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Osteocytes and homeostasis of remote organs

Bone-buried osteocytes talk to remote organs

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Abstract

The study of bones has attracted researchers from many medical fields. To understand bone-organ interactions, hematologists were challenged to investigate bone marrow (BM), the core of bone where hematopoiesis takes place. Through studies of the hematopoietic stem cells niche, hematologists contributed to the discovery of unexpected functions of bone-forming osteoblasts and bone-buried osteocytes. Especially, the recent findings about osteocytes, as the regulatory system of lympho-hematopoiesis and fat metabolism, highlighted the central role of skeletal tissue in inter-organ communication. The cross-cutting consideration including hematology and many other fields will expand the bone research.

Keywords: Hematopoietic stem cell, Niche, Osteocyte, Thymus, Fat metabolism

Introduction

What are the roles of bone? The first is a physical role: to support the structure of the body and to guard the bone marrow (BM), which is important for hematopoiesis. The second is a physiologic role: to control calcium or mineral metabolism. The third is a sensory role: to perceive gravity and mechanical stimuli. The second role is particularly noteworthy from the perspective of the bone-organ network. The osteoblast is thought to be the main actor, affecting multiple organs, such as the BM, the pancreas, and the testis. It regulates hematopoiesis directly as a hematopoietic stem cell (HSC) niche (1, 2), as well as energy metabolism and male fertility by producing osteocalcin (3-5). However, osteoblasts comprise only a small population of bone's component cells. Bone-buried osteocytes make up 90% to 95% of all bone cells. Osteocytes are terminally differentiated osteoblasts that communicate with one another and form extensive

networks such as neural cells, in bone tissue. Osteocytes play an important role as mechanosensory cells and contribute to keep bone homeostasis caused by bone loading (6, 7). Furthermore, osteocytes may also regulate systemic homeostasis by mechanotransduction. Recently, novel roles of osteocytes were elucidated through “osteohematology.” For example, osteocytes regulate osteoblasts to control not only bone remodeling but also the niche function of HSCs by receiving signals from the sympathetic nervous system (8). Furthermore, osteocytes regulate the microenvironment that supports lymph production by primary lymphoid organs, such as the BM and thymus, and fat metabolism throughout the body (9). In this article, we review the unique roles of bone-buried osteocytes as commanders of the HSC niche and regulators of multiple organs

HSC niche inside bone

First, the study of osteoblasts rather than osteocytes become a trigger for joint research of bone metabolism and hematopoiesis. In 2003, two different groups analyzed mutant mice with conditional inactivation of bone morphogenetic protein receptor type IA (BMPRIA) and osteoblast-specific activated parathyroid hormone (PTH)/PTH-related protein (PTHrP) receptors and showed that osteoblasts support an increase in HSC. The results suggest that osteoblasts constitute a niche as a specific microenvironment in which the quiescence and multipotency of HSCs are maintained (1, 2). Subsequently, the Tie2/angiopoietin-1 signaling pathway was reported to play a critical role in maintaining HSC function in the osteoblastic niche, and osteoblast-ablated mice showed a decrease in HSCs and in hematopoiesis (10, 11). Recently, BM endosteal cell populations characterized by the expression of activated leukocyte cell adhesion molecule (ALCAM) and Sca1, and ALCAM+Sca1⁻ cells in particular, showed robust supportive activity in maintaining HSC quiescence and multipotency (12). Furthermore, osteoblastic gene expression and osteoblast differentiation analyses suggest that ALCAM+Sca1⁻ cells are a population of differentiated mature osteoblasts. These studies demonstrated that osteoblasts is important for not only bone metabolism but also HSCs maintenance.

In vivo imaging of bone and BM in mice showed that HSCs are localized at the endosteal surface (13, 14). Because HSCs express a calcium-sensing receptor, they migrate to the endosteal surface, where active bone modeling and remodeling take place, leading to an increase in extracellular calcium ion concentration (15). HSCs within the

endosteal area have superior proliferative capacity and homing efficiency compared with those isolated from the central BM (16). Although CXCL12-abundant reticular cell (17), nestin-positive mesenchymal stem cells (18), nonmyelinating Schwann cells (19), and stem cell factor (SCF)-positive endothelial cells (20) also were reported to be HSC niche cells, bone, especially osteoblasts, still receives much attention. Furthermore, genetic alteration of osteolineage niche cells has been reported to initiate hematopoietic malignancies (21, 22) and interestingly, Wnt/ β -catenin signaling in osteoblasts versus osteocytes for hematopoiesis is different. β -catenin activation in osteoblasts induced not only skeletal phenotype but also acute myeloid leukaemia with chromosomal aberrations in mice (22). However, β -catenin activation in osteocytes did not affect hematopoiesis and did not cause hematopoietic disorders in mice (23). Thus, activation of canonical Wnt signaling in osteoblasts rather than osteocytes is important for hematopoiesis. There is no doubt that osteoblasts are essential for hematopoietic pathophysiology. Thus, many hematologists consider osteoblasts to be crucial regulators of the hematopoietic system and through these studies they became more interested in the role of bone cells including not osteoblasts but osteocytes for hematopoiesis.

Pulling HSCs out of bone

HSCs not only rest in BM but also move throughout the body. Mobilization of HSCs from the BM to the circulation, which is induced by cytokine granulocyte colony-stimulating factor (G-CSF), is a useful clinical application for transplantation of peripheral blood stem/hematopoietic progenitor cells (HPCs) (24). G-CSF-induced HSC/HPC mobilization has been a good research model for the biology of the hematopoietic microenvironment, because alteration of niche function results in the release of HSCs/HPCs from the niche, which is the first step in mobilization. Mice deficient in uridine diphosphategalactose (UDP-galactose) ceramide galactosyltransferase (Cgt), an enzyme used to synthesize galactocerebroside (GC)—a major glycolipid of the myelin sheaths—are defective in HSC mobilization induced by G-CSF (25). The result reveals that G-CSF induces adrenergic signals that emerge from the peripheral nervous system, and the sympathetic tone suppresses the osteoblasts through β 2-adrenergic receptor to control the trafficking of HSCs/HPCs to their osteoblastic niche. Thus, the osteoblastic niche is regulated by the sympathetic nervous system. To elucidate the molecular mechanism or signaling pathway that contributes to osteoblastic niche regulation by the sympathetic nervous system, we focused on vitamin

D, which is one of the calcium-regulating hormones, and analyzed the osteoblastic niche function in vitamin D receptor knockout (*Vdr*^{-/-}) mice. *Vdr*^{-/-} mice showed severe impairment of G-CSF-induced osteoblast suppression and subsequent HSC/HPC mobilization. We elucidated that in osteoblasts, sympathetic nervous system signals through the β 2-adrenergic receptor led to long-lasting suppressive signals against HSC niches via functionally up-regulated VDR gene expression (26).

Calvi et al. (27) further developed their earlier study (1) on the increase of HSCs in osteoblast-specific activated PTH/PTHrP receptor mice. They showed that PTH injection increased the number of HSCs mobilized into the peripheral blood and protected stem cells from cytotoxic chemotherapy. This study provides evidence that the niche may be an attractive target for drug-based stem cell therapeutics. Furthermore, the authors mentioned the cell population targeted by the 2.3-kb fragment of the mouse collagen I gene promoter, which was used for osteoblast-specific activation of PTH/PTHrP receptors, including osteocytes, and PTH stimulated osteocytes through PTH receptors similar to osteoblasts. To examine the effect of osteocytes for HSCs, the authors used transgenic mice in which a constitutively active PTH1R was targeted to osteocytes by using dentin matrix protein-1 (DMP-1) promoter. In these mice, the osteoblasts, osteoclasts, and trabecular bone increased, but the number and function of HSCs decreased. These results suggest that osteocytes did not mediate the HSC expansion induced by PTH1R stimulation, and osteoblastic expansion was not sufficient to increase HSCs (27).

Osteocyte: niche commander

Do osteocytes, which comprise more than 90% of all bone cells, affect HSCs or the osteoblastic niche at all? Mice lacking *Gsa* in osteocytes showed a dramatic increase in myeloid cells in the BM by enhanced osteocyte-derived G-CSF production and were reported to induce myeloproliferative diseases (28). However, this phenotype is clinically similar to myeloproliferations secondary to G-CSF-producing tumor and different from the myeloproliferative disease induced by genetic mutations in HSCs. Although this study did not show hematopoietic abnormality induced by osteoblastic niche defect, it provides an important message, that “the bone could be a fountain of hematopoietic growth factors and this could be one of the reasons why hematopoiesis takes place inside the bone.”

We attempted to determine the role of osteocytes in HSCs and the osteoblastic niche by

studying HSC mobilization by G-CSF administration. Surprisingly, osteocyte-specific genes, such as E11/gp38, phosphate-regulating endopeptidase homolog X-linked (PheX), neuropeptide Y (NPY), matrix extracellular phosphoglycoprotein (MEPE), and sclerostin (SOST), were suppressed earlier than osteoblast-specific genes in G-CSF-injected mouse bone. Because we showed that sympathetic tone was caused by G-CSF-suppressed osteoblasts through the β 2-adrenergic receptor and induced HSC mobilization, we examined and confirmed β 2-adrenergic receptor expression in osteocytes. Moreover, G-CSF-induced suppression of osteocyte-specific genes was canceled by surgical denervation. These results suggest that osteocytes react faster than osteoblasts to the sympathetic nervous system signals induced by G-CSF. Indeed, osteocyte-specific ablated mice had comparable numbers of HSCs/HPCs in the BM, but they failed to mobilize in response to G-CSF. Thus, G-CSF-induced sympathetic nervous system signals regulate not only osteoblasts but also osteocytes, and osteocyte reaction in the early phase of G-CSF stimulation is important for HSC release from the osteoblastic niche (8). It is likely that osteocytes act as the osteoblastic niche commander.

The role of osteocytes for distant organs

Osteocytes have more unique and deeper roles. They are mechanosensors of bone and contribute to bone homeostasis by converting mechanical stress to biologic signals; therefore, the major players in bone turnover, osteoblasts and osteoclasts, are regulated at the local bone surface (6, 7). For instance, reduced mechanical stress on the bones of astronauts or bedridden patients leads to rapid progression of osteoporosis and impaired immunity (29-32). Microgravity is known to induce local osteoporosis on the hindlimbs in the mouse tail suspension system. We confirmed that local microgravity on the hindlimbs suppressed the osteocyte network formation in bone section stained with CD44 and reduced the number of lymphocytes in the lifted BM.

To elucidate the role of osteocytes in hematopoiesis, immunity, and systemic health, we examined osteocyte-specific ablated mice. We used a transgenic mouse model in which inducible and selective ablation of osteocytes is achieved in vivo by targeted expression of diphtheria toxin receptor (DTR) under the promoter of DMP-1 (33). The 15-week-old wild-type (WT) or Tg mice were injected with diphtheria toxin, and 3 weeks after injection, only the Tg mice became osteocyte-less (OL) mice. Importantly, osteocytes appeared to be damaged from a reduction in nuclear size but were not depleted in the bone section of OL mice. In the OL mice, peripheral blood B and T lymphocytes were markedly reduced, and a BM transplantation assay indicated that B and T lymphopenia

was not the result of HSC abnormality. In the BM, which is the primary lymphoid organ for B-lymphogenesis, B-cell precursors disappeared, and it was demonstrated that this impairment occurred at an early skewing step toward B-lymphogenesis by colony-forming unit (CFU) assays and flow cytometric analysis. Long-term BM culture revealed that severe impairment of B-lymphopoiesis in OL mouse BM was the result of a depletion in B-lymphoid specific stromal cells. Another primary lymphoid organ for T cells, the thymus, was drastically atrophic, and the number of T cells and pro-T cells was markedly decreased in OL mice. Immunohistochemical staining of thymic epithelial cells demonstrated that a reduction in cortical stromal cells, which comprise the thymic microenvironment for T-cell differentiation, might be the cause of thymic atrophy. The question is, how do bone-buried osteocytes cause remote thymic abnormality? To investigate whether it was caused by humoral factors from osteocytes, we used a parabiotic model. CD45.1-congenic WT mice were joined surgically with CD45.2 Tg or WT mice, and it was confirmed that the blood was shared equally. Parabiotic pairs were maintained for 5 weeks after surgery, and each mouse was injected with diphtheria toxin. Three weeks after injection, the thymus of each mouse was evaluated. However, the sharing of circulation with normal mice did not prevent thymic atrophy in OL mice. Furthermore, T-cell progenitors from OL mice differentiated normally in WT thymus, but those from WT mice failed to differentiate in OL thymus. Thus, thymic atrophy due to impairment of the thymic microenvironment was not prevented in our parabiotic model, even with the supply of humoral factors. Therefore, osteocytes regulate remote thymus not through humoral factors.

OL mice have another notable phenotype: fat loss. Previously, mice lacking *Gsa* in osteocytes also showed a significant (>40%) decrease in peripheral fat (Fulzele et al., American Society for Bone and Mineral Research [ASBMR] 2010 Annual Meeting, presentation number 1001), and inactivation of serine protease SKI-1 in osteocytes was reported to lead to a rapid increase in body mass and obesity (Gorski et al., ASBMR 2012 Annual Meeting, presentation number 1182). Thus, a correlation between osteocyte and fat metabolism has been suggested, although the mechanism is unknown. OL mice had a steady decrease in body weight for 3 weeks after osteocyte depletion, and white adipose tissue, such as subcutaneous, mesenteric, or retroperitoneal fat, disappeared. Their food intake was decreased slightly, and fat loss was not prevented by a high-fat diet. It is unlikely that the fat/weight loss in OL mice was caused by digestive abnormalities, because they had normal excrement with no signs of diarrhea or hematochezia. Endocrine malfunctions, such as hyperthyroidism, excess catecholamine secretion, and hyperinsulinemia, were not potential causes, because the heart rate and

serum insulin levels were not impaired. Next, we speculated that fat metabolism was disturbed by osteocyte depletion through central nervous system (CNS) function. Certain areas of the brain, such as the ventromedial hypothalamic nucleus (VMH), are important not only for feeding and regulation of energy balance but also for bone metabolism. After ablation of the VMH, we performed osteocyte depletion and then assessed the fat loss. Fat loss occurred irrespective of VMH ablation; however, the livers of OL mice with ablated VMH became markedly enlarged and whitish. Analysis of fat metabolism-related gene expression in the liver showed that lipogenic genes were not affected but that fat clearance genes were impaired in VMH-ablated OL mice. These results suggest that osteocytes maintain peripheral fat in cooperation with the CNS (9).

Conclusions

Many studies by hematologists revealed a bone–blood–body homeostasis connection, and rediscovered the role of bone. In our bodies is a bone-pinnacled functional orchestration of multiple organs (Fig. 1). Bone-buried osteocytes regulate HSC function by commanding the osteoblastic niche, govern lymphopoiesis by regulating the microenvironment in primary lymphoid organs, and control systemic fat metabolism in cooperation with the brain. Thus, signals in the osteocyte network activated under the sensation of gravity may be indispensable in maintaining multiple distant organs.

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