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REVIEW

Germline mutations in the bone marrow microenvironment and dysregulated hematopoiesis

Lane H. Miller, Cheng-Kui Qu, and Melinda Pauly

Department of Pediatrics, Division of Hematology/Oncology, Aflac Cancer and Blood Disorders Center, Children's Healthcare of Atlanta, Emory University School of Medicine, Atlanta, Georgia 30322

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The relationship between the hematopoietic stem cell (HSC) population and its surrounding bone marrow microenvironment is a rapidly evolving area of research. Normal HSC processes rely heavily on a complex communication network involving various marrow niches. Although leukemogenesis largely results from abnormal genetic activity within the leukemia stem cell itself, mounting evidence indicates a significant contributory role played by marrow niche dysregulation. Furthermore, numerous instances of activating or inactivating germline mutations within marrow microenvironment cells have been shown to be sufficient for development of myelodysplastic syndrome, myeloproliferative neoplasm, and acute myeloid leukemia, even in the context of wild-type HSCs. Recent evidence suggests that targeting aberrant chemokine production from germline-mutated marrow stromal cells can potentially reverse the process of leukemogenesis. This elaborate interplay between the HSC population and the marrow microenvironment allows for a number of unique clinical possibilities in efforts to induce remission, enhance chemosensitivity, manage relapsed disease, and prevent leukemia development, both in de novo and germline mutation-associated leukemias, including the use of targeted cytokine/chemokine inhibitors, immune checkpoint blockade, CXCR4/CXCL12 axis antagonists, and combined allogeneic HSC and mesenchymal stem cell transplantation. In this review, we discuss the pathways underlying normal and abnormal bone marrow niche functioning, the relationship between germline mutations in the stem cell microenvironment and dysregulated hematopoiesis, and future clinical perspectives that may be particularly applicable to prevention and treatment of germline-associated leukemias.

The relationship between the hematopoietic stem cell (HSC) population and its surrounding bone marrow microenvironment, which is generally thought of as a collection of functional "niches," is a rapidly evolving area of research. Gains and losses of function within the microenvironment compartment have been repeatedly linked to leukemic and myelodysplastic conditions affecting adjacent HSCs or more differentiated marrow progenitor cells. In this review, we discuss the interactions between HSCs and the marrow microenvironment in hematopoietic dysfunction and chemotherapy resistance, as well as the therapeutic implications, including targeted agents, HSC transplantation, mesenchymal stem cell (MSC) infusions, and genetic modification, for dysregulated hematopoiesis associated with germline mutations.

Offprint requests to: Dr. Lane H. Miller, Pediatric Hematology/Oncology Fellowship Program, Emory University School of Medicine, Aflac Cancer and Blood Disorders Center, 2015 Uppergate Drive, 4th floor Atlanta, Georgia 30322; E-mail: lane.miller@choa.org

Normal bone marrow niche functioning

It has been well established that HSCs require a tightly regulated and conserved set of cooperative interactions with their neighboring cells in order to carry out the normal processes of dormancy, self-renewal, proliferation, locomotion,

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and differentiation [1–5]. This network of intercommunication relies on direct cell-to-cell cross-talk, as well as the production and release of a variety of cytokines and chemokines from the cells constituting these marrow niches [1,3,4].

Although they comprise only a small proportion of the nucleated cell population within the marrow, HSCs are responsible for the daily production of more than 10^{11} red cells, granulocytes, and platelets in humans [6]. In mice, HSCs account for 0.007% of all marrow cells [7]. HSCs accrue within the trabecular marrow in endosteal and subendosteal regions [8,9], circumscribing the vasculature and sinusoidal spaces in close proximity to mature stromal cells, MSCs, and sympathetic nerve system (SNS) fibers, where they receive signals that promote fate decisions [1,2,5]. Our general comprehension of the functional and anatomic marrow niches influencing HSC behavior is continually being adapted and updated. Much of our current understanding of the spatial and temporal features of the marrow microenvironment has come from various techniques employing microscopic analyses of immunohistochemically stained marrow sections, including the use of 2D and 3D imaging that incorporates fluorescence expression [9]. To date, there is substantial evidence to suggest the presence of discrete and vital endosteal, perivascular, and megakaryocytic niches [1,2,10–29] (Figure, Table 1). Additionally, genetic defects within particular niche cell populations have been demonstrated in mouse models in regard to their roles in routine marrow microenvironment functioning. These first descriptions of the critical roles of these cell types are also described in Table 1 [12,13,17,18,21,28,29].

The endosteal marrow extending into the transition zone contains a modest HSC population at baseline [10], is the anatomic host site for renewed hematopoiesis following myeloablation [11], and is the targeted area of HSC trafficking following HSC transplantation [2,15]. Prior evidence had suggested an "N-cadherin^{+}" osteoblastic niche, referring to a critical population of osteoblasts expressing high N-cadherin levels and, as a result, promoting HSC adhesion and maintenance within the endosteal space [1,2,3,13]. Recent work has cast doubt on this notion, noting undetectable N-cadherin HSC expression through numerous reliable laboratory techniques [30,31] and unchanged HSC

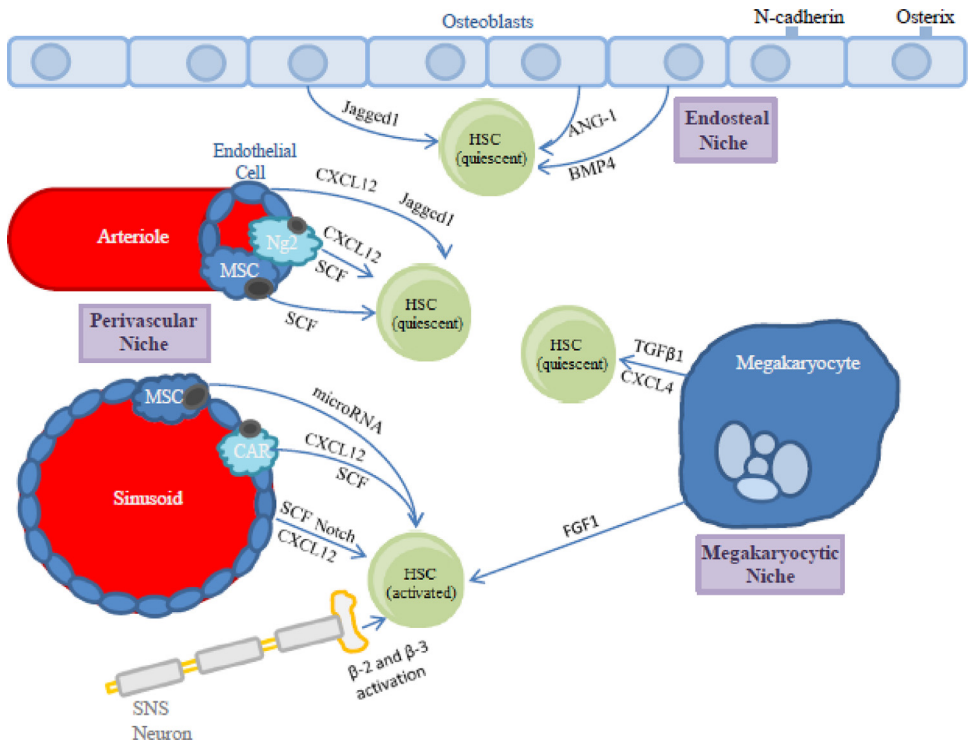

Figure. HSC maintenance requires a tightly regulated, multifaceted series of interactions between bone marrow niche components. Three distinct niche spaces have been convincingly identified: the endosteal, perivascular, and megakaryocytic niches. HSCs accumulate in close proximity with Osterix-expressing osteoblasts and receive signaling through Jagged1, BMP4, and ANG-1, all of which are critical to quiescent HSC self-renewal and protection from differentiation. Quiescent HSCs additionally colocalize with Nestin-GFP^{+} periarteriolar stromal cells, relying on CXCL12, SCF, and Jagged1 signaling. Activated HSCs are found accrue in the vicinity of LEPR^{+} perisinusoidal stromal cells, where they are exposed to CXCL12, SCF, Notch, and microRNA signaling, as well as β-adrenergic activation from the SNS. Finally, the megakaryocytic niche describes both activation and quiescence pathways mediated by FGF1 and TGF-β1/CXCL4, respectively.

Table 1. Summary of murine model findings that have established our current understanding of the functional niche components

Niche	Observations from Murine Models
Endosteal	Hosts a baseline population of quiescent HSCs with a higher concentration of CFUs in close proximity to the endosteal bone surface [10]
	Area of renewed hematopoiesis on day 3 following 5-fluorouracil treatment, although not shown to cluster in the endosteal space at later time points [11]
	PTH/PTHrP receptor stimulated osteoblasts are increased in number and produce high levels of the Notch ligand Jagged1, regulating HSC self-renewal through an increased number of Notch1-activated HSCs [12]
	Positive correlation between number of N-cadherin$^+$ CD45− osteoblastic cells and number of HSCs, mediated through N-cadherin and beta-catenin activity, with bone morphogenic protein receptor type IA (BMPRIA) demonstrated as a key component in this process [13]
	Adherence of Tie2-expressing HSCs to osteoblasts through Ang-1, which results in HSC quiescence, self-renewal, and protection against differentiation [14]
	Area of HSC trafficking 15 hours after syngeneic HSC transplant in nonablated recipients [15] and 5−8 hours following irradiation [16]
	Mature stromal cells produce TGFβ1 that regulates HSC proliferation and apoptosis [17]
	HSCs identified through CD150(+)CD244(-)CD48(-) SLAM cell surface receptor expression and found to be in close proximity to the marrow sinusoidal endothelium [18]
	Endothelial cells attract HSCs as evidenced by reduced HSC numbers following gp130 cytokine receptor deletion in endothelial cells [19]
Perivascular	Most HSCs are found in direct contact with CAR cells and induced CXCR4 deletion results in a considerable reduction in HSC quantity [20]
	Some HSCs localize adjacent to *Nestin*-GFP$^+$ perivascular MSCs [21]
	SNS fibers stimulate HSC locomotion and repopulation through β2- and β3-adrenergic receptor activation [22]
	Endothelial cells release E-selectin, which directs HSC homing and proliferation as evidenced by HSC quiescence induced by E-selectin-deficient endothelial cells [23]
	Reduced HSC frequency is seen in SCF-deficient endothelial perivascular cell models [24]
	Periarteriolar stromal cells co-localize with quiescent HSCs [25]
	Perisinusoidal stromal cells co-localize with activated HSCs [26]
	mRNA and microRNA-containing vesicles released by MSCs regulate HSC division and proliferation [27]
Megakaryocytic	Megakaryocytes localize to sinusoidal spaces and are in direct HSC contact [28]
	Express higher levels of TGFβ1 than neighboring stromal cells, regulating HSC proliferation and apoptosis [28]
	Produce FGF1 during times of stress that promote HSC expansion [28]
	Produce CXCL4 resulting in maintenance of HSC quiescence [29]

SLAM=signaling lymphocyte activation molecule

frequency and function when N-cadherin is deleted in HSCs, osteoblasts, or both [32,33]. Osteoblasts additionally have not been shown to express stem cell factor (SCF), which is essential for HSC maintenance through KIT receptor binding and activation. Nevertheless, quiescent HSCs have repeatedly been demonstrated to accumulate in close proximity with Osterix-expressing endosteal osteoblasts [1]. Additional signals expressed by cells within the endosteal niche, including Jagged1, BMP4, and ANG-1, are also requisite in HSC self-renewal and protection from signals promoting differentiation [2,3,12,14]. Although the conclusive role of osteoblasts in the endosteal niche remains unclear [1], the niche itself appears to be indispensable to the critical functions of quiescent HSC maintenance and development. Much of this role may instead be mediated through distant signaling and widespread crosstalk as opposed to direct interactions [7].

The perivascular niche refers to the substantial majority of HSCs located adjacent to marrow vasculature and the pro-differentiation and proliferation regulatory signals that they receive from nearby mesenchymal cells, endothelial cells, and SNS neurons [1,2,7]. Endothelial cells expressing endoglin (CD105) and Nestin-GFP attract HSCs to these sheltered and protected locations [1,18,19,24], contribute to HSC self-renewal through SCF release, and direct HSC homing and proliferation through E-selectin and P-selectin production [1,3,4,23]. MSCs are multipotent stromal stem cells with the capacity for differentiation into an assortment of stromal tissue cell types, including adipocytes, osteoblasts, chondrocytes, and fibroblasts [2,4,34]. These cells are characterized by positive surface endoglin, CD73, CD90, CD146 [35], and Nestin expression [1] and additionally colocalize with HSCs [21]. Mature CXCL12-abundant reticular (CAR) cells are found concentrated within the perivascular region, supporting comparable HSC functions through, not only CXCL12/CXCR4 axis activation, but also through SCF binding [7,20,34]. MSCs and CAR cells accrue

around the vasculature and sinusoidal spaces and express a variety of genes, most predominantly *scf* and chemokine C-X-C motif ligand 12 (*Cxcl12*) [4,7], the protein product of which serves critical roles in HSC chemotaxis and self-renewal [7,34]. Further evidence suggests separate periarteriolar (Nestin-GFP$^+$) and perisinusoidal (LEPR$^+$) stromal and MSC populations, the former co-localizing with quiescent HSCs [25] and the latter with activated HSCs [7,26,36]. MSCs have also been shown to release vesicles containing microRNA necessary for HSC division and proliferation [27,37,38]. TGFβ1 is a cytokine produced by mature stromal cells to further regulate HSC proliferation and apoptosis [17,37,39]. Finally, SNS fibers organize in the perivascular regions [1,4] and stimulate HSC locomotion and repopulation through circadian β-2 and β-3 adrenergic receptor activation [5,22,37].

Recent work has further delineated a megakaryocytic niche for a subset of HSCs [28,29,40]. Megakaryocytes localize to the sinusoidal spaces [7], are frequently in direct contact with HSCs, and express higher levels of TGFβ1 than neighboring stromal cells [28]. CXCL4 is also produced by megakaryocytes, resulting in HSC quiescence maintenance [29,40]. The relationship between megakaryocytes and HSCs has been demonstrated to be uniquely critical to baseline HSC quiescence and survival, although through FGF1 signaling, megakaryocytes also have the capacity to promote HSC expansion in times of stress [7,28].

Role of the microenvironment in leukemogenesis and dysregulated hematopoiesis

Historically, leukemogenesis had been exclusively viewed as a process intrinsic to the leukemia cell itself, in which genetic aberrations within either an HSC or a more differentiated member of the lymphocyte or myeloid lineage result in dysregulation of normal cell processes and ultimately uncontrolled self-renewal and proliferation, all in the absence of programmed cell death [41]. Recently, however, the bone marrow microenvironment has been shown to be integral to the process of leukemogenesis, largely by providing a supportive environment within which leukemic blasts can thrive and evade chemotherapy-induced cytotoxicity [42–49]. Anecdotal cases have been documented of leukemia occurring in donor-derived stem cells following allogeneic stem cell transplantation in humans, suggesting that the abnormal recipient marrow niche in fact serves as the driver for leukemogenesis in an otherwise healthy HSC population [50].

To date, an abundance of evidence gathered from murine models supports the importance of regulated cellular and extracellular microenvironment functioning in regard to controlling standard hematopoiesis, with a general consensus that discrete genetic aberrations and epigenetic changes within the stromal compartment result in abnormal cytokine signaling and consequently the overall promotion of hematopoietic dysregulation [51]. More specifically, downregulation of the *scf* and *g-csf* genes within myelodysplastic syndrome (MDS) MSCs [52] have been shown to considerably impair normal hematopoiesis. Upregulated CXCL12, CXCR4, and Ang-1 expression [53] within the stromal compartment have been associated with HSC proliferation observed in leukemogenesis. CXCL12 expressed by cells within the marrow niches typically binds to the CXCR4 receptor on the HSC surface, enhancing the cell's adhesion to a secure location within the marrow niche [4]. Dysregulated CXCL12 expression can cause HSCs to lose their ability to locate and take up residence within their normal healthy, supportive microenvironment, a process shown to favor malignant cell expansion in mouse models of chronic myeloid leukemia (CML), for example [45]. Additional decreases in MSC surface adhesion molecule expression, particularly CD44 and CD49e, have also been demonstrated in MDS marrow [48]. Disordered signaling within the Wnt/beta-catenin pathway has been associated with leukemogenesis through resistance to standard HSC differentiation and apoptosis [53]. Passegué et al. [46] identified cooperative interactions between BCR/ABL activity and microenvironment interleukin-6 (IL-6) production, which further contributes to a proinflammatory environment accommodating to CML development and progression.

In terms of the microenvironment's role in chemotherapy resistance, additional work has demonstrated protective effects of MSCs via the N-cadherin receptor against tyrosine kinase inhibitor activity in CML stem cells, suggesting that the niche can serve to shield leukemic stem cells (LSCs) from treatment [47]. Schepers et al. [49] revealed that in the context of myeloproliferative neoplasm (MPN), the endosteal marrow niche is remodeled such that MSCs produce large numbers of proinflammatory osteoblastic-lineage cells that selectively express factors supporting leukemic stem cell retention. Furthermore, in acute myeloid leukemia (AML), excessive release of stromal cell and megakaryocyte-produced TGFβ1 induces quiescence of the LSCs, rendering them protected from cytarabine, an antimetabolite chemotherapy agent [39].

In human samples, the mesenchymal niche cells of patients with MDS and AML demonstrate a number of chromosomal changes [42], epigenetic modifications [43], and transcriptomic variations [44]. Bone marrow samples from newly diagnosed aplastic anemia, CML, and marrow-uninvolved lymphoma patients revealed distinct relative quantities of the various marrow stromal cell components between the different disease states [54]. Altered methylation patterns and

upregulation of Jagged-1 in MDS MSCs have additionally been shown to be associated with dysregulated hematopoiesis [55]. These murine and human examples demonstrate a complex cause-and-effect relationship between the non-transplantable microenvironment and the HSC population in regard to hematopoietic dysregulation and leukemogenesis.

Dysregulated hematopoiesis induced by a mutated stem cell microenvironment

Extensive work has detailed clinically meaningful myelodysplastic, myeloproliferative, or overt leukemic conditions induced by mutations specifically in the marrow microenvironment compartment and not necessarily present within the LSC itself. This induction of malignancy within the donor cells is not universal in the context of germline-mutation-associated syndromes; however, the described experiences are invaluable in understanding the role of the microenvironment in this process and potentially developing therapeutics to prevent leukemogenesis in particular patient subsets. Table 2 details the chronologic progression of these endeavors, the genomic anomalies involved, and the implications of these niche aberrations on the HSC population [56–66].

In 2005, Rupec et al. [56] demonstrated that germline IkB-α deletions resulted in constitutively activated Jagged1 expression and enduring Notch1 activation in granulocytes, prompting development of a severe myeloproliferative premalignant disorder. However, when IkB-α-deficient hepatocytes were cocultured with IkB-α wild-type (WT) hematopoietic cells, this same Jagged1-dependent myeloproliferative process was observed, indicating the ability of nonhematopoietic cells to induce malignant potential within the hematopoietic compartment. In 2014, Kode et al. [63] demonstrated development of AML in mice with activating *β-catenin* mutations in the osteoblast population,

Table 2. Summary of published studies demonstrating dysregulated hematopoiesis in WT HSCs driven by genomic aberrations within the microenvironment compartment

Study	Germline Genetic Aberration	Notable Findings
Rupec et al. (2005) [56]	*IkB-α* deletion	Constitutively activated Jagged1 expression → Notch1 activation → MPN in a setting where *IkB-α* WT HSCs were cocultured with *IkB-α*-deficient hepatocytes
Walkley et al. (2007) [57]	*RAR-γ* deletion	TNF-α-dependent mechanisms → MPN in a setting where *RAR-γ* WT HSCs were cultured in an *RAR-γ*-null marrow microenvironment
Walkley et al. (2007) [58]	*Rb* deletion	Simultaneous deletion of *Rb* in HSCs and marrow microenvironment → MPN, whereas *Rb* deletion in HSC in a *Rb* WT microenvironment did not result in MPN
Kim et al. (2008) [59]	*Mib1* deletion	Defective Notch activation in the marrow microenvironment → MPN in a setting where *Mib1* WT HSCs were transplanted into an *Mib1*-null microenvironment
Raaijmakers et al. (2010) [60]	*Dicer1* deletion	*Dicer1* deletion in osteoprogenitors → underexpression of *Sbds* gene in transplanted WT HSCs → MDS/AML
Zimmer et al. (2011) [61]	*Crebbp* haploinsufficiency	*Crebbp* haploinsufficieny in the marrow microenvironment → stimulation of myeloid differentiation → MPN
Wang et al. (2014) [62]	*RBPJ* deletion	Deletion of DNA-binding motif for *RBPJ* → absent Notch receptor signaling in the marrow microenvironment → upregulation of miR-155 in endothelial cells → κB-Ras1 inhibition → NF-κB activation → MPN
Kode et al. (2014) [63]	*β-catenin* activating mutations	*β-catenin* activating mutations in osteoblasts → increased osteoblastic Jagged-1 expression → activation of Notch signaling in HSCs → AML
Zambetti et al. (2016) [64]	*Sbds* deletion	Transcriptional activation of the p53-S100A8/9-TLR inflammatory signaling axis → mitochondrial dysfunction, oxidative stress, and activation of DNA damage responses in HSCs → MDS/AML in a setting where *Sbds* WT HSCs were transplanted into an *Sbds*-deficient microenvironment
Dong et al. (2016) [65]	*Ptpn11* activating mutations	*Ptpn11* activating mutations in MSCs and osteoprogenitors → CCL3 production → monocyte recruitment → monocytic IL-1β production → HSC hyperactivation → MPN in a setting where *Ptpn11* WT HSCs were transplanted into a *Ptpn11*-mutated marrow microenvironment
Zhou et al. (2017) [66]	*Fancc* and *Fancg* DKO	Increased TNF-α, increased reactive oxygen species, and decreased IL-6 marrow microenvironment production → MDS in a setting where *Fancc* and *Fancg* WT HSCs were transplanted into a *Fancc* and *Fancg* DKO microenvironment

resulting from increased Jagged-1 osteoblastic expression and Notch signaling activation in HSCs. That same year, Carlesso et al. [62] additionally described a murine model in which the DNA-binding motif for recombinant signal binding protein (RBPJ) is deleted, resulting in absent Notch receptor signaling within the marrow microenvironment. Therefore, the microRNA miR-155 is upregulated in the endothelial cells, leading to nuclear factor-kappa beta (NF-κB) activation through inhibition of κB-Ras1. This too led to cytokine release and advancement to MPN.

Similarly, in 2007, Walkley et al. [57] observed myeloproliferative marrow changes in mice that were deficient for retinoic-acid receptor gamma (RAR-γ), a nuclear hormone receptor shown to be requisite in HSC self-renewal and differentiation when liganded with all-*trans* retinoic acid. When WT HSCs were transplanted into lethally irradiated mice with an RAR-γ-null microenvironment, this same myeloproliferative syndrome was observed, largely mediated by a tumor necrosis factor-alpha (TNF-α)-dependent mechanisms. Related findings were shown in several additional studies. Raaijmakers et al. [60] created a mouse model with a *Dicer1* deletion in the osteoprogenitors not seen in mature osteoblasts. As a result of this deletion, the *Sbds* gene characteristically mutated in Shwachman–Diamond syndrome (SBS), was underexpressed and in time gave rise to myelodysplasia and AML. Furthermore, Zambetti et al. [64] revealed that stromal cells in mice with SBS generate an inflammatory signaling cascade responsible for HSC DNA and mitochondrial damage and ultimately MDS.

In 2016, we published results demonstrating the critical role that *Ptpn11* activating mutations within the marrow niche play in leukemogenesis [65]. Noonan's syndrome (NS), occurring in one in 1000–2500 live births, is characterized by short stature, facial dysmorphism, and congenital cardiac defects and carries a predisposition for developing juvenile myelomonocytic leukemia (JMML), a JMML-like syndrome, or AML [67]. Approximately half of children with clinical NS [68] possess germline mutations in the *Ptpn11* gene and the incidence of MPN development among those children is more than 5% [69]. The *Ptpn11* gene encodes the protein tyrosine phosphatase Shp2, a positive regulator of the RAS signaling pathway. Germline *Ptpn11* mutations result in activating Shp2 mutations that have been shown repeatedly to induce a JMML-like syndrome through intracellular mechanisms [65]. Our study demonstrated that *Ptpn11* activating mutations in the mouse marrow microenvironment contribute additionally to the development and progression of HSC myeloproliferation. The presence of the *Ptpn11* activating mutations in the MSCs and osteoprogenitors results in overwhelming CCL3 chemokine production and recruitment of monocytes to the area occupied by HSCs, giving rise to stem cell hyperactivation by monocyte-produced IL-1β and ultimately development of MPN. This was even demonstrated in donor HSCs following transplantation, with MPN development reversed by administration of a CCL3 receptor antagonist [65]. These findings confirmed that dysfunctional marrow microenvironment settings may contribute to leukemogenesis in NS patients.

Most recently, Zhou et al. [66] published the results of their exploration into the interplay between HSCs and the bone marrow niche in Fanconi anemia (FA). Double-knockout (DKO) mice that were lethally irradiated and transplanted with WT bone marrow cells showed a litany of aberrations within the marrow, including dysplastic phenotypes, expanded granulocyte–macrophage progenitor compartments, and reduction in number of colony-forming unit cells. Dysplastic changes were additionally confirmed in the spleens and peripheral blood of these mice. Upon further examination, these DKO mice reconstituted with WT bone marrow cells showed significantly reduced marrow cobblestone area-forming cell numbers, increased percentages of $Gr1^+/Mac1^+$ cells, increased TNF-α concentrations, reduced IL-6 concentrations, and enhanced reactive oxygen species production upon exposure to H_2O_2, all suggesting dysfunctional hematopoietic supportive activity, enhanced myeloid differentiation, and altered paracrine secretion by the DKO mesenchymal stem/progenitor cells [66].

Future clinical perspectives

The interplay between the HSC population and the surrounding marrow microenvironment allows for abundant clinical possibilities either involving targeting of the somatically altered niche activity seen in leukemic, myelodysplastic, and myeloproliferative processes to aid in HSC transplantation and chemosensitization or in prevention of a germline-mutated niche from inciting leukemogenesis in otherwise healthy HSCs. Patients with constitutively aberrant functioning within the microenvironment in particular should be considered for these therapeutic options.

The aforementioned evidence suggests that patients with germline-mutation-associated leukemias cannot be securely managed with HSC transplantation alone. In these cases, concurrent targeting of the aberrant microenvironment may be instrumental in achieving cure and should be a research focus for potential therapeutics. Clinically, immune checkpoint inhibitors of PD-L1 on AML blasts, PD-1 on marrow stromal cells, and CTLA-4 expressed on activated $CD4^+$ and $CD8^+$ T cells have each been explored in regard to surmounting the marrow microenvironment's complicit role in assisting leukemic blast evasion of immune surveillance [70–72].

tThese agents have been or are actively being assessed in patients with AML in remission [73] and patients refractory to conventional chemotherapeutic protocols [74], all with varying results. However, to our knowledge, checkpoint inhibition has not been explored as an adjunct for patients undergoing myeloablative chemotherapy and HSC transplantation for MDS or AML [70,71]. Conceivably, this form of therapy could be useful in germline-mutation-associated leukemias as an adjunct to HSC transplantation and should be further explored. In addition, we suggest that targeted therapies directed at microenvironment cytokine and chemokine overexpression and cell cycle inhibition seen in specific germline-mutation-associated leukemias be investigated in the HSC transplantation setting. Examples of these agents are discussed in further detail later within this section.

Plerixafor, a CXCR4 antagonist, has been used in combination with chemotherapy to disrupt the HSC–bone marrow niche relationship in an effort to enhance chemosensitivity of the LSC in both pediatric and adult leukemias [75–77]. The objective of this regimen is to mobilize the LSC from the marrow and altogether remove it from its supportive niche, as opposed to blocking abnormal microenvironment signaling. Although these regimens have all been well tolerated and shown evidence of increased mobilization of the LSC into the blood, the clinical responses were not improved [75–77]. Nevertheless, inhibition of the CXCR4/CXCL12 axis should continue to be explored as an adjunct to conventional chemotherapy regimens as well as with biologics. Additional adhesion molecule inhibitors, including VLA-4-blocking agents and CD44-specific monoclonal antibodies, have recently been approved by the Food and Drug Administration, although current clinical trials are focused on solid tumors and autoimmune diseases [78].

In murine models, the TGF-β antagonist 1D11 successfully reversed the LSC cycle inhibition typically seen with elevated niche TGFβ1 levels and, when given in combination with cytarabine, resulted in pronounced apoptotic AML cell death. The addition of leukemia cell migration inhibition via plerixafor induced even further decreases in tumor burden [39]. Antisense oligonucleotide pharmaceuticals, anti-TGF-β cancer vaccines, monoclonal antibodies, and TGF-β receptor kinase inhibitors have each been explored in various Phase 1, 2, and 3 human trials in the treatment of high-grade CNS gliomas, non-small-cell lung cancer, melanoma, renal cell carcinoma, mesothelioma, and hepatocellular carcinoma with varying results [79]. Although TGFβ1 blockade continues to be explored in in vitro leukemia models, to our knowledge, these therapeutics have yet to be applied to human subjects with leukemia.

Although we continue to improve in our ability to induce sustained remissions and cures in childhood leukemia, little is known about leukemia prevention. Our work suggests that early inhibition of abnormal microenvironment signals could potentially prevent leukemogenesis in a select group of patients. Using a CCL3 antagonist, we showed an ability to prevent myeloproliferation in healthy HSCs exposed to a *Ptpn11* germline-mutated niche actively overexpressing the CCL3 chemokine [65]. Microenvironment cytokine antagonists could feasibly play a useful supplemental role in treatment of the relapsed, chemotherapy-resistant HSC. We believe that further laboratory and clinical research should focus on chemokine targeting in regard to preventing leukemogenesis in patients with germline mutations within the marrow niche as well as enhancing remission induction and maintenance and managing resistant relapsed disease in all patients.

Allogeneic MSC infusions could also be considered as therapy to abate abnormal microenvironment signaling. This procedure, in conjunction with high-dose chemotherapy and HSC infusion, has been shown to be safe and clinically useful in acute graft-versus-host disease [80] and in enhancing engraftment following allogeneic bone marrow transplant (BMT) [81,82]. Along with their effects on HSCs, MSCs have ancillary anti-inflammatory, anti-proliferative, and immunosuppressive attributes within the marrow. Therefore, MSC infusions have been proposed as therapy for various autoimmune illnesses such as Crohn's disease and multiple sclerosis [83]. However, sustained donor-derived MSC engraftment is rare, even in patients experiencing prolonged benefits from allogeneic MSC administration [84], suggesting that this procedure may not be a viable option for preventing leukemogenesis in patients with germline genetic alterations. For instance, Lazarus et al. [85] showed complete absence or scarce evidence of donor MSCs in bone marrow aspirate chimerism analysis at 6 months after transplantation. In another cohort of patients with osteogenesis imperfecta who underwent allogeneic BMT followed by an allogeneic MSC infusion, polymerase chain reaction-based chimerism analyses of mature marrow stromal cells between 18 and 34 months after transplantation showed <1% donor genetic markers [86]. In a 2004 study using non-invasive imaging techniques, MSCs infused peripherally demonstrated distribution predominantly in the lungs and liver and were nearly undetectable in the bone marrow [87]. Coadministration of a combined allogeneic HSC and MSC graft directly into the bone marrow cavity has been shown to be both feasible as a procedure and successful in sustaining donor MSC marrow engraftment [88]. We suggest that cotransplantation of allogeneic HSCs with allogeneic MSCs should be studied in depth as a potential therapy for patients

with genetic syndromes and known germline mutations within the niche population. This modality carries the same profound risks as a traditional allogeneic transplantation (myeloablative chemotherapy, HLA mismatch [89]), but could prove a viable approach in particular cases when the risk of leukemic transformation is sufficiently high. Further research directed toward refining this combined procedure and sustaining MSC engraftment is necessary. Given these potential shortcomings of MSC transplantation, particularly in regard to engraftment and sustained chimerisms, gene correction therapies should additionally be taken into consideration in patients with high-risk germline aberrations. Significant laboratory-based and translational work in recent years has focused on manipulation of MSC genetics using CRISPR/Cas9 genome editing [90,91]. Although this system carries a great deal of potential, further work will certainly be required to improve the effectiveness of these techniques, reduce immune rejection, and reduce toxicities prior to in vivo utilization.

Conclusions

In summary, we believe that these recently reported discoveries of abnormal hematopoiesis being driven by altered regulation of the marrow microenvironment will continue to guide future research toward unlocking the role of the marrow microenvironment in leukemogenesis in other genetic disorders such as Down syndrome, pure familial leukemia syndromes (e.g., CEBPA mutation), bone marrow failure syndromes (e.g., Diamond–Blackfan anemia, dyskeratosis congenita), DNA repair gene syndromes (e.g., Bloom syndrome, ataxia telangiectasia), and additional tumor suppressor gene syndromes (e.g., Li–Fraumeni syndrome, neurofibromatosis 1). An understanding of the microenvironment and HSC relationship in these syndromes is imperative to developing methods of preventing and treating germline-mutation-associated leukemia.

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