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Review

Vitamin D signaling in osteocytes: Effects on bone and mineral homeostasis

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ABSTRACT

The active form of vitamin D [1,25$(OH)_2$D] is an important regulator of calcium and bone homeostasis, as evidenced by the consequences of 1,25$(OH)_2$D inactivity in man and mice, which include hypocalcemia, hypophosphatemia, secondary hyperparathyroidism and bone abnormalities. The recent generation of tissue-specific (intestine, osteoblast/osteocyte, chondrocyte) vitamin D receptor (*Vdr*) null mice has provided mechanistic insight in the cell-specific actions of 1,25$(OH)_2$D and their contribution to the integrative physiology of VDR signaling that controls bone and mineral metabolism. These studies have demonstrated that even with normal dietary calcium intake, 1,25$(OH)_2$D is crucial to maintain normal calcium and bone homeostasis and accomplishes this primarily through stimulation of intestinal calcium transport. When, moreover, insufficient calcium is acquired from the diet (severe dietary calcium restriction, lack of intestinal VDR activity), 1,25$(OH)_2$D levels will increase and will directly act on osteoblasts and osteocytes to enhance bone resorption and to suppress bone matrix mineralization. Although this system is essential to maintain normal calcium levels in blood during a negative calcium balance, the consequences for bone are disastrous and generate an increased fracture risk. These findings evidently demonstrate that preservation of serum calcium levels has priority over skeletal integrity. Since vitamin D supplementation is an essential part of anti-osteoporotic therapy, mechanistic insight in vitamin D actions is required to define the optimal therapeutic regimen, taking into account the amount of dietary calcium supply, in order to maximize the targeted outcome and to avoid side-effects. We will review the current understanding concerning the functions of osteoblastic/osteocytic VDR signaling which not only include the regulation of bone metabolism, but also comprise the control of calcium and phosphate homeostasis via fibroblast growth factor (FGF) 23 secretion and the maintenance of the hematopoeitic stem cell (HSC) niche, with special focus on the experimental data obtained from systemic and osteoblast/osteocyte-specific *Vdr* null mice.

This article is part of a Special Issue entitled "The Osteocyte".

Contents

Abbreviations: 1,25$(OH)_2$D, 1,25$(OH)_2$ vitamin D; ANK, progressive ankylosis; COLL, collagen; DMP, dentin matrix protein; ENPP, ectonucleotide pyrophosphatase phosphodiesterase; FGF, fibroblast growth factor; G-CSF, granulocyte colony-stimulating factor; HSC, hematopoietic stem cells; NPT, sodium-dependent phosphate transporter; OC, osteocalcin; PPi, pyrophosphate; PTH, parathyroid hormone; RANK, receptor activator of nuclear factor NF-κβ; RANKL, RANK ligand; TNAP, tissue non-specific alkaline phosphatase; VDR, vitamin D receptor.

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Introduction

Systemic inactivation of the vitamin D receptor (*Vdr*) in mice has irrefutably demonstrated that the active form of vitamin D, $1,25(OH)_2D$, is key to maintain normal calcium and bone homeostasis [1]. Several strains of *Vdr* null mice were generated and they all display mineral and hormonal abnormalities that are associated with bone defects including rickets and osteomalacia ([2–5], Table 1). These abnormalities are the consequence of impaired $1,25(OH)_2D$-mediated calcium absorption in the intestine. Indeed, the *Vdr* null phenotype is almost completely prevented by genetic reintroduction of intestinal *Vdr* expression [6] or by overruling the lack of $1,25(OH)_2D$-mediated intestinal calcium transport by dietary calcium/lactose supplementation [7,8]. Tissue-specific *Vdr* inactivation has revealed that $1,25(OH)_2D$ also exerts defined actions in other, extra-intestinal target tissues including the fine-tuning of bone remodeling by osteoblasts [9,10], the regulation of bone and phosphate metabolism by chondrocytes [11], the modulation of parathyroid hormone (PTH) levels by the parathyroid gland [12], and the control of cardiac hypertrophy by cardiomyocytes [13] (Table 1). Moreover, the targeted deletion of the *Vdr* in osteocytes (and mature osteoblasts) (*Dmp1*-Cre, [14]) has provided compelling evidence that osteoblastic/osteocytic *Vdr* signaling is crucial to suppress bone matrix mineralization, an activity that is required to maintain normocalcemia during a negative calcium balance [15]. When, on the other hand, sufficient calcium is acquired from the diet, $1,25(OH)_2D$ activity in the mature osteoblasts/osteocytes is not essential for bone remodeling (see 'The role of osteoblastic/osteocytic VDR signaling in bone homeostasis') [15] (Table 1). In addition to its involvement in bone homeostasis per se, systemic *Vdr* inactivation also suggests that VDR signaling in osteocytes contributes to calcium and phosphate homeostasis by stimulating the production of fibroblast growth factor (FGF) 23 (see 'The role of osteoblastic/osteocytic VDR signaling in mineral homeostasis') and that VDR action in osteoblasts influences the maintenance of the hematopoietic stem cell (HSC) nice (see 'Effects of VDR signaling in osteoblasts on the hematopoietic stem cell niche').

We will give an overview of the effects of VDR signaling in the osteoblastic lineage with special focus on osteocytes, based on the in vivo data obtained from analyzing systemic and when available osteoblast/osteocyte-specific *Vdr* null mice. The latter were generated by crossing mice with a floxed *Vdr* gene with mice that express the CRE-recombinase under the control of the *Dmp1*-promoter [14,15]. Since *Vdr* is not only inactivated in osteocytes in this model but also in mature osteoblasts [16], we ascribe, for correctness, the observed phenotype to a lack of *Vdr* signaling in osteocytes as well as in mature osteoblasts. However, certain skeletal functions have been assigned to a specific osteoblastic differentiation stage, and one may therefore speculate that particular VDR actions are preferentially exerted in osteocytes, as opposed to osteoblasts, or vice versa. More specifically, osteocytes are the primary cells in the regulation of FGF23 secretion [17] and in the mobilization of calcium from bone in a process that is referred to as osteocytic osteolysis [18]. In contrast, mature osteoblasts will play a more substantial role in the suppression of bone matrix mineralization [19] and the functioning of the HSC niche [20]. Calcium release from bone is likely regulated by both cell types, i.e. osteocytes are the main RANKL (receptor activator of nuclear factor NF-κβ ligand)-producing cell types with age, whereas osteoblasts are more important in growing mice [16]. Univocal delineation of the in vivo contribution of VDR signaling in mature osteoblasts versus osteocytes requires the generation of a new transgenic mouse model in which the CRE-recombinase is exclusively expressed in osteocytes.

The role of osteoblastic/osteocytic VDR signaling in bone homeostasis

The impact of osteoblastic/osteocytic VDR signaling on bone homeostasis largely depends on calcium balance. A positive calcium balance is achieved when dietary calcium acquisition equals or exceeds the bodily calcium use, storage and loss. $1,25(OH)_2D$ is essential to maintain a positive calcium balance when dietary calcium levels are normal to low and VDR actions will enhance intestinal calcium absorption. Consequently,

Table 1
Brief description of the phenotype of systemic and tissue-specific *Vdr* null/overexpression mice. *In this model, the *Vdr* is overexpressed as opposed to the other transgenic mice in which the *Vdr* is deleted. nl, normal; ↑, increased; ↓, decreased.

Mouse model of *Vdr* deletion/overexpression	Mineral homeostasis (serum levels)	Hormonal homeostasis (serum levels)	Bone phenotype	Other features	Ref.
Systemic deletion	↓ calcium ↓ phosphate	↑↑ PTH ↑↑ $1,25(OH)_2D$ ↓↓ FGF23	Rickets, osteomalacia, small reduction in mineralized bone mass	↓ Intestinal calcium absorption	[2–5]
Intestinal-specific deletion (*Villin*-CRE mice)	nl calcium nl phosphate	↑ PTH ↑ $1,25(OH)_2D$	Manifest reduction in skeletal calcium levels characterized by (i) bone loss due to increased bone resorption, and (ii) suppression of bone matrix mineralization due to an increased abundance of mineralization inhibitors No growth plate abnormalities	↓ Intestinal calcium absorption	[15]
Chondrocyte-specific deletion (*Coll2*-CRE mice)	nl calcium ↑ phosphate	nl PTH ↑ $1,25(OH)_2D$ ↓ FGF23	Transient increase in bone mass due to delayed invasion of blood vessels and osteoclasts No growth plate abnormalities	↓ Secretion of a chondrocyte-derived factor that controls FGF23 levels	[11]
Deletion from the immature osteoblast stage onwards (*Coll1*-CRE mice)	Unknown	Unknown	Increase in bone mass due to a reduction in bone resorption, without an effect on bone formation		[9]
*Overexpression from the mature osteoblast stage onwards (*Oc*-promoter)	nl calcium	nl to ↑ PTH nl to ↑ $1,25(OH)_2D$	Increased bone mass due to an increase in bone formation and a reduction in bone resorption	Less susceptible to trabecular bone loss caused by calcium restriction	[10,21]
Deletion from osteocytes and mature osteoblasts (*Dmp1*-CRE mice)	nl calcium nl phosphate	nl PTH nl $1,25(OH)_2D$	No bone phenotype	Protected against $1,25(OH)_2D$-induced mineralization defects	[15]
Cardiomyocyte-specific deletion (*myosin light chain*-CRE mice)	nl calcium nl phosphate	nl PTH	Unknown	More pronounced cardiac hypertrophy after isoproterenol treatment	[13]
Parathyroid-gland-specific deletion (*Pth*-CRE)	nl calcium nl phosphate	↑ PTH nl $1,25(OH)_2D$	Increase in bone resorption without an effect on bone formation	No parathyroid gland hyperplasia nor changes in calcium sensitivity	[12]