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OSTEOCYTES AND THEIR ROLE IN BONE REMODELING

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Abstract

Osteocytes are former osteoblasts that become entombed during the process of bone deposition and are regularly distributed throughout the mineralized bone matrix. Osteocytes are the most abundant cells in bone comprising more than 90% of cells within the matrix or on the bone surfaces. Increasing evidence supports the notion that osteocytes coordinate the function of osteoblasts and osteoclasts in response to both mechanical and hormonal stimuli. Osteocytes produce and secrete factors (such as sclerostin) that affect other bone cells by paracrine/autocrine mechanisms. In addition, osteocytes produce and secrete hormones (such as FGF23) that affect other tissues by endocrine mechanisms. This review summarizes the current understanding of osteocyte functions and the role of these cells in bone remodeling.

Key words: osteocytes, bone remodeling.

Resumen

OSTEOCITOS Y SU ROL EN EL REMODELADO ÓSEO

Los osteocitos son osteoblastos que se convierten en osteocitos durante el proceso de deposición de hueso y se distribuyen regularmente a lo largo de la matriz ósea mineralizada. Son las células más abundantes del hueso y comprenden más de 90% de células en la matriz o en las superficies óseas. La evidencia sostiene que los osteocitos coordinan la función de los osteoblastos y los osteoclastos en respuesta a estímulos mecánicos y hormonales. Los osteocitos producen y secretan factores (como esclerostina) que afectan a otras células óseas por mecanismos paracrinos/autocrinos. Además, los osteocitos producen y secretan hormonas (tales como FGF23) que afectan a otros tejidos por mecanismos endócrinos. Esta revisión resume los conocimientos actuales sobre las funciones de los osteocitos y el papel de estas células en el remodelado óseo.

Palabras clave: osteocitos, remodelado óseo.

Osteocyte morphology and functions

Osteocyte bodies are individually encased in lacunae and exhibit cytoplasmic dendritic processes that run along narrow canaliculi within the mineralized matrix. Osteocyte morphology is dictated by the expression of genes involved in dendrite formation and branching, such as E11/gp38, CD44, and fimbrin, which

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are also expressed in neurons and give osteocytes their characteristic morphology *in vivo* as well as in culture.[1] Quantitative analysis using microscopy determined that each osteocyte exhibits an average of 50 cytoplasmic projections emerging from its body.[2] Projections from neighboring osteocytes touch each other and establish communication through gap junctions within canaliculi. Canaliculi also reach both periosteal and endocortical bone surfaces in cortical bone as well as surfaces adjacent to the bone marrow in cancellous bone. The lacunar-canalicular system also allows the transport of proteins that are produced and secreted by osteocytes and exert their action on cells on the bone surface or the bone marrow. Osteocytes are the main bone cells that produce sclerostin, the product of the Sost gene.[3] As expected for an osteocyte-derived secreted protein, high levels of sclerostin are detected in canaliculi. Sclerostin inhibits bone formation by preventing activation of Wnt signaling and also antagonizes the actions of proteins of the bone morphogenetic protein (BMP) family.[4]

Today, it is accepted that osteocytes are the primary mechanosensory cells in bone.[1] Osteoblasts and osteoclasts are present on bone only transiently, in low number, and in variable locations. On the other hand, osteocytes are present in the entire bone volume and are long-lived. Osteocytes are the core of a functional syncytium that extends from the mineralized bone matrix to the bone surface and the bone marrow, which also reaches the blood vessels. Osteocytes' strategic location permits the detection of variations in mechanical signals (either through strain or fluid flow), as well as levels of circulating factors (ions or hormones), and allows amplification of the signals leading to adaptive responses of the skeleton to environmental changes.[5]

Increasing evidence demonstrates that osteocytes regulate the function of osteoblasts and osteoclasts. In response to mechanical and hormonal cues, osteocytes produce and secrete factors (such as sclerostin, RANKL, OPG) that affect other bone cells by paracrine or autocrine mechanisms, and hormones (such as FGF23) that affect other tissues by endocrine mechanisms.[6]

Osteocytes detect fatigue-induced microdamage and signal to osteoclasts to induce replacement of damaged bone through remodeling.[7] They also respond to changes in mechanical load by inducing local changes in bone mass and geometry through modeling.[8,9] Furthermore, osteocytes detect alterations in the levels of circulating hormones and respond by changing the rate of bone formation and resorption.[1]

Osteocytogenesis and osteocyte maturation

Between 5 to 20% of mature osteoblasts become entombed in the matrix that they generate and that subsequently mineralizes. The process of osteocyte formation was long thought to be stochastic. However, it is now recognized that some osteoblasts might be prompted to extend cytoplasmic projections and to contact with already embedded cells, resulting in their differentiation into osteocytes. In particular, expression of the membrane-associated proteins E11 and metalloproteinase MMP14 is required for the formation of osteocyte dendritic processes and canaliculi. This evidence supports the notion that osteocytogenesis is an active process driven by changes in gene expression. However, the mechanisms that determine which osteoblasts will become osteocytes remain obscure.

Osteocyte formation is one of the three possible fates of mature osteoblasts, the other two being becoming lining cells or undergoing apoptosis.[10] It is then expected that stimuli that alter an osteoblast's fate would impact osteocyte formation. Consistent with this notion, inhibition of osteoblast apoptosis by intermittent administration of parathyroid hormone (PTH) leads to increased osteocyte

density.[11] However, it is still unknown whether this effect of the hormone is accompanied by changes in the expression of genes required for the osteoblast-osteocyte transition.

Osteocytes express most of the genes expressed by osteoblasts, including osteoblast-specific transcription factors and proteins, although the levels of expression may slightly differ.[1] Thus, alkaline phosphatase and type I collagen expression is lower whereas osteocalcin expression is higher in osteocytes. Keratocan, an extracellular matrix protein that belongs to the small leucine rich proteoglycan family, has emerged as an osteoblast marker because its expression is greatly reduced in osteocytes.

Osteocytes are richer than osteoblasts in genes related to mineralization and phosphate metabolism, including phosphate-regulating neutral endopeptidase (Phex), dentin matrix protein 1 (DMP1), matrix extracellular phosphoglycoprotein (MEPE) and FGF23. Osteocytes also express high levels of the inhibitor of bone formation Dkk1; and the Sost gene encoding the Wnt antagonist and bone formation inhibitor sclerostin is expressed in osteocytes but not in osteoblasts.

Osteocyte apoptosis: consequences and regulation

Osteocytes are long-lived cells. However, like osteoblasts and osteoclasts, osteocytes die by apoptosis; and decreased osteocyte viability accompanies the bone fragility syndromes that characterize glucocorticoid excess, estrogen withdrawal, and mechanical disuse.[10] Conversely, preservation of osteocyte viability might explain at least part of the anti-fracture effects of bisphosphonates, which cannot be completely accounted for by increases in bone mineral density.[12,13]

Preservation of osteocyte viability by mechanical stimuli

Osteocytes interact with the extracellular matrix (ECM) in the pericellular space through discrete sites in their membranes, which are enriched in integrins and vinculin, as well as through transverse elements that tether osteocytes to the canalicular wall. Fluid movement in the canaliculi resulting from mechanical loading might induce ECM deformation, shear stress, and/or tension in the tethering elements. The resulting change in circumferential strain in osteocyte membranes is hypothesized to be converted into intracellular signals by integrin clustering and integrin interaction with cytoskeletal and catalytic proteins at focal adhesions. Physiological levels of mechanical strain imparted by stretching or pulsatile fluid flow prevent apoptosis of cultured osteocytes.[14] Mechanistic studies indicate that the transduction of mechanical forces into intracellular signals is accomplished by molecular complexes assembled at caveolin-rich domains of the plasma membrane and composed of integrins, cytoskeletal proteins and kinases including the focal adhesion kinase FAK and Src, resulting in activation of the ERK pathway and osteocyte survival. Intriguingly, a ligand-independent function of the estrogen receptor (ER) is indispensable for mechanically-induced ERK activation in both osteoblasts and osteocytes.[15] Accordingly, mice lacking the ERα and ERβ exhibit a poor osteogenic response to loading.[16]

In vivo mechanical forces also regulate osteocyte life span. Apoptotic osteocytes are found in unloaded bones or in bones exposed to high levels of mechanical strain. In both cases, increased apoptosis of osteocytes was observed before any evidence of increased osteoclast resorption, and apoptotic osteocytes accumulated in areas that were subsequently removed by osteoclasts.[5] These findings suggest that dying osteocytes in turn become the beacons for osteoclast recruitment to the vicinity and the resulting increase in bone resorption. In support of this notion, targeted ablation of osteocytes in transgenic mice is sufficient to induce osteoclast recruitment and resorption leading to bone

loss.[17] Whether living osteocytes continually produce molecules that restrain osteoclast recruitment or whether in the process of undergoing apoptosis osteocytes produce pro-osteoclastogenic signals remains to be determined. Taken together with the evidence that osteocyte apoptosis is inhibited by estrogens and bisphosphonates, these findings raise the possibility that preservation of osteocyte viability contributes to the anti-remodeling properties of these agents.[7]

Aging and osteocyte apoptosis

One of the functions of the osteocyte network is to detect microdamage and trigger its repair. During aging, there is accumulation of microdamage and a decline in osteocyte density accompanied by decreased prevalence of osteocyte-occupied lacunae, an index of premature osteocyte death.[18] Reduced osteocyte density might be a direct consequence of increased osteoblast apoptosis, whereas increased osteocyte apoptosis might result from the decline in physical activity with old age leading to reduced skeletal loading, accumulation of reactive oxygen species (ROS) in bone and/or increased levels of endogenous glucocorticoids with age (as will be discussed below). In view of the evidence on the role of osteocytes in microdamage repair, age-related loss of osteocytes could be partially responsible for the disparity between bone quantity and quality that occurs with aging.

Hormonal regulation of osteocyte life span

Estrogen as well as androgen deficiency lead to increased prevalence of osteocyte apoptosis.[19] Conversely, estrogens and androgens inhibit apoptosis of osteocytes as well as osteoblasts. This anti-apoptotic effect is due to rapid activation of the Src/Shc/ERK signaling pathway through non-genotropic actions of the classical receptors for sex steroids. This effect requires only the ligand-binding domain of the receptor, and unlike the classical genotropic action of the receptor protein that require its nuclear functions, the survival effect of sex steroids is eliminated by nuclear targeting of the receptors.

Increased glucocorticoid action in bone may also contribute to induction of osteocyte apoptosis.[20] This might result from treatment with the steroids, which have immunosuppressive effects, from endogenous elevation of the hormones with age, or from increased expression in bone of 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1), the enzyme that amplifies glucocorticoid action by converting inactive into active steroids. The apoptotic effect of glucocorticoids is reproduced in cultured osteocytes and osteoblasts in a manner strictly dependent on the glucocorticoid receptor (GR).[22] Induction of osteocyte and osteoblast apoptosis by glucocorticoids results from direct actions of the steroids on these cells, as overexpression of the enzyme that inactivates glucocorticoids 11β-HSD2 specifically in osteoblastic cells abolishes the increase in apoptosis. The pro-apoptotic effect of glucocorticoids in cultured osteocytic cells is preceded by cell detachment due to interference with FAK-mediated survival signaling generated by integrins.[21] In this mechanism, Pyk2 (a member of the FAK family) becomes phosphorylated and subsequently activates pro-apoptotic JNK signaling.[22] In addition, the pro-apoptotic actions of glucocorticoids may involve suppression of the synthesis of locally produced anti-apoptotic factors including IGF-I and IL-6 type cytokines, as well as MMPs, and stimulation of the pro-apoptotic Wnt antagonist SFRP-1.

Regulation of bone formation by osteocytes: sclerostin

Osteocytes express sclerostin, the product of the Sost gene, which binds to LRP5/LRP6 preventing canonical Wnt signaling and also interacts with some BMPs.[23,24] Wnt and BMP signaling is critical for osteoblastogenesis and bone mass acquisition. Loss of Sost expression in humans causes the high bone mass

disorders Van Buchem's disease and sclerosteosis.[25] In addition, administration of an anti-sclerostin antibody increases bone formation and restores bone lost upon estrogen deficiency and other bone catabolic conditions.[26] Conversely, transgenic mice overexpressing Sost exhibit low bone mass.[27] Taken together, these lines of evidence have led to the conclusion that sclerostin derived from osteocytes, the most differentiated cell of the osteoblastic pathway, exerts a negative feedback control on osteoblast generation and activity.

Regulation of bone resorption by osteocytes: RANKL and OPG

The cues that signal bone resorption are not completely understood. One important factor in the regulation of remodeling appears to be the apoptosis of osteocytes following local bone damage or microdamage, which signals to osteoblast lining cells to form the bone remodeling compartment (BRC). Apoptotic osteocytes could regulate the recruitment of osteoclast precursors and their differentiation in two ways. Osteocyte apoptosis may indirectly stimulate osteoclastogenesis by inducing stromal/osteoblastic cells to secrete RANKL. In addition, osteocytes can directly secrete RANKL. Indeed, *in vitro*, purified osteocytes express higher levels of RANKL than osteoblasts and bone marrow stromal cells. The severe osteopetrotic phenotype observed in mice lacking RANKL in osteocytes and their resistance to bone loss induced by tail suspension, supports the idea that osteocytes are a major source of RANKL *in vivo*.[28] Further, osteocytes secrete OPG, which competes with RANKL for its receptor on osteoclasts. In osteocytes, as in osteoblasts, OPG secretion is regulated by the Wnt/β-catenin pathway and mice lacking β-catenin in osteocytes are osteoporotic due to increased osteoclast numbers.[29] In addition, emerging experimental evidence points to osteocytes as an additional source of secreted M-CSF in bone. Together, these new findings suggest that osteocytes control the bone remodeling process through direct and indirect regulation of osteoclast and osteoblast differentiation and function.

Regulation of bone mineralization by osteocytes

Approximately 50-70% of the bone matrix is mineral. As mature osteoblasts are surrounded by the collagenous matrix and differentiate into osteocytes, mineral is deposited to transform osteoid into mineralized bone. Studies using genetically modified mice have demonstrated that osteocytes actively participate in the regulation of bone mineralization. In particular, DMP1 and MEPE, proteins of the small integrin-binding ligand, N-linked glycoprotein (SIBLING) family, are produced by late stage osteoblasts and osteocytes and can be detected in the canalicular and lacunar walls.[30,31] DMP1 appears to be dispensable for bone mineralization during development, but adult DMP1-deficient mice show defective osteocyte morphology and altered bone mineralization.[32] In contrast, MEPE appears to be an inhibitor of mineralization as MEPE deficient mice exhibit increased bone density and ASARM, a cleavage product of MEPE, can block mineralization *in vitro* and *in vivo*. Both DMP1 and MEPE are mechanoresponsive genes and changes in their expression might be responsible for the reduced mineralization of the matrix surrounding osteocytes induced by mechanical stimulation.

Osteocytes also express and secrete FGF23, a hormone that regulates phosphate reabsorption in the kidney, and that by changing circulating levels of phosphate, also affects bone mineralization.[33] FGF23 also directly activates intracellular signaling in osteocytes and osteoblasts mediated through binding to the FGFR1/KLOTHO receptor complex and has been shown to suppress osteoblast differentiation and matrix mineralization in vitro, suggesting a role for FGF23 not only in the regulation of sys-

temic phosphate levels, but also in the local control of bone mineralization.

Role of osteocytes in the actions of PTH

PTH inhibits the expression of the osteocyte-derived inhibitor of bone formation sclerostin.[3,34] These findings provided the basis for a novel mechanism by which the hormone could affect skeletal homeostasis through effects on osteocytes.[6]

PTH exerts its inhibitory effect on Sost/sclerostin expression downstream of the PTH receptor (PTHR1) and activation of the cAMP pathway. This is demonstrated by the fact that PTHrP, the other ligand of this receptor, and stable analogs of cAMP mimic the effects of PTH on Sost. However, Sost downregulation appears not to depend on transcription factors of the cAMP responsive element binding protein (CREB) family. Instead, transcription factors of the myocyte enhancer factor (MEF2) family mediate the effect of PTH on Sost expression.[35] Nevertheless, the exact molecular mechanism of this regulation remains unknown.

Expression of a constitutively active PTHR1 in osteocytes in transgenic mice is sufficient to downregulate Sost and to reduce sclerostin levels in vivo. This is associated with increased Wnt activation, marked stimulation of bone formation and increases in bone mass.[36] Bone formation and bone mass are reversed to wild type levels in double transgenic mice also expressing Sost in osteocytes, demonstrating that the requirement of Sost downregulation is needed to induce of bone anabolism induced by PTHR1 signaling activation in osteocytes.[27]

Furthermore, mice with constitutive activation of the PTHR1 also exhibit elevated rate of bone resorption, enhanced osteoclasts and increased expression of RANKL.[27] Together with the evidence that osteocytes are a major source of RANKL, these findings raise the possibility that at least part of the pro-resorptive effects of PTH are due to osteocytic RANKL regulation by the hormone.

The findings that activation of PTH receptor signaling in osteocytes is sufficient to mimic the most recognized actions of PTH on the skeleton demonstrate that osteocytes are crucial target cells of hormone in bone.[6]

Osteocytes and the bone remodeling compartment (BRC)

Lining cells play an important function in initiating bone remodeling by retracting from quiescent bone surfaces and creating a canopy over osteoclasts and osteoblasts in the bone multicellular unit. On the endocortical surface, this canopy presumably encases bone marrow osteoblast precursors and is penetrated by blood vessels that provide hematopoietic osteoclast progenitors. The lining cell canopy, associated capillaries, osteocytes, osteoclasts and osteoblasts form a compartment named the bone remodeling compartment (BRC), which is separated from the rest of the marrow and which can potentially sequester molecules that regulate the cells that remodel bone.[37] Premature apoptosis of osteocytes has been shown to precede osteoclast accumulation and resorption, raising the possibility that osteocytes release molecules that induce lining cell retraction facilitating access of osteoclast precursors to bone surfaces. However, the molecular entities responsible for this purported osteocytic function remain unknown. As discussed above, osteocytes express M-CSF, which stimulates proliferation of pre-osteoclasts, and RANKL, the master cytokine inducer of osteoclast differentiation, both of which could reach the BRC. Factors released from the bone matrix upon resorption, in turn, stimulate osteoblastogenesis. It is also likely that osteocyte-derived sclerostin, reaching the BRC through the canalicular system, influences the rate of bone formation, providing an additional level of control of osteoblast activity. Based on these lines of evidence, the BRC might provide a supportive

environment for differentiation of osteoclast and osteoblast progenitors. Thus, regulation of the bone remodeling rate by hormonal and mechanical stimuli could be exerted by controlling the balance between resorption and formation within the BRC through the regulation of osteocytic molecules including sclerostin, RANKL and OPG (Figure 1).

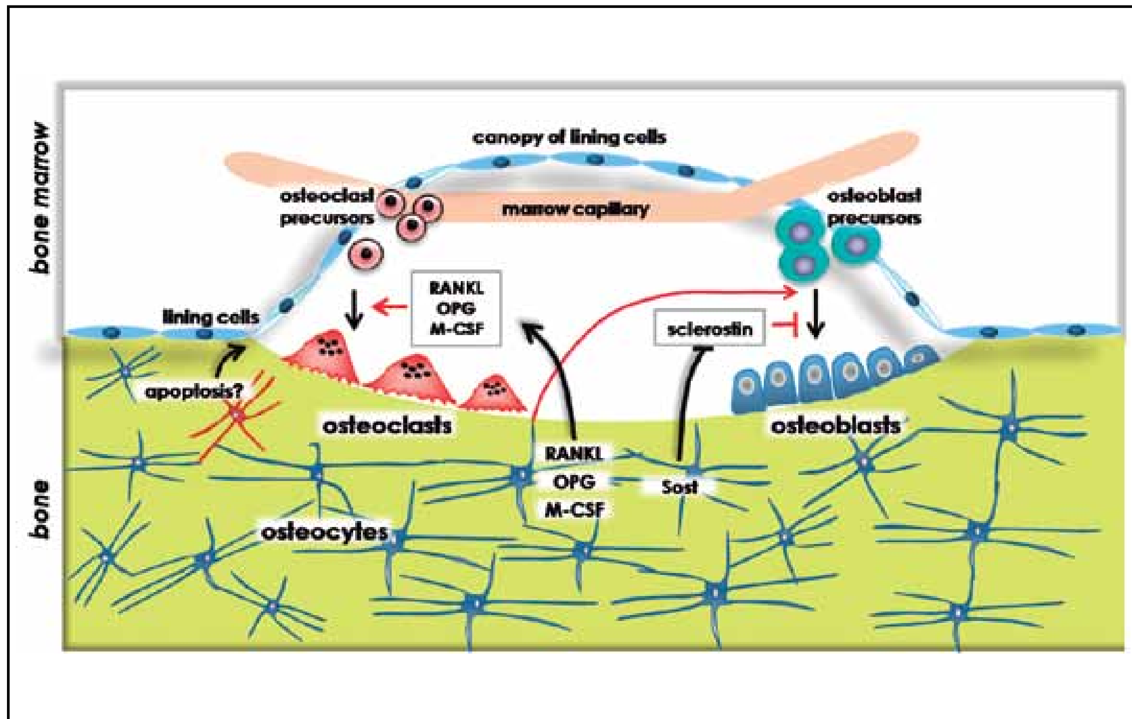

Figure 1. Osteocytes and bone remodeling: Osteocytes sense the need for bone resorption (may be mediated by increased osteocyte apoptosis) and send signals to lining cells, which retract from the bone surface and form a canopy under which remodeling occurs, named bone remodeling compartment (BRC). Osteoclast precursors are transported to the BRC by marrow capillaries, differentiate to mature osteoclasts under the influence of pro- and anti-osteoclastogenic cytokines (RANKL, M-CSF and OPG) derived from osteocytes, and initiate bone remodeling. Osteoblast precursors recruited from the bone marrow or the circulation differentiate into mature, bone synthesizing cells in response to factors released from the bone matrix by resorption. Differentiation and function of osteoblasts is controlled by molecules derived from osteocytes, including sclerostin.

Conflict of interest

The author has no conflict of interest to declare.

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References

1. Bonewald LF. The amazing osteocyte. *J Bone Miner Res* 2011; 26:229-38.
2. Marotti G, Cane V, Palazzini S, Palumbo C. Structure-function relationships in the osteocyte. *Ital J Min Electrol Metab* 1990; 4:93-106.
3. Bellido T, Ali AA, Gubrij I, et al. Chronic elevation of PTH in mice reduces expression of sclerostin by osteocytes: a novel mechanism for hormonal control of osteoblastogenesis. *Endocrinology* 2005; 146:4577-83.
4. Piters E, Boudin E, Van Hul W. Wnt signaling: a win for bone. *Arch Biochem Biophys* 2008; 473:112-6.
5. 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 Min Res* 2006; 21:605-15.
6. Bellido T, Saini V, Divieti Pajevic P. Effects of PTH on osteocyte function. *Bone* 2012; pii: S8756-3282(12):1245-8.
7. Bellido T. Osteocyte apoptosis induces bone resorption and impairs the skeletal response to weightlessness. *Bonekey-Osteovision* 2007; 4:252-6
8. Bonewald LF. Mechanosensation and transduction in osteocytes. *Bonekey-Osteovision* 2006; 3:7-15.
9. Turner CH, Warden SJ, Bellido T, et al. Mechanobiology of the skeleton. *Sci Signal* 2009; 2:t3
10. Jilka RL, Bellido T, Almeida M, et al. Apoptosis in bone cells. In *Principles of Bone Biology*. Bilezikian JP, Raisz LG, Martin TJ, editors. Academic Press. San Diego, San Francisco, New York, London, Sydney, Tokyo. 2008 p:237-261
11. Jilka RL, Weinstein RS, Bellido T, Roberson P, Parfitt AM, Manolagas SC. Increased bone formation by prevention of osteoblast apoptosis with parathyroid hormone. *J Clin Invest* 1999; 104:439-46.
12. Bellido T, Plotkin LI. Novel actions of bisphosphonates in bone: Preservation of osteoblast and osteocyte viability. *Bone* 2011; 49:50-5.
13. Plotkin LI, Weinstein RS, Parfitt AM, Roberson PK, Manolagas SC, Bellido T. Prevention of osteocyte and osteoblast apoptosis by bisphosphonates and calcitonin. *J Clin Invest* 1999; 104:1363-74.
14. Plotkin LI, Mathov I, Aguirre JI, Parfitt AM, Manolagas SC, Bellido T. Mechanical stimulation prevents osteocyte apoptosis: requirement of integrins, Src kinases and ERKs. *Am J Physiol Cell Physiol* 2005; 289:C633-43.
15. Aguirre JI, Plotkin LI, Gortazar AR, O'Brien CA, Manolagas SC, Bellido T. A novel ligand-independent function of the estrogen receptor is essential for osteocyte and osteoblast mechanotransduction. *J Biol Chem* 2007; 282:25501-8.
16. Lee K, Jessop H, Suswillo R, Zaman G, Lanyon L. Endocrinology: bone adaptation requires oestrogen receptor-alpha. *Nature* 2003; 424:389.
17. Tatsumi S, Ishii K, Amizuka N, et al. Targeted ablation of osteocytes induces osteoporosis with defective mechanotransduction. *Cell Metab* 2007; 5:464-75.
18. Manolagas SC, Parfitt AM.. What old means to bone. *Trends Endocrinol Metab* 2010; 21:369-74.
19. Kousteni S, Bellido T, Plotkin LI, et al. Nongenotropic, sex-nonspecific signaling through the estrogen or androgen receptors: dissociation from transcriptional activity. *Cell* 2001; 104:719-30.
20. Weinstein RS. Clinical practice. Glucocorticoid-induced bone disease. *N Engl J Med* 2011; 365:62-70
21. Bellido T. Antagonistic interplay between mechanical forces and glucocorticoids in bone: a tale of kinases. *J Cell Biochem* 2010; 111:1-6.
22. Plotkin LI, Manolagas SC, Bellido T. Glucocorticoids induce osteocyte apoptosis by blocking focal adhesion kinase-mediated survival: evidence for inside-out signaling leading to anoikis. *J Biol Chem* 2007; 282:24120-30.
23. Winkler DG, Sutherland MK, Geoghegan JC, et al. Osteocyte control of bone formation via sclerostin, a novel BMP antagonist. *EMBO J* 2003; 22:6267-76.

24. 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.
25. Balemans W, Van Hul W. Human genetics of SOST. *J. Musculoskelet Neuronal Interact* 2006; 6:355-6.
26. Paszty C, Turner CH, Robinson MK. Sclerostin: a gem from the genome leads to bone-building antibodies. *J Bone Miner Res* 2010; 25:1897-904.
27. Rhee Y, Allen MR, Condon K, et al. PTH receptor signaling in osteocytes governs periosteal bone formation and intra-cortical remodeling: divergent role of Sost and the Wnt pathway. *J Bone Min Res* 2009; 24:S78.
28. Xiong J, O'Brien CA. Osteocyte RANKL: New insights into the control of bone remodeling. *J Bone Miner Res* 2012; 27:499-505.
29. Kramer I, Halleux C, Keller H, et al. Osteocyte Wnt/beta-catenin signaling is required for normal bone homeostasis. *Mol Cell Biol* 2010; 30:3071-85.
30. Rowe PS. Regulation of bone-renal mineral and energy metabolism: the PHEX, FGF23, DMP1, MEPE ASARM pathway. *Crit Rev Eukaryot Gene Expr* 2012; 22:61-86.
31. Quarles LD. FGF23, PHEX, and MEPE regulation of phosphate homeostasis and skeletal mineralization. *Am J Physiol Endocrinol Metab* 2003; 285:E1-9.
32. 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.
33. White KE, Larsson TE, Econs MJ. The roles of specific genes implicated as circulating factors involved in normal and disordered phosphate homeostasis: frizzled related protein-4, matrix extracellular phosphoglycoprotein, and fibroblast growth factor 23. *Endocr Rev* 2006. 27:221-41.
34. Keller H, Kneissel M. SOST is a target gene for PTH in bone. *Bone* 2005; 37:148-58.
35. Leupin O, Kramer I, Collette NM, et al. Control of the SOST bone enhancer by PTH using MEF2 transcription factors. *J. Bone Miner Res* 2007; 22:1957-67.
36. 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.
37. Eriksen EF. Cellular mechanisms of bone remodeling. *Rev. Endocr Metab Disord* 2010; 11:219-27.