The Role of the Osteocyte in Bone and Non-bone Disease

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Synopsis

When normal physiological functions go awry, disorders and disease occurs. This is universal, even for the osteocyte, a cell embedded within the mineralized matrix of bone. It was once thought that this cell was simply a place-holder in bone. However, within the last decade, the number of studies of osteocytes has dramatically increased leading to the discovery of novel functions of these cells. But with the discovery of novel physiological functions came the discoveries of how these cells can also be responsible for not only bone diseases and disorders, but also those of kidney, heart, and potentially muscle.

Keywords

osteocyte; bone disease; sclerostin; FGF23; therapeutics

Introduction

Before osteocytes were recognized as active essential bone cells necessary for bone health, it was assumed that all the action took place on the bone surface and not within the bone. Osteoblasts and osteoclasts were the major players, osteoblasts making bone and osteoclasts resorbing bone to maintain bone homeostasis. It was assumed that osteoblasts and osteoclasts were regulated by external factors such as parathyroid hormone, PTH or 1,25 dihydroxyvitamin D3, and other external regulatory factors. It has also been proposed that osteoblasts make factors that regulate osteoclast activity and conversely that osteoclasts make factors that could regulate osteoblast activity. Therapeutics were generated that would target either osteoclasts or osteoblasts. Osteocytes were left out of the picture.

With new technology and new tools, it became possible to study osteocytes. The normal functions of osteocytes expanded rapidly to include: regulation of osteoblast and osteoclast activity to control bone remodeling, as regulators of both phosphate and calcium homeostasis, as mechanosensory cells that coordinate the skeleton's response to loading or

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unloading, and as endocrine cells targeting other tissues such as kidney. These cells are also one of the longest lived cell types in the body, some living for decades, therefore survival and normal function is paramount. (See box 1). A number of pathologic or disease conditions can now be ascribed to abnormal or missing osteocyte functions including sclerosteosis, hypophosphatemic rickets, osteoporosis, necrotic bone, aging and others. (See Figure 1). Now therapeutics are being generated that target osteocyte factors. (For reviews see[1,2])

Normal osteocyte functions

Mechanosensation

When early histomorphomists peered through their microscopes and began to visualize osteocytes in bone, the morphology and connectedness suggested a network perhaps similar to the neural system. One of the earliest functions ascribed to osteocytes was mechanosensation based on Julius Wolff's descriptions of the capacity of bone to adapt to mechanical loading or lack of loading by adding or removing bone[3]. Over a century later, experiments have been performed supporting the hypothesis that osteocytes are responsible for bone adaption in response to loading. By performing targeted deletion of osteocytes in mice expressing the diphtheria toxin receptor specifically in osteocytes, it was shown that these mice were resistant to unloading-induced bone loss[4].

The osteocyte and its dendritic processes are constantly exposed to canalicular fluid that flows through the lacunocanalicular system. A baseline flow of canalicular fluid flow is driven by the extravascular pressure and intermittent mechanical loading superimposes rapid alterations in canalicular fluid flow[5]. This results in the cells being exposed to different types and magnitudes of fluid flow shear stress. Almost every cell responds to mechanical loading, however osteocytes appear to be most sensitive when compared to osteoblasts and fibroblasts[6,7].

In both primary osteocytes and MLO-Y4 osteocyte-like cells, fluid flow shear stress has been shown to have numerous sequential effects[2]. The first event is the release of intracellular calcium followed by the release of nitric oxide, ATP and prostaglandins, and the opening of connexin 43 hemichannels enhancing gap junction functions. Soon after the rapid change in calcium signaling (within seconds) nitric oxide, ATP, and prostaglandin (within seconds to minutes) are released. Deleting any one of these three early small molecules will inhibit bone's anabolic response to loading. Shear stress has also been shown to induce the bending of osteocyte cilia, and to initiate signaling pathways such as the wnt/β-catenin and PKA pathways. Shear stress also activates gene transcription and translation, and promotes dendrite elongation. One very important effect of fluid flow shear stress is the protection of osteocytes against apoptosis and cell death. Ideally, it would be important to identify early regulators of anabolic signaling in osteocytes in addition to calcium, nitric oxide, ATP, and PGE_2 in order to develop new therapeutics. (For review see[8].

A major source of prostaglandin in the body appears to be from bone. Osteocytes are prodigious producers of prostaglandin in response to loading[9], PGE_2 has paracrine effects on osteocytes to enhance gap junction function[10], it protects and maintains osteocyte viability[11]

and PGE_2 appears to be one of the key initiators of anabolic bone formation. Administration of prostaglandin increases bone mass and inhibitors of prostaglandin production, such as indomethacin, block the effects of anabolic loading[12,13]. One of the most important effects of prostaglandin released in response to loading may be to activate a very important signaling pathway in the osteocyte, the Wnt/β-catenin signaling pathway.

Fluid flow shear stress activates the Wnt/β-catenin signaling pathway through the rapid release of prostaglandin which acts through EP receptors to bypass Low Density Lipoprotein receptor, LRP receptor, activation[14,15]. Components of the β-catenin pathway are essential for osteocyte viability, mechanosensation and transduction, and release of important factors essential for bone homeostasis. The central molecule through which all molecules must go is β-catenin. β-catenin regulates expression of both the positive activators of this pathway, the wnts, and the negative regulators of this pathway, sclerostin and Dkk1 (For review see[16]). Global deletion of b-catenin is embryonically lethal, but deletion in osteocytes using the Dmp1-Cre results in dramatic bone loss characterized by perforated cortises[17]. Interestingly, deletion of only one allele in osteocytes results in mice with a normal skeleton but a completely abrogated response to anabolic loading[18]. β-catenin plays an important role in bone integrity, osteocyte communication, osteocyte viability, but also in bone response to loading. This extends to other components of this signaling pathway.

Two of the most famous and well-studied components of this pathway are the Lrp5 receptor and the negative regulator of the β-catenin pathway, sclerostin encoded by the gene *Sost*. Lrp5, a major co-receptor for Wnt signalling is expressed by many cells in the body, but sclerostin is relatively osteocyte specific. Deletion of Lrp5 results in mice with impaired response to anabolic loading[19]. As Sclerostin is expressed in mature osteocytes and mechanical loading reduces sclerostin levels downregulation of sclerostin most likely creates a permissive environment in which Wnt proteins already present can activate the Wnt/β-catenin pathway. The role of the β-catenin pathway in disease is discussed below.

It has been shown that osteocyte specific or selective genes are regulated by loading or unloading. These genes include the markers for early osteocytes, E11/gp38, Phosphate Regulating Neutral Endopeptidase on Chromosome X, (PHEX), Dentin Matrix Protein 1, (DMP1), and the markers for late osteocytes, sclerostin, Matrix Extracellular phosphoglycoprotein, (MEPE), and Fibroblast Growth Factor, (FGF23). (The function of these and their relationship to disease will be discussed below.) Regulators of mineralization and phosphate homeostasis such as Phex, MEPE, and DMP1 are upregulated in response to mechanical loading[20–22] as is E11/gp38, a marker for the early osteocyte[23]. It would be expected that genes involved in bone formation would be upregulated in response to anabolic load and genes responsible for resorption would be downregulated. It has been shown that unloading increases RANKL, an essential promoter of osteoclast formation, in osteocytes[24], that may be responsible for the bone loss associated with unloading. *Sost*/sclerostin, a marker for the late osteocyte and an inhibitor of osteoblast function, is down-regulated by anabolic mechanical loading and is increased in response to hindlimb unloading[25,26].

In summary, the osteocyte's response to mechanical loading may be one of the major cellular mechanisms responsible for the positive effects of exercise not only on bone but on the function of other tissues and organs in the body.

Calcium homeostasis

Probably the earliest proposed function of osteocytes was a capacity to remove their perilacunar matrix, a process referred to by Belanger as 'osteocytic osteolysis'[27]. In 1910, over 100 years ago, Von Recklinghausen described enlarged lacunae in patients with rickets or osteomalacia suggesting 'pericellular digestion'[28]. Belanger created the term "osteocytic osteolysis" for the enlarged lacunae induced by parathyroid hormone or a by low-calcium diet. 'Osteocytic osteolysis' was viewed as being a feature of pathological conditions, especially due to high or continuous PTH.[27,29,30]. The stimulating effects of parathyroid hormone on lysosomal vesicles in osteocytes was described in the 1970s[31,32] and in 1977, "perilacunar osteolysis" was described in rats sent into space[33] and alveolar bone of hibernating ground squirrels[34]. However, the number of publications began to decrease for various reasons[35] until technology had advanced sufficiently to address critics of this concept. Baylink and Wergedal had described Tartrate Resistant Acid Phosphatase, TRAP, activity in osteocytes in 1969[36], which was criticized as being a diffusion artifact from osteoclasts but later validated by *in situ* hybridization, a technology no available in the 60s and 70s[37].

Baylink also showed tetracycline binding to the perilacunar matrix, which led to the hypothesis that osteocytes can replace their perilacunar matrix[38] which was later reproduced in egg-laying hens[39]. This suggested that under non-pathological conditions osteocytes could remove and replace their perilacunar matrix. Qing and colleagues proposed that the term 'perilacunar modeling' be used in place of 'osteocytic osteolysis' for non-pathological conditions such as lactation[40]. These investigators showed an increase in lacunar area with lactation, that the PTH type 1 receptor was responsible and described a return to normal lacunar area with weaning. They showed that genes thought to be osteoclast specific such as TRAP and Cathepsin K were elevated in osteocytes during lactation and returned to normal with weaning. This study shows that *healthy* osteocytes can both remove and replace their perilacunar matrix thereby playing a role in mineral homeostasis during calcium demanding conditions. Recently it has been shown that the Calcitonin Receptor may also play a role by inhibiting perilacunar remodeling with lactation[41].

As the PTH type 1 receptor is most highly expressed in osteocytes, the osteocyte may be the target of PTH in hyperparathyroidism and conversely, the positive effects of intermittent PTH on bone formation may also be due to effects on the osteocyte. The target of the therapeutic *Forteo* may be osteocytes in the mature skeleton.

Bone Repair

Repair of microdamage and bone fatigue in bone is a normal, physiological process. Bone is constantly sustaining damage in the form of microcracks that is repaired by osteoclasts targeting the damaged bone. Osteoclasts are responsible for initiating a cutting cone, but how does the osteoclast know the location of the microdamage? It appears that the osteocyte

sends signals to the osteoclast providing information on where to resorb and where not to resorb bone (For review see[42]). Microdamage and bone fatigue is associated with of osteocyte apoptosis where an anti-apoptotic factor, BAX, is found in osteocytes around the cutting cone, while the pro-apoptotic factor, Bcl2, is found in osteocytes in the path of the cutting cone[43]. The suggests that the osteocytes in the path are undergoing programmed cell death, while those in the periphery are preserving viability[44]. A number of *in vitro* studies using MLO-Y4 osteocyte-like cells have investigated potential mechanisms. Apoptotic bodies are released by MLO-Y4 cells and primary osteocytes, but not osteoblasts[45], serum starved MLO-Y4 cells will secrete soluble RANKL which is necessary for osteoclast formation[46], and damaged MLO-Y4 cell networks in 3 dimensional gels express elevated RANKL and lower osteoprotegerin, OPG, an inhibitor of the RANK receptor[47].

Pathological osteocyte cell death is associated with thiazolidinediones[48], high dose alcohol[49], and methotrexate used for cancer treatment[50]. Osteocytes express markers of apoptosis in response to withdrawal of estrogen[51], to oxygen deprivation as occurs during immobilization[52], and in response to glucocorticoid treatment[53]. TNFα and Interleukin-1 (IL-1) are potent inducers of osteocyte apoptosis[54]. Osteonecrosis, or dead bone, is due to osteocyte cell death but the mechanisms responsible are still debated. Aging is associated with increased numbers of empty osteocyte lacunae (See below). Therefore, a major research focus has been on osteocyte viability and approaches to prevent osteocyte cell death.

Osteocytes are endocrine cells

Potentially osteoblasts have the capacity to release factors into the circulation, but they compose approximately 3–5% of bone cells compared to 1% osteoclasts, whereas 90–95% of bone cells in the adult human skeleton are osteocytes. It has not been appreciated that the total mass of osteocytes and their dendritic processes in bone that are equivalent to or greater than the mass of the brain[55], therefore these cells are most likely a major source of circulating bone factors. Bone is highly vascularized and secretes factors such as FGF23 into the bloodstream to affect distant targets[56], it must be defined as an endocrine organ[2]. Interestingly, FGF23 is also able to act on the parathyroid gland to decrease PTH secretion, identifying the parathyroid gland as another endocrine target of osteocyte signaling[57,58]. The vascular system has a close, connecting association with the osteocyte lacuna-canalicular system with its bone fluid. Osteocytes also produce other circulating factors such as sclerostin. Osteocytes may also target muscle (See below). It has recently been shown that two factors, prostaglandin E$_2$ and Wnt3a, both produced by osteocytes in response to shear stress support myogenesis and muscle function[59–62]. Therefore, mechanical loading of the skeleton especially in the form of exercise is important to ensure that osteocyte factors are released into the circulation.

In addition to cross talk with muscle, osteocytes may also send signals to hematopoietic cells. Studies showed that osteocytes and GPCR signaling were important in controlling myeloid cells proliferation[63] and mice lacking osteocytes were shown to have defective hematopoietic stem cell mobilization and lymphopenia[64,65]. Osteocyte may also have a role in regulating fat. Using a mouse model in which osteocytes can be ablated by use of

diphtheria toxin, it was shown that osteocytes may also regulate adipose tissue[65]. Studies have shown that sclerostin may play a role in inducing adipocyte differentiation[66]. Osteocytes may be an important reservoir of factors that target other unknown organs and tissues.

Role of osteocytes in bone disease

Osteoporosis

Estrogen deficiency, glucocorticoid treatment, oxidative stress caused by disuse and oxidative stress with aging may be responsible for osteocyte cell death and therefore bone fragility and osteoporosis[67]. As described above, osteocyte cell death is important for repair of damaged bone[43,44,68] so any condition that compromises osteocyte health and function most likely compromises the skeleton. A number of factors and cytokines have been shown to induce osteocyte cell death including glucocorticoids, IL-1 and TNFalpha and conversely, a number of molecules have been shown to protect osteocytes from cell death such as estrogen, Parathyroid Hormone, bisphosphonates (For review see[69–71]), and secreted muscle factors[59]. Osteocyte viability is crucial not only for the normal functioning of the skeleton but for other organs such as kidney as discussed above and muscle as discussed below. As osteocytes appear to have multiple, very important functions, it is important to maintain normal viability and function of these cells[2].

Aging

The osteocyte is a long lived, non-dividing, aging cell headed for senescence. While some osteocytes are removed from bone with remodeling many osteocytes can reside in human bone for decades in contrast to osteoblasts and osteoclasts that live for only days or weeks. Recently it has been shown that primary osteocytes from old 22 mo mice have 6 fold more telomere dysfunction-induced foci than osteocytes from young 6 mo old mice[72]. Senescent osteocytes predominately develop the Senescence Associated Secretory Phenotype, SASP, compared to other bone cells types which may contribute to age related bone loss. Once the cell dies, micropetrosis can result where mineral fills the lacuna resulting in a cell that becomes a 'living fossil'[73]. Fewer numbers of osteocyte lacunae were found in patients suffering from fractures compared to controls[74] and an age-dependent decrease occurs in osteocyte lacunar density with an increased amount of hypermineralized calcium phosphate occlusions caused by micropetrosis[75]. The aging and dying osteocyte in a compromised lacuno-canalicular system is less likely to produce secretory factors, less likely to repair bone, and less likely to respond to anabolic load. Therefore, it is important to maintain osteocyte viability with age.

Hypophosphatemic Rickets

Several phosphate regulating hormones and enzymes, Phosphate Regulating Neutral Endopeptidase on Chromosome X, PHEX, Dentin Matrix Protein 1, DMP1, Matrix Extracellular phosphoglycoprotein MEPE and Fibroblast Growth Factor 23, FGF23, are mainly produced by late osteoblasts and by early and late osteocytes. PHEX is the earliest regulator of phosphate homeostasis to be expressed in the late osteoblast/early osteocyte. PHEX is the mutated gene in the Hyp mouse, which is also widely used as a model of X-

linked hypophosphatemic rickets[76] which leads to increased levels of FGF23 and hypophosphatemia[77]. Hypophosphatemic Rickets in humans is caused by inactivating mutations of *Pex (HYP Consortium Nat Gen 1995)*. PHEX also interacts with DMP1 to regulate phosphate homeostasis by mechanisms that still have not been clearly elucidated. DMP1 is produced by early osteocytes and mice lacking DMP-1 are hypophosphatemic and have increased FGF23 levels[56]. Two teams of investigators independently showed that Autosomal Recessive Hypophoshatemic Rickets (ARHR) was caused by a mutation in DMP1 that affected FGF23 circulating levels[56,78]. Both PHEX and DMP1 are negative regulators of FGF23 but again the mechanisms have not been clearly determined. MEPE is predominantly expressed by osteocytes[79] and is not believed to act on FGF23 directly but through Phex[80]. The ASARM peptide of MEPE can bind to PHEX inhibiting its activity which results in an increase in FGF23[80,81].

The molecule at the center of phosphate regulation is FGF23. FGF-23 was identified in 2000[82] as the phosphate-regulating hormone responsible for Autosomal Dominant Hypophosphatemic Rickets (ADHR), and for phosphate-wasting in Tumor-Induced-Osteomalacia (TIO) and X-linked hypophosphatemia (XLH). Osteocytes are also a main source of FGF23 and recent work showed that targeted ablation of FGF23 in bone cells, recapitulates the hypophosphatemia observed in FGF23 null mice[83]. Clinkenbeard[84,85] targeted FGF23 deletion using Col2.3-cre in osteoblasts and using DMP1-cre in early osteocytes and showed that most likely both osteoblasts and osteocytes are the physiological source of FGF23. FGF23 is not normally expressed at high levels in osteocytes in the healthy state but is dramatically upregulated in both DMP1 and PHEX associated hypophosphatemic rickets[77] and osteocytes appear to be the main source of the elevated circulating levels of FGF23.

Genetic High and Low Bone Mass Diseases

Many of the mutations resulting in high or low bone mass are due to mutations in components of the wnt/b-catenin signaling pathway. One of the most well known are mutations in SOST which is highly expressed in mature osteocytes, but also expressed in articular chondrocytes (Hinton, 2009, Chan et al,2011). As stated above, the protein, sclerostin, is an inhibitor of bone formation and the gene, *Sost* is increased in response to unloading, decreased in response to loading, but also decreased in response to PTH[86]. Patients with sclerosteosis carry a point mutation in the SOST gene whereas patients with van Buchem disease are characterized by a 52kb deletion downstream of the gene[87]. Recently, craniodiaphyseal dysplasia, a rare and severe bone dysplasia characterized by sclerosis of the skull and facial bones has also been linked to a "de novo" mutation in the SOST gene[88]. The pathological role of mutations in sclerostin has been reproduced in knock-out and transgenic animal models that also show the high-bone mass phenotype of sclerosteosis and van Buchem patients[26,89]. Sclerostin binds to low-density lipoprotein (LDL)-related protein 5, 6 and 4 to inhibit Wnt/β-catenin signaling[90].

Genetic mutations in the receptors for sclerostin have also been shown to result in bone disease. Loss-of-function of LRP5 results in the condition Osteoporosis Pseudoglioma, OPPG[91]. The condition is homozygous recessive and affected individuals had a Z-score of

−4.7. Conversely, gain-of-function of LRP5 results in highly increased bone mass[92]. The condition is autosomal dominant and individuals had BMDs 5–8, yet had normally shaped bones. These individuals never broke bones but could not float in water. Mutations in Lrp4 have also resulted in bone overgrowth[93]. Genetically modified mice have replicated these human phenotypes.

Role of osteocytes in non-bone disease

Chronic Kidney Disease

FGF23 secreted from osteocytes plays a pathological role in Chronic Kidney Disease, CKD. FGF23 is elevated in osteocytes in CKD[94] and serum levels of FGF23 are increased, particularly in the later stages of the disease[95,96]. FGF23 levels predict cardiovascular events before but not after dialysis and FGF23 is a risk factor for adverse outcomes in patients with CKD and End Stage Renal Disease (for review see[97]). Therefore, considerable effort is being put towards blocking or reducing FGF23 levels in these patients.

Cardiac Function

High circulating levels of FGF23 have negative effects on cardiac muscle (For review see[98]). Elevated levels of circulating FGF23 have been linked to increased risk of heart disease and independently associated with left ventricular hypertrophy in human population studies[99,100]. Increased serum FGF23 has also been linked with impaired vascular function[99], vascular calcification[101] and increased fat mass[102].

Sarcopenia?

It is not clear if osteoporosis and sarcopenia are concurrent or if one precedes the other. Dogma has been that the main interaction between muscle and bone is the mechanical loading of bone through muscle contraction. Osteocytes secrete factors that regulate muscle mass and function. MLO-Y4 osteocyte-like cells and primary osteocyte factors induce muscle myogenesis and activate the Wnt/βcatenin pathway[6061]. Two factors produced by osteocytes in response to shear stress, PGE_2 and Wnt3a, were found to enhance myogenesis and ex vivo primary muscle function[62]. The hypothesis that osteocytes can support myogenesis and muscle function is now supported by several lines of evidence.

Very recently, *in vivo* data has been published to support the concept that bone regulates muscle mass and function. In 2015 it was shown that osteocalcin partially restored muscle mass in a model of deletion of Cx43 in osteocytes[103]. In 2016 it was shown that osteocalcin can have positive effects on muscle mass[104]. Also in 2015, it was shown that cancer induced release of Transforming Growth Factor beta, TGFβ from bone was responsible for muscle cachexia[105]. That same year it was shown that deletion of a protease, MBTPS1, in osteocytes had little effect on bone but significantly increased muscle mass and function with aging[106]. A reduction in members of the TGFβ superfamily was observed. This suggests that bone produces osteocalcin which has positive effects on muscle, but also that bone and in particular osteocytes can produce negative regulators of muscle. It is not known if osteocyte dysfunction will play a role in sarcopenia.

Conversely, muscle also appears to secrete factors that affect osteocyte viability and function. In 2007 Pedersen coined the term 'myokines,' for muscle secreted factors opening the door to new concepts regarding muscle interaction with bone. Conversely, secreted factors from electrically stimulated skeletal muscle and from myotubes but not from myoblasts was shown to protect MLO-Y4 osteocytelike cells from dexamethasone induced cell death[107]. Other muscle factors are being identified that have positive effects on bone.

Therapeutics targeting osteocyte factors

Anti-sclerostin antibody

In animal studies, anti-sclerostin antibody has consistently been shown to increase bone mass. Inhibition of sclerostin by monoclonal antibody increases bone formation, bone mass, and bone strength in aged male rats[108]. Neutralizing antibody to Sclerostin is being developed as a therapeutic to treat osteoporosis (For review see[109]). The antibody blocks or reduces bone loss and supports bone formation-promotes fracture healing[110] and is a potential therapeutic for a number of conditions of low bone mass such as OI[111]. Romosozumab (AMG 785) has been tested in Phase 1 and II and Blosozumab has been tested in Phase 1 clinical trial with good results. These antibodies increase bone mass to a greater extent than any previously developed therapeutic for osteoporosis including bisphosphonates, Forteo, and Denosumab. Phase III clinical trials in progress.

Anti-RANKL antibody

MLO-Y4 osteocyte like cells support osteoclast formation[112] and apoptotic bodies released from MLO-Y4 cells express RANKL[113]. Primary osteocytes express RANKL[114]. Osteocytes express greater amounts of RANKL than osteoblasts and are better supporters of osteoclast formation[115,116]. Deletion of RANKL using the 10kb Dmp1-Cre results in mice with increased bone mass. This suggests that anti-RANKL antibody is mainly targeting osteocytes. Human anti-RANKL monoclonal antibody, Denosumab, is now available for treatment of osteoporosis. The antibody decreases osteoclast differentiation, function and survival. It reduces risk of spine, hip and nonvertebral fractures and does not require dose adjustment for decreased kidney function. For treatment of osteoporosis, SQ dosing every 6 months is applied and the effect is reversible within 6–12 months of stopping[117].

Anti-FGF 23 antibody

There are several conditions that could potentially benefit from treatment with anti-FGF23 antibody. In bone, Autosomal dominant hypophosphatemic rickets, caused by gain of function mutations in FGF23 that prevent proteolytic cleavage[118] and homozygous mutation in FAM20, a regulatory molecule of FGF23, resulting in hypophosphatemic osteomalacia[119] may benefit from anti-FGF23 antibody. In kidney, as FGF23 is elevated in osteocytes[94] and in serum in Chronic Kidney Disease, CKD[95,96], these would also benefit from reducing FGF23 circulating levels. In heart disease, studies have linked raised levels of circulating FGF23 to an increased risk of heart disease, left ventricular hypertrophy in human population studies[100,120], impaired vascular function, vascular calcification, and increased fat mass[101]. All of these conditions could potentially benefit from FGF receptor inhibitors and anti-FGF23 antibody. Treatment with FGF23 antibody restored serum phosphate levels

and corrects bone defects in the Hyp mouse and Dmp1 null model[121] and treatment with anti-FGF23 antibody KR23 increases serum phosphate in X-linked hypophosphatemic rickets[122]. (For review see[123]).

Other therapeutics to treat bone disease

Could other therapeutics targeted to either osteoclasts or osteoblasts be also having effects on osteocytes? The is a distinct possibility. Calcitonin, bisphosphonates (Fosamax, Boniva, etc.), Anti-RANKL (Denosumab) and Cathepsin K inhibitors (odanacatinb) have been developed to target osteoclasts. However, bisphosphonates have also been shown to reduce osteocyte apoptosis[69], osteocytes can also express Cathepsin K under certain conditions[40], and as shown above, the anti-RANKL antibody may be targeting osteocytes. Hormone replacement therapy, Selective Estrogen Receptor Modulators (Evista), and Parathyroid Hormone peptides (Forteo) have been thought to mainly target osteoblasts, but these could also be having significant effects on osteocytes.

Are there other osteocyte factors?

Much attention has focused on osteocalcin produced by osteoblasts. This bone specific factor has been shown to have effects on glucose metabolism, fertility, calcification and others[124]. The osteoblast has been ascribed the production of osteocalcin and the osteoclast as the releaser of uncarboxylated, the 'active' form of osteocalcin from the bone matrix to target other tissues. However, in the adult skeleton, osteoclasts make up less than 1% of bone cells, and osteoblasts less than 5%. Osteocytes have been shown to also produce osteocalcin so it will be interesting to see if osteocalcin production by osteocytes has a role in normal physiology and pathophysiology. Gene arrays have been performed on primary osteocytes and osteocyte cell lines. As with any gene arrays analysis, it is difficult to identify specific genes. It is likely that there are osteocyte factors responsible for normal function that may also play a role in disease that have not yet been identified.

Summary

When normal physiological functions go awry, disorders and disease occurs. This is universal, and as discussed here, even for the osteocyte, a cell thought to not be in contact with the rest of the body. It was once thought that this cell was simply a place-holder in bone. The early functions proposed for this type of bone cell were mechanosensation and the capacity to remove their perilacunar matrix called "osteocytic osteolysis". Considerable skepticism existed even for these ascribed functions for decades. Within the last decade, the number of studies of osteocytes has dramatically increased leading to the discovery of novel functions of these cells. Along with these discoveries came the discoveries of how these cells can also be responsible for not only bone diseases and disorders, but also those of kidney, heart, and potentially muscle. Osteocytes have entered the realm of therapeutic targets for bone disease and now potentially kidney disease. It will be important not to overlook these cells with regards to the health of other systems and organs.

Acknowledgments

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Key Points

- Within the last decade, the number of studies of osteocytes has dramatically increased leading to the discovery of novel functions of these cells.

- Along with these discoveries came the discoveries of how these cells can also be responsible for not only bone diseases and disorders, but also those of kidney, heart, and potentially muscle.

- Osteocytes have entered the realm of therapeutic targets for bone disease and now potentially kidney disease.

- It will be important not to overlook these cells with regards to the health of other systems and organs.

Box 1

Normal Functions of Osteocytes

➤ Control mineralization through **Phex**[126], **Dmp1**[56], and **MEPE**[127,128]

➤ Regulate phosphate homeostasis through **FGF23**[56,129]

➤ Play a role in calcium homeostasis in response to PTH/PTHrP[40,130]

➤ Can recruit osteoclasts through expression of **RANKL** with or without cell death[112,115,116]

➤ Can regulate osteoblast activity through **Sclerostin**[131,132]

➤ Are mechanosensory cells through β-catenin signaling[133,134]

➤ Have autocrine/paracrine effects through **prostaglandin** production[135–137].

➤ Under calcium restriction, osteocytes remove calcium from bone through the Vitamin D Receptor[138]

➤ Osteocytes regulate myelopoiesis/hematopoiesis through **G-CSF**[63]

➤ **G-CSF** targets osteocytes that mediate mobilization of Hematopoietic Stem/Progenitor Cells and is prevented by surgical sympathectomy[64]

➤ Osteocytes regulate primary lymphoid organs and fat metabolism[65]

➤ Osteocytes can dedifferentiate to become a source of matrix-producing osteoblasts[139]

➤ Can increase muscle myogenesis and muscle function[60,62,140] and can inhibit muscle mass with aging[141]

➤ Can have effects on heart[123,142] and liver[143] through FGF23.

➤ Play a role in fracture healing through IGF-1[144,145]

➤ Regulate bone formation through Bmpr1a signaling[146], Notch activation[147], and ERα signaling[148,149]

➤ Suppress breast cancer growth and bone metastasis[150]

Data from references 1, 2, 125.
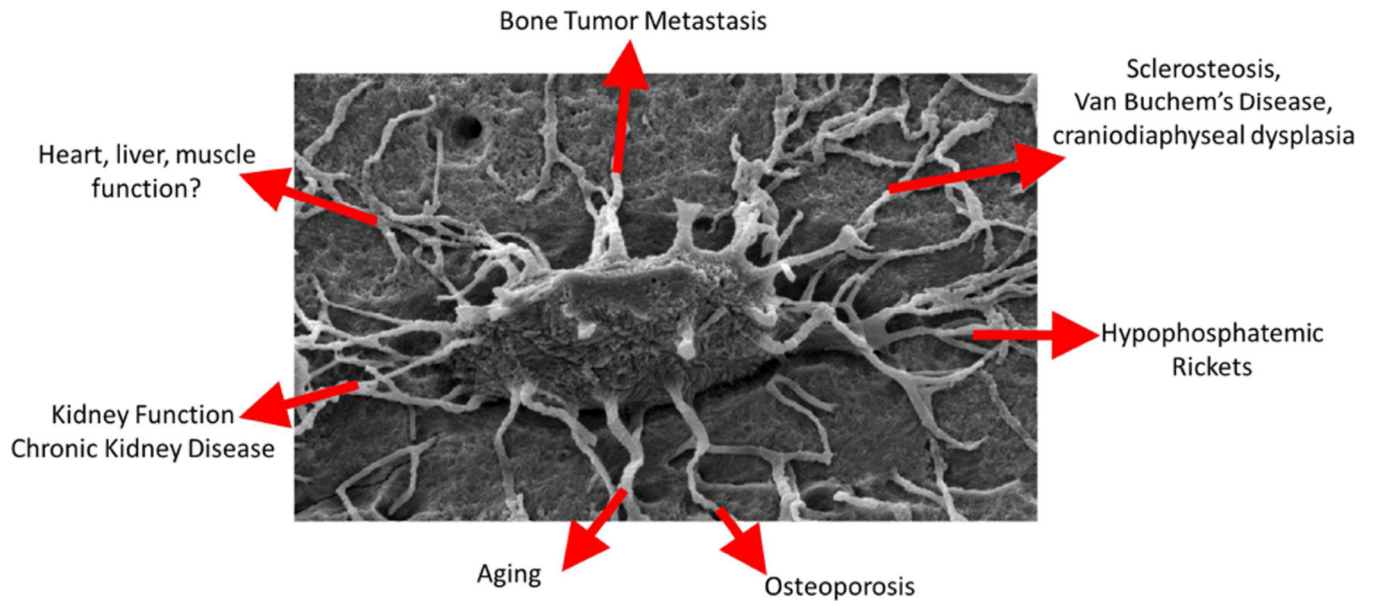
Figure 1.
Defective Osteocyte Function and Disease.