A mysterious triangle of blood, bones, and nerves

Asada, Noboru
Katayama, Yoshio

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Authors: Noboru Asada[1] and Yoshio Katayama[2]

Affiliations of authors:

[1.] Department of Hematology and Oncology, Okayama University Hospital, Okayama, Japan

[2.] Division of Hematology, Department of Medicine, Kobe University Hospital, Kobe, Japan

Correspondence:

Noboru Asada

Department of Hematology and Oncology, Okayama University Hospital, 2-5-1 Shikata-cho, Kita-ku, Okayama 700-8558, Japan

Tel: +81-86-235-7227; Fax: +81-86-232-8226

email: nasada@okayama-u.ac.jp

or

Yoshio Katayama

Division of Hematology, Department of Medicine, Kobe University Hospital, 7-5-2 Kusunoki-cho, Chuo-ku, Kobe 650-0017, Japan

Tel: +81-78-382-6912; Fax: +81-78-382-6910

email: katayama@med.kobe-u.ac.jp

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Abstract: The relationship between bone tissue and bone marrow, which is responsible for hematopoiesis, is inseparable. Osteoblasts and osteocytes, which produce and consist of bone tissue, regulate the function of hematopoietic stem cells (HSC), the ancestors of all hematopoietic cells in the bone marrow. The peripheral nervous system finely regulates bone remodeling in bone tissue and modulates HSC function within the bone marrow, either directly or indirectly via modification of the HSC niche function. Peripheral nerve signals also play an important role in the development and progression of malignant tumors (including hematopoietic tumors) and normal tissues, and peripheral nerve control is emerging as a potential new therapeutic target. In this review, we summarize recent findings on the linkage among blood system, bone tissue, and peripheral nerves.

Introduction

Blood circulates throughout every organ in the body for as long as we live. To the naked eye, it appears to be just a red liquid, but it is an organ that contains various blood cells, cytokines, and other signaling molecules that are constantly changing to maintain systemic homeostasis. Bone marrow, as its name implies, is surrounded by bone, a hard tissue that forms the body's skeleton. Because of the close physical proximity between bone tissue and bone marrow, research on the roles of bone tissue in regulating bone marrow hematopoiesis has been conducted early on, and much knowledge has accumulated.

In contrast, the nervous system networks organs throughout the body, directing and controlling activity at the organ and cellular levels. Abundant peripheral nerves are distributed in bone tissue and meticulously control bone tissue activity, especially bone remodeling. Furthermore, the seemingly tenuous linkage mechanism between the nervous system and hematopoiesis is gradually becoming clear. Although it has been known since the early days of modern medical research that the nervous system innervates organs throughout the body, the innervation of the bone marrow remained unknown until 1968, when Calvo first reported its details [1]. Subsequent histological studies in laboratory animals demonstrated that autonomic (sympathetic and parasympathetic) and sensory nerves are distributed in bone tissue and bone marrow and significantly influence the regulation of these organ functions. We will review the latest findings on organ linkage by the mysterious triangle consisting of

bone tissue, nervous system, and hematopoietic tissue.

1. Bone cell and hematopoiesis

Osteoblast progenitors, osteoblasts, and osteocytes regulate hematopoiesis differentially in the bone marrow

Hematopoietic stem cells (HSCs), which produce all blood cells, reside in the HSC niche (niche), a specialized microenvironment in the bone marrow, at steady-state conditions and are regulated by various signals (niche factors) from the niche [2]. These niche cells include bone lineage cells located at the boundary between bone marrow and bone tissue, vascular endothelial cells, and perivascular stromal cells [3]. Among these cell types, bone-forming osteoblasts can maintain hematopoietic progenitor cells in vitro, and intravenous administration of in vitro-labeled hematopoietic progenitor cells to mice confirmed their abundant distribution around the endosteum in the bone marrow [4]. These findings suggested that bone lineage cells at the endosteum play indispensable roles in HSC regulation.

Two subsequent studies, in which osteoblasts were expanded in vivo by either genetic modulation of bone morphogenetic protein receptor or parathyroid hormone administration, simultaneously reported that HSC number in the bone marrow increased along with osteoblasts, suggesting that progenitors of osteolineage cells control the HSC pool in the bone marrow [5,6]. However, several studies have shown that the number of osteoblasts does not necessarily correlate

with the number of HSCs in the bone marrow [7,8]. Furthermore, mice lacking osteoblast-specific C-X-C motif chemokine ligand 12 (CXCL12) and stem cell factor (SCF), which are essential niche factors for HSC maintenance, showed little change in the number of HSCs in the bone marrow, suggesting that these niche factors from osteoblasts are not necessarily required for the maintenance of HSCs [9,10]. However, osteoblasts may regulate HSCs through the indirect action of secreted factors, such as osteopontin, which negatively regulates the HSC pool in adult and fetal bone marrow [11,12].

A line of evidence shows that osteoblasts play essential roles for lymphocyte differentiation in the bone marrow. CXCL12 deletion from osteoblasts reduced the number of common lymphoid progenitors (CLPs) [13], suggesting their function as a niche cell for specific hematopoietic progenitors. The Notch ligand DLL4 derived from osteoblasts is indispensable for the generation of CLPs with T cell potential in the bone marrow [14].

Osteoblasts are differentiated from mesenchymal stem cells via committed osteoblasts (osteoprogenitors) that express the zinc finger-containing transcription factor osterix (Osx) [15]. Mice in which CXCL12 or insulin-like growth factor 1 was deleted from osteoprogenitors using Cre recombinase driven by Osx promotor revealed the depletion of B cell progenitor in the bone marrow [9,16]. Not only for lymphopoiesis, osteoprogenitors also participate in the regulation of erythropoiesis. Osx expressing osteoprogenitors have been found to produce erythropoietin and

modulate erythropoiesis in HIF signaling dependent manner [17].

Osteoblasts are not the final form of bone lineage cells; some osteoblasts are destined to differentiate further and embedded in bone tissue to become osteocytes. Osteocytes are embedded in hard tissues, but their neighboring cells are densely connected by cellular processes, forming a network similar to neurons [18]. In vivo ablation of osteocytes by using dentin matrix protein 1 (Dmp1) Cre-inducible diphtheria toxin receptor transgenic mice led to a dramatic decrease of B cell progenitors in the bone marrow, and more interestingly, an atrophy of thymic stromal cells accompanied with T cell lymphopenia in the peripheral blood [19]. These results indicate that osteolineage cells at different stages of maturation contribute in different way to the regulation of hematopoiesis.

Osteoblasts play roles in the pathogenesis of hematopoietic malignancies

Leukemia is caused by malignant cell transformation at the HSC level and, like the normal hematopoietic system, comprises a tumor-maintaining hierarchy and a small number of leukemic stem cells (LSCs) [20,21]. LSCs are regulated by the microenvironment in the similar way as normal HSCs [22]. In human acute leukemia xenograft models, chemotherapy-resistant leukemia cells are located near the endosteum, suggesting that a distinct microenvironment for LSCs exists around endosteal area [22]. Kode et al. demonstrated that osteoblasts are involved in the evolution of acute myeloid leukemia. The constitutive activation of β-catenin in

mature osteoblasts stimulated the expression of Notch ligand jagged 1 in osteoblasts, which in turn led to the activation of Notch signaling in HSCs, causing malignant transformation of HSCs to leukemic cells [23]. In the mouse chronic myeloid leukemia model, ablation of mature osteoblasts resulted in accelerated leukemia progression with increased LSC proliferation, suggesting the important roles for osteolineage cells in the regulation of leukemia [24] **(Figure 1)**.

2. Regulation of bone cells by the peripheral nervous system (PNS)

The roles of sensory nerve for the bone regulation

In bone tissue, autonomic and sensory nerves are widely distributed throughout the perichondrium, outer periosteum, Folkman's canal that penetrates the outer periosteum into the bone marrow, and the growth plate, trabecular bone, and cortical bone [25-28]. It has become clear that sensory nerves distributed in bone tissue not only transmit afferent external stimuli, such as pain and pressure on the bone, but also regulate bone remodeling, which is coupled by osteoblasts and osteoclasts.

Sensory nerves distributed in bone tissue consist of myelinated Aδ and unmyelinated C fibers and express the neuropeptides calcitonin gene-related peptide (CGRP), substance P (SP), and the tropomyosin receptor kinase A (TrkA), a high-affinity receptor for nerve growth factor (NGF) [25,29]. NGF is a pain transmitter that transmits bone pain via TrkA and promotes bone formation after bone injuries, such as fractures [30,31]. Stimulated sensory nerves release neuropeptides,

such as CGRP and SP, from nerve endings, and these neuropeptides act on cell surface receptors on osteoblasts, osteoclasts, and osteocytes to modulate bone remodeling. CGRP interact with a dimeric receptor complex of the G protein-coupled calcitonin receptor-like receptor (CALCRL) and receptor activity modifying protein 1(RAMP1). In αCGRP knockout (KO) mice, bone loss was observed due to decreased bone formation [32]. In vitro studies showed that adding CGRP induced the differentiation of bone marrow stromal cells into osteoblasts and inhibited osteoblast apoptosis and osteoclast differentiation, suggesting that CGRP promotes bone formation [33,34].

SP, a neuropeptide belongs to the tachynin family, stimulate proliferation of osteoblasts through neurokinin 1 receptor in vitro. SP also facilitated osteoclastogenesis from bone marrow macrophages and bone resorption by mature osteoclasts [35,36]. In vivo study in which mice lacking SP suggested that SP promotes the proliferation of osteoblasts and bone resorption by osteoclasts simultaneously, but the bone formation-promoting effect seems to exceed the bone resorption-promoting effect [37].

Semaphorin 3A is a potent chemorepellent and plays essential roles for axion guidance [38]. Sema3a$^{-/-}$ mice display skeletal abnormalities, including fusion of cervical bones, partial duplication of ribs, and poor alignment of the rib-sternum junction, indicating its contribution of skeletal development [39]. Indeed, Hayashi et al. reported that Sema3a$^{-/-}$ mice exhibited severe osteoporosis due to

the enhanced osteoclast differentiation and inhibited bone formation by osteoblast. Importantly, exogenous administration of Sema 3A improved osteoporosis in ovariectomized mice, indicating that the Sema 3A is a potential therapeutic target for bone diseases [40]. A subsequent more recent study highlighted the osteocytes, terminally differentiated osteolineage cells, as a main producer of Sema 3A responsible for the osteoprotective roles of estrogen against osteoporosis after ovariectomy [41]. Another study demonstrated that Sema 3A exerts its function on bone not only through direct mechanism but also through sensory nerve guidance into the bone. In this study, the researchers showed that the specific deletion of Sema3a from neurons led to bone loss, accompanied by a significant decrease of sensory nerves in the bone, while osteoblastic deletion of Sema3a by using Osx-Cre mice displayed normal bone mass [42].

Autonomic nervous system coordinately regulates bone remodeling

The autonomic nervous system consists of sympathetic and parasympathetic nerves, and it is generally believed that these two nervous systems exert opposing effects to unconsciously regulate the balance of organ functions throughout the body. Sympathetic nerves release norepinephrine (NE) from nerve endings, stimulating α- and β-adrenergic receptors (AR), and parasympathetic nerves release acetylcholine (ACh), acting on nicotinic ACh receptors (nAChR) or muscarinic ACh receptors (mAChR). Bone tissue contains both sympathetic and parasympathetic nerves, most

of which run along blood vessels, and their main function is to regulate bone remodeling by osteoblasts and osteoclasts [43,44] **(Figure 2)**.

Osteoblasts and osteoclasts express AR on their cell surface, and the balance of bone remodeling is tilted toward bone resorption by NE stimulation released from sympathetic nerves. Osteoblasts highly express β2-AR among AR subtypes, and administration of isoproterenol, a nonselective β-AR receptor agonist or β2-AR selective agonist, caused bone loss due to the suppression of osteoblast function and increase in osteoclasts [45]. Conversely, in mice lacking β2-AR in osteoblasts, an increase in bone mass was observed due to increased osteoblasts and bone formation rate, indicating that sympathetic signaling regulates bone remodeling via β2-AR in osteoblasts [46,47]. Furthermore, as a molecular mechanism by which sympathetic nervous signals promote osteoclast differentiation, β2-AR stimulation increases the production of receptor activator of nuclear factor-κB ligand from osteoblasts, which in turn promotes osteoclast differentiation [47].

The effects of ACh released from parasympathetic nerve endings on bone tissue are also being elucidated. In vitro experiments have revealed that osteoblasts and osteoclasts express subunits of nAChR and mAChR and that the addition of nicotine or ACh induces cell proliferation in osteoblasts and apoptosis in osteoclasts [43,48,49]. In mice lacking α2nAChR, a subunit of nAChR, an increase of osteoclasts accompanied by reduced bone mass was observed [43], whereas α7nAChR-KO or α7β2nAChR-KO increased bone mass [50-52], suggesting that the function may

differ by the AChR subtype. In addition, mice lacking M3R, a subtype of the muscarinic receptor, showed decreased bone mass, suggesting that M3R-mediated signaling may increase bone mass, but this effect is not directly on osteoblasts, but is exerted by stimulating M3R on neurons to suppress sympathetic nerve activity [53].

Clinical relevance of PNS dysfunction to bone

Charcot-Marie-Tooth disease (CMT) is the most common hereditary motor sensory neuropathy characterized by abnormal development of the PNS. CMT patients experience slowly progressive motor-sensory symptoms starting from the feet, resulting in characteristic deformities of the ankles and toes [54]. Patients with CMT have been reported to have a higher risk of fracture [55] and microarchitecture deterioration in the tibia [56], which can be explained by falls due to foot deformity and muscle weakness, as well as reduced loading on the tibia. The relationship between the impaired peripheral nerve function distributed to the bone and microarchitecture deterioration in the tibia is unclear.

Hereditary sensory and autonomic neuropathies (HSANs) are a heterogeneous genetic disease that predominantly degenerate unmyelinated and small peripheral nerve fibers, leading to distal sensory loss and autonomic dysfunction [57]. Patients with familial dysautonomia (FD), a form of HSAN, have reduced bone mineral density compared to healthy individuals [58]. Patients with HSAN are known to have low

plasma CGRP levels, suggesting that BMD may be reduced in HSAN due to the loss of the bone protective effect of CGRP [59]. However, the cause of BMD reduction due to HSAN is multifactorial, involving dietary deficiency, phosphorus malabsorption, and inadequate loading on bone, and the direct relationship to neuropathy is unknown [58].

3. Regulation of hematopoiesis by the PNS

Sympathetic nervous signaling exerts HSC mobilization by G-CSF treatment

Anatomical studies in laboratory animals have shown that the bone marrow, the primary site of hematopoiesis in adult mammals, is abundantly innervated by autonomic and sensory nerves [25,27,28]. Sympathetic nerves enter the bone marrow along the nutrient arteries. Many of which wrap around the arteries, and some of which leave the arteries and project their nerve endings to the bone marrow parenchyma [27]. Adrenergic signals inhibit osteoblast function and tilt bone remodeling toward bone resorption but also alter the niche function within the bone marrow to regulate HSC movement **(Figure 3)**. HSC mobilization from the bone marrow into the blood circulation by administration of supraphysiological doses of granulocyte colony-stimulating factor (G-CSF) has become a common method of HSC collection for HSC transplantation. G-CSF-induced exertion of adrenergic signals suppresses osteoblasts via β2-ARs and HSC niche function, leading to HSC mobilization into the blood [60]. Furthermore, this catecholamine-stimulated

inhibition of osteoblasts and the subsequent mobilization of HSC requires vitamin D receptors in osteoblasts [61].

Because G-CSF treatment disrupts the cell projection network of osteocytes, terminally differentiated osteolineage cell, we focused on the role of osteocytes in the G-CSF-induced mobilization mechanism [62]. In mice in which the osteocyte network was disrupted in vivo, G-CSF-induced mobilization of HSCs was severely impaired, although the number of HSCs in the bone marrow was unchanged. In surgically denervated bone, G-CSF did not suppress osteocytes, whereas sham-operated bone reacted to G-CSF signals. Together with β2-AR expression in osteocytes, these data suggested that osteocytes play important roles in the mobilization mechanisms mediated by sympathetic nervous signaling [62] **(Figure 3)**.

The mechanism of sympathetic stimulation by G-CSF has also been studied. Lucas et al. identified that sympathetic nerves express G-CSF receptors and that direct stimulation with G-CSF enhances local sympathetic signaling by reducing the efficiency of NE reuptake at sympathetic nerve endings [63]. Fever and back pain are well-known side effects during G-CSF induced HSC mobilization in clinical settings. Kawano et al. reported that these side effects are caused by the release of prostaglandin E_2 from marrow neutrophils due to sympathetic nerve stimulation induced by G-CSF via β3-AR [64].

The roles of sympathetic nervous system for HSC regulation in steady state

Sympathetic nervous signaling regulates HSCs and niche cells not only under stress, such as during G-CSF-induced HSC mobilization, but also at steady-state **(Figure 3)**. Transforming growth factor-β (TGF-β) is an important factor for maintaining HSCs in quiescence. Yamazaki et al. examined the distribution of activated TGF-β in the bone marrow. They found that Schwann cells, which do not form a myelin sheath, a glial cell of the sympathetic nervous system, specifically express activated TGF-β. Approximately 20% of HSCs reside in the vicinity of these non-myelinated Schwann cells, and in mice with surgically resected sympathetic nerves, a decrease in HSCs was observed simultaneously with a decrease in non-myelinating Schwann cells, demonstrating that the non-myelinating Schwann cells themselves function as HSC niches [65].

Dopamine, another neurotransmitter released from the sympathetic nerves, is reportedly involved in the regulation of hematopoiesis. Liu et al. demonstrated that dopamine signals via D_2-type receptors on HSCs regulates the kinase Lck, which is required for activation of ERK signaling by c-Kit in response to niche factor SCF, leading to the maintenance of HSCs in the steady state and proliferation of HSCs after bone marrow transplantation [66].

Although most HSCs reside in the niche in the bone marrow, a small number of HSCs circulate in the peripheral blood following the constant diurnal fluctuation. This diurnal variation is caused by a change in niche factors from niche cells due to

sympathetic nerve stimulation emitted from the pituitary gland by light stimulation from the eyes, acting on β3-AR on perivascular stromal cells (nestin-positive niche cells) in the bone marrow [67,68].

Repeated anticancer therapy for hematopoietic malignancies is known to delay hematopoietic recovery. As a cause of this, neurotoxic drugs, such as cisplatin, cause sympathetic nerve injury in the bone marrow, indirectly reducing HSC function by dampening the niche protection of sympathetic nervous signaling [69].

The roles of the PNS in the hematopoietic aging

All living multicellular organisms experience cellular senescence and senescence of the entire organism, referred to as “aging”. Aging phenotypes observed in blood cells include myeloid-biased hematopoiesis at the expense of lymphoid cell production. These alterations in the blood cells are based on the aging in HSCs, primarily due to intrinsic cellular changes, including differential transcriptional and epigenetic profiles, increased ROS levels, and DNA damage [70]. However, recent findings suggest that alterations in HSC niche cells extrinsically promote aging of HSCs. Maryanovich et al. demonstrated that aging mice have decreased adrenergic nerve fibers in the bone marrow, accompanied by an increase in nestin-positive niche cells with reduced HSC niche function. Surgical sympathectomy resulted in a specific expansion of myeloid-biased $CD41^+$ HSCs, a phenotype reminiscent of chronologically aged HSCs, in young mice only in the bone marrow on the side

where the procedure was performed. Intriguingly, administration of β3-AR agonist partially rejuvenated niche cells and restored repopulating ability of aged HSCs. Furthermore, young mice lacking β3-AR in the bone marrow microenvironment exhibited premature aging of HSCs, indicating that loss of β3-AR signaling in niche cells promotes HSC aging [71]. However, a conflicting report has emerged arguing that increased adrenergic signals in the aged mice promote IL-6 release from the microenvironment through β2-AR, leading to the expansion of megakaryocytes, one of the aging phenotypes in the bone marrow. The authors concluded that although β3-AR signaling on niche cells ameliorates aging phenotypes in HSC, β2-AR-mediated effects outweigh β3-AR-mediated favorable effects, resulting in HSC aging [72]. Aside from the differences in results in these two reports, they at least indicate that alterations in sympathetic signaling in the bone marrow, in addition to cell-intrinsic changes in HSCs, contribute to hematological aging.

Similar to sympathetic nerves, sensory nerves distributed in bone and bone marrow have been shown to decrease with aging. Quantitative analysis using fluorescent immunostaining revealed that the number of $CGRP^{+}$ nerve fibers in the periosteum of aged mice is reduced compared to young mice, while the density of nerve fibers in the periosteum is conversely increased due to the reduced thickness of the periosteum [44]. A recent study reported that CGRP levels in the bone and bone marrow decrease with aging, and CGRP supplementation promotes osteogenic potential and simultaneously inhibits the adipogenic differentiation

potential of bone marrow stromal cells in vitro. Furthermore, the study revealed that CGRP treatment promoted bone formation in aged mice and ovariectomized mice, indicating the potential approach for the treatment of age-related osteoporosis [73]. The role of sensory nerves in hematopoietic changes with aging remains to be elucidated and requires further study.

Parasympathetic signaling cooperatively regulate HSCs with sympathetic signaling

The role of the parasympathetic nervous system in regulating hematopoiesis has been less well understood than that of the sympathetic nervous system, but recent studies have gradually revealed its role. In the mechanism of G-CSF-induced HSC mobilization, cholinergic signaling via hypothalamic muscarinic receptors has been shown to regulate corticosterone production via the hypothalamic-pituitary-adrenal pathway and maintains bone marrow corticosterone at optimal levels for HSC mobilization [74].

Garcia-Garcia et al. examined the roles of the parasympathetic nervous system on the circadian fluctuation of blood HSCs (increase in blood HSCs during the day and decrease during the night). They observed that sympathetic stimulation causes HSCs to home to the bone marrow at night by increasing adhesion molecules on bone marrow vascular endothelial cells via β2-ARs and during the day by decreasing niche factor expression on bone marrow niche cells via β3-AR, leading

to HSC efflux into the peripheral blood [75]. Furthermore, analysis of parasympathetic-deficient mice revealed that central parasympathetic stimulation reduces the outflow of HSCs from the bone marrow by suppressing sympathetic activity at night [75]. Namely, the rhythmic migration of HSCs in accordance with circadian rhythms is finely tuned by a sympathetic-parasympathetic balance. A recent study has also reported that ACh signaling may induce secretion of the niche factor CXCL12 via α7nAChR on nestin-positive niche cells during hematopoietic recovery after bone marrow transplantation or chemotherapy, maintaining HSC quiescence and self-renewal capacity [76].

Sensory nerve signaling regulate HSC mobilization

The periosteum, the outer surface of the bone, is densely innervated by sensory nerves, the afferent nerves that transmit nociceptive stimuli applied to the bone toward the brain. These sensory nerves also play an important role in bone remodeling [26]. In contrast, although sensory nerves expressing the neuropeptides CGRP and SP are also distributed within the bone marrow [77], their function has long been unknown.

In a recent study, Gao et al. uncovered the function of sensory nerves in regulating hematopoiesis **(Figure 3)**. Mice in which either sympathetic or sensory nerves were denervated did not demonstrate any change in the bone marrow HSC counts, whereas mice in which both nerves were denervated simultaneously

exhibited an increase in HSCs. In addition, HSC mobilization by G-CSF was impaired in mice with sensory nerve denervation by chemicals or using $Na_v1.8$-Cre transgenic mice. HSC mobilization impairment was mitigated when these mice were supplemented with CGRP. These results suggested that CGRP from sensory nerves is indispensable for G-CSF-induced HSC mobilization. Furthermore, the authors have demonstrated that CGRP acts on the CALCRL/ RAMP1 heterodimeric receptor on HSCs and promotes HSC chemotaxis by activating downstream Gαs-adenylyl cyclase-cAMP signaling. More interestingly, diets containing capsaicin, which stimulates sensory nerves, increased the efficiency of HSC mobilization in mice [78]. These observations suggested that sensory nerve stimulation may be a novel strategy to improve G-CSF-induced HSC mobilization.

Peripheral nerve function in hematopoietic malignancies

Angiogenesis is necessary for the growth and survival of malignant tumors, and increased vascular density is one of the typical histological changes observed in tumors. Peripheral nerves run parallel to blood vessels, and in fact, as a cancer progresses from precancerous lesion to overt cancer, the nerve density increases to almost double that of nonneoplastic tissue [79]. In the mouse MLL-AF9 acute leukemia model, in contrast to most non-hematopoietic malignancies, sympathetic innervation of the bone marrow is reduced. Nestin-positive niche cells lose their quiescence and proliferate abnormally, and niche factors important for maintaining

normal HSCs are reduced, resulting in the extinction of normal HSCs [80]. In other words, leukemic cells may manipulate their microenvironment to favor their survival by damaging sympathetic nerves in the bone marrow. A leukemia model transplanted into mice lacking β2-AR in the bone marrow microenvironment showed an accelerated increase of LSCs, and administration of a β2-AR-selective agonist decreased LSCs, suggesting that improvement of the microenvironment through sympathetic nerve stimulation may hinder LSCs [80]. Autonomic nerve signaling also plays an important role in the pathogenesis of myeloproliferative neoplasms. In a mouse model of myeloproliferative tumors caused by mutations in Janus kinase 2 in hematopoietic cells, interleukine-1β secreted by abnormal HSCs causes damage to sympathetic nerves–in bone marrow, which in turn leads to a decrease in normal HSCs by reducing the function of nestin-positive niche cells **(Figure 1)**. In this model, β3-AR agonist treatment restored nestin-positive niche cells and deterred tumor progression by reducing LSCs [81]. Reports from these leukemia model mice suggested that sympathetic nerve signaling may have an inhibitory effect on the tumor, but the role of parasympathetic and sensory nerves has not been fully investigated.

Concluding remarks

It has become clear that osteoblasts regulate the differentiation and function of hematopoietic cells in various ways. Bone marrow contains osteoblastic cells at all

differentiation stages, and recently developed single-cell transcriptome analyses have enabled more detailed cell characterization [82,83]. In the future, spatial transcriptome analysis with each blood cell controlled by these bone lineage cells may provide new insights [84].

The tripartite relationship among bone tissue, hematopoiesis, and the PNS is gradually becoming clear. The relationship among the three organs, as currently understood, is that the nervous system skillfully regulates the functions of the other two tissues and contributes to maintaining individual homeostasis at steady-state and under stress condition. However, the mutual regulatory function of these three organs is not yet fully understood. For example, the effects on the PNS from bone tissue and hematopoietic tissue, or the function of these organs on the central nervous system, are a mystery. In bone-related diseases such as osteoporosis, osteoarthritis, and bone tumors, crosstalk between PNS and bone cells may be a target for improving bone metabolism and relieving pain in these conditions. However, the potential applications of intervention in the complex crosstalk between the bone and PNS for treating disease and regeneration of tissues are still beyond the reach and require further research.

Peripheral nerve signaling is deeply involved in the mechanisms of development and progression of malignant tumors, including hematopoietic malignancies, but actual therapeutic agents or novel therapies based on nerve signaling regulation have yet to be developed. The PNS is distributed throughout the

body, making it difficult to exert effects only on target organs without affecting other organs, which is a problem in systemic drug administration. The development of methods to enable the local control of neural signal balance in only one organ is a topic for future research and may lead to the achievement of the treatment of malignant tumors through peripheral nerve control.

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Figure legends

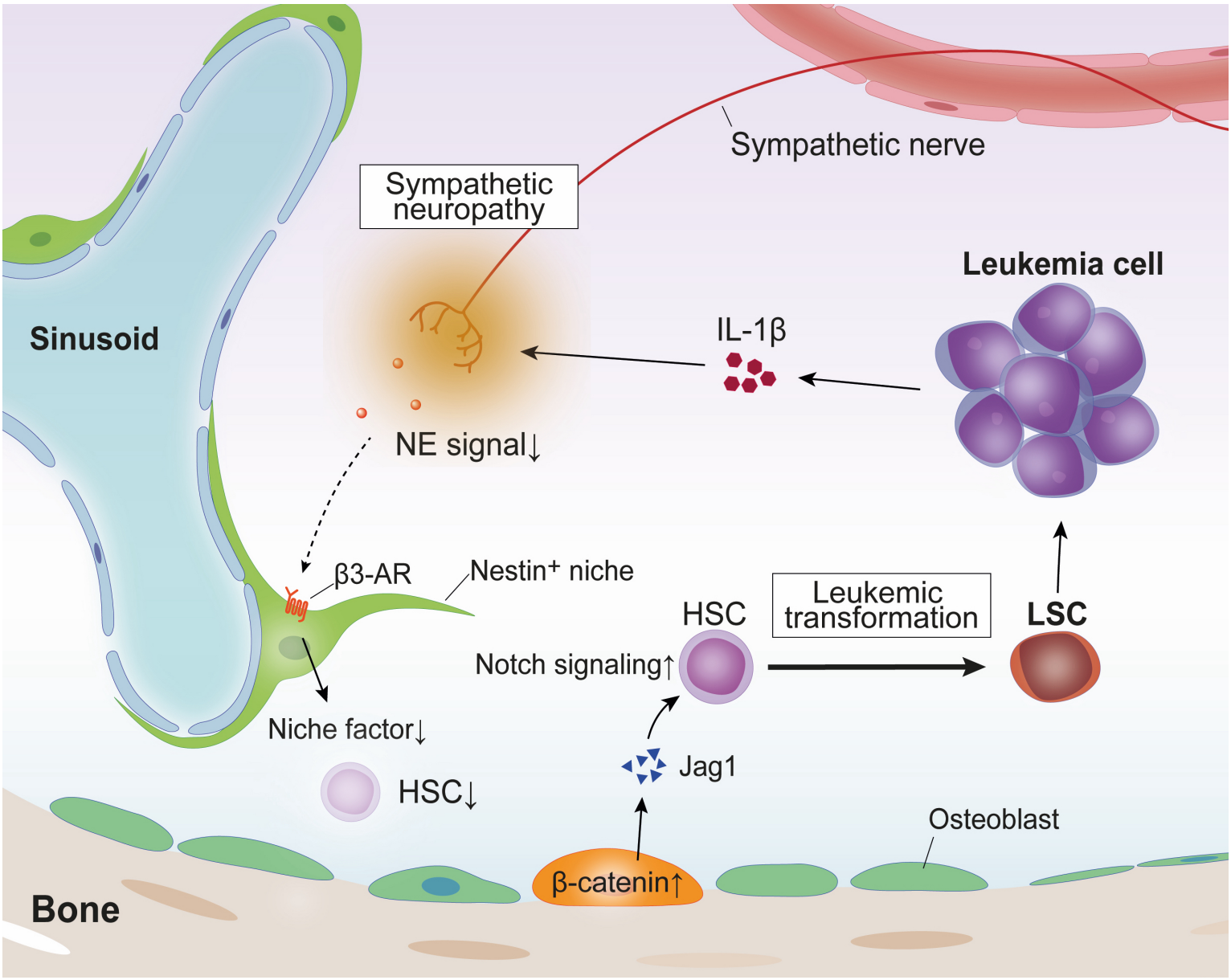
Figure 1. The roles of bone cells and PNS in hematologic malignancies. Constitutive activation of β-catenin in mature osteoblasts enhances the production of the Notch ligand jagged 1(Jag1) in osteoblasts, which leads to the activation of Notch signaling in HSC and promotes the leukemic transformation. Leukemic cells release IL-1β, an inflammatory cytokine, causing the sympathetic neuropathy in the bone marrow, resulting in the decrease of NE signals. Attenuated NE signaling via

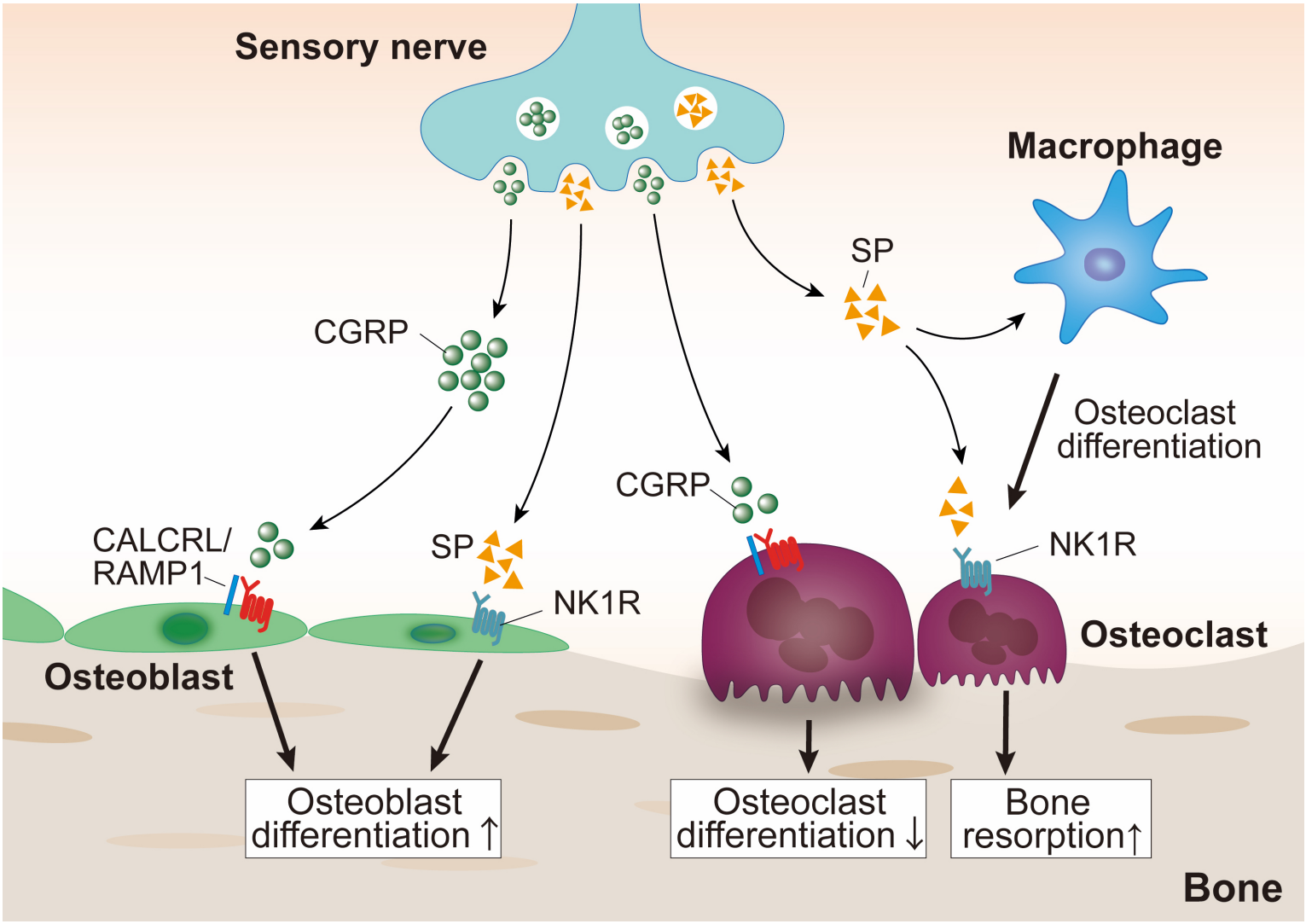
β2-AR on nestin-positive niche cells downregulates the expression levels of niche factors essential for HSC maintenance, which turn in results in the normal HSC distinction in the leukemic bone marrow. LSC, leukemic stem cell.

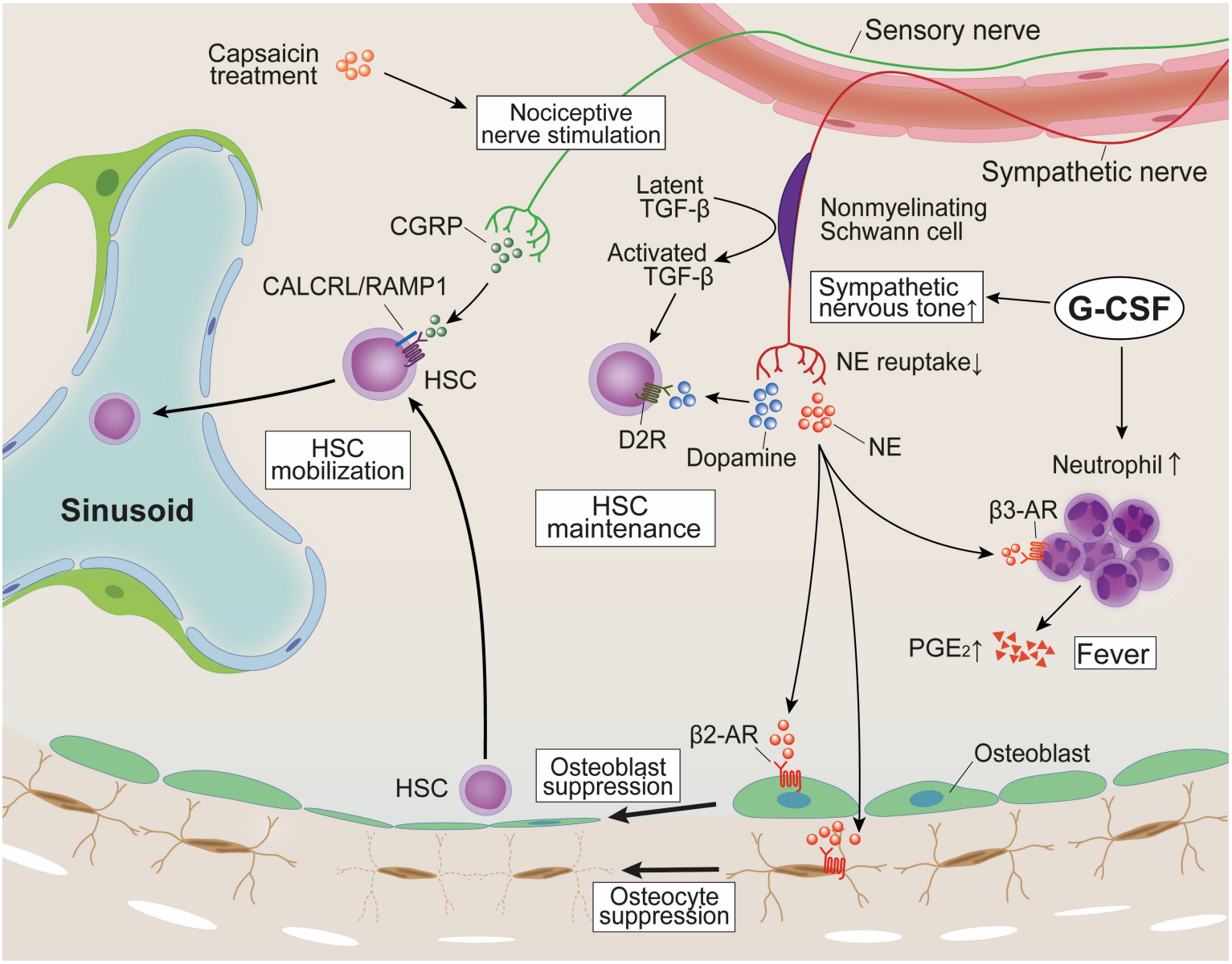
Figure 2. Roles of the sensory nerve in bone remodeling. CGRP from sensory nerve induce osteoblast differentiation via CALCRL/RAMP1 receptor complex and suppresses the differentiation of osteoclast, resulting in the facilitation of bone formation. Substance P (SP) promotes macrophage differentiation into osteoclasts, which promotes osteoclast bone resorption function, but also promotes osteoblast function, which in turn contributes to bone formation. NK1R, neurokinin 1 receptor.

Figure 3. Hematopoietic regulation by PNS in the bone marrow. G-CSF treatment elicits the NE surge in the bone marrow by suppressing NE reuptake at sympathetic nerve endings, which in turn the suppression of both osteoblasts and osteocytes via β2-AR. NE-β2-AR signaling also stimulates the production of PGE_2 from neutrophils during the G-CSF-induced HSC mobilization, leading to a febrile side effect. Dopamine also contributes to the HSC maintenance via D_2-type receptor (D2R) signaling. Nonmyelinating Schwann cells, wrapping the sympathetic nerves, function as niche cells by transforming TGF-β from latent form into activated form. CGRP released from sensory nerves binds to CALCRL/RAMP1 receptor complex on HSCs and promotes HSC chemotaxis by activating downstream signaling.

Capsaicin containing diet, which stimulates nociceptive nerve activity, increases the efficiency of HSC mobilization.


Sympathetic nerve
Sympathetic neuropathy
Leukemia cell
Sinusoid
IL-1β
NE signal↓
β3-AR
Nestin+ niche
HSC
Leukemic transformation
LSC
Notch signaling↑
Niche factor↓
Jag1
HSC↓
Osteoblast
β-catenin↑
Bone


Sensory nerve
Macrophage
SP
CGRP
Osteoclast
differentiation
CGRP
CALCRL/
RAMP1
SP
NK1R
NK1R
Osteoclast
Osteoblast
Osteoblast
differentiation ↑
Osteoclast
differentiation ↓
Bone
resorption↑
Bone


Capsaicin treatment
Nociceptive nerve stimulation
Sensory nerve
Sympathetic nerve
Latent TGF-β
Activated TGF-β
Nonmyelinating Schwann cell
CGRP
CALCRL/RAMP1
HSC
Sympathetic nervous tone↑
G-CSF
NE reuptake↓
D2R
Dopamine
NE
Neutrophil↑
HSC mobilization
Sinusoid
HSC maintenance
β3-AR
PGE2↑
Fever
β2-AR
Osteoblast
HSC
Osteoblast suppression
Osteocyte suppression