The Regulation of Normal and Leukemic Hematopoietic Stem Cells by Niches

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Abstract The origin and propagation of normal and leukemic hematopoietic cells critically depend on their interplays with the hematopoietic microenvironment (or so-called niche), which represent important biological models for understanding organogenesis and tumorigenesis. Nevertheless, the anatomic and functional characterizations of the niche cells for normal hematopoietic stem cells (HSCs) have proved a formidable task. It is uncertain whether the combinational effects of a few sets of molecular niche elements, behind the long-sought cellular architectures with preferred anatomic locations, actually meets the functional definition of HSC niche. Moreover, even much less is known about the niche components for numerous types of leukemia-stem cells (LSCs) that originate via discrete cellular and molecular transforming mechanisms. However, one interesting scenario is emerging, i.e., the leukemia cells can positively remodel the hematopoietic microenvironment favorable for their competition over the normal hematopoiesis that co-exists within the same ecosystem. This property probably represents a previously unappreciated essential trait of a functional LSC. Obviously, the further exploration into how the hematopoietic microenvironment interplay with normal or malignant hematopoiesis will shed light onto the designing of novel types of niche-targeting therapies for leukemia.

Keywords Niche · HSCs · LSCs · Interplay

Introduction

The non-random transition pattern of major hematopoietic sites along with ontogenic development indicates that certain specific hematopoietic microenvironments, supplied by a few particular tissues or organs, must exist to induce and also confine the colonization and proliferation of normal hematopoietic stem cells (HSCs) and progenitors. It is believed that a collaborative development of hematopoietic microenvironment and hematopoietic cells themselves underlies the formation of nascent hematopoietic tissues or organs, such as bone marrow (BM) and spleen.

Schofield proposed the term—hematopoietic niche more than 30 years ago, assuming the existence of putative hematopoietic stromal cells that anatomically associate with HSCs and thus instruct HSCs to undergo self-renewal expansion [1]. Indeed, the self-renewal expansion or/and long-term maintenance of apex HSCs, out of a variety of distinctive developmental processes of hematopoiesis, seems critically dependent on the supporting activity from a niche structure that is not easy to simulate in vitro. A single freshly isolated HSC from BM, once inoculated into a proper recipient, has demonstrated a robust capability of re-initiating and subsequently sustaining a hematopoietic hierarchy encompassing whole hematopoietic lineages throughout life, while in striking contrast, even a lot of HSCs, once put in the ordinary in vitro culture, will inevitably bias to differentiate, usually toward myeloid directions, ending up with only a transient hematopoietic activity. Nevertheless, despite the highly invested research activities over last decade, the accurate cellular identities and molecular profile of the HSC niche still remain elusive.

Although even much less is known about the putative niches for leukemic hematopoiesis, they, in most instances, definitely play an essential role in the sustained survival and proliferation of the malignant hematopoietic cells. Analogue

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to the case of HSCs, in most instances, no leukemia cells, once removed from patients' body and cultivated in vitro, can proliferate and/or survive more than 3 weeks. Only occasionally a subset of rare leukemia cells (for example the original LSCs or the non-LICs that have experienced substantial reprogramming) contained within the freshly isolated hematopoietic samples from the most malignant cases, do have obtained the unusual ability to lead an almost complete autonomous growth in a niche-absent in vitro culture conditions [2].

A Variety of Niche Cells are Found for HSCs

It is becoming obvious that even for the apex HSC subset alone, numerous types of niche cells may exist. Moreover, frequently the observations and claims from the different reports are conflicting. The total candidate niche cell types for HSCs or/and progenitors residing in BM may add up to >10 (Table 1). Except for the BM stromal cells that have been documented in many studies, it is worth of mentioning that many HSC progenies, such as macrophage, osteoclasts and T cells, have also been shown to play an active niche-like role, indirectly or even directly, especially in the conditions of inflammation [3–6]. For the sake of maintaining homeostasis of ongoing hematopoiesis, it is understandable that the proliferation and differentiation of HSCs and progenitors towards a specific direction should be regulated via a feedback mechanism, directly or indirectly, to monitor the accumulation of discrete progenies [7]. Adding to this complexity, numerous insoluble or soluble molecular factors such as the extracellular matrix fibers, nerve transmitters, hormones, nutrients, and even metabolites, besides these local

live regulators, not surprisingly, will also exert crucial influence on HSC and progenitor behaviors.

As the HSC niche basically bears an anatomic implication, the putative HSC niches have been arbitrarily classified by their locations. Up to now, at least two major kinds of niches—endosteal osteoblastic niches and peri-vascular niches have been described within the adult BM.

A hematopoietic niche role of endosteal osteogenic cells other than synthesizing bone matrix was postulated by reasoning why a supposedly non-random migration of the definitive hematopoietic site finally settles within the endosteal osteoblasts-lined bone marrow cavity [8, 9]. And this notion was also supported by the early observation that the hematopoietic progenitors were most enriched within the subendosteal region [10], and by the in vitro co-culture experiments showing that osteoblasts are able to secrete hematopoietic cytokines to facilitate the proliferation and survival of hematopoietic progenitors [11]. Later, the in vivo experimental evidence supporting osteoblasts as a putative HSC niche element was provided by Zhang, et al. and Calvi, et al. works that were based on the analyses of two genetically modified mouse models [12–14].

Although the subsequent attempts to further characterize the niche-like role of endosteal osteogenic cells (including not only the mature osteoblasts but also a portion of osteoblastic progenitors), especially on HSCs, have made the conflicting observations (see next paragraph), there is indeed experimental evidence supporting the existence of an endosteal niche. The prominent ones include: 1) it is repeatedly shown by different groups that at least a portion of

Table 1 The key niche-related molecules that regulate HSCs or/and LSCs

Molecule	Relevant stromal cells	Regulation on HSCs	Regulation on non-HSC hematopoietic cells	Regulation on acute leukemia cells	References
N-cadherin	Endosteal osteoblasts	Homophilic tethering	?	Homophilic tethering	[13, 14, 18, 36, 42, 43, 70–72]
Fibronectin		Integrin α v β 3	CD11b/NK cells	Vla-4/AML	[55, 57, 73, 74]
Jagged-1/2, DLL1	MSCs, osteoblasts, endothelium	Notch1,2	Notch/T cells, Megakaryocyte	Notch/B-ALL	[12, 29, 61, 75–78]
SCF	Endothelium, peri-vascular stromal cells, osteoblast, leukemia cells	c-Kit	c-Kit/mast cells, B cells, T cells	c-Kit/AML	[15, 23, 66, 79–82]
SDF-1 α	CAR cells, osteogenic progenitors, osteoblasts	CXCR4	CXCR4/leukocytes	CXCR4/ALs	[3, 4, 22, 33, 48–54, 60, 65, 83]
Angiopoietin-1	Endothelial cells, mesenchymal progenitors, hematopoietic cells	Tie2		Tie2/AML	[16, 84, 85]
TPO		MPL	MPL/thrombopoiesis	MPL/AML	[74, 83, 86–88]
Wnt/DKK1	Osteoblast, mesenchymal progenitors	Fz/LRP5/6 co-receptor	Fz/LRP5/6 co-receptor/Myeloid progenitors, T cells	Fz/LRP5/6 co-receptor/AML	[47, 71, 77, 89–93]
VEGFA	HSC/leukemia cells	Flk1		Flk1/AML?	[41, 94]
Hedgehog	?	Ptch-Smo	Ptch-Smo/T cell, B cell, erythroid progenitors	Ptch-Smo/ALL, BCR-ABL myeloid leukemias	[95–101]
TGF β	GFAP ⁺ Nonmyelinating Schwann cells	TGFBR	TGFBR/T cells, DCs		[31, 102–106]
CD44-ligands				CD44/AML	[56, 58, 59]
Prostaglandin E ₂ (PGE ₂)	Osteoblasts, osteoclasts, endothelial cells	COX-1 and COX-2	WBC and platelets		[107], [108–110]

putative HSCs, especially those being intravenously transplanted, reside close to the endosteal region [6, 13, 15, 16]; 2) endosteal osteogenic cells express or secrete many HSC-regulating factors such as SDF-1, c-Kit and angiopoietin-1 (ang-1) (see Table 1); 3) the osteogenic progenitor-specific (by osterix promoter-coupled Cre) *Dicer*- or *Sbds*-targeting manipulation that impaired osteoblastic differentiation coincidentally perturbed the proliferation, survival and differentiation of HSCs and the hematopoietic progenitors [17]. Moreover, there are also reports indicating the existence of the endosteal niches that co-localize with the sinusoidal endothelial cells that constitute a major component of the perivascular niche (see following paragraphs) [18].

On the other hand, in the biglycan-deficient or osterix promoter-guided *Rac*-knockout mice wherein reductions in osteoprogenitor and osteoblast numbers were documented, no quantitatively correlated alterations to HSCs and their progenies were detected [14, 19]. So it seems still an open question as to whether endosteal osteogenic cells play an essential niche role in regulating HSC behaviors. If they do, probably it is osteogenic progenitors rather than the mature osteoblasts that exert meaningful influence on a small portion of HSCs [17]. It is difficult to reconcile these contradictory observations. Nevertheless, perhaps only a particular property of osteogenic cells rather than the whole osteogenic cells, or only a portion of redundant osteogenic cells or relevant properties, is vital for providing a sufficient HSC niche function, while these subtle attributes are differentially altered or can be compensated in the different gene-targeting models.

Later, it was realized that the primitive self-renewing osteogenic progenitors that seed the formation of bone and BM might also account for why the final location of major hematopoietic site goes to the bone [20]. In line with this, an early observation showed that co-transplantation of MSCs facilitated the hematopoietic recovery by infused HSCs [21]. The general picture is that the mesenchymal stem cells (MSCs) or other MSCs-like cells, as a kind of peri-vascular cells reside in close neighborhood with the sinusoidal endothelial cells, constitute an essential element of so-called perivascular (sinusoid) niche. The location of the perivascular niche is not necessarily close to the endosteal region. Several reports indicate that probably more HSCs locate close to this perivascular niche than to the endosteal osteoblastic niche mentioned above [15, 20, 22–25].

Several types of mesenchymal stromal cells with overlapping phenotypic or functional attributes have been reported to participate in the formation of the peri-vascular niche. The one first described was human CD146⁺ adventitial reticular cells (ARCs) that reside as a kind of mural cell expressing Ang-1 [20]. Interestingly, ARCs belongs to a subset of self-renewing osteogenic progenitor, seeding the formation of BM-containing heterotopic ossicle in the immunocompromised

mice by sequentially recruiting host-derived vasculature and hematopoiesis, wherein ARCs account for 3 % of human derived cells. Theoretically, ARCs are able to directly signal to not only HSCs but also endothelial cells that ARCs surround with their bodies, which in turn relays signals to HSCs. Similar to ARCs, a portion of mouse CXCL-12/SDF-1 abundant reticular (CAR) cells that account for 0.27 % of BM nucleated cells, as determined by flow cytometry assay, were also reported of attaching to the sinusoid with their body or long processes [22, 23]. CAR cells were envisioned to provide a basement facilitating HSC division and accumulation. A marked decrease of total HSC pool (to about 30 %) or the competitive repopulating units (to about 45 %) was observed after CAR cell depletion. Interestingly, like ARCs, they also harbor the differentiation potentials of maturing into the endosteal osteoblast or adipocytes. Moreover, the detectable expressions of SCF, PDGFR α/β , Runx2, PPAR γ , and osterix together indicate an identity of mesenchymal progenitor. The third one is the sympathetic fiber-innervated Nestin⁺ MSCs that account for about 0.08 % of total murine BM nucleated cells. Apparently being distinct from osterix-expressing pre-osteoblasts and N-cadherin-expressing osteoblasts, they also show a preferred peri-vascular distribution [24]. As its name suggests, like ARCs and CARs, they contribute to the physiological bone remodeling in vivo by differentiating into the mature chondrocyte, and osteoblasts. Interestingly, Nestin⁺ MSCs express multiple hematopoietic regulatory factors such as CXCL12/SDF-1, SCF, IL-7, Ang-1 and Vcam-1, and contribute to HSC pool size maintenance seemingly by preventing HSCs and other primitive progenitors from egressing into the extramedullary sites such as spleen. The depletion of Nestin⁺ MSCs results in about 50 % loss of HSC pool size. Moreover, the niche role of Nestin⁺ MSCs is subject to G-CSF or β -3AR stimulation. The fourth MSC-like cells were a distinctive subset of SCF-expressing LepR⁺ perivascular stromal cells (although SCF-expressing cells also include a portion of endothelium and osteoblasts) that account for about 0.027 % of total mouse BM cells, as measured by flow cytometry [15]. The LepR promoter-Cre induced *SCF* deletion results in a significant reduction in HSC pool (up to 80 %). Interestingly, like three other types of perivascular cells mentioned above, these cells also express numerous MSC-associated markers such as PDGFR α/β , CXCL12, ALP, Vcam-1. Interestingly they do not express Nestin.

As all these HSC niche-related MSC-like cells are distributed as perivascular cells, probably the vascular endothelium represents the most basic component of the so-called perivascular HSC niche [25]. Actually, ontogenic studies of hematopoiesis have already shown that definitive HSCs bud as cellular clusters from the hematogenic endothelium in situ [26], such as the surrounding vascular endothelium is implicated in supporting the early HSC expansion. Moreover, during ectopic bone formation initiated by donor osteogenic

progenitors the establishment of functional HSCs-containing BM tissue is preceded by a prior VEGF secretion and vasculature formation [27]. In accordance, the hematopoietic recovery following a myelo-suppression was preceded by a vasculature restoration [28]. In an anatomic viewpoint, sinusoidal endothelial cells are the portals for the egress or ingress of traveling HSCs out of or into BM, therefore, a specific interaction between HSCs and sinusoidal endothelial cells is not out of expectation. Moreover, many other supporting evidence exist. Firstly, some HSCs and/or progenitors, either in the steady state of BM or after being transplanted, locate close or attach to sinusoidal endothelial cells, although it is not clear whether this preferred anatomic association is actually due to a possibility that a lot of HSCs/progenitors are just passing endothelial portal. Secondly, endothelial cells express certain HSCs-regulating factors such as Angptl3 and SCF, and blockage of these factors produced by endothelial cells resulted in a reduction in HSC pool size (see Table 1). Thirdly, in vitro co-culture experiments showed that the endothelial cells facilitate the HSC self-renewal expansion in a contact-dependent manner, at least partially by supplying Notch-ligands [29, 30].

Intriguingly, apart from the Nestin⁺ peri-vascular MSCs that probably belong to the derivatives of the neuro-crest cells, the peri-vascular niche architecture has also found another member of neuro-crest derived cell lineages: GFAP⁺ Nonmyelinating Schwann cells [31]. GFAP⁺ cells carry the phenotypes of Nestin⁺Itgb8⁺PDGFR α ⁺, express CXCL12, SCF, Angiopoietin-1, and Tpo, and account for about 0.005 % of BM cells (as compared to PDGFR α ⁺ cells, 0.05 %; nestin⁺ cells, 0.026 %). They act by providing the active TGF- β to hibernate HSCs.

As mentioned at the beginning, it has long been suspected that hematopoietic cells, in a reverse manner, regulate the development and function of osteogenic cells, which in turn influence their niche-related roles. As such, recent works suggest that HSC progenies such as macrophages within the BM or endosteal trophic macrophages facilitate the growth and activity of osteoblasts, which in turn influences BM retention of HSCs. The well-known agonist of HSC mobilization, G-CSF, promotes the egress of HSCs out of BM by directly decreasing the activity and number of these supporting macrophages, resulting in a reduced production of SDF-1 α by osteoblasts [3, 4]. Likewise, BM CD169⁺ macrophages were shown to signal peri-vascular Nestin⁺ cells to secrete SDF-1 α , thus preventing HSCs from egressing out of BM [3]. Most recently, it was shown that SDF-1 α -secreting niche cells recruit Treg cells to cluster to the endosteal region, thus co-localizing with the transplanted allogenic HSCs/progenitors and shielding them from immuno-rejection [6].

Finally, a much less explored while otherwise an important biological setting for understanding HSC niche is how

HSCs and progenitors interact with microenvironmental factors to initiate and constitute an extramedullary hematopoiesis outside of BM, which occurs during adulthood when the primary BM hematopoietic microenvironment is disrupted by pathological processes. The establishment of extramedullary hematopoiesis seems not an accidental process, rather it needs the reactivation of niche-like roles of certain extramedullary stromal cells back to an embryonic hematopoietic status [32]. Very little is known about the nature of extramedullary HSC niche cells. Nevertheless, in both spleen and liver [25, 33], it was observed that the putative HSCs were found attached to a portion of sinusoidal endothelial cells, although numerous mature hematopoietic cells also distributed into this region. This localization was at least partly determined by SDF-1 α secretion from the extramedullary sinusoidal endothelium.

HSC Niches are Hypoxic

In line with a widely accepted observation that at least a portion of HSCs are staying at cell-cycle dormant status, the evidence is accumulating that HSCs are located within a blood circulation low-perfused area [34], thus undergoing a relatively anaerobic metabolism [35], and harboring low levels of ROS [36, 37]. Moreover, it is suspected that this distal location of HSCs from circulation also helps prevent the loss of certain niche cells-produced local soluble factors. Notably, results that indicate a basically hypoxic status for both endosteal osteoblastic cells and sinusoidal endothelial cells (but not capillary endothelial cells) within the BM have been reported [38, 39]. In accordance, hypoxia inducible HIF-1 α was shown of essential for maintaining the long-term self-renewal ability of HSCs at several biological settings [35, 40, 41].

The Molecular Niche Elements

The niche cells interplay with HSCs via adhesion molecules and soluble factors, and increasing numbers of such niche molecules or the relevant molecular pathways are being discovered. As mentioned above, in many studies the preferential expression of certain HSCs-regulatory molecules has been used as the identity label for the putative niche cells. Worthy of mentioning, the expressions of many regulatory molecules are not restricted to one or two types of niche cells, and rarely the regulatory effects of a given niche molecule is only restricted onto HSCs. Although the current data that characterize the expression pattern and function of many niche-related molecules are not totally in harmony, dozens of molecules as the highly relevant molecular niche elements are documented in Table 1.

At least for certain molecules or pathways listed in Table 1, a niche role for HSCs remains undetermined. This situation is

well illustrated in the case of N-cadherin that expresses on the endosteal osteoblasts. While a homophilic-tethering of N-cadherin between osteogenic cells and hematopoietic cells seems not essential for maintaining a physiological hematopoiesis, as indicated by osteogenic or hematopoietic-specific *N-cadherin* targeting experiments [42, 43], the siRNA or dominant negative mutant-expression experiments demonstrate a potential hematopoiesis-regulatory effect by manipulating N-cadherin expression [44]. For SCF, although the Lepr-Cre mediated targeting of SCF in perivascular stromal cells affects HSC pool size [15], the ectopic BM formed by SL/SL donor cells contains the same amount of HSC as that produced by WT donor [27]. Actually, for many signaling pathways including Wnt/ β -catenin, Notch and hedgehog, inconsistent conclusions were drawn from the different “gain of function” and “loss of function” studies (see Table 1 for references). It seems that the dosage-effect of gene alterations has to be taken into account. Moreover, the fact that depletion of a single pathway results in no significant influence on HSC maintenance or recovery probably can not preclude an involvement of this pathway. It is possible that the redundant HSCs-maintaining signaling pathways are available, while only a combinational effect of a portion of them is sufficient to maintain the homeostasis or recovery of a functional HSC pool.

The Niche for Malignant Hematopoietic Cells

It is gradually accepted recently that perhaps the final transformation steps for a majority of various types of LSCs do not occur to the HSCs, but rather to different types of immature hematopoietic progenitors being distributed over a long range of differentiation path down an imaginary hierarchy. So it is not surprising to find that different types of LSCs as well as their progeny each propagates by depending on the particular supports from discrete microenvironmental factors. In a striking case, the same LSC clone may interplay with the different microenvironments to produce either acute myeloid leukemia (AML) or acute lymphoid leukemia (ALL) phenotype [45]. We will highlight a few cases about the putative niche cells or molecules for AML and ALL in the following paragraphs.

AMLs: an earlier report showed evidence that LSCs-enriched CD34⁺ cells of human AML (M4) preferentially localized to the metaphyseal endosteal region of *NOD/SCID/IL2 γ ^{null}* mice after transplantation [46]. However, after being intravenously injected to the lethally-irradiated GFP osteoblast report mice, the mouse AML LSCs, originated from the viral vector-mediated transduction of oncogenic MLL-AF9, were found of homing to the BM places away from the endosteal osteoblasts, where instead a preferred distribution by normal HSCs or pre-LSCs was observed [47]. In line with the expectation that an increasingly

autonomous status will be gained by LSCs, the activation of a key self-renewing pathway, Wnt- β catenin axis, in MLL-AF9 AML, was no longer dependent on the BM niche-derived Wnt signals [47]. At molecular mechanism level, as a serial studies have continued to highlight, the SDF-1 α -CXCR4 axis not only maintains the retention of AML cells within BM, but also provides survival-promoting signal, which in turn protects AML cells from chemotherapy or tyrosine kinase inhibitor-induced apoptosis [48–54]. On the other hand, the role for leukemic expression of V α -4 in protecting LSCs seems still controversial [55–57]. And interestingly, CD44 signaling (its ligands in microenvironment include hyaluronan, osteopontin, fibronectin and selectin) on AML LICs might otherwise promote their differentiation rather than maintaining leukemogenic stemness, and also inhibit LICs' homing ability into both BM and spleen [58, 59].

ALL: a human pre-B ALL cell line Nalm-6 cells were observed of homing to certain specific BM vasculature domain that express SDF-1 and E-selectin soon after being intravenously inoculated, and then they diapedased and proliferated around the peri-vascular area [60]. The in parallel experiments showed that this specific vasculature domain was exactly the portal that allowed entry of normal HSCs and progenitors into BM from circulation. On the other hand, quite surprisingly, unlike what has been documented for Notch signaling in favoring T cell development versus B lymphopoiesis, a recent study suggest that Notch signaling activation within B-ALL cells by BM mesenchymal stromal cells protected these malignant B cells from apoptosis [61].

Acute Leukemia Cells Prepare a Favorable Niche to Strive

Intriguingly, experimental evidence accumulates that the primary alterations in hematopoietic microenvironment may contribute to the initiation of leukemogenesis [62]. In corroboration of this notion, one recent report documented that up to 16 % of patients with AML/MDS harbored chromosomal abnormalities in BM mesenchymal stromal cells, and these abnormalities were different from the genetic abnormalities harbored by leukemic cells themselves [63]. However, in most cases, the primary genetic defects intrinsic to the hematopoietic cells probably are already sufficient to create LSC within hematopoietic microenvironment otherwise with a normal genetic background. Nevertheless, the “interplay theory” predicts that even in the latter, the critical epigenetic alterations occurring to the hematopoietic stromal cells, as induced by a small population of starting LSCs at the initial of stage of malignancy, pave the avenue for the further leukemic propagation that ultimately results in clinical manifestation of overt leukemia.

Leukemia belongs to a clonal disease that originates from the expansion, refractory differentiation and probably also

an enhanced survival of a single LSC. It is possible that not all transformed hematopoietic cells, once created in situ or inoculated from outside, will immediately embark on a seemingly unlimited leukemic propagation, which will lead to an overt leukemic phenotype. For example, certain newly generated LSCs may be guided by the microenvironment to stay at a dormant status. Moreover, it is reasonable to take into account of the competitive occupancy and usage of hematopoietic niches by normal HSCs and progenitors that greatly outnumber LSCs at the initial stage of leukemogenesis. However, for all the cases that lead to an overt leukemia, a mini malignant hematopoiesis starting from one single LSC will surpass a giant normal hematopoietic activity within the same eco-system. The resultant depression of normal hematopoiesis virtually represents the direct and major pathological alterations leading to clinical mortality [64]. The observation that many solid malignant growths such as multiple myeloma and solid tumor metastasis within BM usually result in a much less severe depression of normal hematopoiesis indicates that the gross physical compression on the hematopoietic microenvironment is not the sole vital mechanism. It seems reasonable to hypothesize that a niche-related delicate alteration may have to be implicated in this lead-overtake process (Fig. 1). This scenario was well illustrated by a recent observation that chronic myeloid leukemia cells secrete G-CSF to reshape the cytokine-expression profiles

of BM stromal cells, skewing the supporting activity towards LSCs [65]. As also shown in xenograft model of human B-ALL cells [60], the established proliferative peri-vascular foci of B-ALL cells would reshape the original SDF-1-expressing vasculature domain into SDF-1⁻ domain afterwards. This newly created leukemic cell bed then relocated the normal HSCs and progenitors from their physiological niches by secreting higher levels of SCF, inhibited mobilization of, and also reduced the proliferation of HSCs and progenitors [66]. In addition, it was shown that in the in vitro co-culture system, human FAK⁺ CD34⁺CD38^{-/lo}CD123⁺ AML LSCs-enriched cells would upregulate the expressions of SDF-1, IL-6, IL-8, Ang-1 and Wnt5a from the mesenchymal stromal cells [67]. In a mouse AML model, leukemia cells displaying an inhibiting ability on osteoblast production and function [68]. Thus, rather than passively receiving the supports from a basically normal microenvironment, leukemia cells (not necessarily restricted to LICs) might be able to positively reprogram the normal microenvironment, so that the limited microenvironmental resources will be greatly biased to the malignant hematopoiesis at the expense of normal hematopoiesis (supposedly the malignant hematopoiesis may exploit a niche-providing mechanism different from that used by normal hematopoiesis).

Fig. 1 A proposed dynamic interplays between the hematopoietic microenvironment factors with the normal hematopoietic cells or leukemic cells. A seduced or/and enhanced micro-environmental support exploited by leukemic cells underlies the establishment of leukemia predominance over normal hematopoiesis

Final Remarks and Questions

Obviously, these new developments in niche-related research have brought out more questions than ones they may have solved. We are tempted to conclude this review by elaborating three following issues.

The Functional Attributes of HSC Niche The most conducted hematopoietic microenvironment studies have focused on characterizing a putatively specialized niche for HSCs at cellular and molecular levels. Nevertheless, it is temporarily unclear what a HSC is doing right upon its occupying a specialized niche or being away from the niche? It is generally assumed that one HSC could conduct a self-renewal expansion or an asymmetric division that reserves a progeny as HSC, or simply staying at dormant status to survive or/and to avoid differentiation. As such, in complementary to these distinct HSC behaviors, a niche may supply an inductive platform for HSCs to conduct symmetric or asymmetric division, or alternatively serve as an architectural sanctuary hosting and preventing HSC from differentiation or apoptosis. Unfortunately these fundamental questions remain to be clarified. It is even not determined whether all these stemness-preserving processes of HSCs absolutely need the supports from a specialized architectural complex. And if it is, do these different HSC behaviors need to elaborate with different kinds of niches, or with a single type of niche with versatile attributes?

Adding to this complexity, the putatively specific interactions existing between HSCs and the microenvironment are dynamic and reciprocal, as frequently in both physiological and pathological conditions, HSCs egress out and home back to the potential niches distributed within a few different hematopoietic tissues or organs. In this respect, these different kinds of niches may be regarded as the portals, apartment/hotel or even a temporary sanctuary where these hematopoietic tenants or travelers are staying over a transient or longer time. From this viewpoint, it is interesting to ask whether these niches are also available for other hematopoietic cells when HSCs are emptied out.

After all, the cell fate of a HSC or its immediate post-division progeny finally depends on the folding and unfolding of certain intrinsic programs that decide developmental fate of one cell. So understanding how intrinsic mechanisms regulate the behaviors of HSC will provide crucial clues onto what niches are able or need to do to preserve HSC stemness. That is to say, what exterior regulation mechanisms can interplay with the interior machinery? Actually the studies on the intrinsic mechanisms governing HSC stemness have represented a hot focus of recent studies, although basically only a corner of the whole picture has been unveiled. Nevertheless, one emerging theme is that keeping a HSC at quiescence seems an effective way to prevent it from exhaustion, especially at biological settings where repopulation ability of HSCs are exposed to stress conditions such as being transplanted into a hematopoietic ablated recipient.

HSC Niche Components are Shared by Non-HSCs As indicated in the extended discussion above, against a simple assumption that a few specialized niche elements are exclusively reserved for the privileged HSCs, the majority of HSC niche cell types and almost all the molecules or signaling pathways are also implicated in regulating the proliferation and differentiation of hematopoietic progenitors. For example, a specific niche role of osteogenic progenitors or osteoblasts on regulating early B lymphopoiesis has been indicated [17, 69]. The studies demonstrate that BM sinusoidal endothelial cells are a kind of supporting niche cells for the differentiation of megakaryocytic progenitors. At the molecular level, the proliferation or/and survival of B cell and erythroid progenitors, to an extent more than that in HSCs, depends on the existence of CXCL12-expressing cells (not necessarily CAR cells). During the in vitro co-culture, endothelium simultaneously balances the expansion differentiation of HSCs. These observations raise a critical question as to by which means a specialized HSC niche function is achieved. One possibility is that the niche is basically a hub comprising numerous elements. The specific and architectural combinations of several elements from the total pool specialize the formation of individual niches (like a molecular barcode). So a particular niche is alternatively used by different HSCs or/and even progenitors in an economical way. Actually we don't know whether there are enough anatomic niche units for simultaneously hosting every HSC and progenitors.

The Leukemic Niche Elements as the Therapeutic Targets There are accumulating evidences that the microenvironmental factors confer the leukemia cells the resistance to the clinical treatments. Therefore, mobilization of LSCs from these sanctuary niches represents a potential strategy to sensitize LSCs to differentiation or clearance by therapeutic reagents, such as by interfering SDF-1-CXCR4 axis. Nevertheless, as AML LSCs may distribute differently within BM from normal HSCs, they might also respond distinctly to a given environmental regulatory cue. It is important to explore into these unknown differences and using them to design safe LSCs-targeting therapies.

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