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LIGAND INDEPENDENT ACTIONS OF VITAMIN D RECEPTOR

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Summary (142/150 words)

The bioactive form of vitamin D ($1\alpha,25$ -dihydroxyvitamin D_3) ($1,25(OH)_2D_3$) exerts pleiotropic effects, including the control of serum calcium levels and bone integrity, through binding to the Vitamin D Receptor (VDR). VDR also exerts ligand-independent actions to maintain hair follicle homeostasis. The observation that rickets might be more severe in $1,25(OH)_2D_3$ -deficient mice than in VDR-null mice indicated that unliganded VDR is not a silent receptor. Recent data generated in mice expressing $1,25(OH)_2D_3$ -binding deficient VDRs demonstrate that unliganded VDR represses the expression of many genes, and induces skeletal and mineral ion defects that are more severe than those of VDR-null mice, even though only the latter are alopecic. The possibility to restore with vitamin D analogs the transcriptional activity of a point-mutated VDR that cannot bind $1,25(OH)_2D_3$ in mice opens new avenues to develop treatments for patients bearing VDR mutations that impair $1\alpha,25$ -dihydroxyvitamin D_3 binding.

Keywords (5-10): Rickets, calcium homeostasis, repression, nuclear receptor, point-mutation, ligand-binding domain, DNA-binding domain, gemini ligand, VDR.

I) The vitamin D receptor

The Vitamin D receptor (VDR; NR111) is a ligand-dependent transcription factor member of the nuclear receptor superfamily, which mediates the biological function of the vitamin D derivative $1\alpha,25$ -dihydroxyvitamin D_3 [$1,25(OH)_2D_3$ or calcitriol] [1-3]. Like other nuclear receptors, VDR contains 6 functional domains, including a DNA Binding Domain (DBD) formed by two zinc-finger motifs, and a Ligand Binding Domain (LBD) composed of 12 helices [helix 1 (H1) to 12 (H12)] and one β -sheet, that are arranged in a three-layered α -helical sandwich [4,5]. VDR dimerizes with any of the three retinoid X receptor (RXR) isoforms (RXR α , NR2B1; RXR β , NR2B2; and RXR γ , NR2B3), and binds to direct repeats of 2 hexameric core binding motifs (5'-RGKTCA-3'; R = A or G; K = G or T) with 3 intervening nucleotides, termed vitamin D response elements (VDREs), located in enhancer regions of target genes [6,7]. Depending on the cell types, ~1100 to ~13000 VDR binding sites have been identified, and 35 to 95 % of these sites are occupied only after calcitriol treatment (see additional chapters in Section II) [8]. The classical model proposes that unliganded VDR (apo-receptor) interacts with co-repressors (CoR), such as the Nuclear receptor CO-Repressor 1 (NCOR) and Silencing

Mediator for Retinoid and Thyroid hormone receptors (SMRT), resulting in a transcriptionally silent complex, although the evidence for this mode of regulation for the VDR has never been proven (see additional chapters in section II). Ligand binding to VDR induces conformational changes of the LBD, including a rearrangement of key amino acids in H6, H7 and H11, and a reorientation of H12 encompassing the transcriptional Activation Function 2 (AF-2), that release CoRs and promote coactivator (CoA) binding to stimulate target gene transcription (see Chapter “Structural basis for ligands, N. Rochel”)[9-11].

II) Skeletal defects induced by impaired VDR signalling

VDR is expressed in a large number of cell types and tissues [1,3,12]. VDR loss-of-function mutations in the human syndrome termed Hereditary Vitamin D Resistant Rickets (HVDRR) or Vitamin D-Dependent Rickets type-II (VDDR-II), and in genetically modified VDR-null mice exhibit skeletal deformities, osteomalacia, hypocalcemia, hypophosphatemia and secondary hyperparathyroidism (see Chapter 72 “Hereditary 1,25(OH)₂D₃ resistant rickets: VDR mutation, D. Feldman”) [13-16]. Moreover, lack of functional Cyp27b1 (the enzyme that catalyzes the hydroxylation of 25-hydroxyvitamin D to generate calcitriol) induces typical hallmarks of rickets [Pseudovitamin D-Deficiency Rickets (PDRR), also termed Vitamin D-Dependent Rickets type-I (VDDR-I)] (see Chapter 71 “Pseudo-vitamin D deficiency: Cyp27b1 mutations, F. Glorieux”) [16-20]. Thus, calcitriol-mediated VDR activities play a key role in bone and mineral ion homeostasis.

III) Ligand-independent role of VDR in hair cycle

Humans and mice with loss-of-function mutations in the Cyp27b1 gene or those expressing a point mutated VDR that is impaired in 1,25(OH)₂D₃ binding, but not in DNA binding, have a normal hair cycle [17,20-22]. In contrast, patients carrying VDR mutations inducing loss-of-expression [13-15,23] or DNA-binding deficiency [24], as well as VDR-null mice become alopecic (Figure 1). Importantly, hair cycling is restored in VDR-null mice by selective expression in keratinocytes of a wild-type VDR or a ligand-binding deficient VDR [25, 26]. Humans and mice carrying a loss-of-function mutation in the Hairless gene (Hr), a co-regulatory protein highly expressed in skin and brain [27], are also alopecic [28,29]. Hr binds to VDR in the absence and presence of 1,25(OH)₂D₃, and represses VDR-dependent transcription [30,31]. Reduced transcript levels of the VDR target gene LL-37 (cathelicidin antimicrobial peptide; CAMP) after Hr knockdown in human keratinocyte-derived HaCaT cells indicates that Hr might not only act as a CoR, but also as a CoA [32]. As Hr interacts with VDR/RXR heterodimers, but not with RXR homodimers [30], and as mice with a selective ablation of RXR α in keratinocytes are alopecic [33], Hr/VDR/RXR α complex controls hair follicle homeostasis in a calcitriol-independent manner (Figure 1 and see Chapter 30 by M. Demay”).

IV) Unliganded VDR repressive activities induce severe skeletal defects

Genetic and pharmacological experiments in mice indicated that the expression of the VDR target gene encoding the cytokine Thymic stromal lymphopoietin (TSLP) is increased in keratinocytes through a relief of a transcriptional repression mediated by VDR/RXR heterodimers [34]. In agreement with possible repressive activities of unliganded VDR, phenotypic analyses of Cyp27b1- and VDR-null mice suggested that bone and mineral homeostasis alterations might be more pronounced in 1,25(OH)₂D₃-deficient mice than in VDR-deficient mice [19,20]. Moreover, VDR-null transgenic mouse lines bearing a human genomic region encompassing the VDR locus with a mutation of the L233 codon, and expressing a ligand binding-defective VDR (hVDR-L233S) protein at various levels, exhibit hallmarks of rickets, but have no hair follicle defects [22]. Interestingly, hyperparathyroidism symptoms are more severe in these lines than in VDR-null mice, and the line expressing the highest hVDR-L233S protein levels has skeletal defects that are worse than those of VDR-null mice [22]. Moreover, the duodenal transcript levels of VDR target genes involved in calcium homeostasis are slightly lower in VDR-null mice expressing the higher hVDR-L233S levels than in the two other transgenic mouse lines. In agreement with these results, parathyroid hormone levels are higher in mice expressing an AF-2 deleted VDR than in VDR-null mice, whereas their femur calcium content is decreased [35]. Strikingly, mineral ion and bone homeostasis is more impaired in VDRgem mice, that express a calcitriol-binding deficient VDR, generated by substitution of Leucine 304 (located in H7 of VDR LBD) by a Histidine, than in VDR-null mice, raised under similar conditions, even though only the latter develop alopecia [21] (Figure 2). More than 100 genomic regions are bound by VDRgem in the mouse duodenum, and the transcript levels of many VDR target genes are lower in VDRgem than in VDR-null mice (Figure 3 A and B) [[21], and our unpublished results]. In agreement with these results, duodenal transcript levels of several VDR target genes are lower in Cyp27b1-null than in VDR-null mice [36]. As selective ablation of VDR in intestinal epithelial cells does not affect mineral ion homeostasis [37], while selective expression of VDRgem in such cells induces hypocalcemia (our unpublished data), unliganded VDR are hypothesized to exert repressive activities which impair intestinal calcium absorption.

V) Towards improved diagnosis and treatments of HVDRR patients

Alopecia in HVDRR patients is thought to be associated with severe skeletal and metabolic abnormalities [38]. However, as mice expressing a ligand-binding deficient VDR are not alopecic, even though their bones are more altered than those of VDR-null mice, it will be important to determine whether the severity of rickets in 1,25(OH)₂D₃-unresponsive non-alopecic patients bearing a point-mutated VDR-LBD is greater than in alopecic HVDRR patients, to improve the diagnosis of this orphan disease.

The treatment of HVDRR patients resistant to supra-physiological levels of 1,25(OH)₂D₃ is based on intravenous calcium administration, to correct hypocalcemia and secondary hyperparathyroidism [38]. As this therapy is

constraining, it is highly desirable to develop alternative treatments for these patients. We have recently shown that vitamin D analogs of the Gemini family induce VDR_{gem} transcriptional activity and restore mineral ion homeostasis in VDR_{gem} mice (Figure 4 A and B) [21], thereby providing a proof of principle that synthetic ligands can improve clinical signs of rickets caused by mutations in the VDR LBD that abolish 1,25(OH)₂D₃ binding. As overall more than twenty LBD-mutated VDRs have been identified in patients with rickets [16], and more than 3000 vitamin D analogs have been synthesized [39], it might be possible to identify compounds that induce the transcriptional activity of such receptors and thus provide personalized treatments.

VI – Conclusion and perspectives

Vitamin D₃ is known for many years to exert pleiotropic effects via VDR. However defects induced by 1,25(OH)₂D₃ deficiency do not fully overlap with those induced by VDR-deficiency. Whereas calcium homeostasis was shown to be regulated by liganded-VDR, that of hair follicles is controlled by VDR in a ligand-independent manner. Recent data in mice demonstrating that unliganded VDR is not a silent receptor, but has repressive activities that strongly impair calcium homeostasis (Figure 5), shed new light into VDR-mediated signaling. The repressive activities of apo-VDR are reminiscent of those of unliganded Thyroid Receptor (TR). Ligand-dependent VDR activities and TR-mediated gene repression require RXR as a heterodimeric partner [40] [41]. Thus, it is likely that RXRs are also required for apo-VDR-mediated repression. Interestingly, lithocholic acid has also been shown to bind to the canonical VDR ligand binding pocket, but with a much lower affinity than that of 1,25(OH)₂D₃, as well as to an alternative site located on the surface of VDR LBD [42] and induces VDR transcriptional activity [43]. It remains to be determined whether and/or how it modulates the activity of 1,25(OH)₂D₃-liganded and/or-unliganded VDR in vivo. The identification genome-wide of apo-VDR target genes and of the associated CoRs in various cell types might provide further insights into defects induced by ligand-unresponsive VDR or by low vitamin D serum levels.

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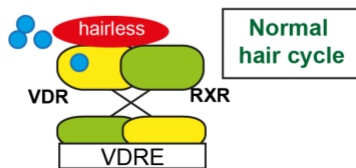
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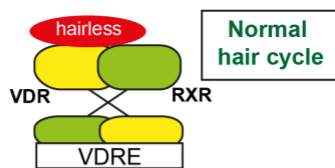
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Physiological $1,25(\text{OH})_2\text{D}_3$ levels



$1,25(\text{OH})_2\text{D}_3$ -deficiency



Physiological $1,25(\text{OH})_2\text{D}_3$ levels



Figure 1

Figure 1: VDR-mediated activities in hair cycle homeostasis. In the absence or presence of $1,25(\text{OH})_2\text{D}_3$, VDR dimerizes with members of the RXR family and binds to vitamin D response elements (VDRE). The complex recruits hairless and modulates VDR target gene expression in keratinocytes that control hair cycling. In the absence of VDR or RXR, postmorphogenic hair cycle is impaired, resulting in alopecia.

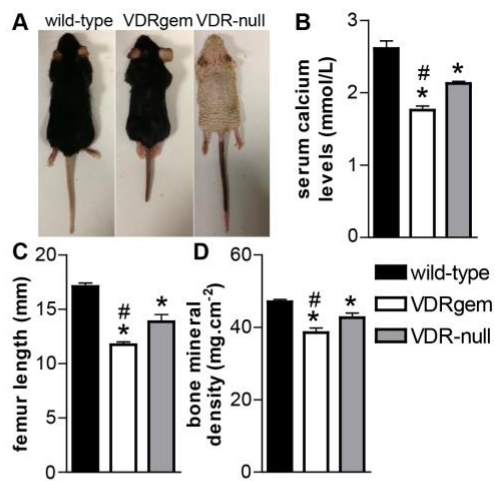


Figure 2

Figure 2: Bone and mineral ion homeostasis is more impaired in VDRgem mice than in VDR-null mice. (A) Representative picture of a 25 week-old wild-type, VDRgem and VDR-null mouse. Serum calcium levels **(B)**, femur length **(C)**, and bone mineral density **(D)** in 10 week-old wild-type, VDRgem and VDR-null mice. * $p < 0.05$ vs. wild-type. # $p < 0.05$ vs. VDR-null.

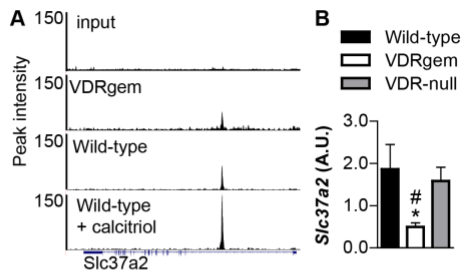


Figure 3

Figure 3: VDRgem binds to VDREs and represses VDR target gene expression. (A) Peak density at the *Slc37a2* locus generated with the MACS2 software, corresponding to DNA segments sequenced after immunoprecipitation of intestinal-chromatin from untreated wild-type and VDRgem mice, and of a wild-type mouse treated for 1.5 h with 1 μ g/kg calcitriol, with a VDR antibody, and the corresponding input. **(B)** Relative duodenal transcript levels of *Slc37a2* at basal level in 10 week-old wild-type, VDRgem and VDR-null mice. * $p < 0.05$ vs. wild-type. # $p < 0.05$ vs. VDR-null.

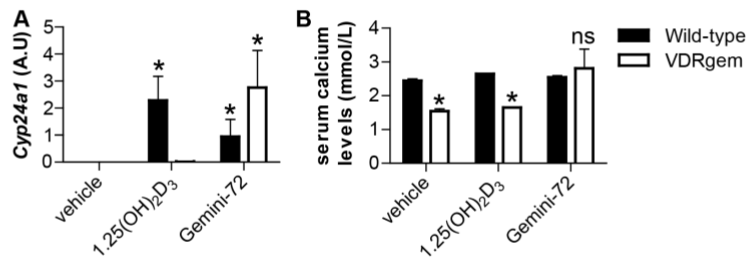


Figure 4

Figure 4: Duodenal Cyp24a1 transcript levels and serum calcium levels in VDRgem mice after VDR agonist administration. Quantification of relative duodenal 1,25(OH)₂D₃-24 hydroxylase (Cyp24a1) transcript levels (A) and of serum calcium levels (B) of 16 week-old VDRgem mice and control littermates subjected to oral administration every second day of vehicle (oil), 0.3 µg/kg 1,25(OH)₂D₃ or Gemini-72 for 3 weeks, and 3 µg/kg 1,25(OH)₂D₃ or Gemini-72 6 h before sacrifice. *p < 0.001. ns, not significant.

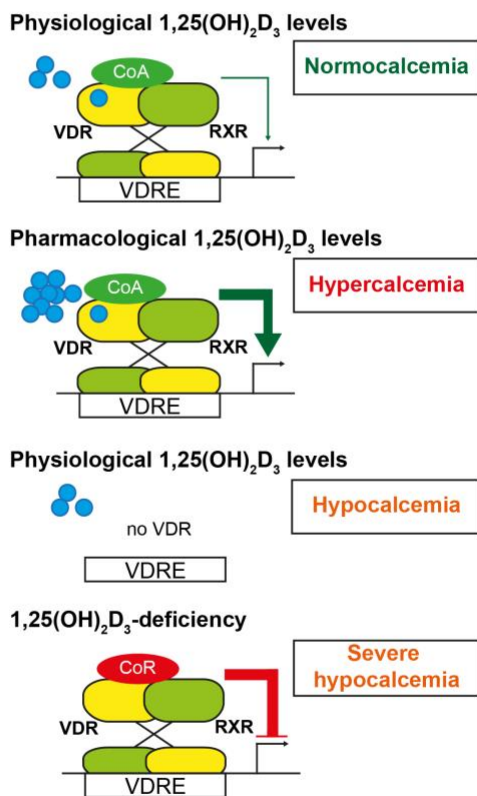


Figure 5

Figure 5: VDR-mediated activities in intestine. In presence of physiological $1,25(\text{OH})_2\text{D}_3$ levels, VDR/RXR heterodimers recruit co-activators and induce the expression of VDR target genes. At pharmacological calcitriol levels, these heterodimers bind to a higher number of VDREs, some of them being located in additional genes, and VDR target gene expression is enhanced. Intestinal calcium absorption and serum calcium levels are increased. In absence of calcitriol, VDR/RXR heterodimers bind to VDREs and recruit corepressors. VDR target genes are repressed, resulting in hypocalcemia that is more severe than that induced by VDR deficiency.