Osteocytes, bone remodeling and parathyroid hormone

Nabanita S. Datta

Department of Internal Medicine,
Endocrinology, Cardiovascular Research
Institute, Karmanos Cancer Institute,
Wayne State University School
of Medicine, Detroit, MI, USA

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.^{1,2} Being ten times more abundant than osteoblasts, osteocytes have the potential to live long.^{3,4} The loss of osteocytes from human bones is associated with the ageing process.^{5,6}

Osteocytes contain a nucleus and a thin ring of cytoplasm. The space that an osteocyte occupies is called a lacuna (15-20 μ m, 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.⁷ 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 osteoclasts) 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.⁸ 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 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.⁹ DNA methylation as a contributor of osteoblast to osteocyte transition has also been suggested very recently.¹⁰

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.¹¹⁻¹³ 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 resorption 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.¹⁴⁻¹⁶ Osteocytes may produce a single, mechanically derived signal to control

Correspondence: Nabanita S. Datta, Associate Professor of Medicine, Internal Medicine, Endocrinology, Wayne State University School of Medicine, 1107 Elliman Building, 421 East Canfield Avenue, Detroit, Michigan 48201, USA. Tel. +1.313.7454008 - Fax: +1.313.5778615. E-mail: ndatta@med.wayne.edu

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osteoblast and bone lining cell functions and thereby regulate bone modeling and remodeling process (Figure 1).^{17,18} 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.^{19,22}

Osteocytes also send signals to activate bone resorption.²³ 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.²⁴ 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.²⁵ 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.²³ 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 mechano-transduction. 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.^{14,30-33} In human trabecular bone, mechanical forces also regulate osteocyte viability and responsible for osteoclast recruitment resulting increase in bone resorption and bone loss.^{23,24,32} Pro-clastogenic signals released from osteocytes such as conditioned media from apoptotic MLO-Y4 *in vitro* are consistent with pro-clastogenic signals observed *in vivo* studies implying increased osteoclastogenesis with increased osteocyte apoptosis.^{14,26,27,32-34} 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*)³⁵ 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.^{4,36,37} 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.*^{4,12} 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 feedbacks 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 κ B ligand (RANKL) and macrophage colony stimulating factor (MCSF) thus activating osteoclastogenesis.⁴⁷⁻⁵² Using genetic tools and gain-of-function and loss-of-function studies very recent report provided evidence that the major source of RANKL is osteocytes (Figure 2).^{53,54} New insights for osteocyte RANKL in controlling bone remodeling has been also nicely discussed.⁵⁵

Osteocytes recognition as master regulators of bone formation and localized bone remodeling led to studies revealing osteocytes importance in the PTH anabolic effects. The expression of PTH1R in osteocytes was suggested several years ago.⁵⁶ This paper reported something more important though than localization, the binding of radiolabelled hormone to receptors on osteocytes after administration *in vivo*. The proof that osteocytes are exposed to the PTH from the circulation was a remarkable and important finding that led the later osteocyte specific PTH1R KO studies. Targeted ablation of PTH/PTHrP receptor specifically in osteocytes under the control of Dmp-1 promoter, direct osteocyte-specific expression of 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 osteocytic cells 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 (CPTHrRs).^{66,67} Divieti *et al.* reported that osteocytes expressing CPTHrR 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 Bringhurst.⁶⁵

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.

SOST/sclerostin

The osteocyte marker gene *SOST* encodes sclerostin, a secreted glycoprotein and bone formation inhibitor.⁶⁸⁻⁷⁰ 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.⁷¹⁻⁷⁴ Loss of *SOST* function in mice results in decreased osteocyte apoptosis.⁷⁴ *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.⁷⁵ Sclerostin also stimulates osteocyte support of osteoclast activity by a RANKL-dependent pathway.⁷⁶ Regulation of circulating sclerostin levels by sex steroids and its contribution to post menopausal osteoporosis has been a subject of investigation in recent years.⁷⁷ These studies suggest a novel mechanism of hormonal control of sclerostin production in osteocyte mediated osteoblastogenesis 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.^{78,79}

SOST is a target gene for PTH in bone (Figure 2).⁸⁰ Evidence that PTH regulates sclerostin expression comes from human and animal studies at both the cellular and molecular level.⁸⁰⁻⁸³ 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.⁸⁴ Sclerostin levels are primarily reduced in the presence of PTH.^{77,85,86} 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.⁸² In osteocyte-containing cultures of murine calvarial cells PTH1R activation exhibit decreased *SOST* mRNA expression, regulates FGF-23 signaling via cAMP and Wnt-dependent mechanisms thus potentially modulating endocrine and auto/paracrine functions of osteocytes by.⁸⁷ The elevated FGF23 expression noted in whole bones and osteocytes from DMP1-caPTH1R mice is corrected in double transgenic mice overexpressing *SOST* in osteocytes.⁸⁷ 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- caPTH1R transgenic mice, or both in osteoblast and osteocyte (2.3 col-caPTH1R mice), express significantly reduced levels of *SOST* mRNA in calvaria or vertebral

and tibial bone.^{80,82,88,89} and display high bone mass.⁹⁰ 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.*^{73,92} 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.⁹³⁻⁹⁸ Sclerostin also inhibits PTH-stimulated cAMP production further suggesting neutralization of sclerostin as a potential therapy for skeletal diseases on enhancing bone formation.⁹⁹

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.^{100,101} 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.¹⁰² Activation of *Wnt* pathway involves association of two membrane receptors, serum frizzled-related proteins (Fzd) and lipoprotein receptor-related proteins (LRP5/6).¹⁰³ 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.¹⁰⁴ 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.¹⁰⁵ Recent advances and discrepancies in how Wnt/Lrp5 signaling regulates osteoblasts and osteocytes has been reviewed by Monroe *et al.*¹⁰² This review also discusses *Wnt* signaling in osteoclastogenesis, new play-

Figure 2. Parathyroid hormone (PTH) signaling in osteocytes and bone remodeling. PTH signaling in osteocytes leads to increased bone mass via suppression of *SOST*/Sclerostin and activating LRP5/6 Wnt signaling in osteoblasts. Osteocyte derived RANKL favors osteoclastogenesis over RANKL derived from osteoblasts. BMSC, bone marrow stromal cells; HSC, hematopoietic stem cells.

ers in this pathways that have important roles in bone development, and progresses made 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.¹⁰⁶ Using mouse strain *Axin2^{LacZ/LacZ}* 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,³⁸⁻⁴⁰ activating *Wnt* signaling in osteoblasts,¹⁰⁸ 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 LRP6 in PTH signaling via positive feedback mechanism (Figure 2).⁹⁹ It was also suggested that PTH1R signaling in osteocytes increases bone mass and the rate of bone remodeling through LRP5-dependent and -independent mechanisms, respectively.⁹⁰ Multiple signaling molecules including sclerostin are utilized by PTH which cross talk and work together to modulate *Wnt*/ β -Catenin signaling pathway and promote bone formation.¹¹⁰ 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.

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 governor 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.

References

- Bonewald LF. The amazing osteocyte. *J Bone Miner Res* 2011;26:229-38.
- Bonewald L. The holy grail of high bone mass. *Nat Med* 2011;17:657-8.
- Parfitt AM. The cellular basis of bone turnover and bone loss: a rebuttal of the osteocytic resorption-bone flow theory. *Clin Orthop Relat Res* 1977:236-47.
- Qing H, Bonewald LF. Osteocyte remodeling of the perilacunar and pericanalicular matrix. *Int J Oral Sci* 2009;1:59-65.
- Dunstan CR, Somers NM, Evans RA. Osteocyte death and hip fracture. *Calcif Tissue Int* 1993;53Suppl1:S113-7.
- Frost HM. In vivo osteocyte death. *J Bone Joint Surg Am* 1960;42-A:138-43.
- Donahue HJ. Gap junctions and biophysical regulation of bone cell differentiation. *Bone*. 2000;26:417-22.
- Qing H, Ardehshirpour L, Pajevic PD, et al. Demonstration of osteocytic perilacunar/canalicular remodeling in mice during lactation. *J Bone Miner Res* 2012;27:1018-29.
- Dallas SL, Bonewald LF. Dynamics of the transition from osteoblast to osteocyte. *Ann N Y Acad Sci* 2010;1192:437-43.
- Delgado-Calle J, Sanudo C, Bolado A, et al. DNA methylation contributes to the regulation of sclerostin expression in human osteocytes. *J Bone Miner Res* 2012;27:926-37.
- Jacobs CR. The mechanobiology of cancellous bone structural adaptation. *J Rehabil Res Dev* 2000;37:209-16.
- Jacobs CR, Temiyasathit S, Castillo AB. Osteocyte mechanobiology and pericellular mechanics. *Annu Rev Biomed Eng* 2010;12:369-400.
- Stoltz JF, Wang X. From biomechanics to mechanobiology. *Biorheology* 2002;39:5-10.
- Zhao S, Zhang YK, Harris S, et al. MLO-Y4 osteocyte-like cells support osteoclast formation and activation. *J Bone Miner Res* 2002;17:2068-79.
- Bonewald LF. Establishment and characterization of an osteocyte-like cell line, MLO-Y4. *J Bone Miner Metab* 1999;17:61-5.
- Hoey DA, Chen JC, Jacobs CR. The primary cilium as a novel extracellular sensor in bone. *Front Endocrinol (Lausanne)* 2012;3:75.
- Martin RB. Toward a unifying theory of bone remodeling. *Bone* 2000;26:1-6.
- Bonewald LF. Osteocyte biology: its implications for osteoporosis. *J Musculoskelet Neuronal Interact* 2004;4:101-4.
- Mori S, Harruff R, Ambrosius W, Burr DB. Trabecular bone volume and microdamage accumulation in the femoral heads of women with and without femoral neck fractures. *Bone* 1997;21:521-6.
- Kamijou T, Nakajima T, Ozawa H. Effects of osteocytes on osteoinduction in the autogenous rib graft in the rat mandible. *Bone* 1994;15:629-37.
- Marotti G. The structure of bone tissues and the cellular control of their deposition. *Ital J Anat Embryol* 1996;101:25-79.
- Marotti G, Farneti D, Remaggi F, Tartari F. Morphometric investigation on osteocytes in human auditory ossicles. *Ann Anat* 1998;180:449-53.
- Kennedy OD, Herman BC, Laudier DM, et al. Activation of resorption in fatigue-loaded bone involves both apoptosis and active pro-osteoclastogenic signaling by distinct osteocyte populations. *Bone* 2012;50:1115-22.
- Mann V, Huber C, Kogianni G, et al. The influence of mechanical stimulation on osteocyte apoptosis and bone viability in human trabecular bone. *J Musculoskelet Neuronal Interact* 2006;6:408-17.
- Gu G, Mulari M, Peng Z, et al. Death of osteocytes turns off the inhibition of osteoclasts and triggers local bone resorption. *Biochem Biophys Res Commun* 2005;335:1095-101.
- Tatsumi S, Ishii K, Amizuka N, et al. Targeted ablation of osteocytes induces osteoporosis with defective mechanotransduction. *Cell Metab* 2007;5:464-75.
- Aguirre JI, Plotkin LI, Stewart SA, et al. Osteocyte apoptosis is induced by weightlessness in mice and precedes osteoclast recruitment and bone loss. *J Bone Miner Res* 2006;21:605-15.
- Ikeda K. Osteocytes in the pathogenesis of osteoporosis. *Geriatr Gerontol Int* 2008;8:213-7.

29. Bellido T. Antagonistic interplay between mechanical forces and glucocorticoids in bone: a tale of kinases. *J Cell Biochem* 2010;111:1-6.
30. Cardoso L, Herman BC, Verborgt O, et al. Osteocyte apoptosis controls activation of intracortical resorption in response to bone fatigue. *J Bone Miner Res* 2009;24:597-605.
31. Herman BC, Cardoso L, Majeska RJ, et al. Activation of bone remodeling after fatigue: differential response to linear microcracks and diffuse damage. *Bone* 2010;47:766-72.
32. Al-Dujaili SA, Lau E, Al-Dujaili H, et al. Apoptotic osteocytes regulate osteoclast precursor recruitment and differentiation in vitro. *J Cell Biochem* 2011;112:2412-23.
33. Shandala T, Shen Ng Y, Hopwood B, et al. The role of osteocyte apoptosis in cancer chemotherapy-induced bone loss. *J Cell Physiol* 2012;227:2889-97.
34. Giuliani N, Ferretti M, Bolzoni M, et al. Increased osteocyte death in multiple myeloma patients: role in myeloma-induced osteoclast formation. *Leukemia* 2012. Epub 2012/02/01.
35. Kulkarni RN, Bakker AD, Everts V, Klein-Nulend J. Inhibition of osteoclastogenesis by mechanically loaded osteocytes: involvement of MEPE. *Calcif Tissue Int* 2010;87:461-8.
36. Doty SB. Morphological evidence of gap junctions between bone cells. *Calcif Tissue Int* 1981;33:509-12.
37. Palumbo C, Palazzini S, Marotti G. Morphological study of intercellular junctions during osteocyte differentiation. *Bone* 1990;11:401-6.
38. Datta NS, Chen C, Berry JE, McCauley LK. PTHrP signaling targets cyclin D1 and induces osteoblastic cell growth arrest. *J Bone Miner Res* 2005;20:1051-64.
39. Datta NS, Pettway GJ, Chen C, et al. Cyclin D1 as a target for the proliferative effects of PTH and PTHrP in early osteoblastic cells. *J Bone Miner Res* 2007;22:951-64.
40. Datta NS, Kolailat R, Fite A, et al. Distinct roles for mitogen-activated protein kinase phosphatase-1 (MKP-1) and ERK-MAPK in PTH1R signaling during osteoblast proliferation and differentiation. *Cell Signal* 2010;22:457-66.
41. Datta NS, Samra TA, Mahalingam CD, et al. Role of PTH1R internalization in osteoblasts and bone mass using a phosphorylation-deficient knock-in mouse model. *J Endocrinol* 2010;207:355-65.
42. Mahalingam CD, Datta T, Patil RV, et al. Mitogen activated protein kinase phosphatase-1 regulates bone mass, osteoblast gene expression, and responsiveness to parathyroid hormone. *J Endocrinol* 2011;211:145-56.
43. Datta NS, Samra TA, Abou-Samra AB. Parathyroid hormone (PTH) induces bone formation in phosphorylation deficient PTHrP knock-in mice. *Am J Physiol Endocrinol Metab* 2012;302:E1183-E8.
44. Jilka RL. Molecular and cellular mechanisms of the anabolic effect of intermittent PTH. *Bone* 2007;40:1434-46.
45. Datta NS, Abou-Samra AB. PTH and PTHrP signaling in osteoblasts. *Cell Signal* 2009;21:1245-54.
46. Datta NS. Osteoporotic fracture and parathyroid hormone. *World J Orthop* 2011;2:67-74.
47. Rodan GA, Martin TJ. Role of osteoblasts in hormonal control of bone resorption—a hypothesis. *Calcif Tissue Int* 1981;33:349-51.
48. Takahashi N, Akatsu T, Udagawa N, et al. Osteoblastic cells are involved in osteoclast formation. *Endocrinology* 1988;123:2600-2.
49. Udagawa N, Takahashi N, Akatsu T, et al. The bone marrow-derived stromal cell lines MC3T3-G2/PA6 and ST2 support osteoclast-like cell differentiation in cocultures with mouse spleen cells. *Endocrinology* 1989;125:1805-13.
50. Lacey DL, Timms E, Tan HL, et al. Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. *Cell* 1998;93:165-76.
51. Yasuda H, Shima N, Nakagawa N, et al. Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclastogenesis-inhibitory factor and is identical to TRANCE/RANKL. *Proc Natl Acad Sci U S A* 1998;95:3597-602.
52. Yoshida H, Hayashi S, Kunisada T, et al. The murine mutation osteopetrosis is in the coding region of the macrophage colony stimulating factor gene. *Nature* 1990;345:442-4.
53. Nakashima T, Hayashi M, Fukunaga T, et al. Evidence for osteocyte regulation of bone homeostasis through RANKL expression. *Nat Med* 2011;17:1231-4.
54. Xiong J, Onal M, Jilka RL, et al. Matrix-embedded cells control osteoclast formation. *Nat Med* 2011;17:1235-41.
55. Xiong J, O'Brien CA. Osteocyte RANKL: new insights into the control of bone remodeling. *J Bone Miner Res* 2012;27:499-505.
56. Fermor B, Skerry TM. PTH/PTHrP receptor expression on osteoblasts and osteocytes but not resorbing bone surfaces in growing rats. *J Bone Miner Res* 1995;10:1935-43.
57. Kalajzic I, Braut A, Guo D, et al. Dentin matrix protein 1 expression during osteoblastic differentiation, generation of an osteocyte GFP-transgene. *Bone* 2004;35:74-82.
58. Yang W, Lu Y, Kalajzic I, Guo D, et al. Dentin matrix protein 1 gene cis-regulation: use in osteocytes to characterize local responses to mechanical loading in vitro and in vivo. *J Biol Chem* 2005;280:20680-90.
59. Feng JQ, Ward LM, Liu S, et al. Loss of DMP1 causes rickets and osteomalacia and identifies a role for osteocytes in mineral metabolism. *Nat Genet* 2006;38:1310-5.
60. Lu Y, Xie Y, Zhang S, et al. DMP1-targeted Cre expression in odontoblasts and osteocytes. *J Dent Res* 2007;86:320-5.
61. Powell WF Jr., Barry KJ, Tulum I, et al. Targeted ablation of the PTH/PTHrP receptor in osteocytes impairs bone structure and homeostatic calcemic responses. *J Endocrinol* 2011;209:21-32.
62. Mikuni-Takagaki Y, Naruse K, Azuma Y, Miyauchi A. The role of calcium channels in osteocyte function. *J Musculoskelet Neuronal Interact* 2002;2:252-5.
63. Jilka RL, Weinstein RS, Bellido T, et al. Increased bone formation by prevention of osteoblast apoptosis with parathyroid hormone. *J Clin Invest* 1999;104:439-46.
64. Stanislaus D, Yang X, Liang JD, et al. In vivo regulation of apoptosis in metaphyseal trabecular bone of young rats by synthetic human parathyroid hormone (1-34) fragment. *Bone* 2000;27:209-18.
65. Bringhurst FR. PTH receptors and apoptosis in osteocytes. *J Musculoskelet Neuronal Interact* 2002;2:245-51.
66. Divieti P, Inomata N, Chapin K, et al. Receptors for the carboxyl-terminal region of pth(1-84) are highly expressed in osteocytic cells. *Endocrinology* 2001;142:916-25.
67. Selim AA, Mahon M, Juppner H, et al. Role of calcium channels in carboxyl-terminal parathyroid hormone receptor signaling. *Am J Physiol Cell Physiol* 2006;291:C114-21.
68. Poole KE, van Bezooijen RL, Loveridge N, et al. Sclerostin is a delayed secreted product of osteocytes that inhibits bone formation. *FASEB J* 2005;19:1842-4.
69. van Bezooijen RL, Roelen BA, Visser A, et al. Sclerostin is an osteocyte-expressed negative regulator of bone formation, but not a classical BMP antagonist. *J Exp Med* 2004;199:805-14.
70. van Bezooijen RL, ten Dijke P, Papapoulos SE, Lowik CW. SOST/sclerostin, an osteocyte-derived negative regulator of bone formation. *Cytokine Growth Factor Rev* 2005;16:319-27.
71. Loots GG, Kneissel M, Keller H, et al. Genomic deletion of a long-range bone enhancer misregulates sclerostin in Van Buchem disease. *Genome Res* 2005;15:928-35.
72. Li X, Ominsky MS, Niu QT, et al. Targeted

- deletion of the sclerostin gene in mice results in increased bone formation and bone strength. *J Bone Miner Res* 2008;23:860-9.
73. Kramer I, Loots GG, Studer A, et al. Parathyroid hormone (PTH)-induced bone gain is blunted in SOST overexpressing and deficient mice. *J Bone Miner Res* 2010;25:178-89.
 74. Lin C, Jiang X, Dai Z, et al. Sclerostin mediates bone response to mechanical unloading through antagonizing Wnt/beta-catenin signaling. *J Bone Miner Res* 2009;24:1651-61.
 75. Winkler DG, Sutherland MS, Ojala E, et al. Sclerostin inhibition of Wnt-3a-induced C3H10T1/2 cell differentiation is indirect and mediated by bone morphogenetic proteins. *J Biol Chem* 2005;280:2498-502.
 76. Wijenayaka AR, Kogawa M, Lim HP, et al. Sclerostin stimulates osteocyte support of osteoclast activity by a RANKL-dependent pathway. *PLoS One* 2011;6:e25900.
 77. Modder UI, Clowes JA, Hoey K, et al. Regulation of circulating sclerostin levels by sex steroids in women and in men. *J Bone Miner Res* 2011;26:27-34.
 78. Morse LR, Sudhakar S, Danilack V, et al. Association between sclerostin and bone density in chronic spinal cord injury. *J Bone Miner Res* 2012;27:352-9.
 79. Amrein K, Amrein S, Drexler C, et al. Sclerostin and its association with physical activity, age, gender, body composition, and bone mineral content in healthy adults. *J Clin Endocrinol Metab* 2012;97:148-54.
 80. Keller H, Kneissel M. SOST is a target gene for PTH in bone. *Bone* 2005;37:148-58.
 81. Silvestrini G, Ballanti P, Leopizzi M, et al. Effects of intermittent parathyroid hormone (PTH) administration on SOST mRNA and protein in rat bone. *J Mol Histol* 2007;38:261-9.
 82. Bellido T, Ali AA, Gubrij I, et al. Chronic elevation of parathyroid hormone in mice reduces expression of sclerostin by osteocytes: a novel mechanism for hormonal control of osteoblastogenesis. *Endocrinology* 2005;146:4577-83.
 83. Drake MT, Srinivasan B, Modder UI, et al. Effects of parathyroid hormone treatment on circulating sclerostin levels in postmenopausal women. *J Clin Endocrinol Metab* 2010;95:5056-62.
 84. Gaudio A, Pennisi P, Bratengeier C, et al. Increased sclerostin serum levels associated with bone formation and resorption markers in patients with immobilization-induced bone loss. *J Clin Endocrinol Metab* 2010;95:2248-53.
 85. Mirza FS, Padhi ID, Raisz LG, Lorenzo JA. Serum sclerostin levels negatively correlate with parathyroid hormone levels and free estrogen index in postmenopausal women. *J Clin Endocrinol Metab* 2010;95:1991-7.
 86. van Lierop AH, Witteveen JE, Hamdy NA, Papapoulos SE. Patients with primary hyperparathyroidism have lower circulating sclerostin levels than euparathyroid controls. *Eur J Endocrinol* 2010;163:833-7.
 87. Rhee Y, Bivi N, Farrow E, et al. Parathyroid hormone receptor signaling in osteocytes increases the expression of fibroblast growth factor-23 in vitro and in vivo. *Bone* 2011;49:636-43.
 88. Calvi LM, Sims NA, Hunzelman JL, et al. Activated parathyroid hormone/parathyroid hormone-related protein receptor in osteoblastic cells differentially affects cortical and trabecular bone. *J Clin Invest* 2001;107:277-86.
 89. Bellido T. Downregulation of SOST/sclerostin by PTH: a novel mechanism of hormonal control of bone formation mediated by osteocytes. *J Musculoskelet Neuronal Interact* 2006;6:358-9.
 90. O'Brien CA, Plotkin LI, Galli C, et al. Control of bone mass and remodeling by PTH receptor signaling in osteocytes. *PLoS One* 2008;3:e2942.
 91. Robling AG, Kedlaya R, Ellis SN, et al. Anabolic and catabolic regimens of human parathyroid hormone 1-34 elicit bone- and envelope-specific attenuation of skeletal effects in Sost-deficient mice. *Endocrinology* 2011;152:2963-75.
 92. Kramer I, Keller H, Leupin O, Kneissel M. Does osteocytic SOST suppression mediate PTH bone anabolism? *Trends Endocrinol Metab* 2010;21:237-44.
 93. Rawadi G, Roman-Roman S. Wnt signalling pathway: a new target for the treatment of osteoporosis. *Expert Opin Ther Targets* 2005;9:1063-77.
 94. Baron R, Rawadi G. Wnt signaling and the regulation of bone mass. *Curr Osteoporos Rep* 2007;5:73-80.
 95. Shahnazari M, Yao W, Corr M, Lane NE. Targeting the Wnt signaling pathway to augment bone formation. *Curr Osteoporos Rep* 2008;6:142-8.
 96. Deal C. Potential new drug targets for osteoporosis. *Nat Clin Pract Rheumatol* 2009;5:20-7.
 97. Hoepfner LH, Secretó FJ, Westendorf JJ. Wnt signaling as a therapeutic target for bone diseases. *Expert Opin Ther Targets* 2009;13:485-96.
 98. Galli C, Passeri G, Macaluso GM. Osteocytes and WNT: the mechanical control of bone formation. *J Dent Res* 2010;89:331-43.
 99. Shi C, Li J, Wang W, et al. Antagonists of LRP6 regulate PTH-induced cAMP generation. *Ann N Y Acad Sci* 2011;1237:39-46.
 100. Nusse R, van Ooyen A, Cox D, et al. Mode of proviral activation of a putative mammary oncogene (int-1) on mouse chromosome 15. *Nature* 1984;307:131-6.
 101. Yang Y. Wnt signaling in development and disease. *Cell Biosci* 2012;2:14.
 102. Monroe DG, McGee-Lawrence ME, Oursler MJ, Westendorf JJ. Update on Wnt signaling in bone cell biology and bone disease. *Gene* 2012;492:1-18.
 103. Kikuchi A, Yamamoto H, Kishida S. Multiplicity of the interactions of Wnt proteins and their receptors. *Cell Signal* 2007;19:659-71.
 104. MacDonald BT, Tamai K, He X. Wnt/beta-catenin signaling: components, mechanisms, and diseases. *Dev Cell* 2009;17:9-26.
 105. Tu X, Rhee Y, Condon KW, et al. Sost down-regulation and local Wnt signaling are required for the osteogenic response to mechanical loading. *Bone* 2012;50:209-17.
 106. Minear S, Leucht P, Jiang J, et al. Wnt proteins promote bone regeneration. *Sci Transl Med* 2010;2:29-30.
 107. Zhang W, Drake MT. Potential role for therapies targeting DKK1, LRP5, and serotonin in the treatment of osteoporosis. *Curr Osteoporos Rep* 2012;10:93-100.
 108. Tian Y, Xu Y, Fu Q, He M. Parathyroid hormone regulates osteoblast differentiation in a Wnt/beta-catenin-dependent manner. *Mol Cell Biochem* 2011;355:211-6.
 109. Costa AG, Cremers S, Rubin MR, et al. Circulating sclerostin in disorders of parathyroid gland function. *J Clin Endocrinol Metab* 2011;96:3804-10.
 110. Fei Y, Hurley MM. Role of fibroblast growth factor 2 and Wnt signaling in anabolic effects of parathyroid hormone on bone formation. *J Cell Physiol* 2012;227:3539-45.