Osteocytes, bone remodeling and parathyroid hormone

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Abstract

Osteocytes, the longest living bone cells, have garnered much of the attention in the part they play in skeletal biology to orchestrate bone homeostasis. Although the multifunctional role of osteocytes in bone remodeling has been recognized, knowledge about the activity of important signaling molecules in osteocytes in this process are limited and far from being clearly depicted than osteoblast's and osteoclast's involvement. Stimulating the function of bone-forming osteoblasts and controlling osteoclastic activity are the preferred pharmacological intervention for skeletal repair. A large variety of studies have been conducted in osteoblasts and osteoclasts on recombinant parathyroid hormone (PTH), an anabolic drug which proved to be effective for bone strength and clinical therapy. Studies in relation to newly functioning signaling molecules in osteocytes and PTH's mode of action is becoming an intense area of investigation suggesting further the importance of these cells in bone anabolic action. The goal of this review is to discuss briefly the function and physiological roles of osteocytes and highlight a few recent findings on the PTH's action in osteocyte signaling in health and disease. In this context this review in addition to PubMed searches also borrows many literatures in bone and related fields from various publications.

What are osteocytes

Osteocytes (Figure 1), the star-shaped cells, are terminally differentiated cells of the osteoblast lineage. Some osteoblasts after laying down osteoid matrix harmonize its mineralization and subsequently become embedded in mineralized matrix. These cells known to be instrumental in coordinating osteoblast and osteoclast activity, and more recently has been recognized as an endocrine cell. Being ten times more abundant than osteoblasts, osteocytes have the potential to live long. The loss of osteocytes from human bones is associated with the ageing process. Osteocytes contain a nucleus and a thin ring of cytoplasm. The space that an osteocyte occupies is called a lacuna (15-20 um, Latin for a pit). Osteocytes are networked to each other via long cytoplasmic extensions that occupy tiny canals called canaliculi (250-300 nm in diameter), which are used for exchange of nutrients and waste through gap junctions. Although osteocytes have reduced synthetic activity and, unlike osteoblasts, are not capable of mitotic division, they are actively involved in the routine turnover of bony matrix, through various mechanosensory mechanisms. These cells destroy bone through a rapid and transient (relative to osteoblasts) mechanism called osteocytic osteolysis. Studies also show that osteocytes can remove mineralized bone matrix using mechanisms like osteoclasts and by reversibly remodeling their perilacunar/canalicular matrix. It was suggested that osteocytic remodeling is concurrent with increase in tartrate resistant acid phosphatase activity and cathepsin K expression. The dynamics of the transition to osteocyte from osteoblast activity is being studied by manipulating gene expression in osteocytes in transgenic mouse models carrying lineage reporters for osteoblasts and osteocytes and has been reviewed by Dallas and Bonewald. DNA methylation as a contributor of osteoblast to osteocyte transition has also been suggested very recently.

Functions of osteocytes

Mechanistic understanding of mechanobiology, an interplay between biology and the cellular mechanical environment, is required for both normal physiological function and etiology of diseases. A wide selection of tissues and cells including differentiated cells, stem cells and progenitors are regulated by mechanical signals and loading, and proper regulation of this process is essential in diseases like osteoporosis, atherosclerosis, and osteoarthritis. When bone cells do not experience adequate mechanical stimulation, bone formation ceases and a program of bone remodeling is initiated. Although osteocyte’s function and role in communication have been recognized by bone biologists for several decades, until recently the mechanosensing mechanisms of osteocytes were less studied for these diseases. Osteocytes have a cell body and cytoplasmic extensions, which play an important role in their function as mechanosensors. Osteocytes are for example sensitive to fluid shear stress as a result of bone loading. In addition, osteocytes have a cilium, which might also play an important role in their mechanosensory function and sends signals to regulate a variety of important phenomena in bone biology. Osteocytes may produce a single, mechanically derived signal to control osteoblast and bone lining cell functions and thereby regulate bone modeling and remodeling process (Figure 1). Thus by responding to mechanical strain osteocytes help new bone formation. Having not enough live osteocytes possibly lead to inefficiency in removing microdamage and reduced remodeling activity. Osteocytes also send signals to activate bone resorption. It was hypothesized that osteocyte death is responsible for signaling osteoclasts for bone resorption. Using ex vivo and in vivo animal models studies show that mechanical stimulation controls osteocyte viability thus maintaining apoptotic cell death. Apoptotic osteocytes co-localize with regions of bone resorption. Studying organ culture system of isolated rat calvaria Gu et al. suggested that osteocytes are negative regulator of osteoclast activity and osteocytes may play a major role in triggering local bone remodeling. While osteocyte apoptosis triggers the bone remodeling response to microdamage, the neighboring non-apoptotic osteocytes are the major source of pro-osteoclastogenic signals. Moreover, both the apoptotic and osteoclast-signaling osteocyte populations are localized in a spatially and temporally restricted pattern consistent with the targeted nature of this remodeling response. Osteocyte-ablated mice exhibit fragile bone with intracortical porosity and microfractures, osteoblastic dysfunction,
and trabecular bone loss with microstructural deterioration.26 These mice are resistant to unloading-induced bone loss further suggesting the importance of osteocytes in mechanotransduction. Using osteocytes ablation model it was also shown that the osteocyte apoptosis is sufficient to initiate an osteoclastogenic response and that osteocytes are required for the skeletal adaptation to reduced mechanical forces.27-29 Several other studies support the concept that lack of osteocyte apoptosis do not support bone resorption.14-16 In human trabecular bone, mechanical forces also regulate osteocyte viability and responsible for osteoclast recruitment resulting increase in bone resorption and bone loss.22,30-32 Pro-clastogenic signals released from osteocytes such as conditioned media from apoptotic MO-LY4 in vitro are consistent with pro-clastogenic signals observed in vivo studies implying increased osteoclastogenesis with increased osteocyte apoptosis.14,26,32-34 Future studies will clarify if living osteocytes continually produce anti-osteoclastogenic signals that limit osteoclast recruitment or whether apoptotic osteocytes produce pro-osteoclastogenic signals.

Intracellular cross-talk within the bone ultimately makes the decision of bone formation and resorption. Identifying signaling mediators participating in the communication between osteocytes and osteoclasts and between osteocytes and osteoblasts remains to be fully explored. In regions of high bone loading, the mechano-responsive osteocytes inhibit osteoclastic bone resorption by producing signaling molecules like matrix extracellular phosphoglycoprotein gene (MEPE) suggesting that mechanical loading of osteocytes affects osteocyte-stimulated osteoclastogenesis by involving MEPE gene. Direct cell-cell communication via gap junctions is an important phenomenon by which bone cell activities are coordinated in addition to endocrine, paracrine, and autocrine factors. Abundant gap junctions are present between osteocytic processes, between osteocytes and osteoblasts on the bone surface, and among osteoblasts.5,36,37 The readers are directed to more extensive reviews on functions of osteocyte mechanobiology and pericellular mechanics which has been also discussed extensively by Jacob et al.112 In this review the authors presented the mechanical process in bone by describing signal transduction from organ to cellular and to molecular level which finally feeds back the biological response from molecules to organ. Starting from mechanical behavior, tissue-level behavior to induce changes at the cellular and pericellular level, to intracellular biochemical signaling such as calcium signaling, G-Protein-Mediated signaling, kinase signaling. Gap junctions, nitric oxide eicosinoid, stromal cell derived factor-1, nucleoside signaling and other cell-cell signaling mechanisms that are specific to bone and osteocyte function have been widely discussed in this review.

**Osteocytes in bone remodeling and parathyroid hormone/parathyroid hormone receptors signaling**

The skeleton undergoes continuous remodeling during adult life to maintain skeletal integrity, renew aging bone, and restore injuries. Initially bone remodeling was viewed to be orchestrated by bone-resorbing cells, osteoclasts and bone-forming cells, osteoblasts. It is now clear osteocytes, which are embedded into the mineralized tissue, governs profound regulatory functions on the bone remodeling process. Parathyroid hormone (PTH) exerts classical actions on bone metabolism by activating PTH/PTHrP receptors (PTH1Rs) on target cells. Calcium, phosphorus and skeletal homeostasis are mainly regulated by PTH. Continuous exposure to PTH is associated with catabolic effects, whereas intermittent exposure to low doses of PTH is associated with anabolic effects. PTH and PTH related peptide (PTHrP) signaling in osteoblasts has been studied by us and others.38-45 Its implication in osteoporotic fractures have also been reviewed.46 Earlier studies demonstrated the presence of PTH1R on osteoblast, and that osteoblasts produce osteoclastogenic factor, receptor activator of nuclear factor κβ ligand (RANKL) and macrophage colony stimulating factor (MCSF) thus activating osteoclastogenesis.47, 51 Using genetic tools and gain-of-function transgenes,26,57-60 impairs bone structure and homeostatic calcium response in mice.61 PTH potentiates the osteocytic response and this is different from osteoblast response in relation to mechanotransduction pathways.62 A role for PTH1R in osteocytes and an antiapoptotic effect of vertebral osteocytes was reported using SMR1 mice.63 This antiapoptotic effect by PTH was also noted either in rat distal femoral osteocytes or in vitro cultures of osteocytes MLO-Y4.64,65 Evidence suggest that carboxy (C)-terminal fragments of PTH, which comprise the majority of circulating PTH, do not interact with PTH1R which mediates the classical hormone actions. C-PTH exerts specific effects on calcium homeostasis and bone metabolism via a specific receptor distinct from PTH1R, known as C-terminal PTH receptors (CPTHRs).66,67 Divieti et al. reported that osteocytes expressing CPTH may be the principal target cells for unique actions of intact PTH(1-84) and circulating PTH C-fragments.66-67 PTH receptors and apoptosis in osteocytes have been reviewed by Brinjikji.68 Among the several signaling molecules or family of proteins in recent years those paid greatest attention for therapeutic action are - SOST, Sclerostin and Wnt. In this review I will mainly focus on these molecules and their interconnections with PTH in osteocytes.

![Figure 1. Osteocyte and its function.](image-url)
**SOST/sclerostin**

The osteocyte marker gene SOST encodes sclerostin, a secreted glycoprotein and bone formation inhibitor.\(^{20-22}\) Transgenic mice overexpressing human SOST shows a low bone mass phenotype and SOST knockout (KO) mice exhibit a progressive high bone mass phenotype and increased bone strength.\(^{71,72}\) Loss of SOST function in mice results in decreased osteocyte apoptosis.\(^{73}\) SOST is known to be a bone morphogenic protein (BMP) antagonist, and loss of SOST activates BMP signaling. The pro-osteoblastogenic action of BMPs and Wnts are inhibited by Sclerostin.\(^{74}\) Sclerostin also stimulates osteocyte support of osteoclast activity by a RANKL-dependent pathway.\(^{75}\) Regulation of circulating sclerostin levels by sex steroids and its contribution to post menopausal osteoporosis has been a subject of investigation in recent years.\(^{76}\) These studies suggest a novel mechanism of hormonal control of sclerostin production in osteocyte mediated osteotogenesis or bone resorption. Association between sclerostin and physical activity, age, gender, body composition, and bone mineral content in healthy adults or bone density in chronic spinal cord injury has been reported.\(^{77,78}\)

SOST is a target gene for PTH in bone (Figure 2).\(^{48}\) Evidence that PTH regulates sclerostin expression comes from human and animal studies at both the cellular and molecular level.\(^{48-52}\) Circulating sclerostin levels may be measured in patients with overproduction or deficient PTH, namely hyperparathyroidism or hypoparathyroidism and serve as useful models to further explore the relationship between PTH and sclerostin.\(^{53}\) Sclerostin levels are primarily reduced in the presence of PTH.\(^{79-85}\) Continuous infusion of PTH to mice causes transcriptional suppression of SOST and reduction in sclerostin protein in vertebral bone; and decreased SOST expression in primary osteocyte cultures and in osteocytic MLO-A5 cells.\(^{52}\) In osteocyte-containing cultures of murine calvarial cells PTH1R activation exhibited decreased SOST mRNA expression, regulates FGF-23 signaling via cAMP and Wnt-dependent mechanisms thus potentially modulating endocrine and autocrine functions of osteocytes by.\(^{57}\) The elevated FGF-23 expression noted in whole bones and osteocytes from DMP1-cPTHR1 mice is corrected in double transgenic mice overexpressing SOST in osteocytes.\(^{52}\) Classical models of PTH induced bone formation such as estrogen-deprived (OVX) rats or transgenic mice expressing a constitutively active PTH1R specifically in osteocytes, DMP1-cPTHR1 transgenic mice, or both in osteoblast and osteocyte (2.3 col-cPTHR1 mice), express significantly reduced levels of SOST mRNA in calvaria or vertebral and tibial bone.\(^{59,62,68,70}\) and display high bone mass.\(^{10}\) Using SOST-deficient mice studies also suggest that changes in SOST expression are not required for anabolic effect of intermittent PTH.\(^{91}\) Furthermore, SOST deletion protects trabecular compartments from bone loss induced by high-dose PTH infusion.

The contribution of suppressed sclerostin level in PTH bone anabolic action has been discussed recently by Kramer et al.\(^{75,92}\) This review addresses how the bone anabolic responses by PTH treatment or by sclerostin inhibition overlap and diverge. Based on the striking effect of SOST inhibition in bone formation SOST has been suggested to be a potential target for therapeutic intervention in bone loss and has led to the development of sclerostin inhibitors or anti-sclerostin antibodies, thus opening new possibilities and prospects of effective anabolic therapy for bone regeneration in orthopedics and in the dental field.\(^{93-94}\) Sclerostin also inhibits PTH-stimulated cAMP production further suggesting neutralization of sclerostin as a potential therapy for skeletal diseases on enhancing bone formation.\(^{95}\)

**Wnt**

Following the discovery of Wnt gene in 1984 the Wnt signaling pathway has been a major area of investigation and known for their roles in normal physiological processes.\(^{95-98}\) Since then Wnt/β-catenin signaling pathways are being studied in bone biology because of their importance in skeletal development; bone remodeling, bone mass maintenance, regeneration and repair during a lifespan.\(^{99}\) Activation of Wnt pathway involves association of two membrane receptors, serum frizzled-related proteins (Fzd) and lipoprotein receptor-related proteins (LRP5/6).\(^{100}\) A protein complex consisting of axin, adenomatous polyposis coli, and glycogen synthase kinase 3 (GSK3) is activated following this association leading to phosphorylation of β-catenin. Accumulated β-catenin then translocates to nucleus and regulates gene transcription essential to osteoblast function. In the absence of Wnt signaling, GSK3 phosphorylates β-catenin and targets it for degradation. The Wnt signaling involves a large number of agonists and antagonists that can regulate the production and interaction of Wnt signaling molecules with the receptors on target cells, and the physiological responses of target cells.\(^{101}\) These antagonists bind to Wnt, Fzd, or LRP5/6 and decrease bone formation. Lack of these inhibitors activate Wnt/β-catenin signaling and bone formation.

Osteocytes can control bone formation by modulating the Wnt signaling pathway as they represent the main source of sclerostin, the negative regulator of bone formation. Using transgenic mice engineered to maintain high levels of SOST expression it has been recently demonstrated that downregulation of SOST/sclerostin in osteocytes and activation of Wnt signaling is necessary to augment osteogenesis.\(^{102}\) Recent advances and discrepancies in how Wnt/Lrp5 signaling regulates osteoblasts and osteocytes has been reviewed by Monroe et al.\(^{103}\) This review also discusses Wnt signaling in osteoclastogenesis, new play-
ers in this pathways that have important roles in bone development, and progresses in translating basic studies of Wnt pathways/antagonists to clinical therapeutics and diagnostics. Since Wnt signaling pathway plays a central role in bone development, homeostasis and in promoting bone growth Wnt factors could be used to stimulate bone healing.105 Using mouse strain \textit{Axin2}\textsuperscript{-/-}\textit{Catenin-/-} in which the cellular response to Wnt is increased it was found that bone healing after injury is accelerated in these mice, implying increased proliferation and earlier differentiation of skeletal stem and progenitor cells. It was further shown that purified Wnt3a in liposomal vesicles at sites with skeletal defects stimulates the proliferation of skeletal progenitor cells and accelerates their differentiation into osteoblasts resulting in faster bone regeneration. These studies also suggested that Wnt signaling and protein-based approach may have widespread applications in regenerative medicine. It is now clear that the Wnt signaling pathway is central to regulation of both skeletal modeling and remodeling and potential role for therapies targeting DKK1, LRP5, and serotonin in the treatment of osteoporosis is gaining much attention.97,107

Intermittent PTH promotes osteoblast differentiation partially by its ability to exit from the cell cycle,34-40 activating Wnt signaling in osteoblasts,106 and suppressing expression of the Wnt antagonist sclerostin in osteocytes as observed in mice and in human.92,99,109

PTH suppression of sclerostin in osteocytes increases the availability of LRP5 in PTH signaling via positive feedback mechanism (Figure 2).9 It was also suggested that PTHIR signaling in osteocytes increases bone mass and the rate of bone remodeling through LRP5-dependent and -independent mechanisms, respectively.100 Multiple signaling molecules including sclerostin are utilized by PTH which cross talk and work together to modulate Wnt/Catenin signaling pathway and promote bone formation.101 Extensive studies on the mechanisms of action of PTH on osteocytes will help to identify new pathways that regulate bone formation leading to possible therapeutic interventions.

\section*{Concluding remarks}

Given the central role of osteocytes in bone remodeling it is not surprising that the molecules regulated by osteocyte signaling figure prominently in physiological and pathophysiological pathways of bone. What is surprising is the fact that osteocytes seem to play such an essential role in bone remodeling despite the fact that the signaling molecules governed by these cells are also regulated by bone forming osteoblasts and/or bone resorbing osteoclasts. This observation highlights the importance of osteocytes in serving as a governing general in critical signaling network of bone homeostasis.

New and exciting findings are now emerging, but many questions remain unanswered: i) Does osteocyte signaling or mutational status of signaling molecules/receptors correlate with disease progression and survival? ii) Can we utilize current studies to predict patient outcome? iii) What are the important signaling pathways in osteocytes activated or deactivated in response to different bone diseases? iv) What are the mechanisms that regulate osteocyte activity in cancer metastasis to bone? v) Are osteocytes viable therapeutic target in bone metastasis? Future progress in these areas may open up new avenues of therapeutic interventions for metabolic bone diseases including osteoporosis and cancer metastasis to bone.

\section*{References}


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