



# A mysterious triangle of blood, bones, and nerves

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26 **Keywords:** nervous system, hematopoiesis, bone remodeling, niche

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

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1 **Introduction**

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

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

1 bone tissue, nervous system, and hematopoietic tissue.

2

### 3 **1. Bone cell and hematopoiesis**

#### 4 **Osteoblast progenitors, osteoblasts, and osteocytes regulate hematopoiesis**

#### 5 **differentially in the bone marrow**

6 Hematopoietic stem cells (HSCs), which produce all blood cells, reside in the HSC  
7 niche (niche), a specialized microenvironment in the bone marrow, at steady-state  
8 conditions and are regulated by various signals (niche factors) from the niche [2].  
9 These niche cells include bone lineage cells located at the boundary between bone  
10 marrow and bone tissue, vascular endothelial cells, and perivascular stromal cells  
11 [3]. Among these cell types, bone-forming osteoblasts can maintain hematopoietic  
12 progenitor cells in vitro, and intravenous administration of in vitro-labeled  
13 hematopoietic progenitor cells to mice confirmed their abundant distribution around  
14 the endosteum in the bone marrow [4]. These findings suggested that bone lineage  
15 cells at the endosteum play indispensable roles in HSC regulation.

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

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

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

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

1 modulate erythropoiesis in HIF signaling dependent manner [17].

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

12

### 13 **Osteoblasts play roles in the pathogenesis of hematopoietic malignancies**

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

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

7

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

### 9 **The roles of sensory nerve for the bone regulation**

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

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



1 such as CGRP and SP, from nerve endings, and these neuropeptides act on cell  
2 surface receptors on osteoblasts, osteoclasts, and osteocytes to modulate bone  
3 remodeling. CGRP interact with a dimeric receptor complex of the G protein-coupled  
4 calcitonin receptor-like receptor (CALCRL) and receptor activity modifying protein  
5 1(RAMP1). In  $\alpha$ CGRP knockout (KO) mice, bone loss was observed due to  
6 decreased bone formation [32]. In vitro studies showed that adding CGRP induced  
7 the differentiation of bone marrow stromal cells into osteoblasts and inhibited  
8 osteoblast apoptosis and osteoclast differentiation, suggesting that CGRP promotes  
9 bone formation [33,34].

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

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

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

13

#### 14 **Autonomic nervous system coordinately regulates bone remodeling**

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

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

3 Osteoblasts and osteoclasts express AR on their cell surface, and the  
4 balance of bone remodeling is tilted toward bone resorption by NE stimulation  
5 released from sympathetic nerves. Osteoblasts highly express  $\beta$ 2-AR among AR  
6 subtypes, and administration of isoproterenol, a nonselective  $\beta$ -AR receptor agonist  
7 or  $\beta$ 2-AR selective agonist, caused bone loss due to the suppression of osteoblast  
8 function and increase in osteoclasts [45]. Conversely, in mice lacking  $\beta$ 2-AR in  
9 osteoblasts, an increase in bone mass was observed due to increased osteoblasts  
10 and bone formation rate, indicating that sympathetic signaling regulates bone  
11 remodeling via  $\beta$ 2-AR in osteoblasts [46,47]. Furthermore, as a molecular  
12 mechanism by which sympathetic nervous signals promote osteoclast differentiation,  
13  $\beta$ 2-AR stimulation increases the production of receptor activator of nuclear factor- $\kappa$ B  
14 ligand from osteoblasts, which in turn promotes osteoclast differentiation [47].

15 The effects of ACh released from parasympathetic nerve endings on bone  
16 tissue are also being elucidated. In vitro experiments have revealed that osteoblasts  
17 and osteoclasts express subunits of nAChR and mAChR and that the addition of  
18 nicotine or ACh induces cell proliferation in osteoblasts and apoptosis in osteoclasts  
19 [43,48,49]. In mice lacking  $\alpha$ 2nAChR, a subunit of nAChR, an increase of osteoclasts  
20 accompanied by reduced bone mass was observed [43], whereas  $\alpha$ 7nAChR-KO or  
21  $\alpha$ 7 $\beta$ 2nAChR-KO increased bone mass [50-52], suggesting that the function may

1 differ by the AChR subtype. In addition, mice lacking M3R, a subtype of the  
2 muscarinic receptor, showed decreased bone mass, suggesting that M3R-mediated  
3 signaling may increase bone mass, but this effect is not directly on osteoblasts, but  
4 is exerted by stimulating M3R on neurons to suppress sympathetic nerve activity  
5 [53].

6

### 7 **Clinical relevance of PNS dysfunction to bone**

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

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

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

6

### 7 **3. Regulation of hematopoiesis by the PNS**

#### 8 **Sympathetic nervous signaling exerts HSC mobilization by G-CSF treatment**

9 Anatomical studies in laboratory animals have shown that the bone marrow, the  
10 primary site of hematopoiesis in adult mammals, is abundantly innervated by  
11 autonomic and sensory nerves [25,27,28]. Sympathetic nerves enter the bone  
12 marrow along the nutrient arteries. Many of which wrap around the arteries, and  
13 some of which leave the arteries and project their nerve endings to the bone marrow  
14 parenchyma [27]. Adrenergic signals inhibit osteoblast function and tilt bone  
15 remodeling toward bone resorption but also alter the niche function within the bone  
16 marrow to regulate HSC movement (**Figure 3**). HSC mobilization from the bone  
17 marrow into the blood circulation by administration of supraphysiological doses of  
18 granulocyte colony-stimulating factor (G-CSF) has become a common method of  
19 HSC collection for HSC transplantation. G-CSF-induced exertion of adrenergic  
20 signals suppresses osteoblasts via  $\beta$ 2-ARs and HSC niche function, leading to HSC  
21 mobilization into the blood [60]. Furthermore, this catecholamine-stimulated

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

3           Because G-CSF treatment disrupts the cell projection network of osteocytes,  
4 terminally differentiated osteolineage cell, we focused on the role of osteocytes in  
5 the G-CSF-induced mobilization mechanism [62]. In mice in which the osteocyte  
6 network was disrupted in vivo, G-CSF-induced mobilization of HSCs was severely  
7 impaired, although the number of HSCs in the bone marrow was unchanged. In  
8 surgically denervated bone, G-CSF did not suppress osteocytes, whereas sham-  
9 operated bone reacted to G-CSF signals. Together with  $\beta$ 2-AR expression in  
10 osteocytes, these data suggested that osteocytes play important roles in the  
11 mobilization mechanisms mediated by sympathetic nervous signaling [62] (**Figure**  
12 **3**).

13           The mechanism of sympathetic stimulation by G-CSF has also been studied.  
14 Lucas et al. identified that sympathetic nerves express G-CSF receptors and that  
15 direct stimulation with G-CSF enhances local sympathetic signaling by reducing the  
16 efficiency of NE reuptake at sympathetic nerve endings [63]. Fever and back pain  
17 are well-known side effects during G-CSF induced HSC mobilization in clinical  
18 settings. Kawano et al. reported that these side effects are caused by the release of  
19 prostaglandin E<sub>2</sub> from marrow neutrophils due to sympathetic nerve stimulation  
20 induced by G-CSF via  $\beta$ 3-AR [64].

21

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

2 Sympathetic nervous signaling regulates HSCs and niche cells not only under stress,  
3 such as during G-CSF-induced HSC mobilization, but also at steady-state (**Figure**  
4 **3**). Transforming growth factor- $\beta$  (TGF- $\beta$ ) is an important factor for maintaining HSCs  
5 in quiescence. Yamazaki et al. examined the distribution of activated TGF- $\beta$  in the  
6 bone marrow. They found that Schwann cells, which do not form a myelin sheath, a  
7 glial cell of the sympathetic nervous system, specifically express activated TGF- $\beta$ .  
8 Approximately 20% of HSCs reside in the vicinity of these non-myelinated Schwann  
9 cells, and in mice with surgically resected sympathetic nerves, a decrease in HSCs  
10 was observed simultaneously with a decrease in non-myelinating Schwann cells,  
11 demonstrating that the non-myelinating Schwann cells themselves function as HSC  
12 niches [65].

13 Dopamine, another neurotransmitter released from the sympathetic nerves,  
14 is reportedly involved in the regulation of hematopoiesis. Liu et al. demonstrated that  
15 dopamine signals via D<sub>2</sub>-type receptors on HSCs regulates the kinase Lck, which is  
16 required for activation of ERK signaling by c-Kit in response to niche factor SCF,  
17 leading to the maintenance of HSCs in the steady state and proliferation of HSCs  
18 after bone marrow transplantation [66].

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

1 sympathetic nerve stimulation emitted from the pituitary gland by light stimulation  
2 from the eyes, acting on  $\beta$ 3-AR on perivascular stromal cells (nestin-positive niche  
3 cells) in the bone marrow [67,68].

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

8

### 9 **The roles of the PNS in the hematopoietic aging**

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



1 where the procedure was performed. Intriguingly, administration of  $\beta$ 3-AR agonist  
2 partially rejuvenated niche cells and restored repopulating ability of aged HSCs.  
3 Furthermore, young mice lacking  $\beta$ 3-AR in the bone marrow microenvironment  
4 exhibited premature aging of HSCs, indicating that loss of  $\beta$ 3-AR signaling in niche  
5 cells promotes HSC aging [71]. However, a conflicting report has emerged arguing  
6 that increased adrenergic signals in the aged mice promote IL-6 release from the  
7 microenvironment through  $\beta$ 2-AR, leading to the expansion of megakaryocytes, one  
8 of the aging phenotypes in the bone marrow. The authors concluded that although  
9  $\beta$ 3-AR signaling on niche cells ameliorates aging phenotypes in HSC,  $\beta$ 2-AR-  
10 mediated effects outweigh  $\beta$ 3-AR-mediated favorable effects, resulting in HSC aging  
11 [72]. Aside from the differences in results in these two reports, they at least indicate  
12 that alterations in sympathetic signaling in the bone marrow, in addition to cell-  
13 intrinsic changes in HSCs, contribute to hematological aging.

14         Similar to sympathetic nerves, sensory nerves distributed in bone and bone  
15 marrow have been shown to decrease with aging. Quantitative analysis using  
16 fluorescent immunostaining revealed that the number of CGRP<sup>+</sup> nerve fibers in the  
17 periosteum of aged mice is reduced compared to young mice, while the density of  
18 nerve fibers in the periosteum is conversely increased due to the reduced thickness  
19 of the periosteum [44]. A recent study reported that CGRP levels in the bone and  
20 bone marrow decrease with aging, and CGRP supplementation promotes  
21 osteogenic potential and simultaneously inhibits the adipogenic differentiation

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

6

### 7 **Parasympathetic signaling cooperatively regulate HSCs with sympathetic** 8 **signaling**

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

16 Garcia-Garcia et al. examined the roles of the parasympathetic nervous  
17 system on the circadian fluctuation of blood HSCs (increase in blood HSCs during  
18 the day and decrease during the night). They observed that sympathetic stimulation  
19 causes HSCs to home to the bone marrow at night by increasing adhesion molecules  
20 on bone marrow vascular endothelial cells via  $\beta$ 2-ARs and during the day by  
21 decreasing niche factor expression on bone marrow niche cells via  $\beta$ 3-AR, leading

1 to HSC efflux into the peripheral blood [75]. Furthermore, analysis of  
2 parasympathetic-deficient mice revealed that central parasympathetic stimulation  
3 reduces the outflow of HSCs from the bone marrow by suppressing sympathetic  
4 activity at night [75]. Namely, the rhythmic migration of HSCs in accordance with  
5 circadian rhythms is finely tuned by a sympathetic-parasympathetic balance. A  
6 recent study has also reported that ACh signaling may induce secretion of the niche  
7 factor CXCL12 via  $\alpha 7nAChR$  on nestin-positive niche cells during hematopoietic  
8 recovery after bone marrow transplantation or chemotherapy, maintaining HSC  
9 quiescence and self-renewal capacity [76].

10

### 11 **Sensory nerve signaling regulate HSC mobilization**

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

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

1 exhibited an increase in HSCs. In addition, HSC mobilization by G-CSF was  
2 impaired in mice with sensory nerve denervation by chemicals or using  $\text{Na}_v1.8\text{-Cre}$   
3 transgenic mice. HSC mobilization impairment was mitigated when these mice were  
4 supplemented with CGRP. These results suggested that CGRP from sensory nerves  
5 is indispensable for G-CSF-induced HSC mobilization. Furthermore, the authors  
6 have demonstrated that CGRP acts on the CALCRL/ RAMP1 heterodimeric receptor  
7 on HSCs and promotes HSC chemotaxis by activating downstream G $\alpha$ s-adenylyl  
8 cyclase-cAMP signaling. More interestingly, diets containing capsaicin, which  
9 stimulates sensory nerves, increased the efficiency of HSC mobilization in mice [78].  
10 These observations suggested that sensory nerve stimulation may be a novel  
11 strategy to improve G-CSF-induced HSC mobilization.

12

### 13 **Peripheral nerve function in hematopoietic malignancies**

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

1 normal HSCs are reduced, resulting in the extinction of normal HSCs [80]. In other  
2 words, leukemic cells may manipulate their microenvironment to favor their survival  
3 by damaging sympathetic nerves in the bone marrow. A leukemia model  
4 transplanted into mice lacking  $\beta$ 2-AR in the bone marrow microenvironment showed  
5 an accelerated increase of LSCs, and administration of a  $\beta$ 2-AR-selective agonist  
6 decreased LSCs, suggesting that improvement of the microenvironment through  
7 sympathetic nerve stimulation may hinder LSCs [80]. Autonomic nerve signaling also  
8 plays an important role in the pathogenesis of myeloproliferative neoplasms. In a  
9 mouse model of myeloproliferative tumors caused by mutations in Janus kinase 2 in  
10 hematopoietic cells, interleukine-1 $\beta$  secreted by abnormal HSCs causes damage to  
11 sympathetic nerves-in bone marrow, which in turn leads to a decrease in normal  
12 HSCs by reducing the function of nestin-positive niche cells (**Figure 1**). In this model,  
13  $\beta$ 3-AR agonist treatment restored nestin-positive niche cells and deterred tumor  
14 progression by reducing LSCs [81]. Reports from these leukemia model mice  
15 suggested that sympathetic nerve signaling may have an inhibitory effect on the  
16 tumor, but the role of parasympathetic and sensory nerves has not been fully  
17 investigated.

18

### 19 **Concluding remarks**

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

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

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

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

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

6

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10

### 11 **Declarations**

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13

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16

## 17 **Figure legends**

### 18 **Figure 1. The roles of bone cells and PNS in hematologic malignancies.**

19 Constitutive activation of  $\beta$ -catenin in mature osteoblasts enhances the production  
20 of the Notch ligand jagged 1(Jag1) in osteoblasts, which leads to the activation of  
21 Notch signaling in HSC and promotes the leukemic transformation. Leukemic cells  
22 release IL-1 $\beta$ , an inflammatory cytokine, causing the sympathetic neuropathy in the  
23 bone marrow, resulting in the decrease of NE signals. Attenuated NE signaling via

1  $\beta$ 2-AR on nestin-positive niche cells downregulates the expression levels of niche  
2 factors essential for HSC maintenance, which turn in results in the normal HSC  
3 distinction in the leukemic bone marrow. LSC, leukemic stem cell.

4

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

11

12 **Figure 3. Hematopoietic regulation by PNS in the bone marrow.** G-CSF  
13 treatment elicits the NE surge in the bone marrow by suppressing NE reuptake at  
14 sympathetic nerve endings, which in turn the suppression of both osteoblasts and  
15 osteocytes via  $\beta$ 2-AR. NE- $\beta$ 2-AR signaling also stimulates the production of PGE<sub>2</sub>  
16 from neutrophils during the G-CSF-induced HSC mobilization, leading to a febrile  
17 side effect. Dopamine also contributes to the HSC maintenance via D<sub>2</sub>-type receptor  
18 (D2R) signaling. Nonmyelinating Schwann cells, wrapping the sympathetic nerves,  
19 function as niche cells by transforming TGF- $\beta$  from latent form into activated form.  
20 CGRP released from sensory nerves binds to CALCRL/RAMP1 receptor complex  
21 on HSCs and promotes HSC chemotaxis by activating downstream signaling.

- 1 Capsaicin containing diet, which stimulates nociceptive nerve activity, increases the
- 2 efficiency of HSC mobilization.
- 3
- 4







