) that modulate chromatin structure to activate gene expression (17, 18). These coactivators are speculated to form a complex. More recently, another coactivator complex has been identified as the DRIP/TRAP complex, which has no HAT activity (13, 19, 20). In contrast to coactivators, corepressors, SMRT and NCoR, have been shown to associate with ligand-unbound thyroid receptor (TR) and the all-trans retinoic acid receptor (RAR) to repress their ligand-induced transactivation functions (21, 22) (Fig. 3). However, these corepressors appear not to interact with ligand-unbound VDR (10).

4. Physiological function of VDR in intact animals—Lessons from Vitamin D receptor knock-out mice

Although the function of VDR has been studied intensively in cell culture systems (10, 11), it was unclear whether such findings reflect the function of VDR in intact animals. Especially, the actions of 1α,25(OH)2D3, in bone formation and metabolism have been well described in adult animals, however, the physiological role of VDR in target tissues during development and in intact animals had not yet been established. Moreover, an animal model of vitamin D-dependent rickets type II patients was absent (23).

We, therefore, generated mice deficient in VDR by gene targeting in order to investigate the function of VDR in vivo (24). Like vitamin D, vitamin A has been shown to play a critical role in bone formation and development, especially in skeletal formation during embryogenesis (25). In fact, the inactivation of one (RARγ) of six vitamin A nuclear receptors results in tracheal cartilage malformation and homeotic transformations along the rostral axial skeleton during embryogenesis. However, no bone malformations or overt phenotypic abnormalities were seen in the VDRα heterozygotes (data not shown). Unexpectedly, VDR null mutant mice do not differ from their heterozygous or wild-type littermates in growth rate or behavior, and seem functionally normal after birth until weaning. However, after weaning (about 3 weeks), the VDR null mutant mice display typical features of rickets such as growth retardation and impaired bone formation, and most of them die by 15 weeks due to unknown reasons. However, no overt abnormalities are found in the heterozygotes even at 6 months, in good agreement with type II rickets as a recessive trait in humans (23). By 7 weeks, all of the VDR null mutant mice develop alopecia and poor whiskers as typical features of rickets, and most of them display a flat face with a shorter nose. For null mutant mice between 7 and 13 weeks of age, no apparent abnormalities are found by histological analysis in VDR-expressing tissues other than bone and skin, including intestine, kidney, brain, and spleen, although these tissues are considered to be direct target tissues for the actions of vitamin D (1, 2).

Observations in VDR null mutant mice by us (24) and other (26) are similar to those of a human hereditary, recessive disease, vitamin D dependency type II, in which mutations in the VDR gene have been identified in several families (3), although, unlike in VDR KO mice, this disease is not lethal. As these patients exhibit rickets with hypocalcemia, hypophosphatemia, and elevated serum levels of alkaline phosphatase (ALP), four week-old VDR-KO mice show reduced serum levels of calcium and phosphorous with a markedly elevated serum ALP activity. Radiographic analysis of the skeletal tissues of VDR null mutant mice reveal a loss of bone density. Typical features of rickets are observed in gross appearance and on X-ray analysis of tibia and fibula, including widening of epiphyseal growth plates, thinning of the cortex, and fraying, cupping and widening of the metaphysis. In addition, orderly columns of hypertrophic chondrocytes are lost and the cartilage layers are widened with inadequate mineralization. In cancellous bone adjacent to the growth plates, marked increases in the extent and width of the osteoid seams are noted, and the bone surfaces are surrounded by numerous osteoblastic cells. Most strikingly, the numbers of
osteoclasts do not appear to be affected by VDR inactivation, although it has been well established in in vitro cell culture systems that vitamin D is a most potent inducer of osteoclast differentiation from precursor cells in the spleen (27, 28). Moreover, when the VDR KO mice were fed a high calcium and phosphate diet to rescue the lowered levels of serum minerals, the impaired mineralization was recovered except in the affected cartilage (Yagishita et al., unpublished results). These findings in the bones of VDR KO mice reveal that the direct target of vitamin D action in bone is the chondrocyte, and that the mineralization to form bone is an indirect effect of vitamin D mediated through serum minerals (Fig. 4).

Together, these findings in VDR KO mice establish that most vitamin D actions known to date are mediated through VDR, and that the vitamin D-VDR system is essential only after weaning.

5. 25-Hydroxyvitamin D, lα-hydroxylase [lα(OH)ase] as a key enzyme in vitamin D synthesis

The serum level of lα,25(OH)2D3 is strictly regulated in response to calcium requirement in the body. Several enzymes regulated by several factors including lα,25(OH)2D3 are involved in the synthesis and metabolism of lα,25-(OH)2D3. The activities of 25(OH)D lα-hydroxylase and 24-hydroxylase are regulated negatively and positively by lα,25(OH)2D3 (6). In VDR null mutant mice, a marked increase in serum lα,25(OH)2D and a clear reduction in serum 24,25(OH)2D are seen, suggesting an increased activity of lα(OH)ase and reduced activity of 24-hydroxylase (24). Thus, it is clear that the expression of these enzymes is under the negative control of lα,25(OH)2D-bound VDR.

Indeed, based on experiments using VDR KO mice, we provided the first report of the cloning of the cDNA encoding mouse 25(OH)D lα-hydroxylase by a newly developed expression cloning method (29). This method is based on the principle that only lα,25(OH)2D, not any precursors or metabolites, can activate the transactivation function of VDR by direct binding. Therefore, when 25(OH)D3 is added to the medium, VDR is activated only in cells expressing lα(OH)ase. At the same time as our mouse cDNA cloning in 1997, several groups using RT-PCR methods reported...
the cloning of cDNAs encoding rat and human 1α(OH)ase, confirming the previously described biochemical characterization of 1α(OH)ase as a cytochrome p450 (30, 31). The predicted amino acid sequences revealed that 1α(OH)ase proteins harbor a mitochondrial target signal and two conserved regions (the sterol-binding domain and the heme-binding domain), and show significant homology throughout the entire amino acid sequence with the p450 enzymes. Mouse 1αOHase exhibits the greatest homologies to the vitamin D hydroxylases: 41.7% homology to rat vitamin D3-25-hydroxylase (CYP27), and 31.6% homology to the mouse 25(OH)D3-24-hydroxylase (CYP24). The organization of the human 1αOHase gene comprises nine exons extending over 5 kbp (5), and resembles other p450 enzymes that hydroxylate steroids.

From clinical studies, genetic defects in the enzymes responsible for the biosynthesis of 1α,25(OH)2D3 are considered to evoke vitamin D deficiency. A group of hereditary rickets patients exhibits low serum levels of 1α,25(OH)2D3, while 25(OH)D3 levels are normal or high, and these patients recover with only supplementation of physiological doses of 1α,25(OH)2D3 or 1α(OH)D3. This autosomal recessive disease is referred to as pseudovitamin D-deficient rickets (PDDR), also known as vitamin D-dependency type 1 (VDDR1) (32). As mutations in the 1αOHase gene resulting in the loss of its enzymatic activity were thought to be a cause of hereditary rickets, we tested this idea using cloned human 1αOHase cDNA. FISH analysis showed that this gene lies on chromosome 12q13.3. Interestingly, this locus closely matches the chromosomal localization of a putative gene that had been mapped as responsible for type I rickets by linkage analysis of a group of Canadian patients (33). Furthermore, we found that distinct homozygous missense mutations in the human 1αOHase gene that abolish 1αOHase activity are present four different Japanese type I rickets patients (5), and, more recently, we further identified hetero-compound type mutations in other patients (34). Interestingly, mutation sites that cause a complete loss of enzymatic activity were found widely over the entire region of the 1αOHase protein (5, 30, 34, 35), although the regions essential for the enzymatic activity have been identified by a biochemical approach (36, 37). Thus, these observations establish that an inactive genetic mutation of human 1αOHase causes type I hereditary rickets, and clearly indicate that 1αOHase is a critical enzyme in vitamin D biosynthesis.

6. Negative regulation of 1α(OH)ase gene expression by VDR

The activity of 1αOHase was first identified in kidney homogenates, and kidney was thought to be the sole tissue expressing 1αOHase, and the proximal convoluted tubule cells were identified as its location in kidney (38). Northern blot analysis using the cloned cDNA demonstrated that this gene is expressed abundantly in kidney, while expression was almost undetectable in extra-renal tissues of mice and humans. Quantitative RT-PCR analysis suggests that the 1αOHase gene is expressed in many extrarenal tissues at very low levels (39). As 1α,25(OH)2D3 plays a primary role in calcium homeostasis, the renal activity of 1αOHase is positively regulated by calcitropic hormones, responding to serum calcium levels (40). 1α,25(OH)2D3 has...
Vitamin D Receptor as a Transcription Factor

been well characterized as a negative regulator of the renal activity of 1α,25(OH)2D3 (41). Our study using VDR knock-out mice showed that 1α,25(OH)2D3 acts at the transcriptional level, and this negative regulation requires the liganded-VDR, since the gene expression of mouse la(OH)ase is VDRE in the human la(OH)ase gene promoter. The previously reported nVDREs are shown except 1α,25(OH)2D3 nVDRE, which has not yet been delineated to a core nVDRE motif.

<table>
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<th>nVDRE gene</th>
<th>response element</th>
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<td>Avian PTH</td>
<td>GGCTCA GGA GGCTTG</td>
</tr>
<tr>
<td>Human PTH</td>
<td>GGCTCA GAG CACAGA</td>
</tr>
<tr>
<td>Mouse osteocalcin</td>
<td>GGCCA ATG AGGACA</td>
</tr>
<tr>
<td>Rat bone sialoprotein</td>
<td>AGGGTT TAT AGGCTA</td>
</tr>
<tr>
<td>PKA inhibitor</td>
<td>AAGTTG CTG AGGCTA</td>
</tr>
<tr>
<td>Rat PTHrP-proximal</td>
<td>AGGTTA CTC AGTGA</td>
</tr>
<tr>
<td>Rat PTHrP-distal</td>
<td>GGCTGG AGA GGCTG</td>
</tr>
<tr>
<td>human vitamin D</td>
<td>ccattaaccccac ttggtgtc</td>
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### REFERENCES


### TABLE I. DNA sequences of negative vitamin D response elements (nVDREs)

<table>
<thead>
<tr>
<th>nVDRE gene</th>
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<tr>
<td>human vitamin D</td>
<td>ccattaaccccac ttggtgtc</td>
</tr>
</tbody>
</table>


28. Takahashi, N., Udagawa, N., Akatsu, T. et al. (1991) Deficiency of osteoclasts in osteopetrotic mice is due to a defect in the local microenvironment provided by osteoblastic cells. Endocrinology 126, 1792-1796


