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PERSPECTIVES

Regulation of Bone Resorption and Mineral Homeostasis by Osteocytes

Paola Divieti Pajevic

Endocrine Unit, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts, USA

Abstract

Osteocytes are the most abundant cells in bone, but their relative inaccessibility and (until recently) a lack of good *in vitro* and *in vivo* models have impeded progress in understanding their functional roles. Compelling evidence points to a mechanosensory function whereby osteocytes regulate bone modeling and remodeling in response to shear or strain forces, but their possible role in calcium and phosphate homeostasis is less clear. Moreover, recent work indicates that the ablation of osteocytes leads to the development of osteoporosis, suggesting that these cells are involved in normal bone development and/or remodeling. Several paracrine and endocrine factors, notably parathyroid hormone (PTH), prostaglandins, glucocorticoids and estrogen, have emerged as potential regulators of osteocyte function(s) and/or survival; furthermore, osteocytes synthesize and/or secrete proteins, such as FGF23, PHEX, MEPE and DMP1, that are of critical importance for the regulation of phosphorous homeostasis. As our understanding of these cells expands, it has become clear that they play a critical role in regulating and maintaining normal skeletal and mineral homeostasis. This *Perspective* reviews osteocyte function(s) and the role of osteocytes in bone remodeling and mineral homeostasis. *IBMS BoneKEy*. 2009 February;6(2):63-70.

Introduction

Osteocytes are the most abundant cells in bone, yet their functions have long eluded scientists and bone biologists. From an evolutionary point of view, osteocytes have been present for a very long time. The structure of bone, with its complex osteocytic organization within the mineralized matrix, is very old. Stellate-shaped osteocytic lacunae were present in bones of jawless fish (400 to 250 million years ago) and dinosaurs (80 million years old) (1). This evolutionarily highly conserved structure is strongly suggestive of a central role for osteocytes in bone homeostasis.

Osteocytes derive from osteoblasts that, during the process of mineralization, remain entrapped in the matrix that they are actively synthesizing. During the transition from cuboidal osteoblast to mature ellipsoid osteocyte, the cell gradually loses organelles and its capacity to synthesize extracellular matrix. The nuclear-to-cytoplasmic ratio increases and the cell acquires a number of osteocyte-specific characteristics, including its typical stellate morphology (Fig. 1). The signaling and molecular mechanisms regulating this process are largely unknown.

Osteocytes act as mechanosensors of bone and recent evidence has also indicated a role for these cells in bone modeling and remodeling (2) and mineral homeostasis (3-5). It has been recognized for over a century that mechanical loading is fundamental for bone health and that reduced loading (as in prolonged bed rest or space flight) is invariably associated with bone loss. Exactly how the osteocyte can sense the mechanical forces applied to the bone and then transform this mechanical stimulus into biological signals remains incompletely understood. Studies on osteocytes have been hampered by the inaccessibility of these cells and by the lack of molecular and cell surface markers that could be used to isolate and characterize them. In the last decade, however, our knowledge about osteocytes has expanded dramatically,

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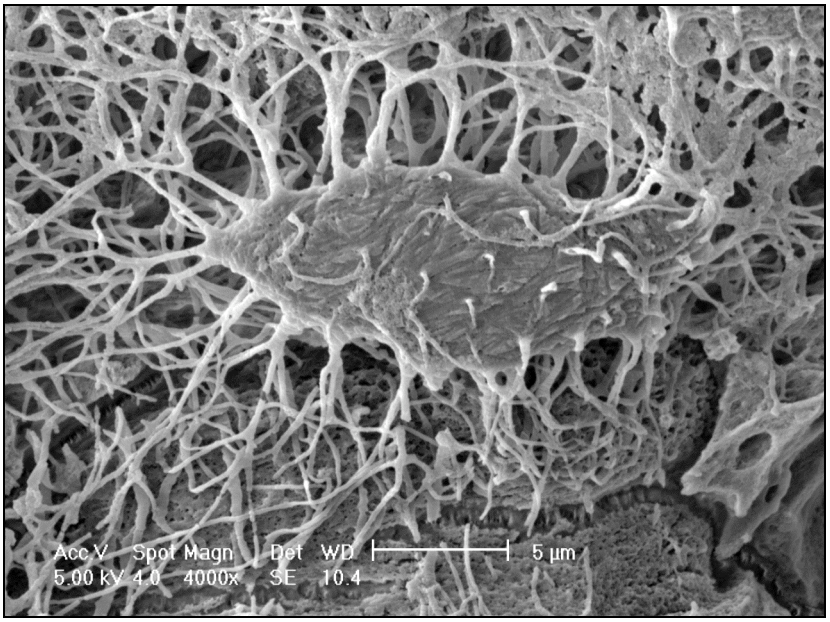

Fig. 1. Scanning Electron Microscopy (SEM) image of an acid-etched, resin-casted, osteocyte-canalicular system from 4-month-old mouse long bone. Note the extensive canalicular system of the cell. This image was kindly provided by Dr. Lynda Bonewald.

mostly as a result of three factors: 1) the identification of several specific osteocytic markers, such as dentin matrix protein 1 (DMP1) (6-8), matrix extracellular phosphoglycoprotein/osteocyte-factor 45 (MEPE/OF45) (9;10), membrane-type matrix metalloproteinase (MT1-MMP), Phex (phosphate regulating gene with homologies to endopeptidases, on the X chromosome) (11;12) and sclerostin (13-15); 2) the identification of disorders of phosphate homeostasis that are caused by mutations in some of these genes; and 3) the isolation and characterization of several osteocytic cell lines, such as HOBIT-1, MLOY-4 (16) and our own cell lines OC14 and OC59 (17) that have allowed, for the first time, more direct analysis of the molecular and cellular biology of osteocytes.

Osteocyte Regulation of Bone Formation and Resorption

Do osteocytes control bone formation and resorption? To answer this question, this *Perspective* will review scientific evidence that ascribes to these cells an important role in regulating skeletal homeostasis mostly by directing the activity of osteoblasts and osteoclasts and by controlling their surrounding milieu. Osteocyte apoptosis and microdamage, osteocyte ablation and its consequences, and finally osteocytic osteolysis are discussed below.

Osteocyte Apoptosis and Microdamage

The osteocytic network, with its highly organized and extensive canalicular system (Fig. 1), is an ideal structure to sense mechanical loading. It has been postulated that osteocytes can send signals of both bone resorption and formation and thus orchestrate a proper cycle of remodeling.

Several studies have demonstrated that osteocyte apoptosis is an early event during bone fatigue-induced microdamage and subsequent intracortical remodeling. Specifically, osteocytes surrounding fatigue microcracks in bone undergo apoptosis, and those regions containing apoptotic osteocytes co-localize precisely with areas subsequently resorbed by osteoclasts (18;19). Elegant studies (20) recently demonstrated that rats treated with a pan-caspase inhibitor exhibit neither fatigue-induced osteocytic apoptosis nor activation of osteoclastic resorption, suggesting that the programmed death of osteocytes is the key event to initiate bone remodeling. These studies demonstrated that osteocytic

apoptosis, and not that of other cells (osteoclasts and bone marrow stromal cells), is the key event in controlling osteoclastic resorption. Although the data clearly showed a requirement for osteocyte death to induce osteoclastic bone resorption, the molecular and cellular mechanisms regulating this phenomenon remain elusive.

Pro-apoptotic factors other than microcracks and fatigue may signal osteocytes to die, and thereby activate osteoclasts (bone resorption), whereas other agents may protect osteocytes from programmed cell death and enable them to activate osteoblasts (bone formation). Glucocorticoid excess, for example, increases the prevalence of osteocyte and osteoblast apoptosis (21;22) and induces bone loss and osteoporosis in part through this mechanism. Bisphosphonates (BPs), on the other hand, not only exert their antiresorptive effect on osteoclasts, but also reduce the rate of osteocyte death. Prevention of osteocyte apoptosis has been suggested to explain the discrepancy between the modest effect of BPs on bone mass and their dramatic reduction in fracture incidence (22-26). Parathyroid hormone (PTH) (27) has also been shown to reduce the rate of osteocyte apoptosis and may exert its anabolic effect on bone partially through this mechanism. Better understanding of this process will greatly benefit our understanding of osteocyte functions and possibly lead to novel therapeutic approaches to osteoporosis.

Osteocyte Ablation and Its Consequences

The most compelling evidence that osteocytes are key regulators of skeletal homeostasis and remodeling comes from recent studies (2) employing a mouse model in which the diphtheria toxin receptor is expressed exclusively in osteocytes. Using this model, the authors ablated osteocytes from the skeleton postnatally and showed that these cells are required to regulate osteoblast and osteoclast activities on the bone surface and are essential for mechanotransduction. Their osteocyte-null mice display a dramatic increase in both osteoclast number and activity as well as bone resorption. The authors interpreted these data as indicating that osteocytes negatively regulate osteoclasts by expressing a tonic osteoclast-inhibitory factor. Alternatively, as recently suggested (28), perhaps the dramatic increase in osteoclast number present in these animals could result from the release, by dying osteocytes, of a pro-osteoclastogenic signal rather than from the loss of an inhibitory factor. Moreover, these “osteocyte-less” mice are resistant to unloading-induced bone loss, providing further evidence for the role of osteocytes in mechanotransduction.

Do osteocytes also regulate bone formation? Several lines of evidence favor this hypothesis. First, preliminary work presented in abstract form at the 2008 ASBMR meeting indicated that osteocyte-specific conditional ablation of β-catenin induces severe osteopenia and osteoporosis, suggesting a critical role for Wnt/β-catenin pathway signaling in supporting a regulatory function for osteocytes in bone remodeling (29). Mice constitutively lacking β-catenin in osteocytes display a severe bone phenotype and die at the age of 2 months. Paradoxically, the phenotype of these mice is more severe than that of mice lacking β-catenin in osteoblasts (30-32), indicating that canonical Wnt signaling in osteocytes might be a particularly key pathway in the control of osteoblast proliferation and function. Moreover, osteocytes synthesize sclerostin, a secreted protein that inhibits bone formation by binding to LRP5/LRP6 co-receptors and blunting Wnt signaling (13-15;33;34). Sclerostin is strongly downregulated by PTH, and it has been postulated that PTH may exert its anabolic effect on bone in part through this mechanism (35;36). Lastly, recent work (27), using a constitutively active PTH/PTHrP receptor (PPR) expressed exclusively in osteocytes, demonstrated that PTH actions on osteocytes control bone modeling and remodeling. Transgenic mice expressing a constitutively active PPR in osteocytes were generated by placing the H223R mutant receptor (37-39) under the control of the 8Kb-DMP1 promoter (named DMP1-caPTHR1). In these mice the levels of sclerostin mRNA in bone were 3-fold lower

than in wild type littermates at 8 weeks of age. Strikingly, DMP1-caPTHR1 mice also exhibited dramatic increases in bone mineral density (BMD) at the spine (42%) and femur (84%), as determined by DEXA (27), suggesting an important role for PPR/cAMP-mediated pathways within osteocytes in the anabolic effect of PTH.

Collectively, these findings point to a critical role of PPRs in osteocytes, though the functions of these receptors in a physiological setting remain to be elucidated.

To better understand the role of PTH signaling through PPRs in osteocytes and to determine the role(s) of these cells in mediating the effects of the hormone on bone, our group has generated mice in which PPR expression is specifically ablated in osteocytes. As reported at the 2008 ASBMR meeting, in these mice there is a decrease in the trabeculae in the primary spongiosa and a delay in the secondary ossification center, suggesting an important role of PTH signaling in osteocytes for endochondral bone development (40). Moreover, when these mice are challenged with a low calcium diet, they display a significantly lower blood calcium level than littermate controls, suggesting a role for osteocyte PPRs in calcium homeostasis. Further work is needed to better define the mechanism of action whereby osteocytic PPRs influence bone remodeling and calcium homeostasis.

Osteocytic Osteolysis

Pioneering studies on osteocytes involving histological analysis of bone specimens raised the possibility that osteocytes could resorb bone and directly contribute to mineral ion homeostasis. The osteocyte surface is indeed 100-fold greater than the trabecular bone surface and has the potential to significantly contribute to mineral-ion homeostasis (41). The finding that in various diseases (renal osteodystrophy, hyperparathyroidism and immobilization) the size of the osteocytic lacunae is increased led to the hypothesis that these cells were indeed capable of modifying the perilacunar mineralized matrix. This theory was quickly abandoned when isolated osteocytes failed to reabsorb bone in a classical osteoclast pit-forming assay (42). The whole concept of “osteocytic osteolysis” was discarded and scientific interest in this phenomenon died out. Recently, however, the idea that an osteocyte might indeed be capable of moving mineral ions into and out of the perilacunar space has been re-analyzed. The current osteocytic osteolysis concept, as proposed recently (43), suggests that osteocytes, unlike osteoclasts, do not remove substantial amounts of bone, but rather modify the matrix mineral composition in the perilacunar areas. It was recently reported (44) that continuous infusion of PTH in rats induced changes in cortical bone osteocytes consistent with osteocytic osteolysis, and similar effects in mice upon prednisone treatment (21) were seen.

This re-examination of osteocytic osteolysis has stimulated renewed interest in this phenomenon and promises to open new perspectives on osteocyte biology and the cellular mechanisms that control bone and mineral ion homeostasis.

Osteocytes and Mineral Ion Homeostasis

Osteocytes appear to be centrally involved in phosphate homeostasis, as suggested by recent work (3;4) reporting that loss of DMP1 in humans and in mice causes rickets, osteomalacia and severe hypophosphatemia. These studies demonstrated that osteocytes play an important functional role in mineral metabolism via DMP1 and that lack of this protein results in defective osteocyte maturation, increased fibroblast growth factor-23 (FGF23) expression and resulting renal phosphate-wasting and pathological changes in bone mineralization. Interestingly there are two additional molecules expressed in osteocytes that play a key role in phosphate regulation: Phex and FGF23. Inactivating mutations in Phex, a peptidase the relevant substrate for which is unknown, lead to suppression of renal phosphate reabsorption and 1α-hydroxylation of 25-hydroxyvitamin D, hypophosphatemia and rickets in Hyp mice and in humans with X-linked hypophosphatemic rickets. FGF23

exerts a direct effect in the kidney to reduce phosphate reabsorption and vitamin D activation, and is overproduced in tumor-induced osteomalacia and, interestingly, by abnormal bone lesions in many patients with fibrous dysplasia/McCune Albright syndrome due to activating mutations of the Gsα signaling protein. Finally, recent work indicates that MEPE induces an increase in DMP1 and Phex by increasing the release of ASARM-peptides (protease inhibitors involved in regulating bone mineralization) (45).

The role of osteocytes in controlling calcium homeostasis is controversial. Osteocytes express receptors for PTH, a known regulator of calcium homeostasis, are distributed widely in bone and are ideally situated to engage in systemic calcium homeostasis. As mentioned above, our group has gathered preliminary data demonstrating that mice lacking the PPR specifically in osteocytes have impaired calcium homeostasis when subjected to a low calcium diet, but further studies are needed to define the mechanisms by which these cells might control calcium homeostasis.

Conclusions and Future Research

From this *Perspective* it is clear that our knowledge of osteocytes and their role in bone and mineral homeostasis, while growing, remains inadequate. Additional studies are needed to better comprehend osteocytes and their functions. We can look forward to witnessing, in these intriguing and amazing cells, the same exponential expansion of knowledge that occurred in osteoblast and osteoclast biology over the past several decades. Areas in need of further understanding are numerous. For example, what are the molecular signals that tell an osteoblast to become an osteocyte? How can osteocytes sense the mechanical forces applied to the skeleton and transform these forces into biological signals? Can osteocytes sense phosphate? How can an osteocyte orchestrate and regulate bone remodeling? And finally, do osteocytes contribute to calcium homeostasis?

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