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REVIEW

The osteocyte plays multiple roles in bone remodeling and mineral homeostasis

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Abstract Osteocytes are the most abundant cells in bone and are the major orchestrators of bone remodeling and mineral homeostasis. They possess a specialized cellular morphology and a unique molecular feature. Osteocytes are a stellate shape with numerous long, slender dendritic processes. The osteocyte cell body resides in the bone matrix of the lacuna and the dendritic processes extend within the canaliculi to adjacent osteocytes and other cells on the bone surface. Osteocytes form extensive intercellular network to sense and respond to environmental mechanical stimulus by the lacunar–canalicular system and gap junction. Osteocytes are long-lived bone cells. They can undergo apoptosis, which may have specific regulatory effects on osteoclastic bone resorption. Osteocytes can secrete several molecules, including sclerostin, receptor activator of nuclear factor κB ligand and fibroblast growth factor 23 to regulate osteoblastic bone formation, osteoclastic bone resorption and mineral homeostasis. A deeper understanding of the complex mechanisms that mediate the control of osteoblast and osteoclast function by osteocytes may identify new osteocyte-derived molecules as potential pharmacological targets for treating osteoporosis and other skeletal diseases.

Keywords Osteocyte · Lacunar–canalicular system · Sclerostin · FGF23 · RANKL · Osteoporosis

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Introduction

Bone remodeling is the fundamental means by which the bone quantity and bone quality are maintained throughout adult life. Bone remodeling is a lifelong process where the old bone is removed by the osteoclast and new bone is formed by the osteoblast. An imbalance in the bone resorption and bone formation results in many metabolic skeletal diseases, such as osteoporosis. Osteoporosis is a systemic disorder characterized by low bone mass and microstructural deterioration of bone tissue with increased fracture risk [1]. Both osteoblasts and osteoclasts function in concert, which requires intimate cross talk with osteocytes. Osteoblasts and osteoclasts, existed on bone surface, are defined by their respective functions of bone formation and bone resorption. Osteocytes are the most abundant cell type in bone. Osteocytes comprise more than 90 % of all cells in the adult bone. They can live for several decades, whereas osteoclasts live only for a few days or weeks and osteoblasts live only for a few months [2]. Osteocytes, embedded within the mineralized bone matrix, are defined mainly by their morphology. Osteocytes are not easily accessible and therefore their function in bone metabolism remains incomplete. With new technology, such as molecular and transgenic approaches, imaging and advanced instrumentation, our understanding of the osteocyte function has expanded dramatically over the last decade or so. Osteocyte is the principal cell type responsible for integrating the mechanical and chemical signals that govern modeling and remodeling and control the onset of both bone formation and bone resorption [2–4]. In addition, more recent studies demonstrate that osteocytes exert regulatory influences beyond the borders of bone by participating in endocrine pathways that regulate phosphate metabolism. This review aims to summarize the current

understanding of the osteocyte morphology and function. We also discuss the possible implications for treating osteoporosis and other related diseases.

Osteocyte differentiation and its selective gene expression

Osteocytes are derived from osteoblasts. The mature osteocyte represents a terminally differentiated stage of the osteoblast lineage. It was reported that about 60–80 % of osteoblasts die via apoptosis [5]. The remaining osteoblasts become either lining cells that cover quiescent surfaces or are entombed individually in lacunae of the mineralized matrix and become osteocytes. In the process of their entombment, osteocytes undergo a dramatic morphologic transformation which includes the development of an average of 50 slender cell processes that radiate from the cell body and give osteocytes the resemblance of neuronal cells [6]. The process during which an osteoblast differentiates an osteocyte involves transition from the plump polygonal to a more stellate shape with numerous long, slender dendritic processes. Osteocyte formation is an active process driven by changes in gene expression. The molecular and genetic mechanisms that regulate osteocyte differentiation are being unraveled. During osteoblast to osteocyte differentiation, osteocytes share many genes with osteoblasts, but they also express unique genes that play key regulatory roles in altering the morphology and function (Table 1). Expression of the transmembrane glycoprotein E11/gp38 and membrane type 1 matrix metalloproteinase (MT1-MMP) is required for the formation of osteocyte dendritic processes and canaliculi [7, 8]. Osteocytes are rich in genes related to mineralization and phosphate metabolism, including dentin matrix protein 1 (DMP1), matrix extracellular phosphoglycoprotein (MEPE), phosphate-regulating gene with homologies to endopeptidases on the X chromosome (PHEX) and fibroblast growth factor 23 (FGF23) [3, 9, 10]. Osteocytes also express genes that affect bone formation, including dickkopf-related protein 1 (Dkk1) and sclerostin, which are primarily expressed in osteocytes [11]. Table 1 summarizes the main marker genes and their function of the osteocyte. These osteocyte-specific genes have multiple functions including mineral regulation, phosphate homeostasis and cytoskeletal arrangement.

Table 1 The main marker gene expression and function of the osteocyte

Protein	Main function
E11/gp38	Formation of cell process
MT1-MMP	Matrix degradation and canalicular formation
PHEX	Regulating phosphate metabolism
DMP1	Regulating phosphate metabolism and mineralization
FGF23	Controlling phosphate metabolism
Dkk1	Inhibiting bone formation
Sclerostin	Inhibiting bone formation
RANKL	Osteoclast differentiation and survival
OPG	Inhibitor of osteoclast differentiation

Morphology of osteocyte, dendritic process and lacunar–canalicular system

Osteocytes are former osteoblasts that entrapped within the mineralized bone matrix. As the osteocyte embeds and further differentiates, there is a reduction in cell volume of up to 70 %. Upon mineralization of the osteoid, there are also changes to osteocyte ultrastructure, such as a reduction in the endoplasmic reticulum and Golgi complex. Osteocyte cell body is enclosed within a lacuna of 15–20 μm in diameter. Osteocyte dendritic processes, ranging from 40 to 100 per cell, pass through the bone matrix through narrow canals called canaliculi, approximately 250–300 nm in diameter (Fig. 1). The osteocyte lacunae and canaliculi are referred as the lacunar–canalicular system (LCS). A fluid travels through the lacunar–canalicular

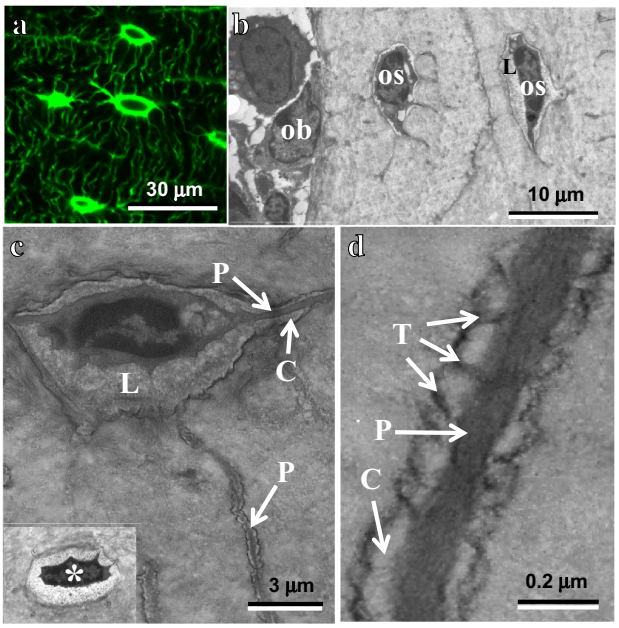

Fig. 1 Photomicrograph showing osteocyte network. **a** Confocal image showing an extensive network of osteocytes. **b–d** Transmission electron micrographs showing osteocyte lacunae (*L*), osteocyte processes (*P*) and canaliculi (*C*). Osteocyte (*os*) connects to other osteocyte and osteoblast (*ob*) at bone surface. Tethering elements (*T*) bridges osteocyte process to the canalicular wall. *Asterisk* apoptotic osteocyte

space and bathes the osteocyte, providing oxygen and nutrients to maintain the viability of the cell in this enclosed environment [3, 4].

Osteocyte dendritic processes in the LCS are surrounded by transverse tethering elements and are directly connected to the canalicular wall at discrete attachment sites that contain β_3 integrin, which were located immunohistochemically on the osteocyte processes [12]. Integrins are transmembrane proteins that link the cell's cytoskeleton to the extracellular matrix and are recognized for their key roles in mechanosensory transduction. When a bone is mechanically loaded, there are several possible stimuli that could be detected by the mechanosensory osteocyte. These include the physical deformation of the bone matrix itself, the load-induced flow of canalicular fluid through the lacunar–canalicular network, which results in fluid flow shear stress, or electrical streaming potentials that are generated from the flow of the canalicular fluid past the charged surfaces of the lacunar–canalicular walls and/or cell membrane. With regard to the temporal sequence of osteocyte responses to mechanical loading, one of the earliest events is the increase in intracellular calcium. Soon after this rapid change in calcium signaling, nitrite oxide (NO), ATP and prostaglandin are released. Deleting or inhibiting any one of these three small molecules will inhibit bone's anabolic response to loading [13].

Osteocytes form an extensive communication system via LCS (Fig. 1). Numerous dendritic processes connect osteocytes to other osteocytes, bone lining cells and osteoblasts [14], endothelial cells and hematopoietic cells near the endosteal surfaces. This characteristic morphology is important for the integration of local and global stimuli, and is essential for maintenance of osteocyte viability and normal function. Disruption of the network can have negative consequences for bone health. At the ends of the dendritic processes, osteocytes form gap junctions to enable them to communicate with one another. Gap junctions permit the direct intercellular exchange of ions, small molecules and second messengers. Gap junctions also act as direct conduits between the cytosol and extracellular fluid [15]. Gap junctions are made up of connexin (Cx) protein monomers. Six molecules of Cx assemble to form a hemichannel. Hemichannels from neighboring cells align to form gap junction channels that allow intercellular communication. Gap junction channels and hemichannels are closed under normal conditions but can open, allowing the passage of molecules from one cell to the other or from cells to the extracellular milieu. Cx43, encoded by the Gja1, is the most abundant gap junction protein expressed in bone. Gap junction channels comprised of Cx43 typically permit the passage of molecules smaller than 1 kDa, including inorganic ions, glucoses, amino acids, nucleotides and vitamins. Cx43 plays a critical role in osteocyte biology. Osteocyte utilizes Cx43 to signal among its network to regulate anabolic and catabolic responses and to maintain cell survival. Conditional deletion of Cx43 from late stage of osteoblast and osteocyte results in increased osteocyte apoptosis [16]. Cx43 can influence cell survival downstream of specific cues. Cx43 is required for anti-apoptotic action of bisphosphonates and PTH on osteocytes and osteoblasts [17].

Osteocyte apoptosis and bone remodeling

Osteocytes are long-lived bone cells, surviving for several decades. It was estimated that approximately 2.5 % osteocytes die each year and that the osteocyte lifespan is 1–50 years [18]. Evidence suggests that a normal rate of osteocyte death is required to maintain bone health. Accumulation of apoptotic osteocytes can be induced by removal of sex steroids, glucocorticoid excess, immobilization, and increased oxidative stress [19, 20]. Aging accelerates osteocyte death. During aging, osteocytes showed degenerative changes and the number of osteocytes declined [21]. Reduced osteocyte density with age might be the increased osteocyte apoptosis due to decreases in physical activity, reduction of the mechanical loading, increases in endogenous glucocorticoids and accumulation of reactive oxygen species in bone [22–24]. Age-related decreases in osteocyte density due to apoptosis could at least partially explain the bone loss with aging. Osteocytes can undergo apoptosis (Fig. 1), which may influence osteoblastic bone formation. It was reported that administration of diphtheria toxin not only induces a dramatic increase in apoptotic osteocytes, but also inhibition of osteoblast maturation [25]. Induction of osteocyte apoptosis disrupts the cell communication between apoptotic osteocyte and the neighbor osteocyte. Immunostimulatory molecules are proposed to be released from lacunae through canaliculi to the bone surface, promoting the production of proinflammatory cytokines, including TNF- α, IL-6 and IL-1 [26]. These cytokines inhibit osteoblast differentiation and maturation [27].

Osteocyte apoptosis has specific regulatory effects on osteoclastic bone resorption. Increased osteocyte apoptosis was shown to play an important role in regulating osteoclast activity. As apoptotic osteocytes were found to colocalize with areas of osteoclastic bone resorption in fatigue-damaged bone, the existence of a link between osteocyte apoptosis and bone resorption. Indeed, osteoclasts have been shown to engulf apoptotic osteocytes microscopically [28]. Apoptotic osteocytes in rats subjected to fatigue loading accumulate in areas where bone resorption occurs [29]. The colocalization of apoptotic osteocytes and osteoclasts was also found in a murine

model of reduced mechanical stimulation [30]. Accumulation of apoptotic osteocytes preceded the increase in osteoclasts, suggesting a cause–effect relationship between dead osteocytes and bone resorption [29, 30]. This relation was confirmed experimentally in which osteocyte apoptosis was blocked using caspase inhibitors, leading to a significant decrease in bone resorption following fatigue loading [31].

Osteocyte apoptosis is a principal trigger for osteoclast resorption (Fig. 2). Evidence for the causal link between osteocyte apoptosis and the initiation of bone resorption is now emerging. Apoptotic osteocytes release factors that instruct neighboring viable osteocytes to produce receptor activator of nuclear factor κB ligand (RANKL) that influences osteoclast differentiation and bone remodeling [32]. RANKL is one of the key molecules essential for osteoclast formation and activation (Fig. 2). RANKL interacts with its receptor RANK, which is expressed by osteoclasts and their precursors. Osteoprotegerin (OPG) is believed to function primarily as a decoy receptor, modulating interactions between RANKL and RANK [33]. The local increase in osteoclasts associated with apoptotic osteocyte is considered to be the result of loss of OPG-producing osteocytes due to cell death, and increased production of RANKL by neighboring viable osteocytes. However, the signals driving from apoptotic osteocytes to neighboring osteocytes have not been identified. One candidate to mediate the recruitment of osteoclasts is the high mobility group box protein 1 (HMGB1), which is released by apoptotic MLO-Y4 osteocytic cells [26]. HMGB1 stimulates the synthesis of RANKL, inhibits the production of OPG, and increases RANKL/OPG ratio to trigger osteoclast recruitment [26].

Endocrine function of the osteocyte

Sclerostin and bone remodeling

Osteocytes are able to sense and respond to mechanical stimulation by secreting several factors, including sclerostin, RANKL, FGF23, nitric oxide, insulin-like growth factors and prostanoids [3]. Since its initial description in 1958, sclerostin has emerged as an important osteocytic secretion with implications for a variety of bone diseases [34]. Sclerostin is a protein encoded by the SOST gene in osteocytes. Loss of function of the SOST gene in humans is associated with sclerosteosis and van Buchem disease [35, 36]. Sclerostin-deficient mice exhibit dramatically increased bone mass, while the overexpression of sclerostin decreases bone strength [37, 38]. Once sclerostin is secreted by osteocytes, it may be transported through the lacunar–canalicular network to the bone surface, where it inhibits osteoblastic bone formation [39].

Sclerostin acts as a link between osteocytic Wnt/β-catenin-mediated osteogenic response, mechanical stimuli and osteoblastic bone formation. One of the most important signaling pathways to regulate bone formation is the Wnt/β-catenin pathway. Wnts comprise a large family of secreted signaling glycoproteins that control cell

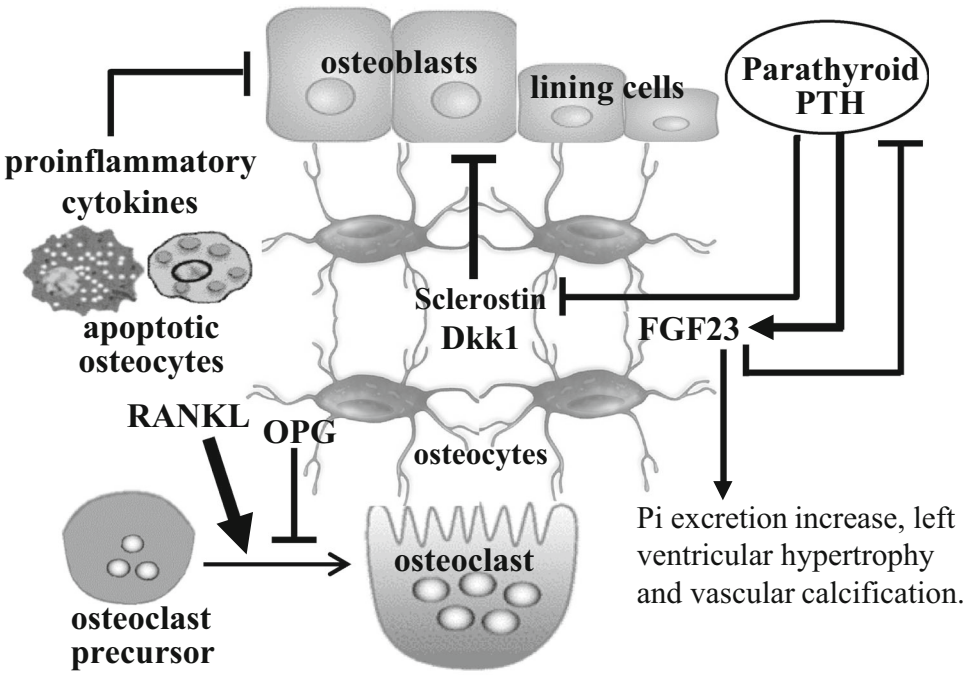

Fig. 2 Simplified diagram showing osteocyte endocrine signaling. Apoptotic osteocytes trigger osteoclast recruitment by increasing RANKL production. Osteocyte-derived RANKL and OPG regulate osteoclastogenesis. Sclerostin and Dkk1 derived from the osteocyte inhibit Wnt/b-catenin pathway, leading to suppression of osteoblast bone formation. FGF23 negatively regulates PTH release, while PTH inhibits sclerostin synthesis and activates FGF23 secretion. FGF23 down-regulates the expression of phosphate transporter NaPi2 and increases phosphate excretion. FGF23 is also associated with left ventricular hypertrophy and vascular calcification

proliferation, differentiation, apoptosis, survival, migration and polarity in a plethora of cell types [40]. Wnt/β-catenin signaling, which is also known as canonical Wnt signaling, is a key signaling pathway required for normal bone formation [41]. Canonical Wnt signaling is initiated by secreted Wnt ligands binding to a receptor-related protein 5 or 6 (Lrp5/6) and the seven-transmembrane domain receptor frizzled. This triggers a downstream signaling cascade leading to inhibition of cytoplasmic glycogen synthase kinase 3β (GSK3β), which in turn relieves β-catenin, the central mediator of canonical Wnt signaling. Wnt/β-catenin signaling stimulates the generation of osteoblasts by promoting commitment and differentiation of pluripotential mesenchymal stem cells toward the osteoblast lineage, while simultaneously suppressing commitment to the adipogenic lineage [42]. Wnt/β-catenin signaling promotes the progression of osterix1-expressing cells to bone-producing osteoblasts. In addition, Wnts prevent apoptosis of mature osteoblasts and thereby prolong their lifespan [43]. Sclerostin is the most potent and best recognized Wnt inhibitor (Fig. 2). It binds to members of a Wnt coreceptor family, the Lrp5/6, thereby limiting their ability to interact with Wnts and their coreceptor frizzled [44].

Osteocytes are sensitive to a variety of factors that change bone mass by altering the expression of sclerostin. Mechanical loading decreases sclerostin expression in association with increased bone formation [45, 46], whereas unloading increases osteocytic secretion of sclerostin and inhibits bone formation [45, 47]. Sex hormones are important regulators of osteocyte sclerostin expression. Compared with premenopausal women, sclerostin level was significantly higher in postmenopausal women [48]. Increased serum sclerostin level was negatively correlated with parathyroid hormone (PTH) and free estrogen index in postmenopausal population [49]. In postmenopausal women receiving estradiol treatment for 4 weeks, a significant decrease in serum sclerostin level was observed compared with a control group [48]. PTH is secreted by the parathyroid gland to act on bone in response to low circulating calcium levels [50]. PTH acts on osteocytes to inhibit sclerostin expression. The anabolic effect of intermittent PTH is possibly attributed to inhibition of sclerostin expression in osteocytes [51]. Sclerostin expression is also regulated by mediators of inflammation. Sclerostin expression was down-regulated by prostaglandin E2 and up-regulated by TNF-α [52, 53]. Sclerostin expression was increased in synovial tissue from rheumatoid arthritis patients [54], suggesting sclerostin may be potential biomarker of inflammatory disease and its progression. The pharmacologic inhibition of the sclerostin using monoclonal antibody has confirmed efficacy in various osteoporosis animal models induced by estrogen deficiency, glucocorticoid and disuse/immobilization [55, 56]. Effects of sclerostin monoclonal antibody have also been reported in human preclinical models of bone loss [57]. The use of sclerostin antibody has shown a consistent ability to increase bone formation, bone mass and bone strength [58]. A human anti-sclerostin antibody was developed and a phase 1 study was conducted. Antibody injection was associated with increases in bone formation and reduction in bone resorption.

Dkk1 and bone remodeling

Osteocytes also produce dickkopf 1 (Dkk1), another inhibitor of the Wnt signaling pathway that binds Lrp4 and Lrp5/6 coreceptors to inhibit canonical Wnt signaling and downstream bone formation [59]. Both sclerostin and Dkk1 can act synergistically to inhibit osteoblast activity. They do not bind simultaneously to Lrp5/6 coreceptors. Dkk1 can displace sclerostin from previously formed sclerostin-Lrp5 complexes [60]. The regulatory mechanism of Dkk1 expression is similar to that of the sclerostin. Mechanical loading decreases Dkk1 expression [46]. Glucocorticoids up-regulate Dkk1 expression and induce bone loss [61, 62]. A significant increase of Dkk1 expression was found in bones of mice treated with prednisolone for 56 days [61]. Interestingly, when mice were treated with prednisolone and PTH, the expressions of Dkk1 were significantly decreased and the glucocorticoid-induced bone loss was reversed compared with prednisolone- or placebo-treated mice [61]. In postmenopausal women on glucocorticoid therapy, the serum sclerostin level was higher, while the serum Dkk1 level was significantly lower compared with non-glucocorticoid-treated patients, suggesting that circulating Wnt inhibitors may be modulated by glucocorticoids in humans [63]. The pharmacologic inhibition of Dkk1 using monoclonal antibody has also demonstrated efficacy in animal models of bone disorders [64]. However, unlike sclerostin antibody, Dkk1 showed no efficacy in estrogen-deficient rats, and a modest improvement in estrogen-deficient rhesus monkeys [65]. Treatment of osteoporosis has advanced significantly, mainly owing to the increased understanding of the mechanisms underlying osteoblast, osteoclast and osteocyte biology. Novel agents, affecting osteocyte-associated sclerostin, calcium-sensing receptor, or Wnt signaling, offer promise for treating bone diseases.

FGF23 and phosphate homeostasis

Since its identification in 2000, FGF23 has emerged as one of the most important osteocyte-secreted endocrine factors [66]. Osteocytes are the main source of FGF-23, which is a key regulator, together with PTH, to regulate phosphate homeostasis via the bone–kidney axis (Fig. 2). Optimal

phosphate balance is important for many physiological functions from cell signaling to energy metabolism to skeletal mineralization. Inadequate phosphate balance disrupts a multitude of physiological processes, and can cause or exacerbate age-associated disorders, cardiovascular calcifications and bone mineralization defects [67]. Osteocytes synthesize and secrete FGF23, which acts on its receptor complex klotho-FGFR1 in its target organs, the renal tubules and the parathyroid gland [68]. In the kidney, FGF23 inhibits the phosphate transporters, NaPi2a and NaPi2c, resulting in a phosphaturia and low serum phosphorus level. Elevated circulating FGF23 level causes increased renal phosphate excretion and subsequent hypophosphatemia [69]. In patients with chronic kidney disease, the serum FGF23 levels were increased, particularly in the later stage of the disease [70]. FGF23 decreases PTH gene expression in the parathyroid gland, and hence serum PTH levels [71]. PTH is produced in response to low levels of serum calcium and secreted PTH acts on the bone and kidney to increase serum calcium level. Low serum calcium levels reduce calcium-sensor receptor signaling and allow active PTH to be secreted, which then binds to the PTH receptor 1, to activate the PKA, PKC, and MAPK pathways in kidney and bone. PTH increases the level of the nuclear orphan receptor, nuclear receptor-associated protein 1 (Nurr1) in the osteocyte. Nurr1 overexpression activates FGF23 promoter activity, and Nurr1 knockdown prevents the effect of PTH on FGF23. PTH activation of Nurr1 is essential for the effect of PTH to increase FGF23 levels [72]. Therefore, FGF23 and PTH mutually regulate each other in a negative feedback loop, where PTH stimulates FGF23 production and FGF23 in turn suppresses PTH synthesis. Several studies have demonstrated a possible link between FGF23 and cardiovascular function [73]. Elevated circulating FGF23 level was found to be associated with left ventricular hypertrophy, impaired vasoreactivity, increased arterial stiffness and vascular calcification [74, 75]. Treatment targeting FGF23 would appear to offer therapeutic potential for skeletal, urinary and cardiovascular homeostasis.

Conclusion

Recent research has greatly increased our knowledge of osteocyte biology. It is becoming clear that osteocyte is not merely a placeholder in the bone matrix, but also an important orchestrator of the bone remodeling. We now know better how osteocyte may trigger the essential signaling pathways to regulate osteoblastic bone formation and osteoclastic bone resorption. Thus, osteocytes represent an attractive target for development of therapeutic strategies to treat osteoporosis and other skeletal diseases. Osteocytes also possess the ability to influence the parathyroid gland, kidney and cardiovascular organs, indicating the importance of the endocrine function of osteocytes. The extensive connectivity of the osteocyte LCS with the blood vessels permits the signal transmission between osteocytes and other tissues. It is expected that other targets of osteocyte signaling will be discovered in the near future.

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