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Review Article

Network anatomy and *in vivo* physiology of mesenchymal stem and stromal cells

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Over the last few years, the mesenchymal stromal compartment of the bone marrow has regained interest. The main reason for this resurgence is the recognition of their immunomodulatory properties and their prominent role in the maintenance and regulation of hematopoiesis. This has simultaneously prompted preclinical and clinical studies trying to take advantage of these properties and, at the same time, basic studies that have tried to dissect the bone marrow stromal compartment and the specific functions that different stromal cells have in the regulation of hematopoiesis and immunity. In this minireview we will summarize our contributions to the functions of mesenchymal stem cells in regulating hematopoietic stem cells. By putting them in a broader context, we will discuss the emerging role of mesenchymal stem cells as key integrators of neuro-endocrine signals, able to couple whole-organism demands to fine-tuned responses in remote stem cell niches.

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Hematopoietic stem cell traffic: ins and outs

Hematopoiesis is migratory throughout life. In adult mammals, hematopoietic stem cells (HSCs) continuously recirculate between the bone marrow and the peripheral circulation. Although the biological significance of circulating HSCs is still unclear, this process represents the basis for modern bone marrow transplantation. In the hematology slang, the term "mobilization" refers to the ability to enforce the egress of hematopoietic stem and progenitor cells (HSPCs) from the bone marrow into peripheral circulation. Current strategies to induce HSC mobilization exacerbate the natural migration of HSCs into circulation. Granulocyte colony-stimulating factor (G-CSF) can elicit robust HSPCs mobilization in 5-10 days. Due to its widespread use, HSC mobilization by G-CSF has served to obtain mechanistic insights into this phenomenon. G-CSF was identified as a factor with proliferative effects mainly on granulopoietic

precursor populations[1]. Prominent advances in molecular biology in the early 1980s triggered the purification and characterization of human G-CSF[2, 3]. In 1991, G-CSF received the approval for clinical use in the United Stated for cancer patients treated with chemotherapy. Setting a representative example of efficient translational research, the first clinical trials started relatively soon after the development of systems that allowed mass culture of recombinant human G-CSF[4-7]. Initially it was used to improve neutropenia, a major side effect of cancer chemotherapy. Since its use led to reduced infections and hospitalization of cancer patients, G-CSF was subsequently approved in many countries for the treatment of myelosuppression after bone marrow transplantation, acute leukemia, severe chronic neutropenia, myelodysplastic syndromes, aplastic anemia and HSPC mobilization[8]. Today millions of patients worldwide have been treated with G-CSF, which is indeed the predominant mobilizing agent currently used. G-CSF elicits HSC mobilization through a variety of mechanisms, including the proteolytic cleavage of adhesion molecules and chemokines, regulation of the CXCL12-CXCR4 chemokine pathway and the sympathetic nervous system (SNS, please see[9] for a review).

Circadian regulation of the HSC niche in the bone marrow

While studying the mechanisms mediating G-CSF-induced HSC mobilization in mice, we noticed an abnormally reduced mobilization in some experiments. Surprisingly, the reason turned out to be a dysfunction of the light system in the animal facilities. Mice that had been exposed to constant light exhibited a 50% reduction in G-CSF-induced HSC mobilization. This observation, together with the recent discovery that the molecular clock regulates bone formation[10], suggested that physiological HSC traffic might be also regulated by the molecular clock, and that these oscillations could represent an ideal setting to study the mechanisms regulating HSC traffic under steady state.

We found that, under standard cycles of 12 h of light and darkness, the number of circulating progenitors oscillates rhythmically, peaking 5 h after the onset of light and reaching a trough 12 h later, at Zeitgeber time 17 (Zeitgeber Time, or ZT, is used in the circadian slang to indicate the number of hours after the onset of light for animals that are kept under normal daily light cycles of 12 hours of light followed by 12 h of darkness). Not only progenitors, but also circulating LSKs (lineage$^-$ Scal$^+$ c-kit$^+$ cells) were 2-3-fold higher at ZT5 than at ZT13. To assess whether circadian rhythms also affected circulating HSCs, we performed long-term competitive repopulation assays using limiting dilutions of blood. These studies conclusively demonstrated that the number of circulating HSCs is not constant but oscillates rhythmically over 24 h. In (nocturnal) mice there are 2.5 more HSCs circulating at ZT5 than at ZT17[11], while in (diurnal) humans the pattern is inverted as compared to mice[12]. Circadian differences in circulating HSCs have been also noted in humans injected with G-CSF[12], suggesting that proper timing of HSC harvest and infusion could increase the transplantation success in patients where mobilization and/or engraftment deem to be insufficient.

In multiple tissues, circadian oscillations are maintained by a peripheral clock that is periodically reset by signals transmitted from the central pacemaker, the suprachiasmatic nucleus, localized in the anterior hypothalamus. The central clock can be synchronized with normal light/darkness cycles through the retinal-hypothalamic tract. This allows for tuned synchronization of all organs to day/night cycles[13]. In mice, circadian fluctuations of HSPC traffic were modulated by light input, since the pattern was arrythmic under constant light. In contrast, the rhythms persisted under constant darkness, consistent with their autonomous regulation by the molecular clock in the absence of light input. A single light disruption was sufficient to alter progenitor trafficking, as shown in mice experimentally subjected to a "jet lag" by shifting 12 h the light cycle. Because of the prominent role of the chemokine CXCL12 in regulating HSC migration, we studied whether CXCL12 was also subjected to circadian control. Indeed, CXCL12 protein and mRNA levels oscillated ryhtmically in the bone marrow in a pattern that closely mirrored circulating HSPCs under all experimental conditions. These results suggested that circadian HSC retention in the bone marrow was triggered by rhythmic expression of Cxcl12[11].

Neural regulation of HSC traffic

The master pacemaker regulates circadian oscillations of different peripheral organs, like the liver[14], via the sympathetic nervous system (SNS). In humans, the major postsynaptic neurotransmitters of the SNS also show circadian levels in plasma and urine[15-17]. In the bone marrow, norepinephrine displays a circadian rhythmicity, peaking at night,

coinciding with an increased number of bone marrow cells in the G2/M and S phases of the cell cycle[18]. Indeed, administration of norepinephrine stimulated bone marrow cell proliferation and rescued mice from chemotherapy[19]. Along this line, norepinephrine was later shown to stimulate the proliferation and migration of human HSCs[20]. Although murine plasma norepinephrine peaks at night, norepinephrine locally released by sympathetic fibres typically shows regional variability, with the sympathetic outflow to some organs being activated but to other regions unchanged or inhibited[21]. In the mouse bone marrow, sympathetic activity has not been directly measured, but has only been inferred from norepinephrine levels. However, plasma or tissue levels of noradrenaline are influenced by complex kinetics including its clearance, reuptake and degradation, and therefore its levels may not directly reflect sympathetic activity[21]. Previous studies have shown that exposure to light acutely induces sympathetic efferent activity and suppresses the parasympathetic tone in various organs, an effect mediated by the suprachiasmatic nucleus. Light exposure acutely induces sympathetic activity of the pancreatic, hepatic, splenic, adrenal and renal branches of the splanchnic nerve and suppressed parasympathetic efferent activity of pancreatic, hepatic and gastric branches of the vagus nerve in rats[22, 23]. In mice, the increase of the renal sympathetic nerve activity, arterial blood pressure and heart rate immediately after the onset of light was accompanied by a rapid suppression of the gastric vagal parasympathetic nerve activity[24]. Further, the induction of sympathetic activity by light in the splanchnic nerve directly stimulated peripheral clock gene expression in the adrenal cortex, leading to enhanced secretion of glucocorticoid hormones[25].

Although circadian oscillations in hematopoiesis and their association with the SNS had been noted before[18], the role of the SNS in regulating HSC trafficking and mobilization was only recently uncovered by serendipitous observations. Different groups investigating the role of selectins in HSPC mobilization reported that the selectin inhibitor fucoidan increases circulating HSPCs independently of selectins' function[26, 27]. The hypothesis was advanced that sulfated glycans expressed in the bone marrow (BM) microenvironment, like sulfatide, might regulate HSCs trafficking. Consequently, the group of Paul Frenette analyzed mice deficient in UDP-galactose:ceramide galactosyltransferase (Cgt), an enzyme required for the synthesis of sulfatide, that is highly expressed in oligodendrocytes and Schwann cells[28]. As a result, myelin production and the activity of myelinated fibres are reduced in *Cgt*$^{-/-}$ mice. G-CSF-induced HSC mobilization was severely compromised in *Cgt*$^{-/-}$ mice. Although these mice exhibited a defect in stromal cells that support lymphopoiesis[29], the defect in mobilization was not found to be caused by the lymphopenia or by the absence of sulfatide, but instead proposed to be the result of impaired sympathetic regulation of osteoblasts[30]. More recently, we found that G-CSF-induced HSC mobilization requires the participation of both β_2- and β_3-adrenergic receptors[31]. However, osteoblasts do not express the β_3-adrenergic receptor, which we later found to regulate physiological HSC release to circulation[11].

Since the SNS relays circadian signals to peripheral organs and participates in G-CSF-induced HSC mobilization, we studied the role of the SNS in circadian HSC traffic using pharmacological and surgical approaches. Chemical sympathectomy caused by administration of 6-hydroxydopamine abolished the normal rhythm of circulating progenitors. To assess whether local norepinephrine release in the bone marrow was involved in this process, we surgically denervated the tibiae of the mice, which resulted in abrogation of CXCL12 oscillations locally in the denervated limb. Altogether, these results indicated that norepinephrine release from sympathetic varicosities in the bone marrow regulated CXCL12 production and HSC attraction. The rapid CXCL12 downregulation in the bone marrow following light onset could be due to increased local norepinephrine release in the bone marrow microenvironment following light exposure, as it has been shown in other settings[22, 23-25]. To investigate the downstream mechanisms, we treated the BM stromal cell line MS-5 with adrenergic agonists and antagonists. Norepinephrine and isoproterenol reduced CXCL12 production in a dose-dependent manner, indicating that this effect was mediated by β-adrenergic receptors. Unexpectedly, it was mediated by the β_3- (low and restricted expression) rather than the β_2-receptor (ubiquitous expression) since it was induced by a β_3 agonist and prevented by a β_3 antagonist, whereas β_2 agonist and antagonist did not significantly affect CXCL12.

Moreover, timed administration of isoproterenol downregulated bone marrow *Cxcl12* but did not increase by itself circulating HSPCs. This suggested that systemic adrenergic stimulation might enhance not only mobilization, but also homing to the bone marrow and/or peripheral tis-

sues. This hypothesis has been explored and confirmed in a recent study that has shown the participation of the SNS in circadian homing of leukocytes and HSPCs to the bone marrow, which occurs preferentially during the night phase in mice[32]. To be able to distinguish the effects on mobilization and homing, we used animals with compromised homing. In mice deficient in E&P selectins in which α_4 integrins were also pharmacologically blocked, isoproterenol administration during the morning phase induced a significant mobilization of murine HSPCs. Moreover, the administration of a β_3 antagonist 1 h before the onset of light prevented the physiological rise of circulating progenitors 2 h later[11].

Circulating progenitors did not oscillate in mice deficient in the clock gene *Bmal-1*, suggesting that clock genes regulate stem cell traffic. We profiled clock genes expression in the BM and observed a trend consistent with the patterns reported in peripheral oscillators under normal cycles, and alterations under continuous light or jet lag, in red, but these patterns were not significant. However, isoproterenol downregulated *Cxcl12* equally in stromal cells derived from control mice and mice deficient in the clock genes *Bmal-1*, *Per1* and *Per2*, suggesting that the molecular clock regulates stem cell traffic principally at the central nervous system level. In summary, we proposed a model where photic cues are transmitted through the retinal-hypothalamic tract to the central pacemaker in the brain, the suprachiasmatic nucleus. The signals are transduced to the bone marrow through the SNS, that releases norepinephrine rhythmically in the bone marrow microenvironment. Norepinephrine binding to the β_3-adrenergic receptor expressed in stromal cells would trigger Cxcl12 downregulation in a circadian manner, leading to rhythmic HSC release to the bloodstream[11].

MSCs regulate HSC traffic

We next tried to characterize this stromal cell targeted by the SNS in the bone marrow and regulating HSC traffic. Predicted requirements for this candidate cell, based on our previous studies, were a peri-vascular location, given that most sympathetic fibres are associated with blood vessels, and enriched expression of Cxcl12 and the β_3-AR. However, these features were not shared by the osteoblast, the cell proposed by the Frenette group[30] and previously shown by other investigators[33-35] to critically regulate HSCs. Based on our previous studies[11], we hypothesized that the stromal cells regulating HSC traffic might be mesenchymal stem cells (MSCs), which were known to express the intermediate filament protein nestin[36]. We noted that GFP expression driven by the regulatory elements of *Nestin*, an intermediate filament protein, was largely restricted to non-hematopoietic ($CD45^-$) cells different from vascular endothelial cells because they did not express CD31 but exhibited a sub-endothelial distribution and were enriched in endogenous nestin. In the bone marrow, *Nestin*-GFP^+ cells represented only 0.08 % of total nucleated cells and 4 % of the stromal $CD45^-$ population. Catecholaminergic nerve fibers were closely associated with sub-endothelial $nestin^+$ cells in the endosteal region and the bone marrow parenchyma (Fig.1). Cell sorting of $CD45^-$ $nestin^{+/-}$ cells showed that $nestin^+$ cells contain enriched transcripts of the β_3-adrenergic receptor and a 50-fold higher expression of Cxcl12, compared to the remaining stromal population. This suggested that $nestin^+$ cells might be candidate cells innervated by the SNS and regulating HSC traffic.

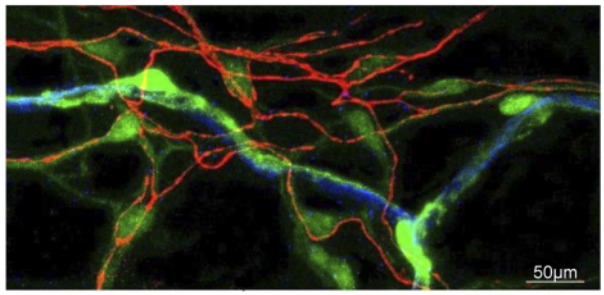


Fig.1 Sub-endothelial nestin+ mesenchymal stem cells are innervated by sympathetic fibers in the bone marrow
Projection stack (~100 μm) of fluorescent images showing the distribution of Nestin-GFP^+ cells (green), $CD31/PECAM^+$ vascular endothelial cells (blue) and tyrosine hydroxylase+ sympathetic nerve fibers (red) after whole mount staining of the skull bone marrow.

Despite the rarity of both *Nestin*-GFP^+ cells and HSCs in the bone marrow (identified as $CD150^+CD48^-Lin^-$ cells) 90% of HSCs were localized within 50 micron from *Nestin*-GFP^+ cells, and 61% of them were directly adjacent to *Nestin*-GFP^+ cells localized in the endosteal or sinusoidal regions. The co-localization of these two rare cells was highly significant[37].

Based on our previous studies[11], we hypothesized that bone marrow $nestin^+$ cells might be bona fide MSCs. Indeed, $nestin^+$ cells contained all the CFU-F or mesenchy-

mal capacity of the bone marrow and robustly differentiated *in vitro* into osteoblasts, adipocytes and chondrocytes. In constrast, nestin$^-$ cells did not generate any differentiated progeny. We devised novel culture conditions to better preserve MSC self-renewal, a stem cell property that has rarely been proven for MSCs[38] due to the lack of proper assays[39]. Unlike other bone marrow stromal cells, nestin$^+$ cells formed clonal multipotent and self-renewing spheres. Over time, these spheres spontaneously tended to differentiate into mesenchymal lineages. When implanted into heterotopic bone ossicles, clonal GFP$^+$ spheres (but not CD45$^-$ GFP$^-$ cells) were able to generate Col2.3$^+$ osteoblasts and transfer the hematopoietic activity to the ossicles. After two months, each primary sphere generated 300 secondary spheres that were subsequently implanted into secondary recipients (1 sphere/mouse). Secondary ossicles were harvested after 8 months. Numerous Col2.3$^+$ donor-derived osteoblasts were observed in the ossicles that also contained hematopoietic (CD45$^+$) cells in contact with *Nestin*-GFP$^+$ cells. More than 8,000 GFP$^+$ spheres were recovered from each secondary ossicle, proving robust *in vivo* self-renewal. These GFP$^+$ spheres were able to generate Col2.3$^+$ osteoblasts, a further proof of their differentiation potential and donor origin[37]. This result represents one of the first to show *in vivo* MSC self-renewal in serial transplantations.

Despite their osteoblastic differentiation potential, *Nestin*-GFP$^+$ cells were distinct from Col2.3$^+$ osteoblasts and N-cadherin$^+$ or osterix$^+$ preosteoblasts. Interestingly, *Nestin*-GFP$^+$ cells were much more quiescent than the remaining bone marrow stromal cells, including the recently reported Mx-Cre$^+$ mesenchymal progenitors[40], but were selectively induced to proliferate after parathormone or 6OHDA treatment, which destroys sympathetic nerve fibres innervating *Nestin*-GFP$^+$ cells. By contrast, G-CSF inhibited proliferation of different stromal populations. The inhibitory effect of G-CSF and the SNS did not only concern cell cycle but also differentiation, because both G-CSF and β_3-adrenergic agonists downregulated genes associated with osteoblastic differentiation selectively in *Nestin*-GFP$^+$ cells. By contrast, parathormone had a stimulatory effect that was direct, because *Nestin*-GFP$^+$ cells have parathormone receptor and sorted cells responded to parathormone by proliferating and differentiating into osteoblasts; moreover, daily injection with parathormone over 5 weeks, a treatment previously shown to expand both the osteoblastic and HSC pools[33, 41], doubled the number of nestin+ cells and favored their osteoblastic differentiation[37].

To gain more insight into the regulation of the HSC niche by G-CSF and the SNS, we analyzed the expression of genes that regulate HSC maintenance and attraction in the BM. The expression of CXCL12, Vcam1, kit ligand, angiopoietin1, osteopontin and IL7 was from 50 to 650-fold higher in nestin$^+$ cells than in the remaining CD45$^-$ population. Moreover, G-CSF treatment significantly downregulated all these genes -except osteopontin- selectively in nestin$^+$ cells, but not in the rest of the CD45$^-$ population[37]. We have recently shown that G-CSF increases norepinephrine bioavailability[42], providing a possible mechanism by which the SNS participates in G-CSF-induced HSC mobilization. The significant co-localization of HSCs and nestin$^+$ cells, together with their high expression of HSC-chemotactic genes in a manner regulated by G-CSF and adrenergic input suggested a role for nestin+ cells in regulating HSC function.

To directly address the role of nestin$^+$ cells in HSC maintenance we crossed a mouse line expressing the diphtheria toxin receptor after a floxed stop codon[43] (iDTR) with *Nestin-Cre^{ER}* mice[44] to selectively deplete nestin$^+$ cells after tamoxifen and diphtheria toxin treatment. Depletion of nestin$^+$ cells did not affect bone marrow or spleen cellularity or content in Lin$^-$ CD48$^-$ progenitors. However, Lin$^-$ Sca-1$^+$ c-kit$^+$ CD48$^-$ cells and CD150$^+$ CD48$^-$ Lin$^-$ Sca-1$^+$ c-kit$^+$ cells, enriched in HSCs, progressively decreased in the bone marrow[37].

We tested whether nestin$^+$ cells were important for the homing of HSCs to the bone marrow in *Nestin-Cre/iDTR*, *Nestin-Cre^{ER}/iDTR* and control *iDTR* mice. Depletion of nestin$^+$ cells using both models reduced bone marrow homing of hematopoietic progenitors between 70 and 90%. To test whether *Nestin*-GFP$^+$ cells directed bone marrow homing of HSCs, 5-11 thousand CD150$^+$ CD48$^-$ LSK cells were sorted from bone marrow of congenic mice, stained with a bright dye and injected in lethally-irradiated *Nestin*-GFP mice. Combined Two-Photon and confocal microscopy of the calvarial bone marrow was performed 2 h later, and the shortest distance between homed HSCs, *Nestin*-GFP$^+$ cells and the bone surface was measured. These experiments showed that HSCs rapidly home near nestin$^+$ cells in the BM[37].

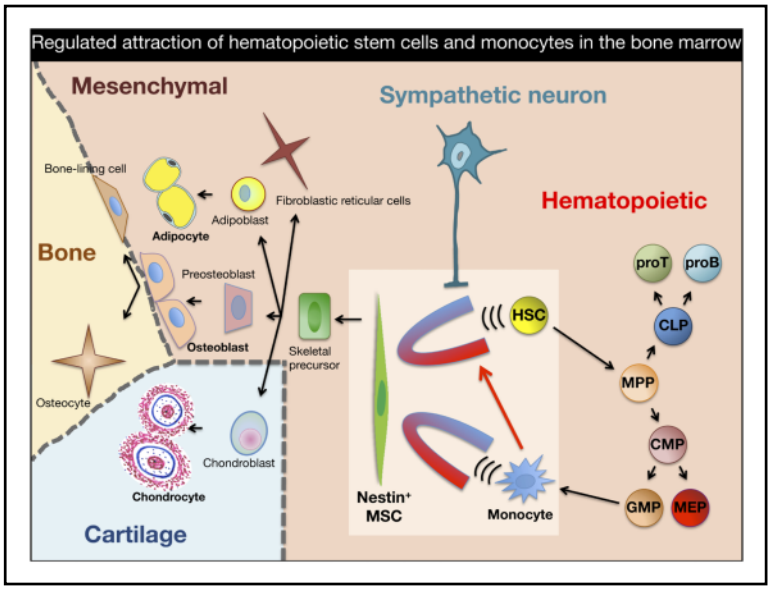
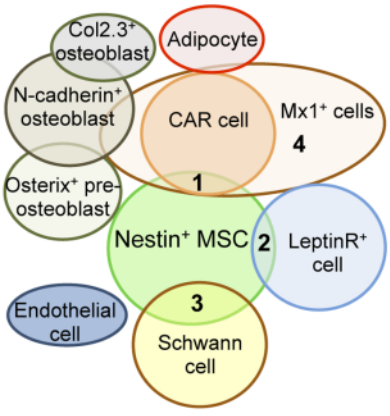


Fig.2 Model of the regulation of HSC and monocyte traffic in the bone marrow

Nestin+ MSCs, which can generate mesenchymal lineages in the bone marrow, regulate the egress of monocytes in response to Toll-like receptor ligands and also the traffic of hematopoietic stem cells (HSC). Both the production of CXCL12 by nestin+ MSCs and these cells' attraction to HSCs are inhibited by sympathetic nerve fibers and stimulated by soluble factors produced by monocytes. CLP, common lymphoid progenitor; CMP, common myeloid progenitor; GMP, granulocyte-macrophage progenitor; MEP, megakaryocyte-erythroid progenitor; MPP, multipotential progenitor.



	Similarities	Differences	Ref
1	• High CXCL12, KITL, VCAM-1	• Size/shape • Higher frequency of CAR cells • CAR cells not only perivascular • CAR cells committed precursors	37 40 49 50
2	• Pericytes • Markers (CXCL12, ALP, VCAM-1, PDGFRα/β) • High KITL, LeptinR mRNA	• High LeptinR expression in LeptinR+ cells • LeptinR+ cell do not express Nestin • Unclear identity of LeptinR+ cells	37 56
3	• Associated to nerve fibers • Expression of Nestin	• Schwann cells are not pericytes • PDGFRα expressed in nestin+ MSCs but not in Schwann cells • Active TGF-β in Schwann cells	37 57
4	• Mesenchymal fate • Contribution to bone turnover (different degree)	• Mx1+ cells are osteoblastic, nestin+ cells are osteochondral • Mx1+ cells more proliferative/ nestin+ cells more quiescent	37 40

Fig.3 Relationships of nestin+ MSCs to other candidate HSC niche stromal cells

Diagram depicting the possible overlap between nestin+ MSCs, CXCL12-abundant reticular (CAR) cells, leptin receptor+ mesenchymal cells, Mx-Cre+ mesenchymal progenitors, osterix+ and N-cadherin+ preosteoblasts, Col2.3+ osteoblasts, adipocytes and non-myelinating Schwann cells. Similarities and differences between nestin+ MSCs and each of those cell populations with a possible overlap.

HSC attraction to nestin+ MSCs is regulated by monocytes

HSC attraction to nestin+ MSCs is also affected by other cells of the bone marrow microenvironment (Fig.2). Others[45, 46] and us[47] have shown that a subset of monocytes promotes the retention of HSCs in the bone marrow. This is at least partially mediated by unknown factors secreted by macrophages that are able to induce CXCL12 expression by MSCs[47]. On the other hand, MSCs regulate not only HSC traffic but also the egress of inflammatory monocytes from the bone marrow. How peripheral infections or inflammation promote monocyte egress from the bone marrow was not clear before. MSCs respond to pro-inflammatory cytokines by producing the chemokine CCL2/MCP1, which directs the egress of these monocytes from the bone marrow compartment toward the peripheral circulation[48]. How different subsets of monocytes crosstalk to MSCs and HSCs and its implications remain to be further investigated.

Relationships between nestin+ MSCs and other candidate HSC niche stromal cells

Other bone marrow stromal cells that have been shown

to regulate hematopoietic stem cell function include CXCL12-abundant reticular (CAR) cells[49, 50], Mx1$^+$ mesenchymal progenitors[40], N-cadherin$^+$ preosteoblasts and osteoblasts[33-35], preosteoblasts identified by the expression of osterix transcription factor[51], adipocytes[52], endothelial cells[53-56], perivascular cells identified by expression of leptin receptor[56] and non-myelinating Schwann cells[57]. Interestingly, some of these cells may represent partially overlapping populations (Fig.3). For instance, nestin$^+$ MSCs, leptinR$^+$ cells and CAR cells express high levels of CXCL12, KITL and VCAM-1. Both nestin$^+$ MSCs and leptinR$^+$ cells are enriched, within the stromal compartment, in the expression of MSC markers, such as alkaline phosphatase, PDGFRα and PDGFRβ. Moreover, a fraction of CAR cells covers the vascular endothelium, like nestin$^+$ MSCs and LeptinR$^+$ cells. However CAR cells are not restricted to a subendothelial location, but rather show a wide distribution over the bone marrow parenchyma reminiscent of fibroblastic reticular cells. Nestin$^+$ MSCs and leptinR$^+$ cells are very rare in the bone marrow, whereas CAR cells are more abundant.

While CAR cells contain mesenchymal precursors (some of them reportedly committed to adipo-osteogenic cell fates), they have not been reported to form CFU-Fs[50]. In contrast, nestin$^+$ MSCs contain all the CFU-F capacity of the bone marrow when separated from the remaining stromal cells, while their progeny robustly differentiates *in vitro* into osteoblasts, adipocytes, and chondrocytes. Moreover, in spite of the striking similarities between nestin$^+$ MSCs and leptinR$^+$ cells, leptinR$^+$ cells have been reported to be nestin-negative, although their identity and differentiation potential remain unclear. However, deletion of KITL in the leptinR$^+$ population markedly reduced bone marrow HSCs in a manner that was not seen when a similar gene deletion was performed using *Nestin-Cre* or *Nestin-Cre^{ER}* mice, although the recombination efficiency in the different strains was not reported. Of note, LepR mRNA expression was remarkably high in the microarray from *Nestin*-GFP$^+$ cells[37].

At least a subset of CAR cells comprises committed preosteoblasts, as shown by their expression of osterix[50]. Moreover, a different subset of CAR cells comprises committed adipoblasts, as demonstrated by their enriched expression in PPARγ. In contrast to nestin$^+$ MSCs, adipocytes are predominantly negative regulators of HSCs[52].

Osterix$^+$ preosteoblasts and N-cadherin$^+$ osteoblasts (enriched in osteoprogenitors) comprise defined cell populations distributed on the endosteum. More primitive mesenchymal cells with osteolineage potential have been traced using ubiquitous Mx1 as a genetic driver[40]. Mx1$^+$ bone marrow stromal cells appear to be more replicative than the more quiescent nestin$^+$ MSC compartment, and seem to play a major role in bone regeneration and dynamic homeostatic replacement of committed osteoblasts.

Sympathetic nerve fibers in the bone marrow are wrapped by nonmyelinating Schwann cells, glial cells with great potential for nerve regeneration and secretion of neurotrophic factors. Interestingly, recent evidence supports a novel functional role of Schwann cells in the bone marrow niche as major players in the activation of TGF-β, growth factor that induces HSC quiescence. TGF-β, as an inactive complex, is widely expressed in the bone marrow by cellular components that include osteoblasts and HSCs. Nonmyelinating Schwann cells of the bone marrow specifically express integrin β_8 that activates the complex, and colocalize with active TGF-β. Nestin$^+$ MSCs are located in perivascular regions, but appear to be directly innervated by sympathetic nervous fibers. Both cell types express nestin; however, the majority of nestin$^+$ cells expressed PDGFRα and were distinct from Schwann cells, suggesting only a partial overlap among these cell populations[37, 57].

Summary

Mesenchymal stromal and stem cells are currently a subject of intense investigation due to their emerging key roles in the regulation of hematopoiesis and immunity and their promising potential in regenerative medicine. Different mesenchymal populations have been proposed to have essential functions in the hematopoietic stem cell niche. Some of these populations might exhibit a significant degree of overlap based on multiple shared features. MSCs are proposed as key integrators of cellular and molecular cues necessary to maintain HSCs and capable of sensing systemic signals and coupling whole-organism demands to specific stem cell responses.

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Conflicts of interest

None

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