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REVIEW

Communication of bone cells with hematopoiesis, immunity and energy metabolism

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The bone contains the bone marrow. The functional communication between bone cells and hematopoiesis has been extensively studied in the past decade or so. Osteolineage cells and their modulators, such as the sympathetic nervous system, macrophages and osteoclasts, form a complex unit to maintain the homeostasis of hematopoiesis, called the 'microenvironment'. Recently, bone-embedded osteocytes, the sensors of gravity and mechanical stress, have joined the microenvironment, and they are demonstrated to contribute to whole body homeostasis through the control of immunity and energy metabolism. The inter-organ communication orchestrated by the bone is summarized in this article.

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Introduction

In mammals, hematopoiesis commences in the fetal liver before birth. At the very near the delivery, the source of all blood cells, namely the hematopoietic stem cell (HSC), migrates from the fetal liver to the bone marrow (BM) located inside the bone tissue. In this context, the bone is first established prior to inhabitation of the BM, and the HSCs rent it for the maintenance of adult hematopoiesis. Other than marrow of the bone, there is no system that strictly regulates the circulating blood cell numbers in steady state and supplies appropriate kinds of cells on demand during an emergency. Extramedullary hematopoiesis observed in patients with myelofibrosis is not an exception because the chromosomal abnormalities in circulating blood cells indicate a neoplasm. Thus, the bone is essential for a well-regulated normal hematopoiesis, and 'the bone equipped with marrow' can be regarded as a distinct internal organ.

In addition to the physical role to support the structure of the body and the physiological role to control mineral metabolism, the bone contributes to whole body homeostasis through another important function: the perception of gravity and mechanical stress. The latter sensory role is mediated by the bone-buried osteocytes. Osteocytes are terminally differentiated osteolineage cells and are now recognized as strong regulators of the conventional players in bone remodeling, such as the osteoblasts and osteoclasts. In the past decade or so, hematologists have studied extensively the function of all these bone cells as modulators of the hematopoietic system. In this review article, I would like to present an overview of the research on bone cells as microenvironments for HSCs and to introduce their unique roles as regulators of multiple organ functions, such as lymphopoiesis and energy metabolism.

Endosteal Microenvironment for Hematopoiesis: Osteoblasts and their Modulators

In the early *in vitro* study, bone-forming osteoblasts are known to have a capacity to support immature hematopoietic progenitor cells (HPCs).[1] *In vivo* studies seeking the specialized place that supports HSCs in the BM have begun with two researches published in 2003.[2,3] One study showed that both transgenic mice with constitutively active parathyroid hormone signaling and normal mice with systemic parathyroid hormone administration had increased the number of HSCs. The other study also demonstrated the positive correlation between the numbers of osteoblasts and HSCs by using mice with conditional inactivation of bone morphogenetic protein receptor type IA. A recent work has confirmed further the impairment of HSC self-renewal in the BM in the absence of osteoblasts.[4] Currently, these endosteal osteoblastic cells can be isolated and subdivided into three populations based on the expression of activated leukocyte cell-adhesion molecule (ALCAM) and

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Sca-1. It is reported that ALCAM + Sca-1 − mature osteoblast fraction harbors a greater capacity to support HSC activity, whereas ALCAM − Sca-1 + mesenchymal progenitors and ALCAM − Sca-1 − pre-osteoblasts mainly support HPCs.[5,6]

Factors that support or attract HSCs in the osteoblastic microenvironment have been reported. The [Ca^{2+}] level is considered high in the endosteum due to bone remodeling, and calcium-sensing receptor expressed on HSCs guides them to the osteoblastic microenvironment.[7] Angiopoetin-1 and thrombopoietin are reported to be osteoblastic factors that promote HSC quiescence through Tie2 and MPL receptors, respectively.[8–10] Non-canonical Wnt signaling is also shown to regulate HSC quiescence at the endosteal region.[11] Adhesion molecules and extracellular matrix proteins, such as N-cadherin and osteopontin, are also reported to be modulators for HSCs.[12,13] However, some studies question the significance of N-cadherin-mediated adhesion to osteoblasts for HSC maintenance.[14,15]

As bone homeostasis is maintained by the coupling of bone-forming osteoblasts and bone-resorbing osteoclasts, osteoclasts are also important players in the endosteal microenvironment for hematopoiesis (summarized in BoneKEy Reports review by Anna Teti[16]). The precursors of osteoclasts are macrophages, and those located near the endosteum, called 'osteomac', are reported to be critical supporters of osteoblasts.[17–19] Deletion of macrophages leads to rapid disappearance of osteoblasts,[17] followed by mobilization of HSCs/HPCs from the BM to the circulation.[19] It is also reported that BM CD169 + macrophages support the mesenchymal stem cells,[20] which suggests that macrophages are positive regulators of a wide spectrum of mesenchymal lineage cells.

In contrast to macrophages, a negative regulator of mesenchymal lineage cells in the BM is the sympathetic nervous system (SNS). The BM is a highly innervated organ, and catecholamine stimulates β2-adrenergic receptor expressed on osteoblasts to suppress their activity.[21] Cytokine granulocyte colony-stimulating factor (G-CSF) is a strong inducer of high sympathetic tone in the BM,[22] which leads to marked suppression of osteoblasts in the endosteum.[23] Catecholamine signal through β2-adrenergic receptor in osteoblasts upregulates strongly the *vitamin D receptor (VDR)* mRNA, which is essential for normal HSCs/HPCs trafficking.[24] It is also reported that mesenchymal stem cells express β3-adrenergic receptor and are suppressed by the SNS,[25] which suggests that the SNS is a negative regulator of a wide spectrum of mesenchymal lineage cells.

Recently, many kinds of perivascular cells have been identified as a supportive population to maintain HSCs.[26–28] Most of them are mesenchymal lineage cells, and, among them, nestin + mesenchymal stem cells and CXCL12-abundant reticular cells (CAR cells) are proven to possess differentiation capacity into osteoblasts.[29,30] From the bone researcher's point of view, the structure of the BM as the hematopoietic microenvironment may be simplified as illustrated in **Figure 1**. Perivascular mesenchymal stem cells differentiate via mesenchymal progenitors, pre-osteoblasts, and bone-forming osteoblasts toward bone-embedded osteocytes. These mesenchymal lineage cells are regulated by the balance between SNS-mediated suppression and macrophage-mediated promotion. This bone-forming system in the BM is utilized by HSCs/HPCs as the hematopoietic microenvironment.

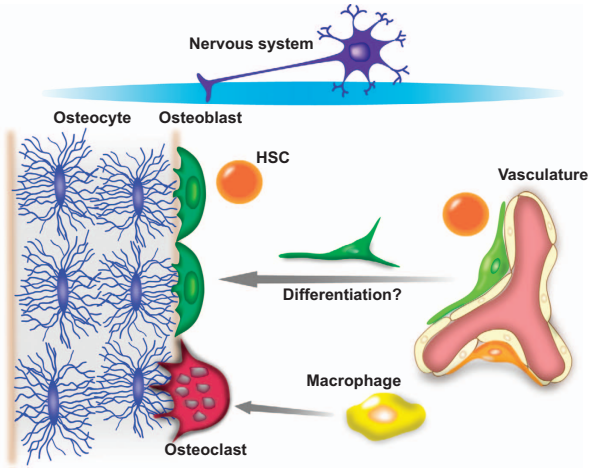

Figure 1 Relationship between bone cell- and blood cell-forming systems. Perivascular mesenchymal stem cells differentiate toward bone-forming cells. These mesenchymal lineage cells are regulated by the balance between sympathetic nervous system-mediated suppression and macrophage-mediated promotion. This bone-forming system in the bone marrow is utilized by hematopoietic stem/progenitor cells as microenvironment.

Endosteal Microenvironment for Malignant Hematopoiesis

Hematopoietic malignancies, such as leukemia, myelodysplastic syndrome and myeloproliferative neoplasm, arise from the BM. Recent reports have revealed the considerable contribution of bone cells in the maintenance of leukemia or even in leukemogenesis.[31,32] Minimal residual disease of human acute myelogenous leukemia cells in immunodeficient mice resides in the endosteal region after chemotherapy.[33] In a murine myeloproliferative neoplasm model, it is reported that leukemic cells remodel the osteoblastic microenvironment by driving the expansion of osteoblastic cells with abnormal function, which is favorable for leukemic cells but unfavorable for normal HSCs.[34] Two reports had demonstrated the osteolineage cell-intrinsic leukemogenesis. The genetic deletion of Dicer1 only in osteoblast precursors in mice caused myelodysplastic syndrome and eventually led to acute leukemia.[35] Constitutive activation of β-catenin selectively in osteoblasts in mice also caused myelodysplastic syndrome/leukemia through the activation of Notch signaling in HSCs/HPCs.[36] Thus, the osteoblastic microenvironment is essential for both normal and malignant hematopoiesis.

Contribution of Osteocytes in Endosteal Microenvironment

Osteocytes are the most abundant osteolineage cells, and they control the balance of activity between osteoblasts and osteoclasts.[37] Network connected with the osteocyte projections communicates directly with endosteal osteoblast.[38] The deletion of Gsα specifically in osteocytes is reported to enhance G-CSF production from the bone tissue (presumably from osteocytes), which leads to myeloid expansion in the BM.[39] This study suggests that bone-embedded osteocytes, which have no direct contact with hematopoietic cells, are potent in the regulation of BM hematopoiesis.

Osteocyte-mediated regulation of the endosteal microenvironment for HSCs/HPCs is also demonstrated. G-CSF administration as aforementioned is often employed in clinic to

induce mobilization of HSCs/HPCs from the BM to the circulation. The collection of these circulating immature hematopoietic cells by apheresis is now a standard graft-harvesting method instead of the traditional BM collection for clinical HSC transplantation as a curable therapy for hematologic malignancies and intractable marrow failures. We had shown previously that G-CSF induced a high sympathetic tone in the BM, and the suppression of osteoblastic microenvironment by catecholamine is one of the pathways to release HSCs/HPCs from osteoblasts.[22] In our subsequent study, the cellular connection between endosteal osteoblasts and bone-embedded osteocytes had been disrupted after G-CSF treatment.[40] However, osteolineage cells do not express G-CSF receptor.[22] As osteocytes express β2-adrenergic receptor, and surgical denervation disrupts the regulation of osteocyte specific genes, it is confirmed that osteocytes are regulated by the SNS. We utilized transgenic mice in which osteocytes were specifically ablated through the targeted expression of a diphtheria toxin receptor under a DMP-1 promoter.[41] Injection of diphtheria toxin in this mouse model generates osteocyte-less (OL) mice. In OL mice, G-CSF-induced HSCs/HPCs mobilization was markedly impaired, whereas their numbers in the BM were unchanged. In addition to the disruption of supporting signals from osteocytes to osteoblasts, the depletion of osteocytes resulted in the disappearance of osteomacs in the BM. Disappearance of osteomacs is also observed in G-CSF-induced mobilization.[19] Thus, as summarized in **Figure 2**, osteoblasts maintain the function of microenvironment for HSCs/HPCs by taking supporting signals from osteomacs and osteocytes in steady state. In G-CSF-induced mobilization, osteoblasts are suppressed through three different pathways: (1) direct suppression by the SNS, (2) loss of supporting signals from osteomacs and (3) loss of supporting signals from osteocytes.[40]

Role of Osteocytes for Remote Organs

Reduced input from mechanical stress, including gravity, on the bones of bedridden patients and astronauts leads to rapid progression of osteoporosis and impaired immunity.[42–45] Osteocytes act as mechanosensors and contribute to bone homeostasis by converting mechanical stress to biological signals.[41] Using the mouse tail suspension system, we confirmed that microgravity on the hind limbs disrupts osteocyte cellular network in the bone tissue and reduces the number of lymphocytes in the suspended BM.[46] To elucidate whether osteocytes were critical for homeostasis of the immune system and even other organs, we used OL mice that manifested osteoporosis with defective mechanotransduction.[41] Ablation of osteocytes led to severe lymphopenia due to the lack of lymphoid-supporting stroma in both the BM for B-cell precursors and the thymus for T-cell precursors, and a marked loss of white adipose tissues. These phenotypes were reversible when osteocytes were replenished within the bone in ~3 months. *In vivo* supply of T-cell progenitors and humoral factors via shared circulation with a normal parabiotic partner did not rescue thymic atrophy in OL mice, which suggested strongly the origin of the lymphopoietic defect from the impaired thymic microenvironment.

Certain areas of the central nervous system, such as the ventromedial hypothalamic nucleus and the arcuate nucleus, control bone metabolism via the SNS in response to leptin signaling.[21,47] To test whether osteocytes cooperated with the central nervous system to regulate fat metabolism, osteocytes

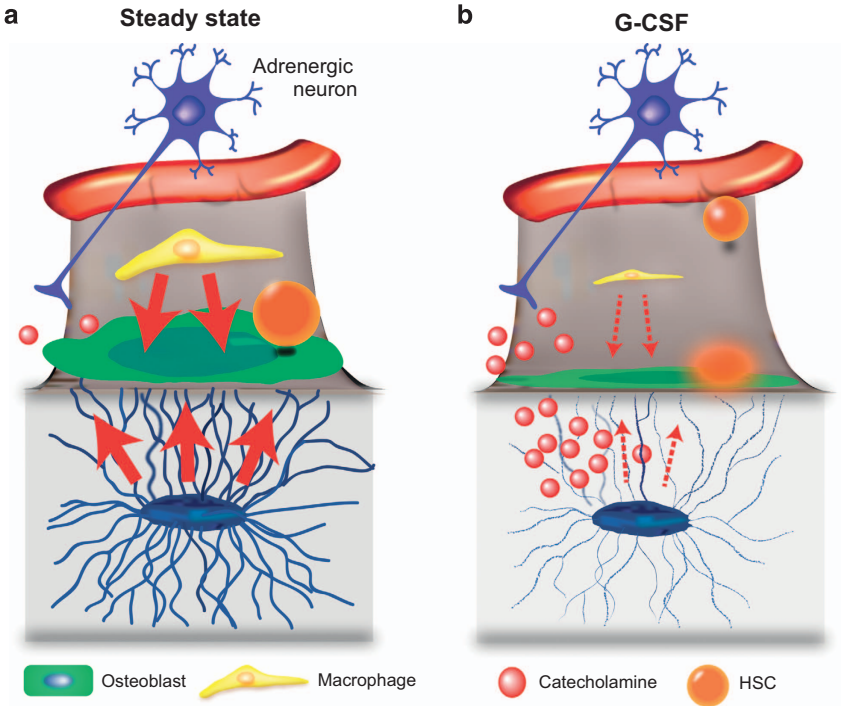

Figure 2 Alteration of endosteal microenvironment by cytokine G-CSF. (**a**) Osteoblasts as microenvironment for hematopoietic stem/progenitor cells are supported by osteomacs (endosteal macrophages) and osteocytes in steady state. (**b**) In G-CSF-induced mobilization, osteoblasts are suppressed through three different pathways: (1) direct suppression by the sympathetic nervous system, (2) loss of supporting signals from osteomacs and (3) loss of supporting signals from osteocytes.

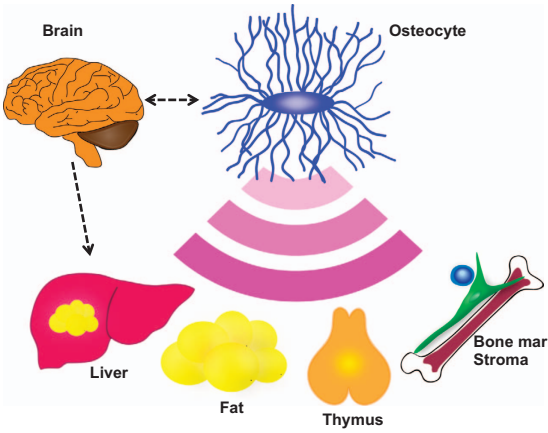

Figure 3 Regulation of remote organs by osteocytes. Bone-buried osteocytes regulate a specific microenvironment in primary lymphoid organs and systemic fat metabolism in cooperation with the brain and liver.

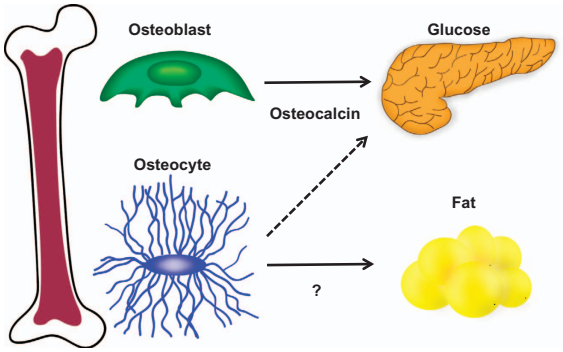

Figure 4 Differential regulation of energy metabolism by bone cells. Osteoblasts and osteocytes may preferentially regulate glucose and fat metabolism, respectively.

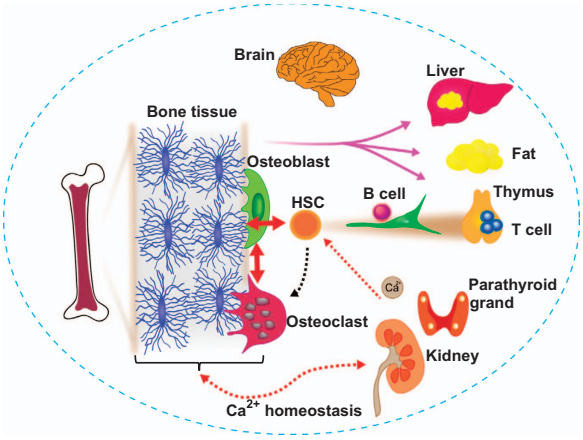

Figure 5 Multiple-organ network. Each organ may be appropriately functional with supportive signals from many other organs through this network where the bone has an essential role.

were deleted in ventromedial hypothalamic nucleus- or arcuate nucleus-ablated mice. In this condition, the mice demonstrated significant obesity with high amount of white adipose tissues before diphtheria toxin injection, and, after osteocyte deletion, they displayed general fat loss with hepatic steatosis. This severe fatty liver was at least partially due to the suppression of fat clearance genes in the liver by hypothalamic and osteocyte ablation. These results revealed that osteocytes maintained peripheral fat in cooperation with the central nervous system.[46] Thus, the bone tissue equipped with osteocytes orchestrates remote organs to maintain whole body homeostasis of immunity and fat metabolism (**Figure 3**).

Recently, osteocalcin produced by osteoblasts has been recognized as a critical homeostatic regulator of glucose metabolism by acting on pancreatic β cells and adipocytes.[48] It is also reported that insulin produced by pancreatic β cells upon osteocalcin stimulation further promotes the osteocalcin production from osteoblasts as a positive feedback mechanism.[49,50] It was reported that, in a mouse model, inducible ablation of osteocalcin-expressing cells resulted in the alteration of both glucose and fat metabolisms. Interestingly, exogenous osteocalcin rescued only the diabetic phenotype but not fat loss in this model.[51] Together with the fact that osteocalcin is highly expressed not only in osteoblasts but also in osteocytes,[40] and with our observations on OL mice in which glucose metabolism is not altered, but fat loss is prominent, mesenchymal lineage cells may control finely the energy metabolism depending on their differentiation stages. Moreover, osteoblasts and osteocytes may regulate preferentially glucose and fat metabolisms, respectively (**Figure 4**).

Concluding Remarks

Although a large number of fish species possess acellular bones, some have cellular bones with osteocytes, the distribution of which is less dense than in mammals.[52] As our ancestors moved from the sea onto the ground in the process of evolution, our body has been stimulated continuously by the 1G gravity. Systems to survive enduring harsh conditions, such as unlimited kinds of infections, unstable temperature and starvation on the ground, may have been refined under the inevitable mechanical stress of gravity. Thus, it seems reasonable that osteocytes as mechanosensory cells regulate critically the hematopoiesis, immunity and energy metabolism. Another important point is that our body responds to these stresses with inter-organ communication (**Figure 5**). Any organ in our body (perhaps including the brain) will not be appropriately functional without supportive signals from other organs. The bone, in particular, has an essential role in this network. Hematologists utilize this fact in clinic already. For example, the mobilization of HSCs/HPCs by G-CSF can be regarded as a transient deviation of this network. It is also possible that the irreversible deviation of this network may lead to diseases and aging.

Conflict of Interest

The authors declare no conflict of interest.

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