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Review

The vitamin D receptor functions as a transcription regulator in the absence of 1,25-dihydroxyvitamin D_3

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ABSTRACT

The vitamin D receptor (VDR) is a critical mediator of the biological actions of 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$). As a nuclear receptor, ligand activation of the VDR leads to the protein's binding to specific sites on the genome that results in the modulation of target gene expression. The VDR is also known to play a role in the hair cycle, an action that appears to be $1,25(OH)_2D_3$-independent. Indeed, in the absence of the VDR as in hereditary 1,25-dihydroxyvitamin D resistant rickets (HVDRR) both skin defects and alopecia emerge. Recently, we generated a mouse model of HVDRR without alopecia wherein a mutant human VDR lacking $1,25(OH)_2D_3$-binding activity was expressed in the absence of endogenous mouse VDR. While $1,25(OH)_2D_3$ failed to induce gene expression in these mice, resulting in an extensive skeletal phenotype, the receptor was capable of restoring normal hair cycling. We also noted a level of secondary hyperparathyroidism that was much higher than that seen in the VDR null mouse and was associated with an exaggerated bone phenotype as well. This suggested that the VDR might play a role in parathyroid hormone (PTH) regulation independent of $1,25(OH)_2D_3$. To evaluate this hypothesis further, we contrasted PTH levels in the HVDRR mouse model with those seen in *Cyp27b1* null mice where the VDR was present but the hormone was absent. The data revealed that PTH was indeed higher in *Cyp27b1* null mice compared to VDR null mice. To evaluate the mechanism of action underlying such a hypothesis, we measured the expression levels of a number of VDR target genes in the duodena of wildtype mice and in transgenic mice expressing either normal or hormone-binding deficient mutant VDRs. We also compared expression levels of these genes between VDR null mice and *Cyp27b1* null mice. In a subset of cases, the expression of VDR target genes was lower in mice containing the VDR as opposed to mice that did not. We suggest that the VDR may function as a selective suppressor/de-repressor of gene expression in the absence of $1,25(OH)_2D_3$.

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Abbreviations: VDR, vitamin D receptor; $1,25(OH)_2D_3$, 1,25-dihydroxyvitamin D_3; PTG, parathyroid gland; PTH, parathyroid hormone; RXR, retinoid X receptor; VDRE, vitamin D response element; ChIP-seq, ChIP-sequencing; RNA-seq, RNA-sequencing; HVDRR, hereditary 1,25-dihydroxyvitamin D resistant rickets; VDDR-1, vitamin D dependency rickets type 1; BAC, bacterial artificial chromosome; qPCR, quantitative polymerase chain reaction.

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1. Introduction

The vitamin D receptor (VDR) is a nuclear factor which mediates the biological actions of 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$). One of the prominent activities of this hormone is to maintain mineral homeostasis in higher vertebrates via the regulation of gene expression by the VDR in intestine, kidney, bone and parathyroid gland (PTG) [1]. Parathyroid hormone (PTH), a calciotropic regulator secreted from the PTG, directly influences $1,25(OH)_2D_3$ target tissues such as kidney and bone but not the intestine. Indeed, PTH profoundly upregulates the expression of renal *Cyp27b1* to increase the production of $1,25(OH)_2D_3$ which in turn activates the VDR [2]. $1,25(OH)_2D_3$-bound VDR modulates the expression of a network of genes to raise calcium absorption in intestine [3] while influencing bone cell differentiation in conjunction with PTH to affect bone remodeling [4]. Increased levels of $1,25(OH)_2D_3$ also exert a suppressive negative feedback loop in the PTG to reduce the production of PTH, although the molecular mechanism of such action remains unclear [5]. In kidney, ligand-activated VDR regulates target genes not only to repress *Cyp27b1* gene expression to control $1,25(OH)_2D_3$ production but also to facilitate calcium reabsorption [2].

The action of the VDR is initiated through its interaction with $1,25(OH)_2D_3$, which results in the formation of a heterodimer with retinoid X receptor (RXR), the binding of this heterodimer to vitamin D response element (VDRE) at target genes, and the recruitment of various molecular machines that are capable of altering chromatin structure [6–10]. Our recent unbiased genome-wide studies using ChIP-sequencing (ChIP-seq) analysis coupled to RNA-sequencing (RNA-seq) analysis in bone cells [11] and mouse small intestine [3] suggest that VDR binding sites are enriched for VDREs but also contain adjacent sequences capable of interacting with additional transcription factors that may be involved. These studies have also revealed that $1,25(OH)_2D_3$/VDR target genes are regulated through multiple enhancers located predominantly within introns and intergenic regions but less frequently in regions near target gene promoters.

Since the syndrome of hereditary 1,25-dihydroxyvitamin D resistant rickets (HVDRR) was first identified [12], the underlying involvement of the VDR was suspected. Further biochemical and genetic studies of patient samples and cells not only supported but eventually confirmed this hypothesis [13], prompting the development of a mouse model in which the VDR was similarly mutated [14–17]. Further understanding of the roles of the $1,25(OH)_2D_3$/VDR system were extended through studies of the syndrome of vitamin D dependency rickets type 1 (VDDR-1), a disease caused by mutations in the *CYP27B1* gene that resulted in a failure of $1,25(OH)_2D_3$ production [18], and the development of cognate *Cyp27b1* null mouse models [19–21]. These human syndromes and mouse models in which $1,25(OH)_2D_3$-mediated transcriptional activity of the VDR was compromised share a similar biological phenotype that includes abnormal mineral homeostasis and skeletal defects that include rickets and growth retardation, indicating the importance of $1,25(OH)_2D_3$-dependent activation of the VDR. This transcriptional activity was also suggested to modulate proliferation and differentiation of epidermal keratinocytes in skin as well [22]. However, prompted by selective differences in the presence or absence of alopecia in humans as well as the observation that *Vdr* null but not *Cyp27b1* null mice are alopecic, the concept emerged that the VDR might function in keratinocytes in the absence of $1,25(OH)_2D_3$. This concept was further supported by studies of VDR null mice in which a mutant human VDR lacking $1,25(OH)_2D_3$ binding activity due to a leucine to serine replacement at amino acid 233 (L233S) was expressed specifically in keratinocytes and led to normal hair growth [23].

We recently generated a humanized VDR mouse model using a bacterial artificial chromosome (BAC) that contained either the entire wildtype human *VDR* gene [24] or a mutant version (L233S) as just documented [25]. Although the wildtype VDR fully rescued transcription, mineral homeostasis and skin abnormalities, the mutant VDR rescued only the alopecia, supporting the concept that the control of hair follicle cycling by the VDR is $1,25(OH)_2D_3$-independent. Interestingly, we found that the presence of this mutant form of the VDR also led to an exaggerated phenotype relative to PTH secretion and lower bone mineral density (BMD) than that seen in the VDR null mouse, suggesting the possibility that $1,25(OH)_2D_3$-independent actions of the VDR might extend beyond the skin [25]. In the present report, we provide data suggesting that the VDR expressed both in the mutant humanized mouse models we generated and in the *Cyp27b1* null mouse model may function to suppress/de-repress specific target genes in the absence of $1,25(OH)_2D_3$ not only in skin but in other mouse tissues as well.

2. Materials and methods

2.1. Animal study

VDR null mice [15] and *Cyp27b1* null mice [21] used in this study were obtained from the Jackson Laboratories. Generation of humanized VDR mice ($hVDR^{WT}/VDR^{-/-}$) and mutant humanized VDR mice ($T805/VDR^{-/-}$, $T806/VDR^{-/-}$ and $T807/VDR^{-/-}$) were previously described [24,25]. Wildtype mice were littermates from C57BL/6 mice (Harlan). All mice were fed standard rodent chow diet (5008; Harlan Teklad) after weaning until sacrifice at 8–10 weeks of age. Mice were exposed to a 12-h light-dark cycle and all animal studies were reviewed and approved by the Research Animal Care and Use Committee of University of Wisconsin-Madison.

2.2. Serum PTH measurement

Blood collection and measurement of serum PTH levels were previously described [24].

2.3. Reverse transcription-polymerase chain reaction (RT-PCR)

Preparation of RNAs and cDNAs from tissues were previously described [24]. Quantitative polymerase chain reaction (qPCR) was performed as previously described [25]. TaqMan primers (Applied Biosystems) for qPCR are available upon request.

2.4. Statistical analysis

Student's unpaired t test was used to identify significant differences between groups ($p < 0.05$).

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