Recent Progress in Osteocyte Research

The Harvard community has made this article openly available. **Please share** how this access benefits you. Your story matters

| Citation         | Divieti Pajevic, Paola. 2013. “Recent Progress in Osteocyte Research.” Endocrinology and Metabolism 28 (4): 255-261. doi:10.3803/EnM.2013.28.4.255. http://dx.doi.org/10.3803/EnM.2013.28.4.255. |
|------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Published Version| doi:10.3803/EnM.2013.28.4.255                                                                                                                                                                      |
| Citable link     | http://nrs.harvard.edu/urn-3:HUL.InstRepos:11879695                                                                                                                                               |
| Terms of Use     | This article was downloaded from Harvard University’s DASH repository, and is made available under the terms and conditions applicable to Other Posted Material, as set forth at http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#LAA |
Recent Progress in Osteocyte Research

Paola Divieti Pajevic

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

The last decade has seen an exponential increase in our understanding of osteocytes function and biology. These cells, once considered inert by-standers trapped into the mineralized bone, has now risen to be key regulators of skeletal metabolism, mineral homeostasis, and hematopoiesis. As tools and techniques to study osteocytes improved and expanded, it has become evident that there is more to these cells than initially thought. Osteocytes are now recognized not only as the key responders to mechanical forces but also as orchestrators of bone remodeling and mineral homeostasis. These cells are the primary source of several important proteins, such as sclerostin and fibroblast growth factor 23, that are currently target as novel therapies for bone loss (as the case for anticalerostin antibodies) or phosphate disorders. Better understanding of the intricate cellular and molecular mechanisms that govern osteocyte biology will open new avenue of research and ultimately indentify novel therapeutics to treat bone and mineral disorders. This review summarizes novel findings and discusses future avenues of research.

Keywords: Osteocytes; Sclerostin; Mineral homeostasis; Bone homeostasis

INTRODUCTION

Our understanding of the function of osteocytes has expanded dramatically over the last decade primarily due to the identification of osteocytes specific markers, such as dentin matrix protein 1 (DMP1) and SOST/sclerostin, that has allowed, for the first time a closer look at the biology of these cells. Osteocytes, the cells deeply entrapped into the mineralized matrix, have emerged as key regulators not only of skeletal and mineral homeostasis, but also hematopoiesis. This review will summarize novel findings in osteocyte biology and future avenues of research.

THE OSTEOCYTES

Osteocytes are postmitotic, terminally differentiated osteoblasts that during the process of matrix mineralization remain entrapped in the mineralizing matrix that they are actively synthesizing. They reside both in the mineralized matrix and in the newly formed osteoid. Earlier work of Gaillard et al. [1] and Rutishauser and Majno [2], described two stages into the life of an osteocyte; an early stage (young osteocyte) in which the cell is smaller in size, reside into the osteoid and do not express alkaline phosphatase and a late stage (mature osteocyte) in which the cell is smaller in size, reside into the osteoid and do not express alkaline phosphatase and a late stage (mature osteocyte) in which the larger cell re-express alkaline phosphatase and is deeply embedded in the mineralized bone. The larger cell will then degenerate and leave an empty lacuna [1]. This classification has been recently revised to include an additional stage of differentiation: according to their spatial localization and gene expression, these cells are now divided into osteoid, mineralizing, and the mature osteocytes [3]. The osteoid, or nascent osteocyte is characterized by a relative proxim-
ity to the endosteal (and possibly periostal) surface and the expression of transcripts such as E11/gp38/podoplain [4], matrix extracellular phosphoglycoprotein (MEPE) and phosphate-regulating gene with homologies to endopeptidases on the X chromosome (Phex). This cell, opposite to the more mature osteocyte, is negative for sclerostin and fibroblast growth factor (FGF)-23 expression but does express DMP1, as elegantly demonstrated by Kalajzic et al. [5] in a recent review. It is not clear, however, if osteoid, mineralizing and mature osteocytes exert different biological functions or if they all work as mechanosensor and skeletal regulators. Indeed recent studies suggest that cortical and trabecular osteocytes might have distinct roles with the former being the sensor of load while the latter being the controller of bone metabolism. Windahl et al. [6], for example, reported that estrogen receptor-α ablation in osteocytes differentially affects trabecular and cortical bone compartments in males mice.

Marotti et al. [7] and Palumbo et al. [8,9] have extensively described the morphological changes that accompany the transformation from a motile osteoblast into an entombed osteocyte. They demonstrate, by histological analysis of newborn rabbit bones, that the formation of osteocyte cytoplasmic processes is asynchronous and asymmetrical and precede the mineralization of the organic matrix. Moreover, osteocytes are evolutionary highly conserved and the organized structure of these cells within a mineralized matrix is present in bone specimens from *Tyrannosaurus rex*, dating back more than 80 million of years ago, clearly indicating an important role for these cells in skeletal metabolism [10].

Osteocytes communicate with each other and with cells at the endosteal and periosteal surface through an extensive and intricate system of canaliculi. These cells are also in close proximity of capillary and vessels, raising the hypothesis that osteocytes might function as an endocrine organ and directly secrete proteins, such as FGF-23, Phex, or sclerostin into the circulation [11]. Moreover, the osteocytic network, with its extensive system, is an ideal structure to sense mechanical loading and control mineral homeostasis. It has been postulated that osteocytes can send signals for both bone resorption and formation and thus orchestrate a proper cycle of remodeling, as discussed in details below.

**OSTEOCYTE FUNCTIONS: MECHANOSENSATION**

What are the functions of an osteocyte? It has been recognized for over 30 years that osteocytes are mechanosensor of bone. They are indeed the cells capable of sensing mechanical forces applied to the skeleton and transform these forces into biological stimuli. It is now widely accepted that the cell perceives forces in the form of shear stress created by the flow of fluid inside the lacuna-canalicular network. It has been proposed that the flow derives from both the compression induced by loading and the extravascular pressure. Efforts to physically measure and quantify this flow are currently ongoing and recent reports suggest that osteocytes are subjected to forces in the order of 5 Pa [12]. Although the theory that osteocytes are the mechanosensor of bone has been around for decades, the definite demonstration of this theory comes from work of Tatsumi et al. [13]. Using a mouse model of targeted osteocytes ablation, they demonstrated resistance to disuse induces bone loss highlighting the central role that osteocytes play in mechanosensation. How an osteocyte can sense the fluid flow in the lacuno-canalicular network is still not completely understood. Primary cilia have been described as important mechanosensor and mice lacking Kif3a or ITF88 (polaris), both key components of the cilium, in bone cells, display skeletal abnormalities. Targeted ablation of Kif3a from osteoblasts, and consequently from osteocytes, using the Col1α 2.3 promoter, affects skeletal anabolic responses to cyclic axial compression, suggesting that primary cilia are needed for proper skeletal mechanosensation [14,15]. Similarly, mice with in which PDK1 is ablated in osteocytes, using the 10kb-DMP1 promoter have impaired anabolic response to mechanical loading, further supporting the importance of cilia in skeletal mechanosensation [16,17].

Osteocytes responses to load, both in vivo and in vitro, include secretion of prostaglandin E2 and nitric oxide and the expression of several mechanosensitive transcripts such as DMP1, MEPE, and Phex [18,19]. Another osteocytic gene highly regulated by mechanical forces is SOST. SOST/sclerostin in suppressed during load whereas it is increased during hindlimb unloading [20]. Moreover mice lacking SOST are resistant to disuse induced bone loss and antisclerostin antibodies can prevent bone loss associated with hindlimb unloading [21]. This demonstrates that osteocytes are key orchestrators not only of skeletal responses to loading but also to unloading, as occurs during paralysis, prolonged bed rest or space flight. Frost [22] postulated that osteocytes are the mechanostat of bone, ascribing to these cells the role of driving skeletal adaptation to mechanical forces. Bone adapts its form to mechanical demands trough a mechanisms known as bone modeling. During modeling, bone resorption,
driven by osteoclasts, and bone formation, osteoblasts-mediated, occurs on different surfaces of the skeleton such as the bone undergoes reshaping to adapt to different loading conditions. Bone remodeling, on the other hand, is the continuous and spatially coupled, resorption and formation of the skeleton to preserve functional integrity. According to Frost [22], the mechanostat distinguishes between bone modeling (shape change) and remodeling (replacement only). Examples of bone adaptation to loading are evident in professional athletes, where the high load applied to the skeleton results in stronger and bigger bones, whereas in astronauts or in paralyzed patients there is severe bone loss due to unloading. As described above, compelling evidences ascribe to sclerostin an important role in this disuse induced bone loss.

**BONE AND MINERAL HOMEOSTASIS**

Osteocytes are not only the sensor of mechanical forces, they are also master controller of bone and mineral homeostasis. These cells are, postnatally, the main source sclerostin and receptor activation of nuclear factor-kB ligand (RANKL). Sclerostin, the product of the gene SOST, has emerged as a powerful inhibitor of bone formation. The protein binds to LRP5 and the related LRP6 and four receptors and block the canonical Wnt-βcatenin signaling pathway. Lack (or mutation) of sclerostin induces sclerosteosis whereas its over expression causes severe osteopenia (for review on Wnts) [23]. Osteocytes are also the main source of FGF-23, a key regulator, to calcium homeostasis [24,25]. Thus, it is not surprising that genetic manipulation of osteocytes induced both skeletal and mineral defects. Mice lacking RANKL in osteocytes have increased bone mineral density and reduced bone remodeling [26,27], as do mice lacking the receptor of PTH (PTH1R). Mice lacking Atg7, an autophagy gene, from osteocytes, display similar skeletal phenotype as aging mice, indicating that these cells are key regulator of skeletal metabolism [28]. Both DMP1-null mice and individuals with inactivating mutation of this gene have rickets and osteomalacia and are hypophosphatemic as a consequence of high levels of FGF-23 [29]. On the contrary, mice lacking FGF-23 are hyperphosphatemic, osteopenic, and dye prematurely. It has been reported that osteocytes are also the major source of insulin-like growth factor (IGF)-1 and IGF-1 produced by these cells is required for proper anabolic responses to mechanical loading. Deletion of IGF-1 in osteocytes, (using the DMP1-Cre promoter) impairs skeletal growth, have reduced periosteal circumference and have a blunted response to mechanical forces. When IGF-1 conditional KO mice were subjected to mechanical loading, there was a significant reduction in osteoblastic bone formation response, indicating that IGF-1 is an important determinant of both bone size and bone strength [30].

Pioneering studies on osteocytes involving histological analysis of bone specimens raised the possibility that osteocytes could reabsorb bone and directly contribute to mineral homeostasis. The finding that in various diseases (renal osteodystrophy, hyperparathyroidism, and immobilization) the size of the osteocytic lacunae was 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 is a classical osteoclast pit-forming assay and few decades later, the concept that osteocytes can indeed reabsorb their perilacunar matrix has been revisited. The current osteocytic osteolysis concept, as proposed by Cullinan [31], suggests that osteocytes do not remove substantial amount of bone (as osteoclast), but rather modify the matrix minerals in the perilacunar areas. This perilacunar remodeling is evident during continuous infusion with PTH [32], treatment with prednisone [33] or during lactation [34]. As described above, osteocytes express receptor for PTH, a known regulator of calcium homeostasis, and they are the ideal candidates for systemic homeostasis regulation. Mice lacking the PTH1R specifically in osteocytes have indeed an impaired calcium homeostasis when subjected to a low calcium diet, indicating these cells also control calcium homeostasis [35]. Lastly, when the vitamin D receptor is ablated from osteocytes, the animals are unable to mobilize calcium from bone under a vitamin D stimulus, indicating that vitamin D signaling in osteocytes is critical for moving calcium from skeletal stores to the blood [36]. In light to recent report indicating that osteocytes are the major source of RANKL and therefore bone remodeling [26,27], we can speculate that the calcemic effect of vitamin D on bone is via osteocyte-derived RANKL although evidences for this pathway are still missing.

**HEMATOPOIESIS**

Hematopoiesis, the process that continuously gives rise blood cells, is tightly regulated not only by intrinsic factors but also by extrinsic clues derived from various cells within the bone marrow microenvironment. The bone marrow is indeed the pri-
mary site for hematopoiesis that is highly orchestrated by interactions between the hematopoietic stem cells (HSCs) and their niches on the endosteal bone surface. The HSC niche consists of cells from the osteolineage, sinusoidal endothelial cells, mesenchymal stromal and stem cells, sympathetic neurons, and the extracellular matrix [37,38]. In these niches, the HSC undergo self-renewal and/or differentiate into hematopoietic lineage with cues from the niche-supporting cells, which provide structural as well as signaling support for the hematopoietic microenvironment. Osteoblasts have been shown as critical regulators of HSC maintenance, proliferation, and maturation [39,40] via various cell-surface proteins such as Notch1 ligand Jagged1 and via secreted cytokines such as CXCL12. Osteoblasts also provide a niche for B-lymphopoiesis in part via interleukin-7 expression through Gsα-signaling [41]. Hypoxic osteoblasts are also a major producer of erythropoietin through which they regulate erythropoiesis [42]. Moreover, osteoclasts has been shown to be potential HSC regulators through degradation of endosteal components and promotion of mobilization of HSCs [43]. In contrast, the role of osteocytes in HSC and progenitor regulation, and hence hematopoiesis, has remained completely unexplored despite the fact that these cells comprise 90% to 95% of all bone cells in the adult skeleton and they produce an extensive canalicular network that reaches the endosteal bone surface, a preferred site for the HSC niche. Osteoblasts and osteocytes express several G-protein coupled receptor (GPCR) and signaling trough these receptors has been shown to control the niche. Interestingly constitutive activation of the PTH/PTHrP receptor in osteoblasts increased the numbers of HSCs [39] whereas its expression in osteocytes does not [44], suggesting that cells early in the osteoblast lineage are critical for maintaining an intact HSC niche. To examine the role of osteocytes and GPCR signaling in regulation of hematopoiesis we recently generated mice lacking Gsa in osteocytes (Ocy-GsaKO). Surprisingly, ablation of Gsa from these cells induces a marked neutrophilia, thrombocytopenia and splenomegaly. Ocy-GsaKO mice displayed a profound myeloproliferative phenotype characterized by a dramatic increase in myeloid cells in bone marrow, spleen, and peripheral blood and marked [45]. Using a novel ex vivo coculture system using osteocyte-enriched bone explants we identified granulocyte colony-stimulating factor as the principal cytokine regulating granulopoiesis in these mice. Interestingly, PTH1R signaling in osteocytes is not responsible for the myeloproliferative phenotype since mice lacking receptor expression in osteocytes (Ocy-PPRKO) have normal hematopoiesis [45]. Moreover, the expression of SOST/sclerostin, a Wnt inhibitor and suppressor of osteoblast proliferation and functions, was increased significantly in these mice. Treatment with antisclerostin antibody partially restored the number of osteoblasts in Ocy-GsaKO mice without rescuing the bone marrow abnormalities demonstrating that the hematopoietic abnormalities present in these mice are independent on osteoblasts or increased SOST/sclerostin expression [45]. On the other hand, SOST/sclerostin can directly control the fate of B-cells, as recently reported [46]. Cain et al. [46] showed that SOST/-/- mice, despite the high bone mass and increased number of osteoblasts, have no differences in the frequency or absolute number of HSCs, common lymphoid progenitors, common myeloid/megakaryocyte erythroid progenitors, or granulocyte/monocyte progenitors, confirming the findings in mice overexpressing the constitutive PTH/PTHrP receptor in osteocytes [44]. Interestingly, in these mice, B cells are significantly reduced in both their frequency and cell number in the bone marrow and the reduction was a consequence of increased apoptosis due to a reduction in Cxcl12 expression in the stromal cells.

FUTURE DIRECTIONS

It is evident that there is a need for further investigations aimed at better understand osteocytes function. Several questions remain unanswered and areas in need of better understanding are numerous. For example, which are the molecular cues that signal an osteoblast to become an osteocyte? How can osteocytes sense mechanical forces applied to the skeleton and what is the role of primary cilia? What control the increased in SOST/sclerostin induced by unloading? How can an osteocyte sense phosphate? What is the role of osteocytes in hematopoiesis? These are many more other unanswered questions will require better tools and in vitro models to study and analyze these cells and advance our understanding of their complex functions even further.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

ACKNOWLEDGMENTS

This work was supported by grants AR060221 and DK079161 (PDP) from the National Institutes of Health.
REFERENCES

1. Gaillard PJ, Talmage RV, Budy AM, te Leiden R; Rice University. The parathyroid glands: ultrastructure, secretion, and function. Chicago: University of Chicago Press; 1965. Osteolysis: an outlook on its mechanism and causation; p. 137-43.

2. Rutishauser E, Majno G. Physiopathology of bone tissue; the osteocytes and fundamental substance. Bull Hosp Joint Dis 1951;12:469-90.

3. Bonewald LF. The amazing osteocyte. J Bone Miner Res 2011;26:229-38.

4. Zhang K, Barragan-Adjemian C, Ye L, Kotha S, Dallas M, Lu Y, Zhao S, Harris M, Harris SE, Feng JQ, Bonewald LF. E11/gp38 selective expression in osteocytes: regulation by mechanical strain and role in dendrite elongation. Mol Cell Biol 2006;26:4539-52.

5. Kalajzic I, Matthews BG, Torreggiani E, Harris MA, Divieti Pajevic P, Harris SE. In vitro and in vivo approaches to study osteocyte biology. Bone 2013;54:296-306.

6. Windahl SH, Borjesson AE, Farman HH, Engdahl C, Moverare-Skrtic S, Sjogren K, Lagerquist JM, Koskela A, Tuukkanen J, Divieti Pajevic P, Feng JQ, Dahlman-Wright K, Antonson P, Gustafsson JA, Ohlsson C. Estrogen receptor-α in osteocytes is important for trabecular bone formation in male mice. Proc Natl Acad Sci U S A 2013;110:2294-9.

7. Marotti G, Ferretti M, Remaggi F, Palumbo C. Quantitative evaluation on osteocyte canalicular density in human secondary osteons. Bone 1995;16:125-8.

8. Palumbo C, Palazzini S, Marotti G. Morphological study of intercellular junctions during osteocyte differentiation. Bone 1990;11:125-8.

9. Palumbo C, Palazzini S, Zaffe D, Marotti G. Osteocyte differentiation in the tibia of newborn rabbit: an ultrastructural study of the formation of cytoplasmic processes. Acta Anat (Basel) 1990;137:350-8.

10. Schweitzer MH, Wittmeyer JL, Horner JR, Toporski JK. Soft-tissue vessels and cellular preservation in Tyrannosaurus rex. Science 2005;307:1952-5.

11. Dallas SL, Prideaux M, Bonewald LF. The osteocyte: an endocrine cell. Endocr Rev 2013;34:658-90.

12. Price C, Zhou X, Li W, Wang L. Real-time measurement of solute transport within the lacunar-canalicular system of mechanically loaded bone: direct evidence for load-induced fluid flow. J Bone Miner Res 2011;26:277-85.

13. Tatsumi S, Ishii K, Amizuka N, Li M, Kobayashi T, Kohno K, Ito M, Takeshita S, Ikeda K. Targeted ablation of osteocytes induces osteoporosis with defective mechanotransduction. Cell Metab 2007;5:464-75.

14. Jacobs CR, Temiyasathit S, Castillo AB. Osteocyte mechanobiology and pericellular mechanics. Annu Rev Biomed Eng 2010;12:369-400.

15. Temiyasathit S, Tang WJ, Leucht P, Anderson CT, Monica SD, Castillo AB, Helms JA, Stearns T, Jacobs CR. Mechanosensing by the primary cilium: deletion of Kif3A reduces bone formation due to loading. PLoS One 2012;7:e333 68.

16. Xiao Z, Dallas M, Qiu N, Nicolella D, Cao L, Johnson M, Bonewald L, Quarles LD. Conditional deletion of Pkd1 in osteocytes disrupts skeletal mechanosensing in mice. FASEB J 2011;25:2418-32.

17. Xiao Z, Zhang S, Mahlios J, Zhou G, Magenheimer BS, Guo D, Dallas SL, Maser R, Calvet JP, Bonewald L, Quarles LD. Cilia-like structures and polycystin-1 in osteoblasts/osteocytes and associated abnormalities in skeletonogenesis and Runx2 expression. J Biol Chem 2006;281:30884-95.

18. Gluhak-Heinrich J, Ye L, Bonewald LF, Feng JQ, MacDougal M, Harris SE, Pavlin D. Mechanical loading stimulates dentin matrix protein 1 (DMP1) expression in osteocytes in vivo. J Bone Miner Res 2003;18:807-17.

19. Harris SE, Gluhak-Heinrich J, Harris MA, Yang W, Bonewald LF, Riha D, Rowe PS, Robling AG, Turner CH, Feng JQ, McKee MD, Nicollella D. DMP1 and MEPE expression are elevated in osteocytes after mechanical loading in vivo: theoretical role in controlling mineral quality in the perilacunar matrix. J Musculoskeletal Neuronal Interact 2007;7:313-5.

20. Robling AG, Nizolek PJ, Baldridge LA, Condon KW, Allen MR, Alam I, Manita SM, Gluhak-Heinrich J, Bellido TM, Harris SE, Turner CH. Mechanical stimulation of bone in vivo reduces osteocyte expression of Sost/sclerostin. J Biol Chem 2008;283:5866-75.

21. Spatz JM, Ellman R, Cloutier AM, Louis L, van Vliet M, Suva LJ, Dwyer D, Stolina M, Ke HZ, Bouxsein ML. Sclerostin antibody inhibits skeletal deterioration due to reduced mechanical loading. J Bone Miner Res 2013;28:865-74.

22. Frost HM. Bone “mass” and the “mechanostat”: a propos- al. Anat Rec 1987;219:1-9.

23. Baron R, Kneissel M. WNT signaling in bone homeostasis and disease: from human mutations to treatments. Nat Med
24. Bergwitz C, Jüppner H. Regulation of phosphate homeostasis by PTH, vitamin D, and FGF23. Annu Rev Med 2010; 61:91-104.

25. Nakashima T, Hayashi M, Fukunaga T, Kurata K, Oh-Hora M, Fung JQ, Bonewald LF, Kodama T, Wutz A, Wagner EF, Penninger JM, Takayanagi H. Evidence for osteocyte regulation of bone homeostasis through RANKL expression. Nat Med 2011;17:1231-4.

26. Onal M, Piemontese M, Xiong J, Wang Y, Han L, Ye S, Komatsu M, Selig M, Weinstein RS, Zhao H, Jilka RL, Almeida M, Manolagas SC, O’Brien CA. Suppression of autophagy in osteocytes mimics skeletal aging. J Biol Chem 2013;288:17432-40.

27. Feng JQ, Ward LM, Liu S, Lu Y, Xie Y, Yuan B, Yu X, Rauch F, Davis SI, Zhang S, Rios H, Drezen MK, Quarles LD, Bonewald LF, White KE. Loss of DMP1 causes rickets and osteomalacia and identifies a role for osteocytes in mineral metabolism. Nat Genet 2006;38:1310-5.

28. Kollet O, Dar A, Shivtiel S, Kalinkovich A, Lapid K, Szainberg Y, Tesio M, Samstein RM, Goichberg P, Spiegel A, Elson A, Lapidot T. Osteoclasts degrade endosteal components and promote mobilization of hematopoietic progenitor cells. Nat Med 2006;12:657-64.

29. Calvi LM, Bromberg O, Rhee Y, Weber JM, Smith JN, Basil MJ, Frisch BJ, Bellido T. Osteoblastic expansion induced by parathyroid hormone receptor signaling in murine osteocytes is not sufficient to increase hematopoietic stem cell niche size. Nature 2003;425:836-41.

30. Calvi LM, Adams GB, Weibrecht KW, Weber JM, Olson DP, Knight MC, Martin RP, Schipani E, Divieti P, Bringhurst FR, Milner LA, Kronenberg HM, Scadden DT. Osteoblastic cells regulate the haematopoietic stem cell niche. Nature 2003;425:841-6.

31. Calvi LM, Bromberg O, Rhee Y, Weber JM, Olsen SP, Murphy J, Lanahan SA, Scadden DT. Osteoblastic cells induce B lymphopoiesis through Gsα-dependent signaling pathways. Proc Natl Acad Sci U S A 2008;105:16976-81.

32. Calvi LM, Adams GB, Weibrecht KW, Weber JM, Olson DP, Knight MC, Martin RP, Schipani E, Divieti P, Bringhurst FR, Milner LA, Kronenberg HM, Scadden DT. Osteoblastic cells regulate the haematopoietic stem cell niche. Nature 2003;425:841-6.

33. Calvi LM, Bromberg O, Rhee Y, Weber JM, Scadden DT. Osteoblastic regulation of B lymphopoiesis is mediated by Gsα-dependent signaling pathways. Proc Natl Acad Sci U S A 2008;105:16976-81.

34. Calvi LM, Bromberg O, Rhee Y, Weber JM, Scadden DT. Osteoblastic regulation of B lymphopoiesis is mediated by Gsα-dependent signaling pathways. Proc Natl Acad Sci U S A 2008;105:16976-81.
cells. Blood 2012;119:2489-99.

45. Fulzele K, Krause DS, Panaroni C, Saini V, Barry KJ, Liu X, Lotinun S, Baron R, Bonewald L, Feng JQ, Chen M, Weinstein LS, Wu JY, Kronenberg HM, Scadden DT, Divieti Pejovic P. Myelopoiesis is regulated by osteocytes through Gsα-dependent signaling. Blood 2013;121:930-9.

46. Cain CJ, Rueda R, McLelland B, Collette NM, Loots GG, Manilay JO. Absence of sclerostin adversely affects B-cell survival. J Bone Miner Res 2012;27:1451-61.