Citation: BoneKEy Reports **3**, Article number: 494 (2014) | doi:10.1038/bonekey.2013.228

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REVIEW

Vitamin D endocrine system and osteocytes

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The physiological role of the osteocyte, the most numerous of the three bone cell types, was significantly underestimated until recently. It is now known that they not only coordinate bone remodeling but also have an endocrine function as part of the regulatory network for calcium and phosphate homeostasis. Vitamin D and osteocytes interact in numerous ways to accomplish these activities. The major source of active vitamin D ($1,25(OH)_2D_3$) is the kidney but there is evidence that osteocytes can produce it as well. Renal $1,25(OH)_2D_3$ regulates osteocyte production of fibroblast growth factor 23 (FGF23), a powerful phosphaturic factor with far-reaching physiological effects. The function of $1,25(OH)_2D_3$ produced by osteocytes themselves is poorly understood and is an area of active research. Osteocytes affect local bone remodeling by producing regulatory factors for osteoblasts and osteoclasts in response to mechanical loading and to endocrine signals such as serum $1,25(OH)_2D_3$. In addition, $1,25(OH)_2D_3$ may inhibit mineralization in osteocyte lacunae. Whether $1,25(OH)_2D_3$ has a role in osteocytic perilacunar remodeling is currently unknown. This short review presents the current state of our knowledge about the physiologically and clinically significant roles of vitamin D signaling in osteocytes.

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Background Osteocytes

The osteocyte is one of three major bone cell types and is by far the most numerous one. The other two cell types (osteoblast and osteoclast) have well-known roles in bone remodeling; however, until recently the role of the osteocyte was poorly understood and greatly underestimated.[1] Osteoblasts actively form bone while osteoclasts resorb it. Osteocytes, which differentiate from osteoblasts, reside within the bone matrix itself, apparently isolated and without a function. Recent research, however, has shown that osteocytes have major physiological roles both within bone and in the body as whole.[2] Osteocytes not only appear to coordinate bone remodeling, at least to some degree,[3,4] they are also the major source of various endocrine factors that are released into the circulation such as fibroblast growth factor 23 (FGF23)[5] or osteocalcin.[1]

Osteoblasts and osteocytes are derived from mesenchymal stem cells, which are multipotent cells that differentiate into various cell types, including osteoblasts and chondrocytes. Mesenchymal stem cells that express Runt-related transcription factor 2 differentiate into either pre-osteoblast cells (expressing Runt-related transcription factor 2 and β-catenin) or pre-chondrocyte cells (expressing Sox9). Pre-osteoblast cells differentiate into osteoblasts, which then may continue to differentiate into osteocytes.[1,6] The osteoblast–osteocyte transition involves both morphological and genetic changes that involve cell structure, position within the bone and gene expression (**Figure 1a**). Pre-osteoblasts migrate to the bone surface and differentiate into osteoblasts and then into embedding osteoblasts that begin to enter the bone. Once in the osteoid, osteoblasts become immature osteoid osteocytes that display the characteristic morphology of osteocytes, most notably the dendrites that are a key element of osteocyte function. As mineralization proceeds, the osteocytes pass through the mineralizing osteocyte stage to the final mature osteocyte stage. Each stage has a unique set of genetic markers expressed during that stage (**Figure 1b**).

Osteocytes are embedded in lacunae inside mineralized bone and have dendritic processes that pass through tunnels called canaliculi that connect to other osteocytes as well as surface osteoblasts and vasculature. The highly ordered system of lacunae and canaliculi—the lacunocanalicular network—contains a fluid called canalicular fluid that flows through the system to allow oxygen and nutrients to reach the osteocytes. This system is a key morphological component of osteocyte functionality (**Figure 2**). The dendrites allow osteocytes to communicate through gap junctions at the tips of the dendrites. It is speculated that they may also be able to communicate with surface cells as well. The dendrites and canalicular fluid provide a mechanism for osteocytes to sense mechanical loading.

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Received 26 July 2013; accepted 11 September 2013; published online 5 February 2014

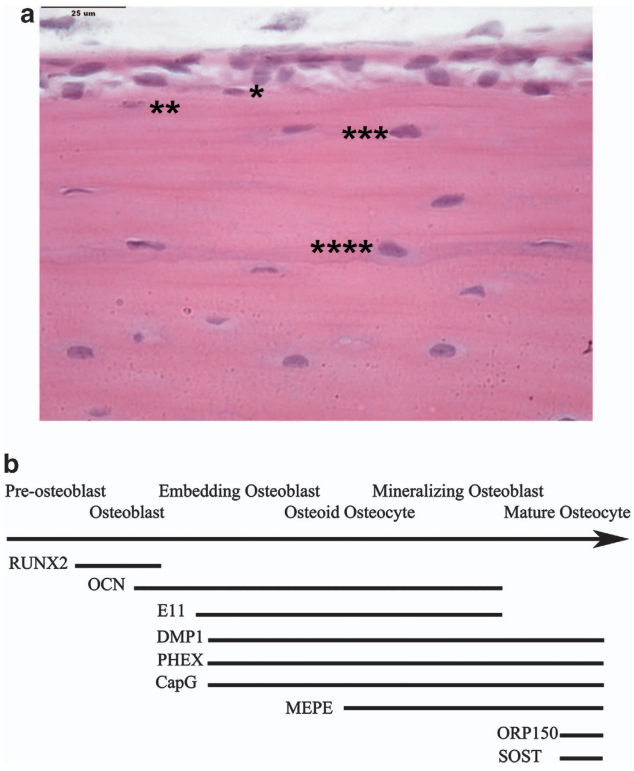

Figure 1 (**a**) Gene expression pattern of bone markers during osteoblast to osteocyte transition. *Runt-related transcription factor 22, OCN; **OCN, E11, DMP1, PHEX, CapG; ***OCN, E11, DMP1, PHEX, CapG, MEPE; ****DMP1, PHEX, CapG, MEPE, ORP150, sclerostin. (**b**) Schematic presentation of gene expression pattern during osteocyte differentiation. The expression of most of these genes is regulated by $1,25(OH)_2D_3$.[30,45–48]

Osteocytes are particularly sensitive to fluid shear stress. The lacunocanalicular network, its canalicular fluid and the ordered arrangement of osteocytes provide an excellent system for detecting mechanical forces and then transmitting signals to surface cells to regulate bone remodeling in order to respond to the loading. Significantly, this system also supports the endocrine function of osteocytes, allowing external endocrine factors such as the vitamin D hormone $1,25(OH)_2D_3$ and parathyroid hormone (PTH) to access osteocytes and providing an outlet for factors produced by the osteocytes such as FGF23.

Osteocytes as Target Cells for $1,25(OH)_2D_3$ Action

Although osteocytes are generally considered to be target cells of $1,25(OH)_2D_3$ action, direct evidence for the presence of the vitamin D receptor (VDR) in osteocytes *in situ* is scarce. Probably the best evidence comes from an ultrastructural immunohistological study in calvariae of neonatal mice and rats, showing the presence of ligand and VDR mainly in the nuclei of osteoblasts and osteocytes.[7] *In situ* hybridization in rat mandibles confirmed the presence of VDR transcripts in osteocytes.[8] There is also good evidence for direct activation of $25(OH)D_3$ in osteocytes by 1α-hydroxlase;[9] however, the physiological role of local production of $1,25(OH)_2D_3$ in osteocytes is currently not known.

In contrast to the vast knowledge about vitamin D target genes in osteoblasts (see other chapter), little is known about

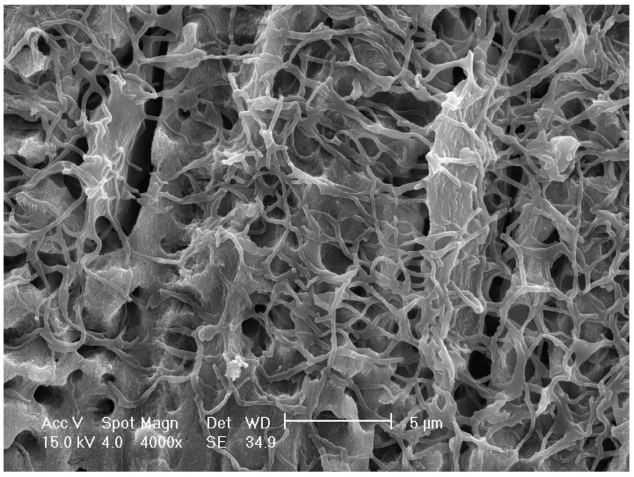

Figure 2 Morphology of an osteocyte. Shown is a scanning electron micrograph of an acid-etched resin-casted mouse cortical bone specimen. The organization of the osteocyte lacunocanalicular system is shown. The numerous dendritic processes connect osteocytes to other osteocytes, osteoblasts and bone-lining cells.

the specific vitamin D-responsive genes in osteocytes. There is evidence that $1,25(OH)_2D_3$ stimulates transcription of receptor activator of nuclear factor kappa-B ligand (RANKL),[10] BMP7[11] and FGF23[12] in osteocytes or osteocyte-like cells. RANKL is one of the master regulators of osteoclastic bone resorption.[13] Moreover, based on what is known from osteoblasts,[14,15] it is likely that $1,25(OH)_2D_3$ regulates the mRNA abundance of genes related to mineralization such as osteocalcin, osteopontin, progressive ankylosis, ectonucleotide pyrophophatase/phosphodiesterase 1 (Enpp1) and Enpp3 also in osteocytes.[14] However, to date a comprehensive analysis of vitamin D-regulated target genes in osteocytes has not been performed.

What is the physiological function of VDR in osteocytes? VDR-ablated mice that are fed a rescue diet enriched with calcium, phosphorus and lactose do not show alterations in bone mineral density or bone histology until old age.[16] In addition, genetic ablation of the VDR in osteocytes/mature osteoblasts (Dmp1 (dentin matrix protein 1)-Cre) has no effect on bone and mineral homeostasis.[14] Thus, a physiologically important role of $1,25(OH)_2D_3$ in osteocytes *in vivo* is questionable under the conditions of a normal dietary calcium supply. However, $1,25(OH)_2D_3$-induced inhibition of bone mineralization in osteoblasts/osteocytes was shown to be important for maintenance of calcium homeostasis when endogenous $1,25(OH)_2D_3$ hormone levels are upregulated in response to an increased calcium demand.[14] The relative contribution of osteoblasts and osteocytes to the latter effect remains uncertain due to the inability of the Dmp1-Cre construct to distinguish between osteocytes and mature osteoblasts.[17]

FGF23, PTH and Vitamin D Interactions

Recently, we have come to understand that bone does not merely have a passive role as a physical framework for the body and a repository for minerals but is also an active endocrine organ. Bone acts together with the kidneys and parathyroid glands to form a regulatory network to maintain mineral ion homeostasis. In this network, PTH from the parathyroid glands, $1,25(OH)_2D_3$ mainly from the kidney and FGF23 from the bone

regulate the intestinal absorption and renal excretion of calcium and phosphate, as well as the storage and removal of these key minerals from the skeleton. This regulatory network is well known and there are a number of excellent reviews that provide far more details than we can present in this review.[18] It is currently thought that osteocytes are a major source of FGF23, and that $1,25(OH)_2D_3$ is one of the most important endocrine regulators of its production. Whether FGF23 is mostly expressed by osteoblasts or osteocytes in healthy individuals is not clear, as its detection is very difficult because of the low abundance of FGF23 under normal conditions. What has become evident, however, is that under pathological conditions with higher levels of FGF23 such as rickets, loss of DMP1[19] or chronic kidney failure,[20] the osteocyte becomes the major source of FGF23.

The existence of an endocrine phosphaturic factor was hypothesized as early as 1959 and was confirmed by the discovery of a humoral factor secreted by tumors that cause osteomalacia and by studies with a mouse model for X-linked hypophosphatemia (Hyp mice) that demonstrated a circulating factor inducing hypophosphatemia and inhibiting $1,25(OH)_2D_3$ production. In 2000, mutations in FGF23 were identified as the cause of autosomal-dominant hypophosphatemic rickets.[21] As a result of this discovery, FGF23 was recognized as the hypothesized, but previously unidentified, phosphaturic factor that is a key to phosphate homeostasis. FGF23 is one of three members of the FGF19 superfamily that have endocrine functions and that require a cofactor for efficient activation of their receptors.[22] The cofactor of FGF23, a form of Klotho (αKl), is primarily expressed in the kidney, parathyroid glands and the choroid plexus of the brain and limits the actions of FGF23 primarily to these tissues. FGF23 acts to lower phosphate levels by downregulating sodium-dependent phosphate co-transporters in the kidney, thus reducing reabsorption of phosphate. It also downregulates the 1α-hydroxylase in the kidney, an enzyme that converts the precursor 25(OH)vitamin D into its active metabolite, $1,25(OH)_2D_3$. Moreover, FGF23 also induces the expression of 24-hydroxylase, an enzyme that catabolizes $1,25(OH)_2D_3$ and thereby reduces $1,25(OH)_2D_3$ actions. $1,25(OH)_2D_3$ is the primary systemic factor for increasing FGF23 production and thus FGF23 acts to self-regulate its production with this negative feedback loop (**Figure 3**).

Other factors such as PTH and serum phosphate may act as stimulants to FGF23 as well but $1,25(OH)_2D_3$ is thought to be the most significant regulator. The mouse and rat Fgf23 gene promoter regions include a vitamin D-responsive element, suggesting that $1,25(OH)_2D_3$ directly stimulates Fgf23 transcription.[23] However, as serum Fgf23 levels in mice deficient for the sodium-phosphate co-transporter (SLC34A1) are low (personal observation) in the presence of very high serum $1,25(OH)_2D_3$, it is not clear whether this vitamin D-responsive element is being activated.[24] Three hypophosphatemic disorders have been associated with dysregulated FGF23 that involve genes expressed in osteocytes. Autosomal recessive hypophosphatemic rickets 1 is caused by mutations in DMP1.[25] X-linked dominant hypophosphatemic rickets results from mutations to an endopeptidase, *phosphate-regulating gene with homologies to endopeptidases on the X chromosome* (PHEX).[26] Finally, autosomal recessive hypophosphatemic rickets 2 is caused by mutations in ENPP1.[27,28] Inactivating

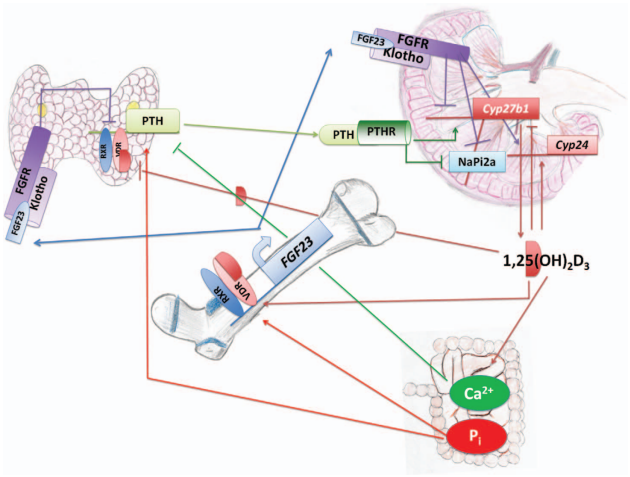

Figure 3 Regulatory mechanisms between bone, kidney, parathyroid gland and gut. FGF23, 1,25-dihydroxyvitamin D3 and PTH regulate each other's expression and secretion and thereby balance mineral ion homeostasis.

mutations in these three factors are known to lead to overproduction of FGF23 and thus hypophosphatemia. The exact mechanisms by which DMP1, PHEX and ENPP1 affect FGF23 are still being investigated.[29] It was originally thought that some of these genes enhance cleavage of FGF23 to an inactive form; however, there is evidence that this may not be the case. It has also been proposed that these factors act in concert to suppress FGF23 transcription. However, it is important to note that at least one of these, PHEX, is known to be regulated by $1,25(OH)_2D_3$.[30] This suggests an indirect mechanism for $1,25(OH)_2D_3$ to regulate FGF23 activity in osteocytes by regulating PHEX.[31]

Osteocytes, Mineralization and Vitamin D

Osteocytes have an impact on mineralization in several ways. FGF23 produced in osteocytes has systemic effects that influence mineralization.[32] In addition, FGF23 may have local effects on mineralization. Osteocytes also directly affect bone remodeling by inhibiting or enhancing osteoblast or osteoclast activity as needed to balance bone homeostasis. All of these actions are affected, at least in part, by $1,25(OH)_2D_3$.

The vitamin D/FGF23 regulatory axis has a major effect on mineralization, and dysregulation of this system in osteocytes has a profound impact on health. Elevated FGF23 production in osteocytes and osteoblasts leads to hypophosphatemia, abnormally low levels of $1,25(OH)_2D_3$ and several skeletal defects. Conversely, loss of FGF23 production results in hyperphosphatemia, elevated $1,25(OH)_2D_3$, ectopic mineralization and skeletal defects.[33] A major factor in the skeletal defects in the presence of abnormally low systemic FGF23 levels is the resulting increase in $1,25(OH)_2D_3$, as shown in experiments with mouse models with multiple gene ablations.[34] Mice ablated for both FGF23 and 1α-hydroxylase to prevent the production of $1,25(OH)_2D_3$[34] or ablated for FGF23 and VDR to block the actions of $1,25(OH)_2D_3$ do not display mineralization issues provided they are fed a high-calcium/phosphate/20% lactose diet.[35]

Osteocytes exert a local effect on both mineralization and on bone remodeling, and there is evidence that FGF23 has a role

in this. The three potential FGF receptors for FGF23 are expressed in bone; however, Klotho—its required cofactor for efficient binding—is only expressed at very low levels. It has been suggested that FGF23 is nevertheless able to weakly bind and affect FGF signaling. Moreover, in order to reverse the high phosphate levels found in Fgf23 null mice, experiments were conducted with mice that were ablated for both type IIa sodium-phosphate transporter (NaPi2a) and Fgf23.[24] These experiments showed that the skeletal phenotype seen in Fgf23 null mice could not be rescued, even though serum phosphate levels had been reversed to very low levels by ablating NaPi2a. Moreover, adult mice with only NaPi2a ablated had no skeletal phenotype. Taken together, these findings also suggest an independent role for FGF23 in bone mineralization.

Finally, osteocytes respond to hormonal and mechanical signals such as $1,25(OH)_2D_3$ and mechanical loading.[3] They then influence bone remodeling to maintain bone homeostasis by regulating both osteoblast and osteoclast activities. Osteocytes express and secrete several factors that affect Wnt signaling and thus modulate osteoblast activity. Osteocytes express prostaglandin E2, nitric oxide and ATP to activate Wnt signaling and promote osteoblast differentiation and bone formation. Moreover, they also express sclerostin, Dickkopf-related protein 1 and Secreted frizzled-related protein 1 that inhibit Wnt signaling to reduce bone formation. Osteocytes also secrete factors that influence osteoclast activity, nitric oxide and osteoprotegerin to inhibit osteoclasts or RANKL and macrophage-colony-stimulating factor to promote osteoclast activity.[1] As mentioned above, $1,25(OH)_2D_3$ is known to regulate RANKL expression in stromal cells and could serve the same role in osteocytes.[10,36,37]

Regulation of Osteocytic Perilacunar Remodeling by Vitamin D

The term 'osteocytic osteolysis' was coined in the 1960s, implying that osteocytes can actively resorb bone in the perilacunar network in response to hormonal signals, especially PTH.[38,39] Although the concept of osteocytic osteolysis was dismissed in the 1980s,[40–42] it was recently shown that osteocytes are indeed able to remodel perilacunar bone in lactating mice by increased PTHrP signaling through the PTH type 1 receptor.[43] It is currently unknown whether $1,25(OH)_2D_3$ signaling can influence osteocytic perilacunar remodeling. However, earlier studies showed that the injection of $1,25(OH)_2D_3$ did not alter the size of osteocytes, organellar development and contour of osteocytic lacunae in thyro-parathyroidectomized rats,[44] suggesting that increased $1,25(OH)_2D_3$ signaling *per se* may not be involved in osteocytic bone resorption in response to an increased calcium demand. Thus, it is currently believed that $1,25(OH)_2D_3$ inhibits bone mineralization in osteocyte lacunae,[14] and upregulates osteoclastic bone resorption through increased expression of RANKL, but does not induce active resorption of bone in osteocyte lacunae or the osteocytic canalicular network.

Conclusions

Our knowledge of the physiological significance of osteocytes in both local and systemic contexts has advanced greatly in the recent decade. We have progressed from the view that osteocytes had a static role in skeletal structure to one of an active paracrine/endocrine cell with far-ranging effects on the body. Osteocytes are active participants in bone mineralization and remodeling. They sense mechanical stress and endocrine signals such as serum $1,25(OH)_2D_3$ and signal nearby osteoblasts and osteocytes to regulate bone remodeling in response to structural and systemic needs. Osteocytes inhibit bone formation by producing Wnt inhibitors sclerostin, Dickkopf-related protein 1 and Secreted frizzled-related protein 1 and promote it by producing Wnt activators prostaglandin E2, nitric oxide and ATP. They also affect bone resorption by producing nitric oxide/osteoprotegerin or RANKL/macrophage-colony-stimulating factor to inhibit or activate osteoclast activity, respectively. Moreover, they may directly affect mineralization. In addition to these paracrine activities, osteocytes produce the phosphaturic, endocrine factor FGF23 as part of a systemic mineral homeostasis regulatory network that involves four organs: bone, kidney, gut and parathyroid gland. Mutations in FGF23 or improper regulation of its production and activity have severe developmental and health consequences.

In concert with the expansion of our knowledge of osteocyte physiology, we are also discovering more about the close relationship between vitamin D and osteocytes. It is well established that $1,25(OH)_2D_3$ is a major contributor to the regulation of FGF23 in osteocytes and osteoblasts—an activity with significant systemic effects. In addition to stimulating the production of FGF23, it has been shown that $1,25(OH)_2D_3$ can regulate PHEX—a putative indirect regulator of FGF23 secretion—and it is possible that it may regulate other such factors in osteocytes. The physiological significance of the presence of VDR and 1α-hydroxylase in osteocytes is only partially understood and is an area of active investigation. Moreover, the importance of $1,25(OH)_2D_3$ interaction with osteocytes is often only apparent under conditions of disturbed mineral homeostasis, and these interactions may have significance for treatment options. Continued research into osteocytes and the actions of $1,25(OH)_2D_3$ on osteocytes is therefore highly significant both scientifically and clinically.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgements

We would like to thank JQ Feng for providing the images for this review. This work was supported by The National Institute of Health (R01 DK097105) to BL and by a grant from the Austrian Science Fund (FWF P24186-B21) to RGE.

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