The alliance between nerve fibers and stem cell populations in bone marrow: life partners in sickness and health

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ABSTRACT: The bone marrow (BM) is the central hematopoietic organ in adult mammals, with great potential to be used as a tool to improve the efficacy of the body’s response to a number of malignancies and stressful conditions. The nervous system emerges as a critical regulatory player of the BM both under homeostatic and pathologic settings, with essential roles in cellular anchorage and egress, stem cell differentiation, and endothelial cell permeability. This review collects the current knowledge on the interplay between the nervous system and the BM cell populations, with a focus on how the nervous system modulates hematopoietic stem and progenitor cell, mesenchymal stromal cell, and endothelial progenitor cell activity in BM. We have also highlighted the pathologies that have been associated with disturbances in the neuronal signaling in BM and discussed if targeting the nervous system, either by modulating the activity of specific neuronal circuits or by pharmacologically leveling the activity of sympathetic and sensorial signaling-responsive cells in BM, is a promising therapeutic approach to tackling pathologies from BM origin.—Leitão, L., Alves, C. J., Sousa, D. M., Neto, E., Conceição, F., Lamghari, M. The alliance between nerve fibers and stem cell populations in bone marrow: life partners in sickness and health. FASEB J. 33, 8697–8710 (2019). www.fasebj.org

KEY WORDS: sympathetic • sensorial • mesenchymal stromal cells • hematopoietic stem cells • endothelial cells

Bone marrow (BM) is the soft tissue found in the hollow spaces of the interior of bones, accounting for ~5% of body weight in humans. It is also the central hematopoietic organ in adult mammals, giving rise to all of the mature blood and immune cells required to address the body’s needs throughout life (1, 2). BM is composed of multiple stem cell populations [including the hematopoietic stem and progenitor cells (HSPCs), mesenchymal stromal cells (MSCs), and endothelial progenitor cells (EPCs)] surrounded by a shell of vascularized and innervated bone (1, 2). These stem cell populations are organized in functional units, or niches, within the BM, a concept first hypothesized by Schofield for the HSPC population (3). Within BM niches, stem cells are functionally associated with other cellular populations, including endothelial, immune, and bone cells, and their cooperative action ultimately dictates stem cell behavior by controlling proliferation, survival, self-renewal, and differentiation, either by a physical interaction between the distinct cells of the niche or by distal communication mediated by secreted soluble factors (4).

Over the last few decades, researchers have significantly improved comprehension of the BM dynamics and functionality and are now exploring its potential as a tool to improve the efficacy of the body’s response to a number of malignancies and stressful conditions (5). Under both biologic scenarios, the nervous system arises as a critical regulatory player, with accumulating evidence pointing to essential roles in modulating processes ranging from cellular anchorage and egress from BM and bone remodeling to HSPC-inflammatory fate (6, 7) (Fig. 1). Sympathetic signaling has been highly associated with the mobilization of HSPCs from BM to circulation, an effect dependent on the activity of mesenchymal stem–resident cells (8–10). In addition, receptors for neuronal signaling have been observed in HSPCs, and different reports have been pinpointing the sensorial nervous system as a critical

ABBREVIATIONS: 6-OHDA, 6-hydroxydopamine; AML, acute myelogenous leukemia; BM, bone marrow; CD, cluster of differentiation; CGRP, calcitonin gene-related peptide; CXCL12, chemokine (C-X-C motif) ligand 12; DRC, dorsal root ganglia; EC, endothelial cell; EPC, endothelial progenitor cell; G-CSF, granulocyte colony–stimulating factor; HSPC, hematopoietic stem and progenitor cells; LSC, leukemia stem cell; MPN, myeloproliferative neoplasm; MSC, mesenchymal stromal cells; NE, noradrenaline; NGF, nerve growth factor; NPY, neuropeptide Y; RAGE, receptor for advanced end-glycation products; SP, substance P; TH, tyrosine-hydroxylase; WT, wild type

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doi: 10.1096/fj.201900454R
regulator of MSC activity in BM (11–13). Interestingly, MSCs can, in turn, secrete neurotrophic factors beneficial for the nervous system microenvironment, with the potential to promote neuroprotection and improve regeneration (14), suggesting a bidirectional crosstalk between the nervous system and BM-resident cells. Importantly, distinct pathologic conditions have been linked to disturbances in the normal neuronal signaling in BM. These can either be due to excessive adrenergic inputs or axonal sprouting, as seen in stress-related pathologies or bone cancer pain, respectively, or due to the destruction of nerve fibers and consequent lack of signaling, as it occurs in patients with diabetes (Fig. 2).

Nonetheless, the communication between the nervous system and BM stem cells has proven difficult to address because an accurate description of the structural organization of the BM is still missing (i.e., the spatial distribution and molecular interactions of its different cellular constituents and the key players in regulating the activity of the stem cell niche). This is mostly because of the difficulty in retaining the histologic integrity of the BM when sectioning the bone, the complexity of the immunostaining techniques required to label stem cells, and the puzzle of isolating specific cellular mechanisms without disturbing the activity of neighboring cells (1).

In this review, we discuss the current knowledge on the bidirectional crosstalk between the nervous system and BM, focusing not only on the response of the different cellular population of the BM to neuronal signaling but also on the evidence that point to a neuronal modulation triggered by marrow resident cells. We also integrate the current knowledge on pathologies that bridge both BM and the nervous system and infer if pharmacologic manipulation of the nervous system is a potential therapeutic avenue either for BM-related diseases or selective mobilization of stem cells to injury sites.

NERVE FIBERS WITHIN THE BM COMPARTMENT

The first histologic evidence of a neuronal component in BM was provided in 1968 by Calvo (15) which showed nerve fibers penetrating the bone through the nutrient foramina and with a distribution pattern similar to the
nutrient artery. Follow-up studies have supported these observations and showed that BM is innervated both by unmyelinated or thin myelinated fibers, with nerve terminals branching and signaling to BM niche resident cells (16).

Most of the innervation of the BM is provided by the autonomic nervous system, with both sympathetic and parasympathetic fibers, contributing with different classes of neurotransmitters and neuropeptides (16). Interestingly, although only a small percentage of the autonomic nerve fibers that reach the bone penetrate the marrow compartment, BM still comprises the higher density of autonomic innervation when compared to the relative volume of the periosteal region and mineralized bone (7, 16, 17). Among the sympathetic branch of the autonomous nervous system, the noradrenergic nerve fibers are the most studied neuronal projections in BM, mainly due to the observations that the activation of its G-protein-coupled α- (α-) and β- (β-) adrenergic receptors by noradrenaline (NE) often results in changes in the hematopoietic cell behavior (as discussed in the section “Role of nervous system in HSPC mobilization to circulation”). In BM of mouse femurs, sympathetic tyrosine-hydroxylase (TH)-positive fibers have been described as having a corkscrew-shaped appearance because they are tightly wrapped around blood vessels (18). Neuropeptide Y (NPY)-expressing neurons have also been reported in the BM of rat tibias and femurs (19), and fibers expressing vasoactive intestinal peptide have been reported to occasionally enter the BM of rat tibias (19). Finally, parasympathetic nerve fibers have also been detected in the intertrabecular regions of the BM in rat femurs (20, 21).

Sensorial innervation is also present in BM microenvironment. Studies have shown the presence of substance P (SP) and, more frequently, calcitonin gene-related peptide (CGRP)-positive projections penetrating the BM (22–24). In mouse femur, CGRP- and NF200-positive sensory fibers have been described as evenly distributed within BM as single or bundled threads with linear morphology generally associated with vasculature (18, 23). Interestingly, most of the sensory fibers present in BM express proteins highly recognized for their role in axonal growth and regeneration, neuronal survival, and sprouting, such as TrkA (23, 25).

Changes in the neuronal composition of the BM have been associated with phenotypes often observed in aging and other pathologic scenarios. Studies have shown that loss of sympathetic nerves in BM trigger the development of aging phenotypes in HSPCs, resulting in HSPC niche function impairment and consequently leading to the development of blood disorders (26). Interestingly, sensory nerve fibers appear not to decline with age, displaying more branch points in BM instead (18).
chemotherapy drugs often cause sensory neuropathy in BM, resulting in impaired hematopoietic regeneration (27). Finally, the presence of sensory terminals in BM can also support nociceptive sensations, usually described as pain from BM origin, and is often reported by patients upon injection of irritants into the medullary cavity or in cases of needle aspiration of the marrow content (28, 29). Likewise, injuries in BM have been correlated with pain in knee osteoarthritis in an epidemiologic study because BM lesions were much more frequent in participants with knee pain than those without it (30).

**HSPCs AND NERVOUS SYSTEM ACQUAINTANCE IN BM**

**HSPCs express receptors for neuronal signaling**

Different reports have described that hematopoietic cells from different sources express receptors for sympathetic and sensorial neurotransmitters, depicted in Table 1 (13). Curiously, to the date, few studies have addressed the role of neurotransmitter-receptor activation in HSPCs. Most studies show that the regulation of HSPC niche dynamics through neuronal signaling is mostly due to the activation of receptors in nonhematopoietic cells, such as MSCs, osteoblasts, macrophages, and endothelial cells (ECs), whose stimulation in turn introduces changes in the activity of the hematopoietic niche in BM. A rare exception was described in 2007 by Spiegel et al. (31) in a study where they examined sympathetic signaling in HSPCs. In this study, they demonstrate *in vitro* that the stimulation with granulocyte colony-stimulating factor G-CSF of a culture enriched with cluster of differentiation (CD) 34+ cells derived from either adult human BM or cord blood up-regulates the surface expression of the dopamine receptors DR3 and DR5 as well as the β2-adrenergic receptors. Importantly, *in vitro* treatment with a dopamine agonist of G-CSF–mobilized CD34+ progenitor cells (*i.e.*, already expressing high levels of dopamine receptors) before transplantation resulted in improved engraftment in mouse BM when compared to untreated control cells, whereas treatment with a dopamine antagonist produced the opposite effect. Also, a direct correlation between adrenergic signaling and HSPC behavior was demonstrated on the motility ability of these cells because the stimulation with NE in a transwell system increased the migration of hematopoietic progenitors, accompanied with increased metalloproteinase activity. Taken together, these studies hint for a direct function for neuronal regulation in HSPCs *in vivo*, namely in augmenting repopulation and in controlling cellular motility, and might represent an important regulatory mechanism underlying stress-induced settings (31).

**Role of the nervous system in HSPC mobilization to circulation**

The sympathetic nervous system has been reported to be a crucial yet indirect regulator of the mechanisms underlying HSPC anchorage and egress from BM (8). Katayama *et al.* (8) published an interesting study showing that the sympathetic nervous system is a critical player in the regulation of the hematopoietic niche, with a role on the mobilization of HSPCs from BM to circulation. In this study, they show that in chemically sympathectomized mice, the number of HSPCs mobilized by G-CSF was dramatically reduced. The same result was obtained when using mice deficient for dopamine β-hydroxylase, the enzyme necessary for the conversion of dopamine into NE, suggesting that this catecholamine is required for the mobilization induced by G-CSF. This hypothesis was further strengthened when pharmacological experiments were performed using β-blockers in wild-type (WT) mice and β2 adrenergic agonists in NE-deficient mice. In this set of experiments, researchers observed a reduction in the circulating levels of HSPCs in WT mice when treated with β-blockers, whereas recovery of the circulating levels of HSPCs was observed in NE-deficient mice in the presence of β2 adrenergic agonists (8).

**Table 1. Neuronal receptors identified in HSPCs**

| Receptor identified | Neurotransmitter | Hematopoietic markers | Source | Reference |
|--------------------|-----------------|-----------------------|--------|-----------|
| GABA<sub>B</sub> | GABA | CD34<sup>+</sup> cells | Human PB | 94 |
| A<sub>B</sub> | ATP | CD34<sup>+</sup> cells | Mouse BM | 95 |
| κ1 and μ1 | Opioids | CD34<sup>+</sup> cells | Human PB | 96 |
| 1F | Serotonin | CD34<sup>+</sup> cells | Mouse BM | 97 |
| Orexin 1 and 2 | Orexin | CD34<sup>+</sup> cells | Human BM | 98 |
| CGRP receptor subunits | CGRP | CD34<sup>+</sup> cells | Human PB | 99 |
| Adrenergic α1-, α2- or β2- | NE | Lin<sup>-</sup> CD17<sup>high</sup> Sca-1<sup>+</sup> cells | Mouse BM | 100 |
| DR3 and DR5 | Dopamine | CD34<sup>+</sup> CD38<sup>high</sup> and Low | Human BM | 101 |
| β2 | NE | CD34<sup>+</sup> CD38<sup>+</sup> cells | Human PB | 102 |
| VPAC<sub>1</sub> | VIP | CD34<sup>+</sup> CD38<sup>+</sup> cells | Mouse BM | 103 |
| P2X and P2Y | ATP | CD34<sup>+</sup> CD38<sup>+</sup> cells | Human BM | 104 |
| RET | GDNF | Lin<sup>-</sup> CD150<sup>-</sup> CD48<sup>-</sup> CD41<sup>-</sup> cells | Mouse BM | 105 |
| NK1 | SP | CD34<sup>+</sup> AC133<sup>+</sup> | Human BM | 106 |

A<sub>B</sub>, adenosine A2B receptor; AC, epitope AC133; CB, cord blood; DR3, dopamine receptor 3; DR5, dopamine receptor 5; GDNF, glial cell line-derived neurotrophic factor; Lin, lineage; P2X, P2X purinoreceptor; P2Y, P2Y purinoreceptor; PB, peripheral blood; RET, rearranged during transfection; Sca-1, stem cells antigen-1; u1, opioid k1 receptor; VIP, vasoactive intestinal peptide; VPAC1, vasoactive intestinal peptide (VIP) receptor type 1.
Also, and in accordance, loss of sympathetic signaling in adult mice, through the administration of 6-hydroxydopamine (6OHD), disrupted both the robust circadian fluctuation observed in the levels of circulating HSPCs, and the correspondent antiphase expression of chemokine (C-X-C motif) ligand 12 (CXCL12) (also known as stromal cell–derived factor 1) in the BM microenvironment (9). In this study, scientists also showed that HSPC mobilization induced by the circadian NE release via sympathetic nerve fibers in BM is mainly due to the activation of β3-adrenergic receptor in stromal cells (9). This conclusion derived from a combination of experiments including the use of selective β3-adrenergic receptor agonists and knockout mice. First, researchers observed that positive modulation of β3- but not β2-adrenergic receptor induced a decrease in the expression of CXCL12 in stromal cells in vitro, whereas the β3-adrenergic receptor antagonist inhibited this effect. Additionally, the treatment with nonselective adrenergic receptor agonists induced significant mobilization of HSPCs in control but not in Adrb3−/− mice. Finally, the administration of a β3-adrenergic receptor antagonist 1 h before the onset of light prevented the morning increase of circulating progenitors, an effect that was also attenuated in Adrb3−/− mice while preserved in Adrb2−/− mice (9).

In accordance, a recent study showed that light-induced NE elevation in BM promotes not only HSPC differentiation and egress but also increases BM vascular permeability via β3-adrenergic receptor. Pharmacological blockade of the β3-adrenergic receptor after onset of light resulted in reduced levels of both common lymphoid and myeloid progenitors in the BM and also decreased the number of circulating HSPCs and diminished BM vascular permeability (32). Importantly, in this study, researchers also showed that 2 daily peaks of HSPC activity occur in BM, which are accompanied and dependent on transient elevations of both NE and TNF. Interestingly, the increase in TNF levels relies on the activation of the β2-adrenergic receptor, and in the consequent increase in the expression of the TNF-converting enzyme. Researchers showed that the pharmacological blockade of β2-, but not β3-, adrenergic receptor resulted in reduced TNF-converting enzyme levels on BM HSPCs and monocytes and decreased TNF levels following light initiation. In addition, mice deficient for the β2- but not β3- adrenergic receptor showed reduced BM TNF levels (32). Overall, these reports show that light initiation and termination trigger stress reactions that activate the sympathetic nervous system resulting in an increase in the levels of NE in BM. Consequently, the activation of β2- and β3-adrenergic receptor in specific BM cellular subtypes controls the daily release of HSPCs into circulation, depicting this mechanism as a possible component of the intrinsic regenerative program of BM niche itself (9, 32, 33).

Role of sympathetic signaling on the behavior of HSPCs in BM pathologies

The activation of the β3-adrenergic receptor in BM has been associated to an increase in the proliferation of hematopoietic progenitors in cases of stress, giving rise to higher levels of disease-promoting inflammatory cytokines, indicating a direct link between chronic stress and chronic inflammation (34). In mice exposed to different stressful procedures, which included cage tilting, isolation followed by crowding, and rapid light-dark changes, the excessive stress-induced release of NE in BM by the sympathetic fibers tuned down the global expression of CXCL12 in BM, promoted HSPC proliferation, and directed HSPC differentiation toward neutrophil and inflammatory monocyte production (34). Also, it has been shown that patients who suffered a blunt or penetrating trauma have elevated levels of both epinephrine and NE for more than a week (35), accompanied with elevated levels of G-CSFs, and increased HSPC progenitor levels in circulation (36). In accordance, rats subjected to stress derived from lung trauma and hemorrhagic shock showed a significant decrease in overall BM cellularity immediately after trauma, which was sustained for at least 7 d, when compared to control rats (37). Interestingly, the use of β-blockers drastically attenuated this outcome, indicating that prolonged NE signaling in BM may be a key factor potentiating BM dysfunction following severe trauma (37).

On a different note, it has been described that the aging of HSPCs (i.e., the decline in their regenerative capacity and multilineage differentiation potential) critically depends on sympathetic nervous system signaling in BM (26). Recently, it has been described that aging causes a profound loss of functional sympathetic signaling in BM, an event that occurs before the appearance of HSPCs with phenotypes of physiologic aging (26). In this study, it is also reported that young mice subjected to surgical sympathetic denervation showed a premature aging-like HSPC phenotype, including expansion of myeloid-biased HSPCs with reduced long-term self-renewal capacity, dramatic reduction of engraftment capacity and lymphoid cell production, and increased engraftment capacity of donor HSPCs in competitive repopulation assays. Importantly, the role of the sympathetic inputs on HSPC aging seems to rely on β3-adrenergic receptor signaling because the supplementation of an agonist to old mice significantly rejuvenated HSPCs and restored their functional properties, whereas the depletion of β3-adrenergic signaling in Adrb3−/− mice promoted premature aging (26).

It is also important to state that the impact of sympathetic signaling in the regulation of the HSPC niche in BM is not restricted to adrenergic signaling. NPY treatment has been described to restore BM function in mice treated with the chemotherapy drug cisplatin, a drug that often induces sympathetic neuropathy characterized by a reduction of TH-positive fibers in BM (27, 38). Also, NPY-deficient mice have been reported to encompass reduced HSPC numbers both in circulation and in BM and impaired BM regeneration upon transplantation of BM cells from control mice (38). Interestingly, these results were neither due to a perturbation of HSPC motility and migration capacity nor their differentiation ability in BM but were instead related to an impairment in HSPC survival, suggesting a role of supportability to NPY signaling in BM. To dissect the mechanisms underlying these observations,
researchers induced HSPC impairments by chemotherapy in mice conditionally depleted for the NPY-Y1 receptor in osteoblasts or macrophages, the 2 cellular populations within bone compartment with higher expression of this receptor. Their results showed that NPY treatment recovered the damage induced by chemotherapy in control- and osteoblast-depleted Y1 receptor animals but not in Y1-macrophage–deficient mice, suggesting that macrophages may be supporting HSPC survival in BM through Y1 receptor signaling (38). Interestingly, although NPY signaling through the Y1 receptor in osteoblasts was not associated with HSPC survival, it may play a role in regulating HSPC mobilization from the BM. In fact, mice deficient for the Y1 receptor in osteoblasts showed impaired HSPC mobilization in response to NPY, an effect that was correlated with a reduction in the activity of matrix metalloproteinase-9 and compromised expression of adhesion molecules responsible for HSPC homeostasis and mobilization from BM, such as CXCL12, Vcam1, and Kitl (39).

Overall, these results suggest that pharmacological manipulation of the sympathetic signaling in BM might be an attractive therapeutic option to target stress, traumatic-related diseases, or tackle abnormal BM outcomes resulting from chemotherapy treatments (34, 37, 38).

Effect of HSPCs on the neuronal functionality

Studies explicitly targeting an effect of HSPCs on peripheral nerve terminals in BM are still scarce. However, reports have hinted that HSPCs may trigger a neuronal response, although through indirect mechanisms. As an example, transplantation of HSPCs has been shown to improve the locomotion deficits of mice with spinal cord injury by decreasing astrogliosis and restoring the levels of neurotrophin-3 and MAPK 1, emerging as a possible therapeutic procedure to tackle spinal cord–related lesions (40).

Also, clinically, the exacerbated inflammatory microenvironment established by HSPCs under diabetic settings has been linked to the neuronal damage often observed in patients with diabetes who developed peripheral neuropathy (41). Diabetes has been shown to induce an injurious inflammatory environment by tempering with the hematopoietic process in the BM and promoting an enhanced inflammatory monocyte profile (41, 42). The link between HSPC niche activity disturbance and neuronal damage is supported by studies showing that the depletion of proinflammatory inducible NO synthase or poly [ADP-ribose] polymerase 1 factors specifically from BM cells prevents the development of tactile allodynia in diabetic animals, indicating that neural dysfunction is a consequence of the inflammatory setting established by a myeloid over-differentiation by HSPCs (43). Furthermore, transplantation of BM cells lacking the receptor for advanced end-glycation products (RAGE) to irradiated diabetic mice promoted axonal regeneration and improved conduction velocity after acute sciatic nerve crush, which was accompanied by an increased number of macrophages with M2 phenotype in the peripheral nerve (44). It has been described that the activation of RAGE by members of the proinflammatory family sustains inflammation and suppresses the repair process (44, 45). Therefore, these results suggest that the blockade of RAGE activity in BM suppresses the HSPC-derived acute inflammatory status often observed in diabetes. Furthermore, these studies also indicate that the development of peripheral neuropathy, a common outcome of diabetes, might have a BM origin, possibly as a consequence of an impaired hematopoietic process.

On a different pathologic scenario, it has been described that the development of acute myelogenous leukemia (AML) is accompanied by sympathetic neuropathy in the BM. In AML, a deregulation of leukemia stem cells (LSCs) occurs, leading to LSC expansion, ultimately resulting in impairment of the normal hematopoietic process. Hanoun et al. (46) have shown that the development of AML induces sympathetic neuropathy in BM at LSC infiltrated sites. The resulting decrease in sympathetic tone is concomitant with not only increased proliferation and accumulation of osteoblast-committed MSCs but also with reduced ability in maintaining healthy HSPCs in BM. Furthermore, in this study, researchers showed that inhibition of β2-, but not β3-, adrenergic receptor dramatically augmented the number of LSCs in BM, presented higher proliferative capacity, and was associated with increased mortality of leukemic mice. The same outcome of increase LSC infiltration in the BM was observed in β2-adrenergic receptor–deficient mice. Overall, this study highlights the importance of the sympathetic signaling in promoting disease progression in a leukemia scenario by regulating BM niche resident cell activity to create a self-sustaining microenvironment (46).

MSCS AND INNERVATION IN BM: A BOND TO ENDURE

The autonomous nervous systems dictate MSC function in BM

MSCs are essential constituents of the stem cell niche of the BM and are defined by their self-renewal capacity and for being able to differentiate into osteoblasts, adipocytes, or chondrocytes (47). Due to these properties, MSCs have been the focus of many studies, either to understand their functional role in the BM or as a potential tool to be used in cell-based therapies (48). With this regard, accumulating evidence has been pinpointing the nervous system as a critical controller of the function of MSCs in BM. Analysis of the BM of NPY-knockout mice showed a reduced number of Sca1+ CD51+ MSCs in the BM, suggesting that NPY signaling may mediate MSC survival (38). Also, in an elegant study by Méndez-Ferrer et al. (10), it was unraveled a structurally unique niche in BM, involving a partnership between MSCs and HSPCs that is tightly regulated by the autonomous nervous system. They show that the proliferative ability of Nestin+–MSCs flushed from BM samples is increased in chemically sympathectomized mice and that differentiation into osteoblasts is impaired in the presence of either nonselective β- or selective β3-adrenergic receptor
agonists, suggesting that proliferation and differentiation of MSCs in BM level is controlled by inputs provided by the sympathetic nervous system. Importantly, they also report that the pharmacological treatment with both β- and β3-adrenergic receptor agonists down-regulates the expression core hematopoietic maintenance and mobilization genes, including CXCL12. These results, combined with the observation that the levels of HSPCs are reduced in the BM in mice deficient for Nestin+-MSCs, suggest that neural mechanisms tightly regulate HSPC niche fate through MSC activity (10).

Mobulation to bone-forming sites and subsequent differentiation into osteoblasts, adipocytes, and chondrocytes is also a feature of MSCs that may be regulated by the nervous system. It has been shown in vivo that sympathetic denervation improved the bone mineral density in a rat model of distraction osteogenesis, which was correlated with a higher tendency of MSCs to migrate from perivascular zones to bone-forming sites (49). These results were further strengthened in vitro, where researchers have demonstrated that NE, via β3-adrenergic receptor, is a negative modulator of both MSC migration and osteoblastic differentiation by reducing the expression levels of osteogenic markers, such as alkaline phosphatase, Runx2, and osteocalcin (49). Overall, these results suggest that the sympathetic nervous system can dictate MSC behavior and control their function in BM, namely proliferation, differentiation, and HSPC mobilization. They also hint that β3-adrenergic receptor might be a critical switch in the regulation of BM activity by the MSCs. When activated by NE in response to a stressful event, it instructs MSCs not to migrate to bone-forming sites and remain in close proximity to HSPCs. This immediacy creates the optimal conditions for the BM to tackle the stressful event by acutely down-regulating the expression of CXCL12, therefore promoting an immediate mobilization of HSPC to circulation (50). Additionally, it ensures that MSCs are not misused in performing not-so-urgent needs, such as migrating to bone-forming sites and differentiating to osteoblasts (Figs. 1, 2).

The sensorial nervous system impact in MSC differentiation to osteoblasts

The sensorial nervous system has been described as a positive modulator of the ability of MSCs to differentiate in osteoblasts, in contrast with the anti-osteogenic role that has been described for the sympathetic innervation (11, 51–56). Fukuda et al. (53) demonstrated in vivo that mice deficient for semaphorin 3A specifically expressed by neurons have lower bone mass, reduced bone formation rates, and abnormalities in osteoblastic differentiation. They also observed that neuron-specific semaphorin 3A-deficient mice had a reduced number of sensory innervations in the bone, whereas the sympathetic nerve fibers remained unchanged. To establish a causality effect between loss of sensory innervation and reduction of bone mass, the authors pharmacologically ablated sensory innervation chemically with capsaicin both in WT and neuron-specific semaphorin 3A-deficient mice and verified that only WT mice presented a decrease in bone mass, whereas the bone mass loss was not aggravated in mice deficient for semaphorin 3A, which had already reduced sensorial innervation. Overall, this study provides evidence that sensorial projections are critical regulators of bone mass, affecting the mechanisms underlying the differentiation of MSCs to osteoblasts (53, 57).

It is important to highlight that most studies, including the one previously described, lack a direct connection between the sensorial signaling and osteoblastogenesis, and do not integrate both systems in their experimental setup. Most studies rely either solely on the effect of the neurotransmitter, neglecting the fact that it might also be expressed by non-neuronal cells, or address the effect on the overall bone mass in the absence or impairment of sensory neurons, disregarding the fact that other cells present in the bone microenvironment might also be targeted by sensorial signaling and have a role in osteoblastogenesis. Examples include studies describing in vitro that mouse BM MSCs express receptors for both the sensorial neuropeptides CGRP and SP throughout osteoblastic differentiation, and treatment with these neuropeptides boosted MSC proliferation, up-regulated the expression of osteoblastic genes, and increased alkaline phosphatase activity and mineralization (11, 12). Also, SP knockout mice showed reduced bone formation rate (51), and capsaicin-induced depletion of SP and CGRP sensory neurons in adult rats is accompanied by bone loss and increased bone fragility, associated with a reduction in the bone formation rate (52). However, a recent study published by Silva et al. (58) has functionally connected sensory neurons and MSCs. In this study, researchers show that rat dorsal root ganglia (DRG) neurons enhance the differentiation of rat BM MSCs toward osteoblastic lineage in a coculture approach using compartmentalized microfluidic devices. By using this in vitro setting, researchers show that the presence of DRG neurons up-regulated the expression of osteoblast-related genes such as Runx2, Sp7, Col1a1, and Bglap, through the activation of the Wnt/β-catenin signaling pathway, when compared to a monoculture setting (58). Nevertheless, and even though this study mimics in minimalistic ways the interaction between the sensory nerves and osteoblasts (59, 60), the sensorial signals triggering the observed effect on osteoblastogenesis were not disclosed (Tables 2 and 3).

The clinical relevance of targeting the nervous system in pathologies from BM origin

The importance of the interplay between the adrenergic signaling and MSCs, in the context of HSPC mobilization, gains additional significance when applied to pathologic scenarios. The studies previously described show that MSCs are responsive to different neuronal signals and behave differently according to the type of neuronal input, which can be advantageous when developing therapies to target specific pathologies.

Pharmacological manipulation of adrenergic receptors, targeting a specific MSC function in BM, might have potential applications in tackling hematopoietic cell
transplantation–based challenges and antitumor immu-
nity (6, 61). As an example, in patients with myelo-
proliferative neoplasms (MPNs), reduced sympathetic innervation and MSC numbers in BM are often observed. MPNs often develop as a result of uncontrolled cell ex-
pansion due to mutations in hematopoietic cells (62). In
MPN mutant mice, researchers have shown that mutant
HSPCs trigger Schwann cell and sympathetic nerve
damage in BM due to an overexpression of the proin-
flammatory mediator IL-1. Also, they observed that
neuronal impairment is followed by an increase of MSC
apoptosis in BM, suggesting a role for sympathetic sig-
naling in MSC survival. Finally, in this study, researchers
also demonstrate that the reduced numbers of MSCs and
consequent decrease in the expression levels of CXCL12
results in an uncontrolled expansion of mutant HSPCs,
exacerbating MPN progression. Importantly, this vi-
cious cycle showed to be reversible through the manip-
ulation of the adrenergic circuit. Systemic treatment of
MPN mutant mice with a positive modulator of the β3-
adrenergic receptor activity blocked MPN progression,
associated with replenishment of Nestin+-MSC numbers
and increased CXCL12 expression levels in BM, in-
directly reducing the number of leukemic cells. Overall,
this suggests that BM neuronal damage compromises
MSC survival and function, therefore critically contrib-
uting to disease progression (63). Also, targeting the
communication between sensorial innervation and
MSCs might be an interesting strategy to improve bone
regeneration and address bone-related diseases such as
familial dysautonomia, a degenerative disorder charac-
terized by the loss of unmyelinated axons, including
sensory nerves, and whose patients often suffer from
osteoporosis and multiple fractures (64).

**BM-derived MSCs impact on neuronal activity**

MSCs isolated from BM have been reported as secreting
neurotrophic factors beneficial for the nervous system
microenvironment, arising as a promising therapeutic
tool with regenerative and neuroprotective potential (14, 65).
Studies have shown that MSCs cocultured with DRG
neurons allow a long-lasting survival and maturation of
neurons otherwise committed to die (66). Others have
demonstrated that MSCs can secrete a broad range of
regulatory factors, including brain-derived neurotrophic
factor, nerve growth factor (NGF), VEGF, and IGF-1, that
promote neurogenesis, neuronal and glial survival, and
neuroprotective actions under pathophysiological con-
texts (14, 67, 68). These factors released by MSCs have been
reported to promote neuronal survival and increased
number and length of neurites in axotomized retinal
ganglion cells cocultured with human BM MSCs in a
transwell system in which there is no direct cell-cell contact
(69). Also, recently, it was reported that MSC secretome
induce peripheral neuropathy (and glutamate (which
mimics the excitatory effect observed in multiple sclerosis).
Conversely, the observed protective effect of MSCs
was not observed in the cortical neuron cultures treated with
the different drugs. The different outcomes in neuronal
survival, when exposed to MSC factors, suggests that the
mechanisms provoking damage induced by the drugs,
and the protection induced by MSCs might be different in
central and peripheral neurons, recognizing MSCs as a
valuable tool to tackle peripheral neuropathic pathologies
(71). In accordance, in vivo treatment of a sciatic nerve
transection with MSCs collected from BM significantly
improved sciatic functionality, allied with an increase in
myelination indexes in regenerated fibers, and increased
number of neurons in DRG, which was correlated with
higher levels of NGF (72). In addition, MSC-secreted exo-
somes (i.e., vesicles containing neurotrophic factors to-
gether with a milieu of other proteins, mRNAs, and
microRNA), proved to be beneficial in regards of neuro-
protection and axonal outgrowth in a rat model of optic

**TABLE 2. Physiologic response of BM to the different neurotransmitters and neuropeptides**

| Neurotransmitter | Physiologic importance in BM | Reference |
|------------------|-----------------------------|-----------|
| Norepinephrine   | G-CSF–induced HSPC mobilization | 8         |
|                  | Circadian HSPC mobilization | 9, 32, 50 |
|                  | HSPC proliferation and differentiation | 9, 32, 34, 36, 37 |
|                  | HSPC aging                  | 26        |
|                  | MSC differentiation to osteoblasts | 10, 49   |
|                  | MSC migration to bone-forming sites | 49 |
|                  | EC survival                 | 27        |
| NPY              | HSPC survival and mobilization | 27, 38   |
|                  | MSC and EC survival         | 38        |
|                  | Vascular permeability       | 84        |
| Semaphorin 3A    | Bone mass regulator        | 53        |
| CGRP and SP      | MSC proliferation           | 11, 12    |
|                  | MSC differentiation to osteoblasts |          |

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| Beta adrenergic receptor | Treatment | Model | Physiologic response | Result | Reference |
|-------------------------|-----------|-------|----------------------|--------|-----------|
| **β2-ADR**              | Agonist   | NE-deficient mice | G-CSF–induced HSPC mobilization | Increased | 8         |
| Agonist                 | Stromal cell line | Adβ2<sup>−/−</sup> mice | CXCL12 expression | No effect | 9         |
| Agonist                 | Adβ2<sup>−/−</sup> mice | Light-induced HSPC mobilization | No effect | 9         |
| Antagonist              | Stressed mice | Adβ2<sup>−/−</sup> mice | Circadian fluctuation of CXCL12 | No effect | 9         |
| Antagonist              | Stressed mice | Adβ2<sup>−/−</sup> mice | Stressed-induced BM outcomes | No protection | 41        |
| Agonist                 | Old mice | Adβ2<sup>−/−</sup> mice | BM circadian TNF levels | Increased | 32        |
| Agonist                 | WT mice | Adβ2<sup>−/−</sup> mice | LSC infiltration in BM LSC | Increased | 46        |
| **β3-ADR**              | Agonist/antagonist | Stromal cell line | CXCL12 expression | Decreased | 9         |
| Agonist                 | WT mice | Adβ3<sup>−/−</sup> mice | Light-induced HSPC mobilization | Decreased | 9, 32     |
| Agonist                 | WT mice | Adβ3<sup>−/−</sup> mice | Light-induced vascular permeability | Decreased | 32        |
| Antagonist              | WT mice | Adβ3<sup>−/−</sup> mice | Light-induced HSPC mobilization | Decreased | 9         |
| Nonselective adrenergic agonist | Control but not Adβ3<sup>−/−</sup> mice | Adβ3<sup>−/−</sup> mice | HSPC mobilization | Increased | 9         |
| Antagonist              | Stressed mice | Adβ3<sup>−/−</sup> mice | Stressed-induced BM outcomes | Increased | 34        |
| Agonist                 | Old mice | Adβ3<sup>−/−</sup> mice | CXCL12 expression | Restored | 34        |
| Antagonist              | WT mice | Adβ3<sup>−/−</sup> mice | HSPC number in the BM | Decreased | 34        |
| Agonist                 | WT mice | Adβ3<sup>−/−</sup> mice | Circulating monocytes and neutrophils | Decreased | 34        |
| Antagonist              | WT mice | Nes-Gfp mice | osteoblastic genes | Down-regulated | 10        |
| Agonist                 | Nes-Gfp mice | Adβ3 siRNA MSC in vitro | HSPC maintenance genes | Decreased | 49        |
| Antagonist              | Leukemic mice | Adrb3 siRNA MSC in vitro | HSPC mobilization genes | Decreased | 49        |

*Adrb2, beta-2 adrenergic receptor gene; Adrb3, beta-3 adrenergic receptor gene; B2-ADR, beta-2 adrenergic receptor; Nes-GFP, nestin-GFP reporter; siRNA, small interfering RNA.*
nerve crush (68, 73). Finally, transplantation of BM-derived MSCs to the injury site at the spinal cord has been reported to promote axonal elongation, to reduce glial scar, and improve hindlimb locomotor function in rats (74, 75).

EPCS AND INNERVATION IN BM: UNDISCLOSED PARTNERS

The nervous system regulation of BM permeability and EC survival in BM

It is widely accepted that the vascular and the nervous system interplay, and their communication controls different biologic functions (76). This field of study has been accredited with additional importance from the observations that ECs in microvasculature receive signals from the nervous system and the evidence that endothelial and neuronal dysfunction often happen simultaneously in the development of pathologies as described in different cardiovascular pathologies (77, 78).

The spatial proximity between blood vessels and nerve fibers in BM indicates that they share or complement each other in maintaining BM homeostasis and functionality (79). BM-derived ECs have been reported to play a critical role in HSPC niche dynamics by monitoring their mobilization to and from the BM and also their viability and proliferation (80–83). The link between these functions attributed to ECs and the nervous system came in follow-up studies. In an exciting study, ECs have been credited with a gatekeeper function for HSPC trafficking from BM, and NPY and CD26 have been identified as central regulators of this mechanism (84). HSPC mobilization to circulation in response to G-CSFs has been associated with increased activity of CD26, an enzyme that is also able to truncate NPY (85). First, the researchers show in vitro that treatment of mouse BM-ECs with the truncated form of NPY restored the enhanced migration of HSPC induced by G-CSFs previously blocked by the pharmacological inhibition of CD26. Secondly, mice deficient for NPY and CD26 showed reduced HSPC mobilization rates in response to G-CSFs, and the in vivo administration of the truncated form of NPY both to NPY−/− and to CD26−/− mice restored the mobilization response to G-CSFs to levels similar to the ones observed in WT mice, reinforcing once again the role of the nervous system in the HSPC mobilization. Importantly, the observed effect on HSPC mobilization from the BM upon NPY administration was correlated with increased permeability at EC junctions. In an interesting approach, researchers measured the gap distances in EC junction and observed that the administration of the truncated form of NPY increased the gap distance to values only observed with G-CSFs alone. Furthermore, this increase in permeability was correlated with changes in the expression of vascular endothelial–cadherin and CD31 in EC gap junctions. These results suggest that NPY signaling regulates the gatekeeper function of ECs in BM by increasing vascular permeability, facilitating this way HSPC egress from BM (84).

NPY-knockout mice have been reported to have lower levels of CD31+ ECs and reduced sympathetic nerve fibers (38). To further understand the cause and effect relationship underlying this observation, researchers induced the destruction of sympathetic fibers in WT mice with 6OHDA. Sympathetic nerve destruction reduced the number of ECs in the BM, suggesting that the sympathetic signaling affected the viability of ECs, possibly due to a disturbance in the EC apoptosis process, which ultimately resulted in an impairment of HSPC survival and reduced HSPC numbers in BM (38). The role attributed to the nervous system in regulating EC survival in BM was strengthened in a study addressing BM dysfunction and neuropathy, outcomes usually observed in cancer therapies. Researchers showed that challenging both WT and 6OHDA-treated mice with 5-fluorouracil, a chemotherapeutic drug that ablates proliferating cells while inducing quiescent HSPCs to repopulate the BM in situ, resulted in reduced levels of both Nestin-GFP+ cells and CD31+ ECs in BM in 6OHDA-treated mice when compared to untreated controls (27). The same result was obtained when mice were treated with a β2- and β3-adrenergic receptor antagonist. Oppositely, treatment with 4-methylcatechol, a drug reported to induce endogenous NGF expression and to protect sympathetic nerve fibers, restored the basal numbers of Nestin-GFP+ and CD31+ ECs in mice exhibiting neuropathy induced by the anticancer drug cisplatin. These results suggest that the sympathetic nervous system protects Nestin-GFP+ cells and ECs from neurotoxicity induced by anticancer chemotherapies, promoting an efficient hematopoietic recovery (27). Overall, these reports strengthen the idea that the interplay between the nervous system and ECs in BM not only is maintained throughout adulthood but also represent an additional point of regulation of the HSPC niche activity, namely regarding mobilization and cell survival, in BM.

Neural regulation of EPCs

Mobilization of EPCs from BM is of relevance in pathologic situations that require new trends of vascularization, such as ischemia, thermal injury, and wound healing. In mouse hind limb ischemic models, researchers have shown that both EPC mobilization to circulation and overall levels in BM are increased upon systemic administration of NE. Importantly, this observation was ablated in the presence of both α- and β2-adrenergic receptor blockers. An increase of VEGF in BM was also reported, and an in vitro promotion of EPC proliferation and migration in the presence of NE were also reported, suggesting that the observed effect was in part due to a specific action of NE in BM (86, 87). Furthermore, the effect of NE on EPC mobilization seems to rely on the activity of eNOS, a known promoter of EPC mobilization from the BM (88). It has been shown in vitro that NE stimulation of EPCs increases not only the phosphorylation of eNOS but also eNOS mRNA levels in BM in mouse models of hindlimb ischemia. Importantly, blockade of eNOS signaling blunted the NE-induced mobilization of EPCs to
circulation, further supporting a role of the sympathetic nervous system in EPC mobilization (86, 87). Finally, patients with diabetes have been described to have fewer circulating EPCs and profound BM neuropathy, a phenotype reproduced in rat models of type 2 diabetes. In this rat model, it was shown that an accumulation of EPCs was trapped in BM, suggesting that the reduced sympathetic signaling in BM impairs standard EPC mobilization mechanisms (89).

Importantly, even though an accurate description of the mechanisms underlying the regulation by the nervous system in the activity of ECs in BM is still missing, some studies in humans in different pathologic situations have linked these 2 systems. Immunohistochemistry and histomorphometry analysis of the BM of patients with critical limb ischemia has shown a reduction in both microvascularization and sympathetic innervation (90). In patients with chronic obstructive pulmonary disease, EPCs from peripheral blood have impaired motility and decreased proliferative capacity when compared to control cells, together with decreased expression of both the β2-adrenergic receptor and CXCR4, suggesting an impairment in both the NE signaling and response to stromal cell–derived factor 1α in the onset of this pathology (91). Also, participants that underwent psychologic stress or β-adrenergic receptor agonist administration exhibited an increase in the circulating EPCs (92). Overall, these studies identify the sympathetic nervous system as a common factor in the regulation of EPC mobilization from BM to circulation under pathologic situations. Even though EPCs do not always fulfill the criteria used to define a progenitor cell because studies have suggested that their vascular repair ability is mostly due to a paracrine action on local ECs and not due to a potential to differentiate into new ECs (93), tackling EPC mobilization in BM might pose a promising therapeutic strategy, to be applied in vascular regeneration, in relieving the symptoms of ischemia, prevention of hypoxia-related tissue damage, and avoidance of vascular complications such as thrombosis, dissection, or capillary leakage.

CONCLUSIONS

This review collects the current knowledge on the interplay between the nervous system and BM cell populations, with a focus on how the nervous system modulates HSPC, MSC, and EPC activity in BM. We have gathered evidence that highlights the nervous system as a critical regulatory player in BM by modulating processes ranging from HSPC anchorage and egress, EC permeability, and bone remodeling. We have also highlighted the pathologies associated with disturbances in the neuronal signaling in BM and suggested that accurately targeting the nervous system in BM, either by modulating the activity of specific neuronal circuits or by pharmacologically targeting the activity of sympathetic and sensorial signaling-responsive cells at BM, is a promising therapeutic strategy to tackle pathologies from BM origin (Tables 2 and 3).

Nevertheless, the communication between the different cellular components of BM is highly complex, and a full description of its functional organization under the nervous system regulation is still missing. An accurate and complete picture of the neuronal fiber composition in the BM and information on how the different BM cellular populations affect innervation patterning, axonal outgrowth, and neuropeptide/neurotransmitter release, both under homeostatic and pathologic conditions, is valuable for researchers because it will allow them to infer if changes in the neuronal component of BM is the cause or a consequence of a pathologic situation. This information, combined with full comprehension of the temporal response profile of the different BM constituents upon a stressful event would also be advantageous for clinicians, to assess if targeting innervation in BM might pose as valuable target for a therapeutic approach to tackle or prevent a specific pathologic outcome by improving or delaying a specific BM response. An accurate and full descriptive image of all the cellular events taking place in BM, together with the technological improvements in drug development and delivery, will pave the way to the development of new, effective, and targeted clinical treatments.

ACKNOWLEDGMENTS

This article is a result of the project NORTE-01-0145-FEDER-000012, supported by Norte Portugal Regional Operational Programme (NORTE 2020), under the PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund (ERDF), and by Portuguese funds through FCT - Fundação para a Ciência e a Tecnologia/ Ministeiro da Ciência, Tecnologia e Inovação in the framework of the financed project PTDC/MED-PAT/30158/2017. L.L and F.C. are recipients of Ph.D. fellowships (SFRH/BD/109686/2015 and SFRH/BD/128771/2017, respectively). D.M.S. is the recipient of postdoc fellowship (SFRH/BPD/115341/2016). The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

L. Leitão wrote the manuscript; and all authors revised the manuscript and agreed with its accuracy and integrity.

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*Received for publication February 15, 2019. Accepted for publication April 2, 2019.*